予防に必要な$\dot{V}O_{2max}$の基準値に達した者である．このような持久力の高い者にとって，最大酸素摂取量の過小評価すること（わずか7.7%）は，公衆衛生上の問題は少ないと考えられる．

現在，生活習慣病発症の予防が注目されており，その対策として重要な点は一定の体力水準を維持することと言われている．そのためには特に全身持久力の向上は重要な要素であり，健康に関する体力の重要な指標の一つである全身持久力を評価し，各個人の体力を把握したうえ，適切な身体活動量を決定することは，重要な事項であることが考えられる．本研究が開発した$\dot{V}O_{2max}$の推定式は20～69歳の健康な成人男性を対象としており，今後女性や様々な年齢の方に応用できる推定式を作成するために，適切な被験者を対象とした研究が必要であると考えられる．

V. ま と め

$\dot{V}O_{2max}$の推定式の作成に用いた独立変数（年齢，身体組成及び3分間歩行距離）は，いずれも$\dot{V}O_{2max}$と有意な相関関係があった．3分間歩行距離は$\dot{V}O_{2max}$を反映する重要な独立した予測因子であることが示唆された．また，それにより得られた推定式の精度は高く，$\dot{V}O_{2max}$を妥当に評価できる測定方法であり，日本人男性の全身持久力を評価する指標として有用であることが示唆された．

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Poor trunk flexibility is associated with arterial stiffening

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[1]*Health Promotion and Exercise Program, National Institute of Health and Nutrition, Tokyo, and* [2]*Waseda University, Saitama, Japan;* [3]*University of North Texas Health Science Centre, Fort Worth, Texas; and* [4]*Ritsumeikan University, Siga, and* [5]*International Pacific University, Okayama, Japan*

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Yamamoto K, Kawano H, Gando Y, Iemitsu M, Murakami H, Sanada K, Tanimoto M, Ohmori Y, Higuchi M, Tabata I, Miyachi M. Poor trunk flexibility is associated with arterial stiffening. *Am J Physiol Heart Circ Physiol* 297: H1314–H1318, 2009. First published August 7, 2009; doi:10.1152/ajpheart.00061.2009.—Flexibility is one of the components of physical fitness as well as cardiorespiratory fitness and muscular strength and endurance. Flexibility has long been considered a major component in the preventive treatment of musculotendinous strains. The present study investigated a new aspect of flexibility. Using a cross-sectional study design, we tested the hypothesis that a less flexible body would have arterial stiffening. A total of 526 adults, 20 to 39 yr of age (young), 40 to 59 yr of age (middle-aged), and 60 to 83 yr of age (older), participated in this study. Subjects in each age category were divided into either poor- or high-flexibility groups on the basis of a sit-and-reach test. Arterial stiffness was assessed by brachial-ankle pulse wave velocity (baPWV). Two-way ANOVA indicated a significant interaction between age and flexibility in determining baPWV ($P < 0.01$). In middle-aged and older subjects, baPWV was higher in poor-flexibility than in high-flexibility groups (middle-aged, 1,260 ± 141 vs. 1,200 ± 124 cm/s, $P < 0.01$; and older, 1,485 ± 224 vs. 1,384 ± 199 cm/s, $P < 0.01$). In young subjects, there was no significant difference between the two flexibility groups. A stepwise multiple-regression analysis ($n = 316$) revealed that among the components of fitness (cardiorespiratory fitness, muscular strength, and flexibility) and age, all components and age were independent correlates of baPWV. These findings suggest that flexibility may be a predictor of arterial stiffening, independent of other components of fitness.

arteriosclerosis; blood pressure; prevention; fitness

ARTERIAL STIFFNESS has been identified as an independent risk factor for mortality and cardiovascular disorders (2, 16, 17, 22). Higher levels of physical fitness, especially cardiorespiratory fitness, appear to delay an age-related arterial stiffening (4, 25). Although flexibility is one of the components of physical fitness, the relationship between flexibility and arterial stiffness remains unclear.

