that is not statistically different from that observed after exhaustive training consisting of 14 bouts of intermittent exercise. These results suggest that nonexhaustive, intermittent exercise is probably safer and more effective in improving glucose metabolism in skeletal muscle.

Materials and methods

Materials

All chemicals were purchased from Sigma Chemical (St. Louis, MO, USA) and Wako Pure Chemical Industries (Osaka, Japan).

Treatment of animals

All rats used for the present investigation were purchased from CLEA Japan (Tokyo, Japan). The animals were housed in rooms lighted from 7 a.m. to 7 p.m. and were maintained on an ad libitum diet of standard chow and water. The room temperature was maintained at 20–22°C. Prior to the swimming exercise and training experiment, all rats were acclimated to swimming exercise for 10 min day^{-1} for 2 days, as described by Ren et al. [14]. All animal experiments were conducted with the approval of the National Institute of Health and Nutrition Ethics Committee on Animal Research.

High-intensity intermittent swimming exercise training

Twenty-four 5-week-old male Sprague-Dawley rats with body weights ranging from 90 to 110 g were randomly assigned to a control group and two exercise training groups [one group undergoing 3 bouts of high-intensity intermittent swimming exercise training (HIT) and one group undergoing 14 bouts of HIT]. During HIT, the rats performed the designated number of 20 s swimming bouts while bearing a weight equivalent to 14 and 15% of their body weight for the first 3 and last 2 days, respectively. A 10 s pause was allowed between exercise sessions. The rats performed the above swimming protocol once a day for 5 days. Each rat performed the swimming exercise alone in a barrel filled to a depth of 25 cm. The water temperature was maintained at 35 ± 1°C during the exercise. Food intake for all rats was restricted to 8 g from 7:00 p.m. on the last day before the experiment. All rats ate all 8 g of food. Approximately 18 h after the last bouts of training exercise, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg 100 g^{-1} body weight), and their EPI muscles, which had been shown to be recruited during swimming exercise [15], were dissected out. Age-matched sedentary control rats were kept in cages until they were sacrificed.

Measurement of GLUT-4 protein content

Immediately after dissection of the EPI muscles, the muscles were clamp-frozen by tongue, cooled by liquid nitrogen and stored in a deep freezer (−80°C) until analysis. EPI muscles were homogenized in 29 volumes of ice-cold 10 mM HEPES, 1 mM EDTA, 250 mM sucrose, 1 mM NaF, 1 mM sodium orthovanadate (Na_3VO_4), and 2 μl ml^{-1} Protease Inhibitor Cocktail (Sigma), pH 7.4, buffer. The homogenate thus obtained was centrifuged for 10 min at 13,000×*g* at 4°C. Protein concentrations were determined by the bicinchoninic acid (BCA) method [16] in triplicate. Aliquots of homogenate (40 μg of protein) were solubilized in Laemmli sample buffer [17], subjected to SDS-PAGE, and electrophoretically transferred to a polyvinylidene difluoride (PVDF) sheet. The sheet was incubated overnight at 4°C with primary monoclonal GLUT-4 antibody (Chemicon, Temecula, CA, USA) diluted 1:5,000 in 5% skimmed milk. After overnight incubation, the sheet was incubated for 1 h at room temperature with anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA, USA). Immunoreactive bands were detected by ECL plus (GE Healthcare UK, England).

Measurement of citrate synthase activity

For the enzyme activity measurements, 10% homogenates were made from the EPI muscles in 175 mM KCl, 10 mM GSH, and 2 mM EDTA, pH 7.4. These homogenates were frozen and thawed four times and mixed thoroughly before measurement of the enzyme activities. The citrate synthase activity in the muscle was measured using Srere's method [18].

Acute bouts of high-intensity intermittent swimming exercise

Twenty-four 5-week-old male Sprague-Dawley rats with body weights ranging from 110 to 130 g were used in the present experiment. The rats performed the high-intensity intermittent swimming exercise (HIE) used for the HIT exercise protocol described previously. Immediately after the exercise, the rats were anesthetized with inhalation of chloroform (diethyl ether) and a subsequent intraperitoneal injection of pentobarbital sodium (5 mg 100 g^{-1} body weight). Their EPI muscles were then dissected out within 5 min after cessation of the exercise. Age-matched sedentary control rats were kept in cages until they were sacrificed.

Measurement of AMPK phosphorylation

The EPI muscles were homogenized with 29 volumes of ice-cold 10 mM HEPES, 1 mM EDTA, 250 mM sucrose, 1 mM NaF, 1 mM sodium orthovanadate (Na_3VO_4), and 2 μl ml^{-1} Protease Inhibitor Cocktail (Sigma), pH 7.4, buffer. The homogenate thus obtained was centrifuged for 10 min at 13,000×*g* at 4°C. Protein concentration was determined by the BCA method [16] in triplicate. Aliquots of homogenate (40 μg of protein) were solubilized in Laemmli sample buffer [17], subjected to SDS-PAGE, and electrophoretically transferred to a PVDF sheet. The sheet was incubated overnight at 4°C with primary phospho-AMPK-α(Thr172)antibody (Cell Signaling, Beverly, MA, USA) diluted 1:500 in 5% bovine serum albumin (BSA). After overnight incubation, the sheet was incubated for 1 h at room temperature with anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch). Immunoreactive bands were detected by ECL plus (GE Healthcare UK, England). Total AMPKα was also determined using the sheets that had been immunoblotted for phosphorylated AMPKα (Thr172). The sheets were incubated with stripping buffer [62.5 mM Tris–HCl (pH 6.7), 100 mM β-mercaptoethanol, 2% SDS] at 50°C for 30 min. The sheets were performed with total AMPKα (Cell Signaling) antibody using the protocol described above.

Measurement of glycogen concentration

Immediately after acute bouts of the high-intensity intermittent exercise, the EPI muscles were dissected and clamp-frozen by tongue, cooled by liquid nitrogen and stored in a deep freezer (−80°C) until analysis. EPI muscles were weighed and homogenized in 0.3 M perchloric acid and then stored for later determination of glycogen concentrations. Glycogen concentrations were determined by enzymatic methods according to Lowry and Passonneau [19] after acid hydrolysis.

Measurement of lactate concentrations in blood

Immediately after the different bouts of high-intensity exercise, the rats were anesthetized with inhalation of chloroform (diethyl ether) and a subsequent intraperitoneal injection of pentobarbital sodium (5 mg 100 g^{-1} body weight), and blood was obtained from the tail vein. Blood samples were analyzed for lactate levels using the YSI 1500 lactate analyzer (YSI Life Sciences, Yellow Springs, OH, USA).

Measurement of GLUT-4 mRNA

Six and 18 h after the exercise (HIE), the EPI muscles were dissected and clamp-frozen by tongue, cooled by liquid nitrogen and stored in a deep freezer (−80°C) until analysis. Total RNA from the EPI muscle was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Because the RNA extraction procedure required a large amount of tissue, two EPI muscles were homogenized together. The DNase-treated total RNA (1 μg) was reverse-transcribed (RT) into cDNA by using random primers and ImProm-II Reverse Transcriptase (Promega, Madison, WI, USA). Aliquots of each RT reaction were added to a PCR master mix (Promega) containing *Taq* DNA polymerase, dNTPs, $MgCl_2$, reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR, and 10 pmol of both sense and antisense primers (forward 5′-GTGTGGTCAATACCGTCTTCACG-3′, reverse 5′-CC ATTTTGCCCCTCAGTCATTC-3′). The reaction medium was subjected to PCR amplification. After the lid was warmed at 94°C for 2 min, the PCR mixtures were subjected to a 40-cycle profile, including denaturation for 60 s at 94°C, hybridization for 60 s at 57°C, and elongation for 60 s at 72°C. In the present investigation, 18S rRNA expression was simultaneously measured as an internal standard using a QuantumRNA 18S Internal Standard Kit (Ambion, Austin, TX, USA). The PCR products were separated by electrophoresis on 2% agarose, stained with SYBR Green (Molecular Probes, Eugene, OR, USA), photographed, and analyzed by densitometry (LAS3000, Fujifilm, Tokyo, Japan). The ratio of GLUT-4 to 18S rRNA standard band densities was then calculated.

Statistical analysis

All values are expressed as mean ± SD. Statistical comparisons were made by one-way analysis of variance (ANOVA) using the Jandel SigmaStat statistical software (Jandel, San Rafael, CA, USA). Whenever the ANOVA indicated significant differences, the Tukey test was used for post-hoc analysis. The statistical significance was defined as $P < 0.05$.

Results

Effects of high-intensity intermittent swimming exercise training with different numbers of exercise bouts on the body weight of rats

The body weight rats did not differ among the sedentary control and the two training groups (Table 1).

Table 1 Effect of high-intensity intermittent swimming training with different numbers of exercise bouts on body weight of rats

	Control	Number of bouts	
		3	14
Body weight (g)	176 ± 8	174 ± 9	175 ± 6

Values are mean ± SD for eight rats

Effects of high-intensity intermittent swimming exercise training with different numbers of exercise bouts on GLUT-4 protein in rat EPI muscle

The GLUT-4 protein content in rat EPI muscle was significantly elevated by 75 and 71%, after 3 and 14 bouts of exercise training, respectively, compared with that of the age-matched sedentary control rats ($P < 0.01$; Fig. 1). There was no difference in muscle GLUT-4 between the two training groups.

Effects of high-intensity intermittent swimming exercise training with different numbers of exercise bouts on citrate synthase activity in rat EPI muscle

Citrate synthase activity in rat EPI muscle after the training with 3 and 14 bouts of exercise was significantly higher than that in the same muscle of the control rats by 23 and 25%, respectively, 18 h after the last bouts of training ($P < 0.05$; Fig. 2). No significant difference in citrate synthase activity was observed among the two training groups with different numbers of high-intensity exercise bouts.

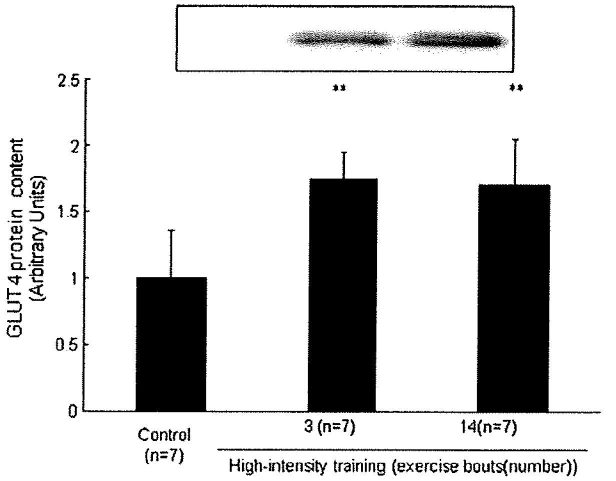

Fig. 1 Effects of high-intensity intermittent swimming training with different numbers of exercise bouts on GLUT-4 protein content in rat EPI muscle. **$P < 0.01$ compared with the control group. Values are mean ± SD for seven muscles

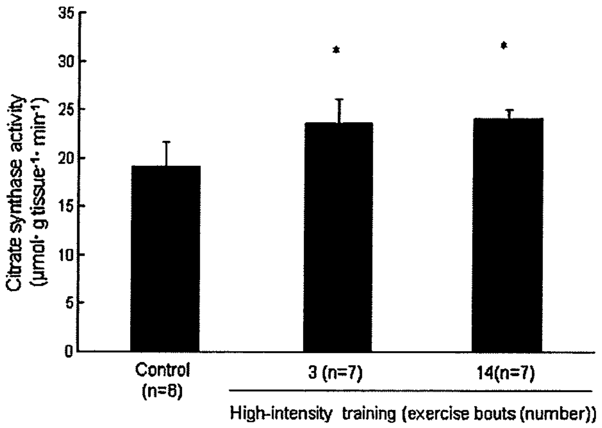

Fig. 2 Effects of high-intensity intermittent swimming training with different numbers of exercise bouts on citrate synthase activity in rat EPI muscle. *$P < 0.05$ compared with the control group. Values are mean ± SD for six to seven muscles

Effects of acute high-intensity intermittent swimming exercise with different numbers of exercise bouts on AMPK phosphorylation in rat EPI muscle

AMP-activated protein kinase phosphorylation in rat EPI muscle was significantly elevated by 33% after 14 bouts of exercise, compared with that of the age-matched sedentary control rats ($P < 0.05$; Fig. 3). However, the P value was near significance level between the controls and those undergoing three bouts of exercise ($P = 0.06$).

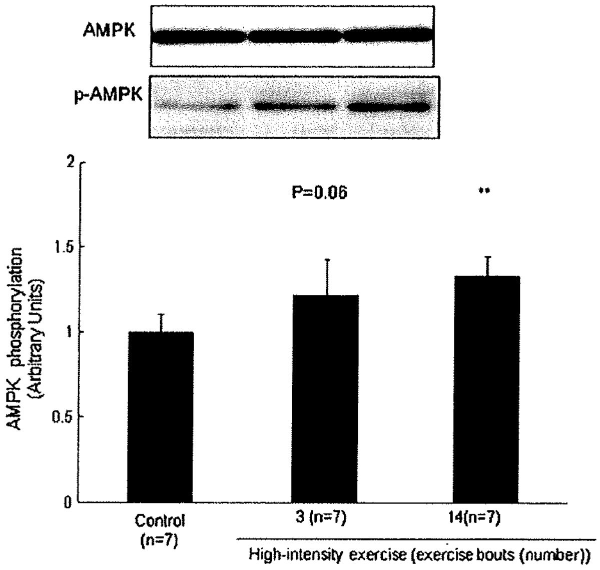

Fig. 3 Effects of high-intensity intermittent swimming with different numbers of exercise bouts on AMPK phosphorylation in rat EPI muscle. **$P < 0.01$ compared with the control group. $P = 0.06$ indicates a nonsignificant difference between the group undergoing three bouts of exercise and the control group. Values are mean ± SD for seven muscles

Table 2 Effect of high-intensity intermittent swimming exercise (HIE) with different numbers of exercise bouts on blood lactate concentration in rats

	Control	Number of bouts	
		3	14
Lactate concentration (mmol l^{-1})	2.5 ± 0.7	$6.8 \pm 1.8^{**}$	$14.4 \pm 2.9^{***,\dagger\dagger\dagger}$

Values are mean ± SD for five to eight rats

$^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control group

††† $P < 0.001$ compared with the group experiencing three HIE bouts

Effects of acute high-intensity intermittent swimming exercise with different numbers of exercise bouts on blood lactate concentrations

The blood lactate concentrations were significantly elevated after 3 (from 2.5 to 6.8 mmol l^{-1}; $P < 0.01$) and 14 (from 2.5 to 14.4 mmol l^{-1}; $P < 0.001$) bouts of exercise compared with those of control rats (Table 2). Blood lactate concentrations after 3 bouts of exercise were significantly lower than those observed after 14 bouts of exercise ($P < 0.001$; Table 2).

Effects of acute high-intensity intermittent swimming exercise with different numbers of exercise bouts on glycogen concentrations in rat EPI muscle

The glycogen concentrations in rat EPI muscle were significantly reduced by 44% ($P < 0.01$) and 79% ($P < 0.001$) after 3 and 14 bouts of the high-intensity intermittent exercise, respectively, compared with those of the nonexercise rats (Table 3). Glycogen concentrations after 14 bouts of exercise were 66% lower than the levels observed after 3 bouts of exercise ($P < 0.05$; Table 3).

Table 3 Effect of high-intensity intermittent swimming exercise with different numbers of exercise bouts on glycogen concentration in rat EPI muscle

	Control	Number of bouts	
		3	14
Glycogen concentration ($\mu\text{mol g muscle}^{-1}$)	25.6 ± 2.9	$14.4 \pm 5.8^{**}$	$5.5 \pm 3.6^{***,\dagger}$

Values are mean ± SD for five to seven muscle samples

$^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control group

† $P < 0.05$ compared with group experiencing three HIE bouts

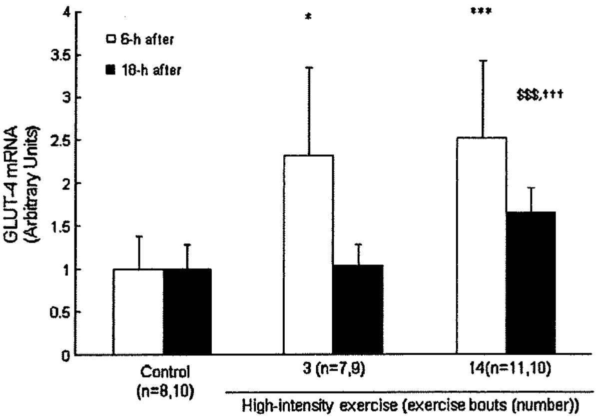

Fig. 4 Effect of acute bouts of high-intensity intermittent swimming exercise with different numbers of exercise bouts on GLUT-4 mRNA in rat EPI muscle. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with the control group at 6 h. $^{\$\$\$}P < 0.001$ compared with the control group at 18 h. $^{\dagger\dagger\dagger}P < 0.001$ compared with the group undergoing three bouts of high intensity intermittent swimming exercise (at 18 h). Values are mean ± SD for 7 to 11 muscles

Effects of acute high-intensity intermittent swimming exercise with different numbers of exercise bouts on GLUT-4 mRNA in rat EPI muscle

The GLUT-4 mRNA in rat EPI muscle was significantly increased by 132% ($P < 0.05$) and 152% ($P < 0.001$) 6 h after 3 and 14 bouts of exercise, respectively, compared with that of the aged matched sedentary control rats (Fig. 4). Eighteen hours after the 14 bouts of exercise, the GLUT-4 mRNA still remained at a level higher than the sedentary control by 65% ($P < 0.001$; Fig. 4). There was no statistically significant difference in GLUT-4 mRNA between sedentary control rats and exercised rats that had performed three bouts of the intermittent exercise at the time.

Discussion

The present investigation demonstrated that exercise training consisting of only three nonexhaustive bouts (20 s × 3 = 60 s) of extremely high-intensity intermittent swimming exercise elevates GLUT-4 protein in rat EPI muscle to levels comparable to those attained after 14 exhaustive bouts of exercise training, which has been regarded as the maximal exercise-related stimulus.

From a physiological perspective, the overall effect of exercise training on recruited muscle during a specific type of exercise training *generally* depends on the exercise intensity and exercise time (number of bouts). Because maximal exercise time is dependent on the exercise intensity, we previously [13] compared changes in GLUT-4 after low- and high-intensity training with exercise during which the rat becomes virtually exhausted. In the previous investigation, a stint of 14 bouts of high-intensity exercise training, as was used for the present investigation, was adopted to compare the effects on GLUT-4 concentration with those induced by low-intensity exercise training (for 6 h/day). It was found that the high-intensity exhaustive intermittent training increased GLUT-4 content to the same levels as those observed after the low-intensity exhaustive training that had been regarded as the maximal stimulus to GLUT-4 expression in rat skeletal muscle.

Since the HIT protocol (8–10 exhausting bouts of 20 s high-intensity exercise with 10 s rest between bouts) adopted in that study was originally developed for elite athletes [20], the protocol is exhaustive, as indicated by the high blood lactate concentrations (11.4 mM [13]), and not thought to be suitable for exercise prescriptions oriented toward health promotion. Therefore, for the purposes of finding the suitable number of high-intensity intermittent training bouts to elevate GLUT-4 concentrations to satisfactory levels, as compared to the levels attained with exhaustive training, we observed the effects of nonexhaustive high-intensity intermittent exercise (three bouts) on GLUT-4 expression in EPI muscle. Consequently, we found that a shorter training period with only three bouts of the same intensity exercise increases GLUT-4 expression to levels comparable to those obtained with high-intensity intermittent training that results in the rats becoming exhausted every day. Since blood lactate concentrations after 3 bouts of exercise were not as high as those observed after 14 bouts of exercise (Table 2), the training is not considered to be exhaustive. Furthermore, the muscle glycogen content after 3 bouts of the high-intensity exercise was not as low as that observed after 14 bouts of exercise (Table 3). Therefore, short-term high-intensity exercise training might be an effective tool for not only experimental animals but also for healthy humans in terms of preventing diabetes safely by increasing the GLUT-4 protein content in skeletal muscle.

The present investigation has demonstrated that exercise training consisting of only three nonexhaustive bouts ($20 \text{ s} \times 3 = 60 \text{ s}$) of extremely high-intensity intermittent swimming exercise elevates GLUT-4 protein in rat EPI muscle to a level comparable to that attained after 14 exhaustive bouts of exercise training, which has been regarded as the maximal exercise-related stimulus to GLUT-4 expression (Fig. 1). These results suggest that the effects of high-intensity training on GLUT-4 expression become saturated after a limited number of bouts. This finding might be explained by two different mechanisms. One is that with the shorter exercise training period, the GLUT-4 expression machinery, possibly including pathways of transcription and translation of the protein, cannot respond to higher levels of exercise-related signal(s), depending on the number of high-intensity exercise bouts. The other possibility is that exercise-related signal(s) related to GLUT-4 expression have already peaked after three bouts of high-intensity exercise.