Structurally, arterial stiffness is determined by the intrinsic elastic properties of smooth muscle and/or connective tissue (e.g., elastin-collagen composition) in the artery (19). Flexibility is also determined by skeletal muscle and/or connective tissue in the tendon, ligaments, and fascia (1). Age-related alterations in the muscles or connective tissues in the arteries may correspond to similar age-related alterations in the whole body (8). Accordingly, we hypothesized that a less flexible body would indicate arterial stiffening. To test our hypothesis, we examined the relationship between flexibility and arterial stiffness in three age categories using a cross-sectional study.

METHODS

Subjects. A total of 526 adults (178 males and 348 females), 20 to 39 yr of age (young), 40 to 59 yr of age (middle-aged), and 60 to 83 yr of age (older), participated in this study. All subjects were nonobese (body mass index < 30) and free of overt chronic diseases as assessed by medical history, physical examination, and complete blood chemistry and hematological evaluation (e.g., plasma glucose concentration < 126 mg/dl, and total cholesterol < 240 mg/dl). Candidates who smoked in the past 4 yr, were taking medications, or had characteristics of peripheral arterial disease [ankle-brachial index (ABI) < 0.9] were excluded. The purpose, procedures, and risks of the study were explained to each participant before inclusion, and all subjects gave their written, informed consent before participating in the study, which was approved by the Human Research Committee of the National Institute of Health and Nutrition. All subjects were recruited to the same site (National Institute of Health and Nutrition). The study was performed in accordance with the guidelines of the Declaration of Helsinki.

To assess the effects of flexibility on arterial stiffness, the subjects in each age category were divided into either poor- or high-flexibility groups on the basis of the mean value of a sit-and-reach test every 10 yr of age in each sex. We attempted to isolate the influence of flexibility as much as possible. To do so, poor- and high-flexibility groups were carefully matched for age, height, weight, and metabolic risk factors.

Before they were tested, the subjects abstained from caffeine and fasted for at least 4 h (a 12-h overnight fast was used to determine arterial stiffness and blood pressure). Subjects also abstained from heavy exercise for at least 24 h to avoid the immediate (acute) effects of exercise. All subjects were tested between 9:00 AM and 12:00 AM.

Arterial stiffness. After 10 min of quite rest in the supine position, subjects were studied in the supine position. Bilateral brachial and ankle blood pressures were simultaneously measured with a vascular testing device (form PWV/ABI; Omron Colin, Kyoto, Japan). Bilateral brachial and ankle arterial pressure waveforms were stored for 10 s by extremity cuffs connected to a plethysmographic sensor and an oscillometric pressure sensor wrapped on both arms and ankles. The brachial-ankle pulse wave velocity (baPWV) was calculated from the distance between two arterial recording sites divided by the transit time (14, 21, 28). The value of baPWV mainly reflects stiffness in the central arteries (21, 28), because baPWV correlates well with the aortic PWV using a catheter tip with a pressure manometer (28). The mean value of right and left baPWV was obtained for analysis. The standard deviation of the differences for interobserver reproducibility was 51 cm/s in our laboratory (3, 28).

In 309 (107 males and 202 females) of the pooled population, brachial, ankle, carotid, and femoral arterial pulse waves were simultaneously measured with the vascular testing device (form PWV/ABI; Omron Colin) for assessing aortic PWV and femoral-ankle PWV (faPWV). Carotid and femoral arterial pressure waveforms were

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stored for 30 s by applanation tonometry sensors attached on the left common carotid and left common femoral arteries. Aortic PWV was calculated from the distance between the carotid and femoral artery sites divided by the transit time. The standard deviation of the differences for interobserver reproducibility was 62 cm/s in our laboratory. The faPWV was calculated from the distance between the femoral and ankle artery sites divided by the transit time. The standard deviation of the differences for interobserver reproducibility was 44 cm/s in our laboratory (3, 28).