The present investigation and previous mechanism-oriented studies might imply the latter, which might be explained by the following rationale. First, AMPK phosphorylation, which has been postulated as an exercise-related signal of exercise-induced GLUT-4 expression in skeletal muscle, is known to be dependent on exercise intensity [11, 21] and exercise duration (and number of exercise bouts) [22]. Therefore, it is assumed that the higher the exercise intensity or the greater the number of exercise bouts, the higher the levels of AMPK phosphorylation and GLUT-4 expression after exercise training. However, the present investigation showed that both GLUT-4 expression after high-intensity training with an increased number of exercise bouts and AMPK phosphorylation after the same bouts of exercise used in the training tended to reach a plateau after three bouts of the exercise protocol (Fig. 3). These results might suggest that at least one signal (AMPK phosphorylation) peaked after the third bout of exercise training and that GLUT-4 protein was expressed according to the signal (AMPK phosphorylation).

Since GLUT-4 protein content is at least partially controlled by transcriptional regulation, mRNA of GLUT-4 presumably increases after any kind of training exercise that elevates the GLUT-4 protein content in recruited skeletal muscle. In the present investigation, GLUT-4 mRNA in rat EPI muscle was significantly elevated 6 h after the 3 bouts of HIE to levels comparable to those attained after 14 exhaustive bouts of HIE (Fig. 4). Since the magnitude of the increase may depend on the exercise stimuli related especially to exercise intensity, it is noteworthy that the mRNA levels of GLUT-4 had increased at 6 h after the HIE to the same level as in the rats that had undergone what is believed to be the maximal stimulus in terms of exercise-induced GLUT-4 protein expression.

Further, GLUT-4 mRNA after the high-intensity exercise with 14 bouts remained higher than that of the sedentary rats, whereas the mRNA of the rats undergoing 3 bouts returned to levels similar to those of the control rats 18 h after the exercise. Therefore, for unknown reasons, stimulation of GLUT-4 mRNA expression after 14 bouts of high-intensity exercise appears higher than that induced by 3 bouts of the same exercise. However, since there was no statistical difference in GLUT-4 content 6 h after the training, these results might suggest that elevation of GLUT-4 mRNA for at least 6 h after three bouts of high-intensity exercise is saturated in terms of inducing GLUT-4 protein after high-intensity exercise in rat skeletal muscle.

Since GLUT-4 and mitochondrial enzymes are often expressed simultaneously, and expression of the two functional proteins is hypothesized to be regulated by the same mechanism [23], we measured citrate synthase activity in the same muscle from the training rats. Consistent with the expression of GLUT-4 protein, we observed that only three nonexhaustive bouts (20 s × 3 = 60 s) of HIT elevated the activity of citrate synthase in rat EPI muscle to a level comparable to that attained after 14 exhaustive bouts of exercise training at the same intensity (Fig. 2).

In conclusion, the present investigation has demonstrated that only three bouts (net exercise time: 60 s) of high-intensity intermittent exercise training increases GLUT-4 content to the maximal level attained with exhaustive high-intensity intermittent exercise in rat skeletal muscle, which has been regarded as the maximal stimuli in terms of exercise-protocol for GLUT-4 expression.

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Classifying household and locomotive activities using a triaxial accelerometer

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ABSTRACT

The purpose of this study was to develop a new algorithm for classifying physical activity into either locomotive or household activities using a triaxial accelerometer. Sixty-six volunteers (31 men and 35 women) participated in this study and were separated randomly into validation and cross-validation groups. All subjects performed 12 physical activities (personal computer work, laundry, dishwashing, moving a small load, vacuuming, slow walking, normal walking, brisk walking, normal walking while carrying a bag, jogging, ascending stairs and descending stairs) while wearing a triaxial accelerometer in a controlled laboratory setting. Each of the three signals from the triaxial accelerometer was passed through a second-order Butterworth high-pass filter to remove the gravitational acceleration component from the signal. The cut-off frequency was set at 0.7 Hz based on frequency analysis of the movements conducted. The ratios of unfiltered to filtered total acceleration (TAU/TAF) and filtered vertical to horizontal acceleration (VAF/HAF) were calculated to determine the cut-off value for classification of household and locomotive activities. When the TAU/TAF discrimination cut-off value derived from the validation group was applied to the cross-validation group, the average percentage of correct discrimination was 98.7%. When the VAF/HAF value similarly derived was applied to the cross-validation group, there was relatively high accuracy but the lowest percentage of correct discrimination was 63.6% (moving a small load). These findings suggest that our new algorithm using the TAU/TAF cut-off value can accurately classify household and locomotive activities.

1. Introduction

The modern lifestyle with reduced physical activity and a dietary intake greater than needed for daily energy expenditure is closely related to an increasing proportion of obese people. Low levels of physical activity are also associated with cardiovascular diseases [1], type 2 diabetes mellitus [2,3], and osteoporosis [4,5]. In order to prevent and control obesity and other diseases, moderate-intensity physical activity is recommended [6–8]. It has been reported that occupational, leisure-time, and household activities are also effective in the prevention of obesity and related diseases [9,10]. In fact, energy expenditure (EE) induced by these activities is much larger than exercise-induced EE when measured throughout the day [11]. In addition, a large inter-individual variation is observed in EE for these activities [10,11]. Therefore, it would be very useful for obesity research to measure both locomotive and household activities accurately.

There are several methods for evaluating short- and long-term physical activities under free-living conditions [12,13]. Accelerometers are currently used by some groups as monitoring tools because they are small, non-invasive, and relatively inexpensive [14]. Although several prediction equations have been developed, a single regression equation based on walking and jogging underestimates the EE of moderate-intensity household activities [15]. In contrast, a single regression equation based on household activity overestimates the EE of sedentary and light activities and underestimates the EE of vigorous activities [15,16]. Therefore, recent studies have attempted to classify physical activity into locomotive and household activities using an accelerometer. Although techniques for correct discrimination have been examined, their validity and usefulness for improving the accuracy of EE prediction have not been sufficiently proven. In addition, household activity comprises some part of total physical activity and non-exercise activity thermogenesis (NEAT). However, most studies have focused on locomotive activities such as walking and jogging, and the degree to which household activity contributes to the total amount (duration and EE) of physical activity under free-living conditions remains unclear.

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Accelerometers with a DC (direct current) response are capable of measuring acceleration due to movement and gravitational acceleration [17]. Therefore, filter processing that removes gravitational acceleration is performed to detect dynamic movements and gravitational acceleration is used to discriminate static postures such as lying and standing. Although the discrimination of posture is important in understanding behavior patterns, it is also necessary to determine what kinds of activities a person is performing under sitting or standing conditions. We hypothesized that information from the gravitational acceleration signal may contribute not only to discrimination of posture but also to classification of physical activity into locomotive or household activity, because household activities tend to involve a change in inclination of the upper body in addition to movement.

The purpose of this study was to develop a new algorithm for quick and accurate classification of physical activity into either locomotive or household activity using a triaxial accelerometer and to compare the accuracy of the algorithm with a previously proposed method.

2. Methods

2.1. Subjects

Sixty-six volunteers (31 men and 35 women) participated in this study. The subjects were separated randomly into a validation group ($n = 44$) and a cross-validation group ($n = 22$). Physical characteristics of the subjects are shown in Table 1. Before measurements, the purpose and procedure of the study were explained in detail. Written informed consent was obtained from all subjects. When we recruited subjects, participants were excluded from the study if they had any contraindications to exercise or if they were physically unable to complete the activities. This study protocol was approved by the Ethical Committee of the National Institute of Health and Nutrition in Japan.

2.2. Protocol

Before testing, height and weight were measured with subjects in light clothing without shoes using a stadiometer and a physician's scale. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index was calculated as weight (kg) divided by height squared (m^2).

All 66 subjects performed 12 sequences of normal daily movements in a controlled laboratory setting while wearing a triaxial accelerometer on the left side of the waist. The testing procedure was the same for all subjects. Participants performed each activity for 3–7 min with a break of a few minutes between each activity. The selected activities were personal computer (PC) work, laundry, dishwashing, moving a small load (5 kg), vacuuming, slow walking (3.3 km/h), normal walking (4.2 km/h), brisk walking (6.0 km/h), normal walking while carrying a bag (3 kg) in the hand, jogging (8.4 km/h) on a track, and ascending and descending stairs at personal normal speed (Table 2). These activities were chosen as representative activities of daily life and were based on our observations for 3 days in free-living conditions. The preliminary study was performed using the activity records of 93 subjects living in the Tokyo metropolitan area.

2.3. Triaxial accelerometer device

In order to perform this experiment, we used a triaxial accelerometer device with 4 GB of memory (Omron Healthcare, Kyoto, Japan) consisting of a MEMS-based accelerometer (LIS3LV02DQ; ST-Microelectronics) which responds to both acceleration due to movement and gravitational acceleration. The sensor is built in a plastic case designed to be clipped onto a waist belt. The device measures 80 mm × 20 mm × 50 mm and weighs 60 g, including batteries. During the experiment, the device was attached at waist level on the left side using an elastic belt. A commercial device (Omron Healthcare, Active Style Pro HJA-350IT) has been developed from the device used in the present study.

Table 1
Physical characteristics of subjects.

	Men	Women	Total
Validation group			
n	21	23	44
Age (years)	42.2 ± 14.4	43.0 ± 13.1	42.6 ± 13.7
Height (cm)	170.2 ± 5.8	159.3 ± 5.4	164.5 ± 7.8
Weight (kg)	68.3 ± 15.1	55.6 ± 9.8	61.6 ± 14.1
BMI (kg/m^2)	23.4 ± 4.2	21.9 ± 3.7	22.6 ± 4.0
Cross-validation group			
n	10	12	22
Age (years)	41.9 ± 14.3	42.0 ± 11.4	42.0 ± 12.8
Height (cm)	170.2 ± 7.5	156.9 ± 5.2	162.9 ± 9.2
Weight (kg)	68.2 ± 11.9	54.9 ± 7.6	61.0 ± 11.8
BMI (kg/m^2)	23.4 ± 3.2	22.3 ± 2.9	22.8 ± 3.1

Values are means ± SD; BMI, body mass index.

Table 2
Household and locomotive activities performed in this study.

PC work: typing with a personal computer (7 min)
Laundry: carrying clothes from a laundry basket and hanging up clothes (6 min)
Dishwashing: washing the dishes (6 min)
Moving a small load: lifting a small load of 5 kg and unloading it after a few steps (5 min)
Vacuuming: cleaning the floor with a vacuum cleaner (6 min)
Slow walking: walking at 55 m/min around a track (6 min)
Normal walking: walking at 70 m/min around a track (5 min)
Brisk walking: walking at 100 m/min around a track (5 min)
Walking while carrying a bag: walking at 70 m/min around a track while carrying a bag of 3 kg (5 min)
Jogging: jogging at 140 m/min around a track (4 min)
Ascending stairs: walking up stairs at a self-selected speed (3 min)
Descending stairs: walking down stairs at a self-selected speed (3 min)

2.4. Analysis of acceleration signal

Anteroposterior (x-axis), mediolateral (y-axis), and vertical (z-axis) accelerations were obtained from the triaxial accelerometer during each activity at a sampling rate of 32 Hz. The acceleration data are expressed relative to g ($1\ g = 9.81\ m/s^2$). With a 12-bit analog-to-digital converter, the maximum scaling of the acceleration data was $\pm 6\ g$ (resolution: 0.003 g). The acceleration data were uploaded to a personal computer. The signals obtained from the triaxial accelerometer were processed as follows. Each of the three signals from the triaxial accelerometer was passed through a second-order Butterworth high-pass filter to remove the gravitational acceleration component from the signal. The cut-off frequency was chosen based on frequency analysis of movements conducted. The power spectrum of each direction was calculated by fast Fourier transform (FFT) for a temporal window that contained 256 samples of the signal. This was normalized to the maximum power of each window, and the normalized power spectrums of the three directions were composited. We calculated the integral of the absolute value of the accelerometer output of each of the three axes using acceleration signals (X, Y, Z) over a 10-s time interval. The interval size was determined based on physiological aspects and the processing performance of the CPU; it has been reported that the use of 10-s epochs does not result in a significant underestimation of high-intensity activity relative to 5-s epochs whereas longer epochs do [18]. Then, the calculated horizontal acceleration in the X–Y plane (horizontal acceleration filtered, HAF) and the calculated total three-dimensional acceleration (total acceleration filtered, TAF) were determined. In addition, total acceleration using an unfiltered acceleration signal (total acceleration unfiltered, TAU) was calculated. Finally, the ratios of unfiltered to filtered total acceleration (TAU/TAF) and of filtered vertical acceleration (VAF) to HAF (VAF/HAF), as proposed by Midorikawa et al. [16], were calculated. When TAU/TAF was calculated, the phases of TAU and TAF were matched in consideration of the phase shift of the high-pass filter. The acceleration signals from six 10-s epochs in the middle of each activity were processed to various acceleration output variables.

2.5. Statistical analysis

Statistical analyses were carried out using SPSS Version 14.0 for Windows (SPSS, Inc., Chicago, IL). All results are shown as the mean ± SD. $P < 0.05$ was considered statistically significant.

To assess the cut-off value for classification of household and locomotive activities, receiver-operating characteristic (ROC) curve analysis was applied to the acceleration data. We calculated the sensitivities and specificities using the TAU/TAF and VAF/HAF ratios. The sensitivity was multiplied by the specificity, and the point with the maximum product of sensitivity and specificity was considered to be the most valid discrimination cut-off value. The triaxial accelerometer signals from the validation group were used to identify the optimum cut-off value of parameters to classify physical activity. This cut-off value was then applied to the cross-validation group and the accuracy of discrimination was evaluated.

3. Results

FFT analysis showed that for locomotive activities, peak power appeared at a frequency of 1.0 Hz or more and the frequency of the peak increased with an increase in walking pace. For household

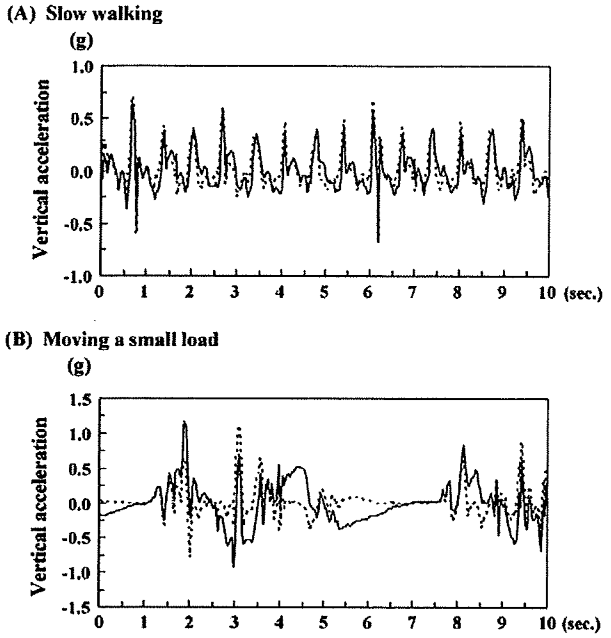

Fig. 1. Typical examples of a vertical acceleration signal during slow walking (A) and while moving a small load (B). Dotted line is before high-pass filtering, and solid line is after high-pass filtering.

activities, peak power appeared at 1.0 Hz or less and the mean frequency of the peak was 0.29 ± 0.19 Hz. Therefore, the cut-off frequency for the high-pass filter was set at 0.7 Hz (mean + 2SD).

Fig. 1 shows typical examples of a vertical acceleration signal before and after high-pass filtering during slow walking (Fig. 1A) and moving a small load (Fig. 1B). TAU during moving a small load (0.34 g) was larger than that during slow walking (0.23 g). TAF during both physical activities was similar (0.22 g and 0.22 g, respectively). Therefore, TAU/TAF during moving a small load (1.55) was larger than that during slow walking (1.04).

Fig. 2A shows TAU/TAF in the validation group. As in the case illustrated in Fig. 1, the average TAU/TAF during locomotive activities was 1.03 ± 0.03 (range 0.96–1.12). In contrast, the average TAU/TAF during household activities was 2.46 ± 0.73 (range 1.19–5.53). The product obtained by multiplying the sensitivity and specificity from the TAU/TAF data was 1.0 when TAU/TAF was between 1.13 and 1.19. Therefore, the discrimination cut-off value was set at 1.16, which is the mid-point for the TAU/TAF data. When the discrimination cut-off value derived from the validation group was applied to the cross-validation group, the percentage of correct discrimination was over 95.5% (Table 3).

The VAF/HAF ratio in the validation group is shown in Fig. 2B. The average VAF/HAF during locomotive activities was 1.13 ± 0.36 (range 0.56–2.58) and that during household activities was 0.55 ± 0.13 (range 0.32–0.99). The largest product of sensitivity and specificity using the VAF/HAF data was 0.74. Table 3 shows the results from applying the discrimination cut-off value to the cross-validation group. Vacuuming, doing laundry, dishwashing, and normal walking while carrying a bag were correctly classified by the VAF/HAF cut-off ratio. Percentages of correct discrimination for other activities ranged from 63.6% to 95.5%.

4. Discussion

Our major finding in this study is that locomotive and household activities can be accurately classified from the analysis of both unfiltered and filtered acceleration signals. We used the ratio of TAU/TAF to classify physical activities into either locomotive or household activities. The TAU/TAF ratios obtained during locomotive activities (around 1.0) and household activities (>1.0) were entirely different. Since the distinction between locomotive, household and light activities is important for accurate estimation of energy expenditure using an accelerometer, various classification methods have been proposed in previous studies [16,19]. Light activities can be discriminated from locomotive or household activities by using only total acceleration, because a lower total acceleration is observed for light activities than for the other two types of activities [16]. However, locomotive and household activities could not be clearly distinguished by a previously reported method [19]. It is very important to estimate EE accurately, including the proportion of time spent in household activities throughout the day, which is a large component of NEAT.

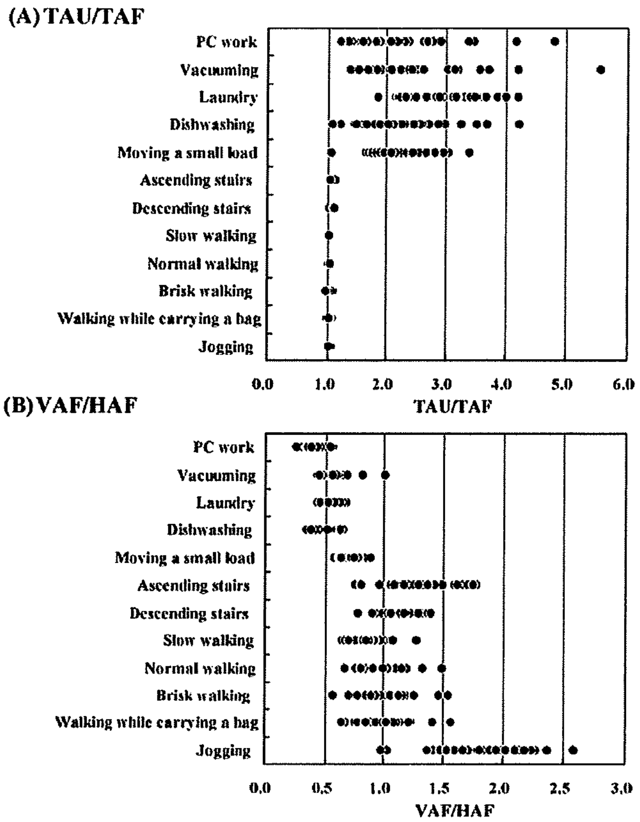

Fig. 2. The ratio of unfiltered to filtered total accelerations (TAU/TAF) (A) and the ratio of filtered vertical to filtered horizontal accelerations (VAF/HAF ratio) (B) during each activity in the validation group ($n = 44$).

Table 3
Percentage of correct discrimination in cross-validation group ($n = 22$).

	TAU/TAF (%)	VAF/HAF (%)
PC work	100	100
Vacuuming	100	100
Laundry	100	100
Dishwashing	100	100
Moving a small load	100	63.6
Ascending stairs	100	90.9
Descending stairs	95.5	90.9
Slow walking	95.5	81.8
Normal walking	95.5	95.5
Brisk walking	100	95.5
Normal walking while carrying a bag	100	100
Jogging	100	100

TAU/TAF, ratio of unfiltered to filtered total acceleration; VAF/HAF, ratio of vertical to horizontal acceleration.

Therefore, our findings are very pertinent to future research in this area.

The compendium of physical activities [20] is a common source of information regarding the intensities of various activities. The EEs of both normal walking and vacuuming are similar according to the compendium listings, whereas in this study the total acceleration for vacuuming was 1/4 that for normal walking. These results are consistent with previous reports that household activities have a higher oxygen cost, at the same total acceleration, compared with walking and running [19,21]. The increased EE during household activities is due to arm movements, lifting and carrying objects, climbing hills and stairs, and changing directions in the horizontal plane [21]. Therefore, different prediction equations are needed for accurate estimation of household and locomotive activities.

The acceleration signal was passed through a high-pass filter to remove the gravitational acceleration component in order to examine the actual relation of acceleration to physical activity [22]. In the present study, total acceleration was calculated from both the filtered and the unfiltered signals. If the acceleration signal is derived from locomotive activity which consists of only dynamic movement, the TAU/TAF ratio is mostly found to be 1.0. In contrast, if the acceleration signal is derived from household activity which consists of dynamic movement and gravitational acceleration, the TAU/TAF ratio is found to be larger than 1.0. The change in the gravitational acceleration component indicates a change in the inclination of the acceleration sensor. Because the acceleration sensor is attached to the waist of the subject, TAU/TAF reflects dynamic changes in body posture. The waist is not in the upper body, but the inclination of the upper body accompanies that of the waist in most instances. Therefore the gravitational acceleration signal at the waist reflects postural changes of the upper body to some degree. The cut-off value for classification was set at 1.16 in the present study, as a slight postural change at the waist seems sufficient to capture the postural changes of the upper body. Previous studies have reported a classification method for physical activity using the gravitational component of the triaxial accelerometer [23,24]. However, most such classification methods only discriminate static postures such as sitting and standing.

The TAU/TAF ratio was around 1.0 during locomotive activities regardless of the speed and was above 1.0 during household activities. This result suggests that there is a characteristic dynamic change in posture, such as inclining the upper part of the body forward, during household activities. While mainly the lower limbs move during locomotive activities such as walking and jogging, movement of the arms while lifting and pushing accompany household activities. Therefore, some researchers have attempted to classify and quantify the different types of physical activities using both trunk acceleration and wrist acceleration [25–27]. However, using multiple sensors can have disadvantages such as increased monetary cost and reduced convenience. If classification can be done with a single acceleration sensor, those disadvantages can be avoided. It has been reported that wrist-worn accelerometer signals can explain only a small part of the variance in EE [25,26]. In addition, it has been reported that the EEs of upper limb movements in activities such as deskwork were not different from the resting level, whereas self-care tasks accompanied by trunk movements approximately doubled the resting level [25]. Therefore, measurements of changes in posture are more important for discrimination of household activity intensity than measurements of upper arm movements.

The percentage of correct discrimination between locomotive and household activities by the VAF/HAF ratio was over 63.6% in the present study. Midorikawa et al. [16] reported that the sensitivity and specificity for discriminating between housework and walking using the VAF/HAF ratio was over 90%. This discrepancy may be due to differences between the protocols. Although 12 activities were chosen in the present study, only four types of activity (sitting, standing, housework, and walking) were performed in the study of Midorikawa et al. [16]. Moving a small load, which is a dynamic activity, complicated by lifting and walking, had the lowest discrimination accuracy of the 12 activities and slow walking had the lowest discrimination accuracy among the locomotive activities. The VAF/HAF ratio reflects the main direction of movement and may be associated with differences in movement between the upper body and the lower body. In the above two activities (moving a small load and slow walking), movement of both the upper and lower body occur to some degree and there may be large inter-individual differences. Therefore, the VAF/HAF ratio may tend to misclassify dynamic household activities and light locomotive activities.

Crouter et al. [19] attempted to distinguish walking and running from all other activities by calculating the coefficient of variation from Actigraph data. Because locomotive activities yielded a consistent minute-to-minute count, the coefficient of variation during locomotive activities was lower than that of other activities. They used six 10-s epochs of data to calculate the coefficient of variation for each minute. Therefore, it is necessary to keep a constant speed for at least 1 min for an accurate discrimination of walking with their method. Although it is possible to maintain an even pace in experimental conditions using a treadmill, speed is more variable in free-living walking because of pauses for traffic lights or walking on curved roads. Therefore, it is preferable that the discrimination be done over shorter time periods. In contrast, we could discriminate with 10-s epochs of data using TAU/TAF. Further research is required to determine the effectiveness of our approach for measurements of daily life EE.

In addition, it is possible that TAU/TAF may increase even during locomotive activities due to soft tissue movement or loosening of the belt that attaches the device to the waist. Since our study included only five subjects whose BMI was more than 30, we do not know the degree to which soft tissue may influence the results. It would be necessary to confirm whether our new algorithm can be adjusted for subjects with abdominal obesity.

In conclusion, we have shown that it is possible to classify locomotive and household activities using a single waist-mounted triaxial accelerometer. By analyzing raw acceleration data, changes in gravitational acceleration could be evaluated. The TAU/TAF ratio during household activities was larger than that during locomotive activities.

Acknowledgments

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Conflicts of interest

I declare that I have no conflict of interest.

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ORIGINAL ARTICLE

Predicting $\dot{V}O_{2max}$ with an objectively measured physical activity in Japanese men

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Abstract The present study investigated the use of the accelerometer-determined physical activity (PA) variables as the objective PA variables for estimating $\dot{V}O_{2max}$ in Japanese adult men. One hundred and twenty-seven Japanese adult men aged from 20 to 69 years were recruited as subjects of the present study. Maximal oxygen uptake ($\dot{V}O_{2max}$) was measured with a maximal incremental test on a bicycle ergometer. Daily step counts (SC) and the amount spent in moderate to vigorous PA (MVPA) and vigorous PA (VPA) were measured using accelerometer-based activity monitors worn at the waist for seven consecutive days. The non-exercise models were derived using hierarchical linear regression analysis, and cross-validated using two separate cross-validation procedures. SC, MVPA, and VPA were significantly related to $\dot{V}O_{2max}$ (partial correlation coefficient $r = 0.58$, $r = 0.42$, and $r = 0.51$, respectively) after adjusting for age. Two models were developed by multiple regression to estimate $\dot{V}O_{2max}$ using data of age, SC, VPA, and either BMI (the coefficient of determination (R^2) $= 0.71$, standard error of estimate (SEE) $= 4.2$ ml kg^{-1} min^{-1}), or waist circumference ($R^2 = 0.74$, SEE $= 3.9$ ml kg^{-1} min^{-1}). All regression models demonstrated a high level of cross-validity supported by the minor shrinkage of R^2 and increment of SEE in the PRESS procedure, and by small constant errors for subgroups of age, SC, and $\dot{V}O_{2max}$. This study demonstrated that combining SC with VPA, but not with MVPA, was useful in predicting $\dot{V}O_{2max}$ variance and improved the ability of the regression models to accurately predict $\dot{V}O_{2max}$.

Keywords Cardiorespiratory fitness · Maximal oxygen uptake · Accelerometer · Prediction models · Physical activity

Introduction

Studies have provided evidence that a low level of cardiorespiratory fitness (CRF), quantified as poor maximal oxygen uptake ($\dot{V}O_{2max}$), was a powerful predictor of the risk for developing coronary heart disease (Hooker et al. 2008), hypertension (Barlow et al. 2006), type 2 diabetes mellitus (Sui et al. 2008), and metabolic syndrome (LaMonte et al. 2005) as well as mortality from cardiovascular disease (Hooker et al. 2008; Katzmarzyk et al. 2004), cancer (Sawada et al. 2003), and all causes of mortality (Katzmarzyk et al. 2004). Although CRF is an important health indicator, CRF assessment is usually not performed in the public health settings because of the absence of feasible and practical assessment methods.

The impracticality of maximal and submaximal exercise tests for the assessment of CRF in the general public is well recognized due to expensive equipment requirements, the

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potential risks of exercise exertion, practical personnel, and time demands. Therefore, a variety of non-exercise (without exercise test) prediction models (Blair et al. 1989; George et al. 1997; Heil et al. 1995; Jackson et al. 1990; Jurca et al. 2005; Malek et al. 2004, 2005; Sanada et al. 2007; Wier et al. 2006) utilizing age, gender, body composition, and physical activity rating or perceived functional ability, have been developed as alternative approaches to fitness assessment and the estimation of $\dot{V}O_{2max}$. An international group of experts in the areas of physical activity and fitness assessment, epidemiology, preventive medicine, and clinical exercise testing reviewed the precision and feasibility of a variety of methods that might be used to quantify CRF in healthcare settings, and concluded that the prediction of CRF from non-exercise regression models would be most appropriate for widespread use in many healthcare settings if sufficient validity was obtained with this method of assessment (Jurca et al. 2005). However, the previous studies reporting non-exercise models have relied on a subjective self-reported physical activity (PA) measure, which, when compared with objectively measured PA, have been shown to have low correlations in the range of 0.14–0.53 and to suffer from social desirability and recall biases (Sallis and Saelens 2000). In addition, perhaps, the greatest limitation of subjective self-reported PA measures is their inability to accurately assess unstructured and incidental ambulatory PA, which may account for a greater proportion of total PA in sedentary people.

Recently, Cao et al. evaluated the validity of objectively measured PA variables, quantified as the pedometer-determined daily step counts (SC) and the accelerometer-determined time spent in moderate to vigorous PA (>3 METs, MVPA) and vigorous PA (>6 METs, VPA), as a predictor variable in estimating $\dot{V}O_{2max}$ in Japanese women (Cao et al. 2009, 2010). They demonstrated that SC, MVPA, and VPA were useful in predicting $\dot{V}O_{2max}$ variance and helped their non-exercise $\dot{V}O_{2max}$ prediction model generate relatively accurate estimations of $\dot{V}O_{2max}$ ($R = 0.86$, SEE = 2.98 ml kg^{-1} min^{-1}); the accuracy of the model was better than the accuracy of the submaximal exercise models. However, in terms of fitness and/or physical activity levels, males are different from females (Katzmarzyk 2006), and it is therefore not known whether the validity of objectively measured PA variables as a predictor variable in estimating $\dot{V}O_{2max}$ also applies to adult men.

The aim of the present study was to determine whether objectively measured PA variables including SC, MVPA, and VPA are potential predictors of $\dot{V}O_{2max}$ in Japanese adult men. More specifically, the purpose of this study was to develop new non-exercise $\dot{V}O_{2max}$ prediction models using SC, MVPA or VPA as the objective PA variables as well as additional covariates, including age and body composition in Japanese adult men.

Methods

The present study consists of three parts: first, we hypothesized that the objectively measured PA variables, including the SC, MVPA, and VPA are potential predictors of CRF in Japanese men. To verify this hypothesis, the relationships between objectively measured PA variables and $\dot{V}O_{2max}$ were investigated. Second, validation procedures were used to develop new non-exercise $\dot{V}O_{2max}$ prediction models that included body composition and objectively measured PA variables as predictor variables. Finally, the accuracy of the new non-exercise prediction models was assessed using two separate cross-validation procedures.

Subjects

One hundred and twenty-seven Japanese men aged from 20 to 69 years old were tested in two independent institutions supervised by two of the coauthors NM and MH. None of the subjects had any chronic diseases or were taking any medications that could affect the study variables. All subjects provided written informed consent according to the local institute policy before the measurement of CRF. The research project was approved by the Ethics Committee of the National Institute of Health and Nutrition. The subjects' characteristics are described in Table 1.

Anthropometrics

The anthropometric variables measured were body mass, height, and waist circumference (WC) in all subjects. The

Table 1 Physical characteristics of the study subjects

Variable	Mean ± SD $n = 127$	Range $n = 127$
Age (years)	45.4 ± 13.9	21–69
Height (cm)	170.4 ± 6.0	155.8–188.0
Body mass (kg)	67.8 ± 8.9	52.1–109.8
BMI (kg m^{-2})	23.3 ± 2.8	18.3–35.3
WC (cm)	82.5 ± 8.1	65.5–116.2
$\dot{V}O_{2max}$ (ml kg^{-1} min^{-1})	36.7 ± 7.6	21.3–58.4
SC (steps day^{-1})	9,076 ± 3,661	1,602–1,9269
MVPA (min)	31.9 ± 22.7	3.2–142.3
VPA (min)	3.8 ± 5.4	0.0–33.9

BMI body mass index, *WC* waist circumference, *SC* daily step counts, *MVPA* moderate-to-vigorous physical activity, *VPA* vigorous physical activity methodology and equipment used in taking these measurements have been described in detail elsewhere (Cao et al. 2009, 2010).

Cardiorespiratory fitness

$\dot{V}O_{2max}$ was measured using a maximal graded exercise test (GXT) with bicycle ergometers [Lode Excalibur (NM), Lode BV, Groningen, The Netherlands; Monark Ergomedic 828E (MH), Varberg, Sweden]. The methodology and equipment used in taking this measurement have been described in detail elsewhere (Cao et al. 2009, 2010). Achievement of $\dot{V}O_{2max}$ was accepted if two of the following conditions were met (Cao et al. 2009, 2010): subject's maximal heart rate (HR) was >95% of the age-predicted maximal HR (220 − age), and the $\dot{V}O_2$ curve showed a leveling off.

Physical activity

Physical activity was measured by the Kenz Lifecorder (LC; SUZUKEN Co Ltd, Nagoya, Japan) which is a recent addition to the growing number of uniaxial accelerometer options; it offers comparable instrument outputs with several potentially attractive features for researchers and practitioners. The LC has displayed reasonable estimates of PA intensity and energy expenditures under controlled conditions on a treadmill (Kumahara et al. 2004), over 24 h of typical daily activities undertaken in a respiratory chamber (Kumahara et al. 2004), and in a free-living environment using doubly labeled water as the criterion method (Yamada et al. 2009). Furthermore, when compared with many other accelerometers, the LC is somewhat more affordable and can potentially simplify the data-interpretation process by reducing the time spent and the need for advanced technical expertise or software programs (McClain et al. 2007). The subjects were taught how to use the instrument, and were told to wear it on their belt or waistband at the right midline of the thigh from the moment they got up until they went to bed except while bathing or swimming, for seven consecutive days (Clemes and Griffiths 2008). The activity monitor was firmly attached to their clothes at the waist by a clip.

Statistical analyses

Measured and calculated values are presented as mean ± SD. Pearson's product correlations were calculated between the independent variables (age, BMI, WC, SC, MVPA, and VPA) and $\dot{V}O_{2max}$. Hierarchical linear regression analysis was used to generate prediction equations for $\dot{V}O_{2max}$. We entered the age, a different body composition measure (i.e., BMI or WC) into the first block, SC into the second block, and MVPA or VPA into the third block. Because the outcome measurements were performed at different institutions, the effect of institution was assessed by adding a dummy-coded institution variable and then applying a multiple regression to determine whether the institution variable provided a significant increase in the explained variance of $\dot{V}O_{2max}$ over the independent variable. The goodness of fit and precision of the regression equations were evaluated using multiple coefficient of determination (R^2) and the absolute standard error of estimate (SEE) and relative SEE (%SEE). The new non-exercise prediction models were assessed using two separate cross-validation procedures, using the predicted residual sum of squares (PRESS) method (Holiday et al. 1995) and various subsamples of the entire sample. The PRESS method is based on the error in prediction for each case when only that case is deleted from the model-generating process. The PRESS adjusted R^2 (R_p^2) can be calculated as 1 − (PRESS/SStotal). The PRESS SEE (SEE_p) can be calculated using the following equation: $SEE_p = \sqrt{PRESS/n}$. The models were further examined for accuracy by dividing the entire sample into subgroupings of age, SC, and $\dot{V}O_{2max}$, and then by comparing the constant errors (CE) among these subgroupings. All analyses were done with SPSS 16.0J for Windows (SPSS Japan Inc., Tokyo, Japan). The statistical significance level was set at $P < 0.05$.

Results

The results from CRF testing for $\dot{V}O_{2max}$, anthropometric variables, and PA variables are presented in Table 1. The highly varied nature of the sample is reflected by the respective physical characteristics data ranges.

Table 2 presents the Pearson correlations matrix of $\dot{V}O_{2max}$ and all independent variables. These correlations between $\dot{V}O_{2max}$ and all independent variables were statistically significant ($P < 0.01$) and ranged from a low of 0.21 for MVPA to a high of −0.66 for age and WC in our sample, indicating that each independent variable was related to $\dot{V}O_{2max}$. The correlation coefficients between $\dot{V}O_{2max}$ and VPA or SC were significantly higher than the correlation coefficient between $\dot{V}O_{2max}$ and MVPA. After statistically controlling for the influence of age using partial correlation analysis, the correlations between $\dot{V}O_{2max}$ and SC, MVPA, and VPA significantly increased to 0.58, 0.42, and 0.51, respectively.

Table 3 shows the multiple regression analysis. All variables used in the model were independently related to $\dot{V}O_{2max}$. Among the BMI and WC prediction models in the current study, the WC model^{VPA} showed the highest multiple correlations and lowest SEE. When estimating $\dot{V}O_{2max}$ with age and body composition, the addition of SC

Table 2 Correlations matrix of $\dot{V}O_{2\max}$ and independent variables ($n = 127$)

	$\dot{V}O_{2\max}$ (ml kg^{-1} min^{-1})	Age (years)	BMI (kg m^{-2})	WC (cm)	SC (steps day^{-1})	MVPA (min)
Age (years)	−0.66**					
BMI (kg m^{-2})	−0.45**	0.22*				
WC (cm)	−0.66**	0.47**	0.84**			
SC (steps day^{-1})	0.31**	0.18*	−0.13	−0.15		
MVPA (min)	0.21*	0.16	−0.08	−0.07	0.84**	
VPA (min)	0.49**	−0.17	−0.10	−0.21*	0.51**	0.42**

BMI body mass index, *SC* daily step counts; *MVPA* moderate-to-vigorous physical activity, *VPA* vigorous physical activity, *WC* waist circumference

* $P < 0.05$, ** $P < 0.001$

Table 3 Multiple regression non-exercise models estimation $\dot{V}O_{2\max}$ (ml kg^{-1} min^{-1})

$\dot{V}O_{2\max}$ (ml kg^{-1} min^{-1})	BMI model (kg m^{-2})		WC model (cm)	
	Coefficients	β	Coefficients	β
Model^{SC}				
Constant	61.838*		71.011*	
Age (years)	−0.371*	−0.678	−0.309*	−0.565
Body composition	−0.677*	−0.246	−0.328*	−0.347
SC (10^3 steps day^{-1})	0.827*	0.397	0.748*	0.359
R	0.826*		0.846*	
SEE (ml kg^{-1} min^{-1})	4.347		4.123	
%SEE	11.852		11.241	
R_p	0.814*		0.835*	
SEE$_p$ (ml kg^{-1} min^{-1})	4.416		4.187	
Model^{VPA}				
Constant	61.925*		70.679*	
Age (years)	−0.338*	−0.618	−0.279*	−0.509
Body composition	−0.698*	−0.254	−0.328*	−0.347
SC (10^3 steps day^{-1})	0.577*	0.277	0.513*	0.246
VPA (min)	0.305*	0.215	0.288*	0.204
R	0.845*		0.862*	
SEE (ml kg^{-1} min^{-1})	4.145		3.931	
%SEE	11.301		10.718	
R_p	0.833*		0.851*	
SEE$_p$ (ml kg^{-1} min^{-1})	4.201		3.988	

%SEE calculated as (SEE/mean of measured $\dot{V}O_{2\max}$ × 100)

BMI body mass index, *SC* daily step counts, *MVPA* moderate-to-vigorous physical activity, *VPA* vigorous physical activity, *WC* waist circumference. β standardized regression weights. *SEE* standard error of estimate, *SEE$_p$* PRESS standard error of estimate, *R_p* PRESS multiple correlation coefficients

* $P < 0.0001$

raised the R^2 from 0.54 to 0.68 for the BMI model^{SC} and from 0.60 to 0.72 for the WC model^{SC}, representing increases of 14% for the BMI model^{SC} and 12% for the WC model^{SC} in the explained variance of $\dot{V}O_{2\max}$. When estimating $\dot{V}O_{2\max}$ with model^{SC}, the addition of VPA significantly increased the explained variance in $\dot{V}O_{2\max}$ by an additional 3% in the BMI model^{VPA} and 3% in the WC model^{VPA} and decreased the SEE by 0.12–0.20 ml kg^{-1} min^{-1}. However, when MVPA as an independent variable was added to the multiple model^{SC}, we found that MVPA was not statistically significant ($P > 0.12$) and produced no appreciable difference in the accuracy of the models ($R^2\Delta < 0.006$, data not shown). When the institution variable as an independent variable was added to the multiple regressions, we found that the institution variable was not statistically significant ($P > 0.27$) and produced no appreciable difference in the accuracy of the models ($R^2\Delta < 0.003$, data not shown). The cross-validation results of the PRESS method are also shown in Table 3. The shrinkage of R^2 and the increment of SEE for each prediction model were minor ($R^2\Delta < 0.02$ and SEE$\Delta < 0.069$ ml kg^{-1} min^{-1}).

In the second stage of the cross-validation analysis, the accuracy of the models was examined by analyzing and comparing the CE and SD of the CE for various subsamples of the validation sample. The results are provided in Tables 4 and 5. Table 4 suggests that model^{SC} is the most accurate in predicting $\dot{V}O_{2\max}$ for individuals who are older (>50 years), less active (SC <7,500 steps day^{-1}), and with intermediate fitness (30–40 ml kg^{-1} min^{-1}). Subgroups of $\dot{V}O_{2\max}$ showed high absolute CE values (>3.4 ml kg^{-1} min^{-1}) in the high fitness subgroup (>40 ml kg^{-1} min^{-1}). When prediction model^{VPA} was applied to the subgroups (Table 5), the CE values (<1.0 ml kg^{-1} min^{-1}) for all ages and SC groupings, except for individuals who were middle aged and for the intermediate fitness subgroup (30–40 ml kg^{-1} min^{-1}), were small. At the extremes of fitness, the model^{VPA} systematically overestimated by 1.57–1.90 ml kg^{-1} min^{-1} and underestimated $\dot{V}O_{2\max}$ by 0.31–1.86 ml kg^{-1} min^{-1}. Figures 1 and 2 illustrate the tendency for model^{SC} and model^{VPA} to consistently underestimate $\dot{V}O_{2\max}$ for individuals with high fitness. Fifteen subjects in the present study were measured with a $\dot{V}O_{2\max}$ >47 ml kg^{-1} min^{-1}, and their

Table 4 Constant error (CE) and standard deviations (SD) for subgroups of the sample

Subgroup	n (%)	BMI model^{SC}		WC model^{SC}	
		CE	SD	CE	SD
Age					
<35 years	36 (28.3)	0.55	5.17	0.48	4.91
35–50 years	39 (30.7)	−1.24	4.11	−1.09	3.93
>50 years	52 (40.9)	0.50	3.61	0.42	3.42
SC					
<7,500 steps day^{-1}	45 (35.4)	0.00	4.39	−0.06	4.15
7,500–9,999 steps day^{-1}	34 (26.8)	−0.08	5.05	−0.13	4.77
≥10,000 steps day^{-1}	48 (37.8)	0.00	3.68	0.08	3.51
$\dot{V}O_{2max}$					
<30 ml kg^{-1} min^{-1}	24 (18.9)	−1.83	3.21	−1.45	2.86
30–40 ml kg^{-1} min^{-1}	66 (52.0)	−1.22	4.17	−1.29	4.09
>40 ml kg^{-1} min^{-1}	37 (29.1)	3.38	3.18	3.23	2.79

BMI body mass index, *WC* waist circumference, *SC* daily step count

Table 5 Constant error (CE) and standard deviations (SD) for subgroups of the sample

Subgroup	n (%)	BMI model^{VPA}		WC model^{VPA}	
		CE	SD	CE	SD
Age					
<35 years	36 (28.3)	0.58	4.89	0.50	4.60
35–50 years	39 (30.7)	−1.30	3.77	−1.16	3.62
>50 years	52 (40.9)	0.55	3.49	0.47	3.35
SC					
<7,500 steps day^{-1}	45 (35.4)	−0.08	4.37	−0.15	4.10
7,500–9,999 steps day^{-1}	34 (26.8)	0.12	4.88	0.07	4.60
≥10,000 steps day^{-1}	48 (37.8)	−0.04	3.15	0.04	3.09
$\dot{V}O_{2max}$					
<30 ml kg^{-1} min^{-1}	24 (18.9)	−1.90	3.05	−1.57	2.74
30–40 ml kg^{-1} min^{-1}	66 (52.0)	−1.04	3.98	−1.12	3.92
>40 ml kg^{-1} min^{-1}	37 (29.1)	0.97	3.11	1.86	2.98

BMI body mass index, *WC* waist circumference, *SC* daily step count

average measured value was 50.8 ± 2.6 ml kg^{-1} min^{-1}. The average $\dot{V}O_{2max}$ estimated by the models for these subjects, however, were, for the BMI model^{SC} and model^{VPA}, 44.7 ± 3.1 and 45.7 ± 4.5 ml kg^{-1} min^{-1}, respectively, and for the WC model^{SC} and model^{VPA}, 45.1 ± 3.0 and 46.2 ± 4.3 ml kg^{-1} min^{-1}, respectively.

Discussion

The results of the present study showed that the PA variables of SC, MVPA, and VPA were significantly related to $\dot{V}O_{2max}$, thus supporting our first hypothesis that the accelerometer-determined PA variables including SC, MVPA, and VPA are potential predictors of CRF in Japanese men. Furthermore, this study indicates that the non-exercise model for the prediction of $\dot{V}O_{2max}$ can be substantially improved by the inclusion of both these objectively measured PA variables of SC and VPA, which are easily and reliably measured using an accelerometer, and, therefore, is applicable to healthcare settings and large epidemiological cohorts.

A positive relationship between the objectively measured intensity of physical activity and CRF has been established in youth (Dencker et al. 2006, 2008; Gutin et al. 2005). However, few studies have reported this relationship in adults. Hebestreit et al. (2006) conducted a study of 71 patients with cystic fibrosis (aged 12–40 years) and found MVPA, as assessed by an accelerometer, to be significantly associated ($r = 0.55$, $P < 0.001$) with $\dot{V}O_{2max}$ as assessed by a GXT with a bicycle ergometer. However, they did not assess the relationship between VPA and $\dot{V}O_{2max}$. Cao et al.'s (2010) study is the first to examine the relationships between accelerometer-determined MVPA and VPA and $\dot{V}O_{2max}$ in healthy adults. They demonstrated that each of the accelerometer-determined PA variables was independently related to $\dot{V}O_{2max}$ in healthy adult women. The correlation coefficient of 0.21 between MVPA and $\dot{V}O_{2max}$ found in this study was smaller than that found in patients with cystic fibrosis (Hebestreit et al. 2006) and the correlation coefficient ($r = 0.46$) in healthy adult women reported by Cao et al. (2010) suggested that the proportion of the variance in CRF explained by MVPA in healthy adult women is larger than that in healthy adult men. The results of the present study also showed a similar good relationship ($r = 0.49$) between VPA and $\dot{V}O_{2max}$ compared with the results in healthy adult women ($r = 0.56$) (Cao et al. 2010) and adolescents ($r = 0.45$) (Gutin et al. 2005). The relationships tended to be stronger for VPA than for MVPA, which is consistent with other findings reported in healthy adult women (Cao et al. 2010) and children (Dencker et al. 2006), suggesting that VPA make a bigger contribution to the variance in CRF as compared to MVPA in men.

Cao et al. (2010) demonstrated that MVPA and VPA were useful in predicting $\dot{V}O_{2max}$ variance and improved the ability of the regression models to predict $\dot{V}O_{2max}$ accurately in Japanese women when compared with the use of age, either BMI or WC, and SC alone. Similar to the Cao et al. (2010) study, the present study came to a similar prediction accuracy and model stability, as evidenced by the similar R^2 and SEE values, and minor shrinkage of the R^2 and increment of SEE in the PRESS procedure. The new non-exercise equations in the present study resulted in a validity coefficient of R ranging from 0.85 to 0.86 for

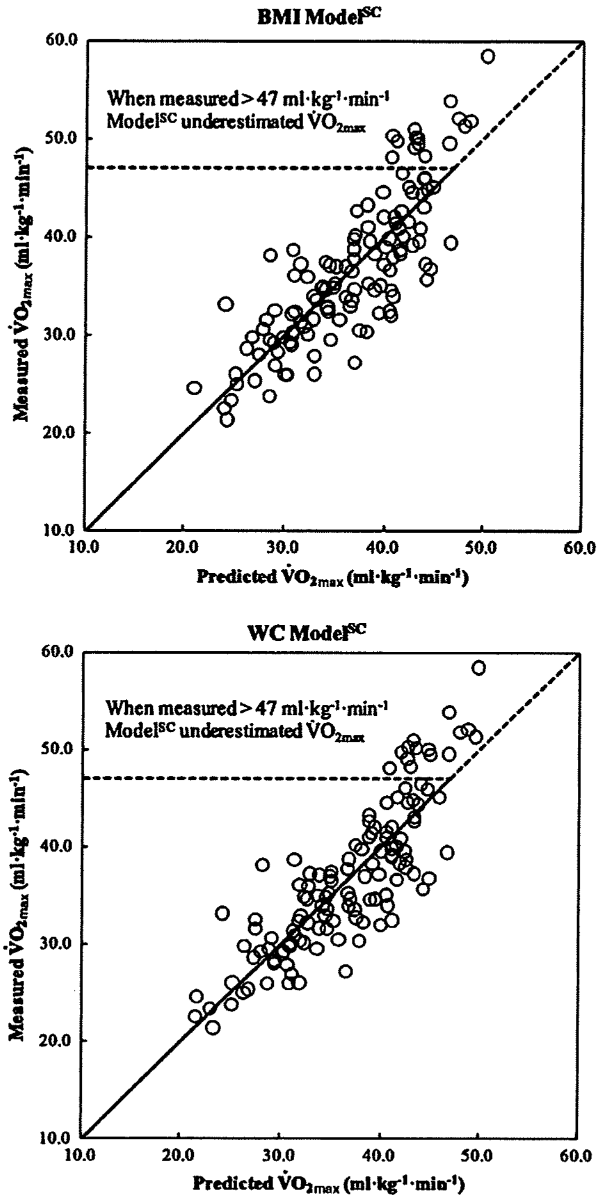

Fig. 1 Relationships between the measured and predicted $\dot{V}O_{2max}$ values for the multiple regression model^{SC} in the entire sample ($n = 127$). The *solid line* is the line of equality (measured $\dot{V}O_{2max}$ = predicted $\dot{V}O_{2max}$). The areas within the *dashed lines* show where the model^{SC} tends to underestimate $\dot{V}O_{2max}$

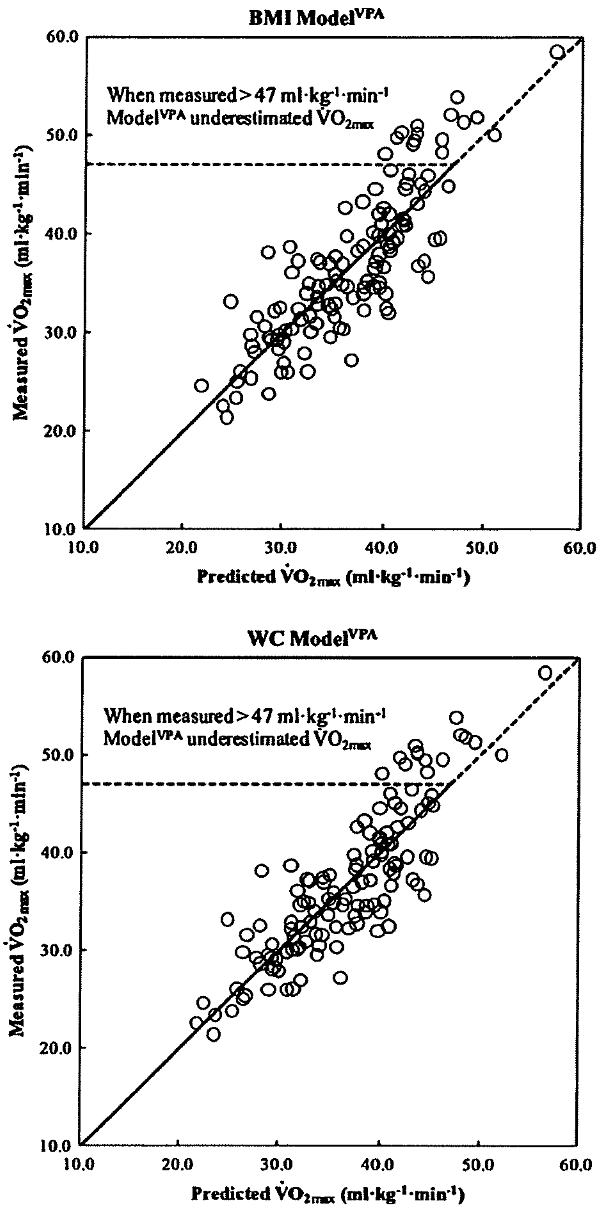

Fig. 2 Relationships between the measured and predicted $\dot{V}O_{2max}$ values for the multiple regression model^{VPA} in the entire sample ($n = 127$). The *solid line* is the line of equality (measured $\dot{V}O_{2max}$ = predicted $\dot{V}O_{2max}$). The areas within the *dashed lines* show where the model^{VPA} tends to underestimate $\dot{V}O_{2max}$

model^{VPA}, and a value of SEE ranging from 3.91 to 4.15 ml kg^{-1} min^{-1} for model^{VPA} (Table 3). Previously published non-exercise test prediction models reported varying success in predicting a measure of CRF, with SEE and R values ranging from 2.98 to 8.63 ml kg^{-1} min^{-1} and 0.46 to 0.88, respectively (Blair et al. 1989; Cao et al. 2009, 2010; George et al. 1997; Heil et al. 1995; Jackson et al. 1990; Jurca et al. 2005; Kohl et al. 1988; Malek et al. 2004, 2005; Plasqui and Westerterp 2005, 2006; Rankin et al. 1996; Siconolfi et al. 1985; Whaley et al. 1995; Wier et al. 2006). Therefore, the SEE and R values determined by the regression model in the present study were within the range of those associated with previous non-exercise methods for estimating $\dot{V}O_{2max}$. In addition, the %SEE for most submaximal exercise methods used to estimate $\dot{V}O_{2max}$ was 10–20% (McArdle et al. 2001). Therefore, the %SEE values (10.7–11.3%) determined by the regression model in the present study were equivalent to those reported for the most accurate submaximal exercise prediction methods for estimating $\dot{V}O_{2max}$. To examine the unique contribution of accelerometer-determined PA intensity variables in addition to the previous objectively measured PA variable, we used hierarchical linear regression to develop the model. In the present study, the regression equation yielded R^2 values ranging from 0.68 to 0.75 when using age, body composition (BMI or WC), and SC. The coefficient of determination increased to R^2 values ranging from 0.71 to 0.74 when VPA was added to the equations. However, unlike in a previously published study (Cao et al. 2010), when MVPA was added to the equations as a surrogate for VPA, MVPA was not found to be statistically significant in predicting $\dot{V}O_{2max}$ and, therefore, was dropped from the regression model. This difference may be explained by differences in the physical activity patterns of subjects (women vs. men) in both studies. In women (Cao et al. 2010), SC is moderately associated with MVPA ($r = 0.48$), whereas in men, MVPA and SC are more strongly related to each other ($r = 0.84$). Thus, when SC and MVPA were combined into one model, neither explained significantly more variation than the other in the present study. Those results suggested that VPA, but not MVPA, substantially improved the accuracy of the estimation of $\dot{V}O_{2max}$ in adult men when compared with the use of age, BMI or WC, and SC alone.

To estimate the prediction model's performance, we conducted two cross-validation analyses based on the PRESS and various subsamples of the sample. For the PRESS procedure, shrinkage of the R^2 (<0.02) and the increment of SEE (<0.06 ml kg^{-1} min^{-1}) for each prediction model were minor (Table 3). In the second stage of the cross-validation analysis, the CE values of both model$^{\text{SC}}$ and model$^{\text{VPA}}$ were small except for individuals at extremes of fitness. The results of two cross-validation analyses provide evidence supporting the validity of the prediction model used in the present study. Our finding of a significant underestimation of $\dot{V}O_{2max}$ among individuals with high fitness (Figs. 1, 2) has been consistently observed in previous studies (Cao et al. 2010; Jackson et al. 1990; Wier et al. 2006). The present study drew on a smaller estimation bias (<2 ml kg^{-1} min^{-1}) compared with the study by Wier et al. (<8 ml kg^{-1} min^{-1}). Wier et al. (2006) pointed out that estimating $\dot{V}O_{2max}$ for highly fit individuals is not a pressing problem for the typical work force because no negative consequences are seen due to high fitness. Furthermore, they suggested that the estimation bias can be corrected by modifying the intercept using the CE value.

This study has several limitations: first, the prediction model we developed may have limited generalizability because it was developed in a group of relatively healthy Japanese men aged from 20 to 69 years. The stability of the predicted $\dot{V}O_{2max}$ values using the present model is unknown in groups of individuals whose characteristics vary substantially from the range of characteristics in our study samples (e.g., children and adolescents, and individuals with metabolic syndrome, and other racial groups) or whose PA were measured by other accelerometers, since the relationship between objectively measured PA and $\dot{V}O_{2max}$ in such groups may have different characteristics than in our study. Further investigation is required to validate our prediction models in these groups. Second, accelerometers do not capture all types of PA, such as cycling or swimming, which may weaken the accuracy of our prediction model when our prediction models are applied in individuals who regularly exercise by riding a bike or swimming. Therefore, further study is needed to validate our prediction models in these individuals.

To our knowledge, this study marks the first attempt to develop new non-exercise $\dot{V}O_{2max}$ prediction models using accelerometer-determined objective PA variables as well as additional covariates including age and body composition in Japanese adult men that can be used in large epidemiological cohorts. This study demonstrated that objectively measured PA variables including SC, MVPA, and VPA are potential predictors of CRF in Japanese adult men. The present study also demonstrated that combining SC with VPA, but not with MVPA, was useful in predicting $\dot{V}O_{2max}$ variance and improved the ability of the regression models to accurately predict $\dot{V}O_{2max}$. In addition, because each of these predictor variables is easily obtained, it is believed that the non-exercise $\dot{V}O_{2max}$ prediction model using SC and VPA as a surrogate for the PA variable can be a routine component of primary healthcare examinations for men in healthcare settings and large epidemiological cohorts.

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ORIGINAL ARTICLE

Age and cardiorespiratory fitness are associated with arterial stiffening and left ventricular remodelling

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Arterial stiffening, hypertension and left ventricular (LV) remodelling are associated with increased risk of cardiovascular disease. Cardiorespiratory fitness is associated with cardiovascular function and reduced risk of cardiovascular disease. This cross-sectional study was carried out to determine the relationships between cardiorespiratory fitness, arterial stiffness, blood pressure (BP) and LV remodelling in women. On the basis of peak oxygen uptake, a total of 159 premenopausal (young) and postmenopausal (older) women were categorized into either low (unfit) or high (fit) cardiorespiratory fitness groups. The arterial stiffness and LV remodelling were measured by brachial-ankle pulse wave velocity (baPWV) and carotid augmentation index (AI) and LV relative wall thickness (RWT). Two-way analysis of variance indicated a significant interaction between age and cardiorespiratory fitness in baPWV, carotid AI, BP and RWT. In the older group, arterial stiffness (baPWV; 1401 ± 231 vs 1250 ± 125 cm s^{-1}, $P<0.01$, AI; 32.9 ± 9.9 vs 24.8 ± 10.1%, $P<0.01$), systolic blood pressure (SBP) (130 ± 22 vs 117 ± 15 mm Hg, $P<0.01$) and RWT (0.47 ± 0.08 vs 0.42 ± 0.04, $P<0.05$) in fit women were lower than in unfit women. In older women, RWT was significantly related to baPWV ($r=0.46$, $P<0.01$), carotid AI ($r=0.29$, $P<0.05$), SBP ($r=0.57$, $P<0.01$) $\dot{V}_{2\mathrm{peak}}$ ($r=-0.32$, $P<0.05$). In young women, they were not significant correlations, except for a weak correlation between RWT and SBP ($r=0.21$, $P<0.05$). These results suggest that higher cardiorespiratory fitness is associated with lower arterial stiffness, BP and RWT in older women.

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Introduction

The cardiovascular system is affected by ageing. Arterial stiffness increases progressively with advancing age even in healthy men and women.[1] This arterial stiffening is associated with future hypertension[2] and death from cardiovascular disease.[3] Moreover, left ventricular (LV) relative wall thickness (RWT) (ratio of wall thickness to chamber radius) also increases with age (LV remodelling).[4] LV remodelling is often associated with LV systolic and diastolic dysfunctions[5] and all-cause mortality.[6] The risk of cardiovascular disease in women increases sharply after menopause,[7] which is associated in part with arterial stiffening, hypertension and LV remodelling.[8] Accordingly, the prevention and treatment of age-related arterial stiffening, hypertension and LV remodelling in women are of great clinical importance.

Cardiorespiratory fitness is strongly associated with risk of cardiovascular disease[9] and high blood pressure (BP).[10] Previous studies have indicated that age-related increases in arterial stiffness were attenuated in higher-fit adults.[11,12] Moreover, arterial stiffness and BP were negatively associated with cardiorespiratory fitness.[13] When considering the pathophysiological implications of vascular stiffening, it is also important not to overlook changes in the heart to which the blood vessels are coupled. In accordance with the concept of 'vascular–ventricular coupling' in that morphological and functional changes in the left ventricle and vasculature are closely coupled, we hypothesized that higher cardiorespiratory fitness is associated with the smaller age-related increases in arterial stiffness and BP, and that smaller increases attenuate LV

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