Flexibility. Flexibility was measured by a sit-and-reach test using a digital flexibility testing device (T.K.K.5112; Takeikiki, Tokyo, Japan) after some stretching. The device displays the distance which the device moved. Subjects sat on the floor, attaching their hip, back, and occipital region of the head to a wall, with legs held straight by a tester. They put both hands on the device, with arms held straight. In the position, zero point of the device was set. They were then asked to bend forward slowly and reach as far forward as possible. The best of two trials was recorded (24). The standard deviation of the differences for interobserver reproducibility was 2.3 cm in our laboratory.

Cardiovascular fitness and muscular strength. Physical fitness is mainly composed of cardiorespiratory fitness, muscular strength and endurance, and flexibility. To examine the relationship among flexibility, cardiorespiratory fitness, and muscular strength in determining arterial stiffness, we measured peak oxygen uptake as an indicator of cardiorespiratory fitness and leg extension power as an indicator of muscular strength. The leg extension power was determined using a dynamometer (Anaero Press 3500; Combi Wellness, Tokyo, Japan) in the sitting position. The subjects were advised to vigorously extend their legs. Five trials were performed at 15-s intervals, and the average of the two highest recorded power outputs (in W) was taken as the definitive measurement (29). The peak oxygen uptake was determined by incremental cycle ergometer exercise (27). The highest value of oxygen uptake during the exercise test was designated as peak oxygen uptake. Because the test requires an incremental cycle exercise to exhaustion, subjects could determine their participation in the test. Three hundred sixteen (95 males and 221 females) subjects of the pooled population participated in the test.

Statistical analysis. All data are presented as means ± SE. The data were analyzed by two-way ANOVA (age × flexibility) and analysis of covariance (ANCOVA) that included sex as a covariate. In the case of a significant F value, a post hoc test with Scheffé's method identified significant differences among mean values. Univariate regression and correlation analyses were used to analyze the relationships between variables of interest. Stepwise multiple regression analysis was used to determine the influences of age, sit-and-reach, peak oxygen uptake, and leg power on baPWV. Differences were considered significant when $P < 0.05$.

RESULTS

Table 1 shows the subject characteristics. In each age category, age, height, weight, and all metabolic risk factors did not differ between high-flexibility and poor-flexibility groups. In middle-aged and older subjects, the systolic blood pressure was higher in poor-flexibility than in high-flexibility groups. In middle-aged subjects, the pulse pressure was higher in the poor-flexibility than in the high-flexibility groups.

Table 2 shows the effects of age and flexibility on baPWV, aortic PWV, and faPWV. In baPWV, two-way ANOVA indicated a significant interaction between age and flexibility in determining baPWV ($P < 0.05$). Within both flexibility groups, baPWV was higher in middle-aged and older subjects compared with young subjects. The baPWV was also higher in older subjects compared with middle-aged subjects. Most importantly, in middle-aged and older subjects, baPWV was higher in the poor-flexibility than in the high-flexibility groups. The differences remained significant after normalizing baPWV for sex when analyzed by ANCOVA. In the young subjects, there was no significant difference between the two flexibility groups. In aortic PWV, two-way ANOVA indicated a significant interaction ($P < 0.05$). In middle-aged and older subjects, aortic PWV was higher in the poor-flexibility than in the high-flexibility groups. The differences remained significant after normalizing aortic PWV for sex when analyzed by ANCOVA. In faPWV, there were no significant differences between the two flexibility groups in each age category.

Figure 1 shows the relationships between sit-and-reach and baPWV (*A*) or aortic PWV (*B*) in each age category. The

Table 1. *Characteristics of the subjects*

	Young		Middle-Aged		Older	
	High	Poor	High	Poor	High	Poor
N	98	92	104	100	71	61
Age, yr	26±1	26±1	49±1*	49±1*	67±1*†	67±1*†
Height, cm	169±1	168±1	161±1*	159±1*	156±1*†	155±1*†
Weight, kg	60±1	61±1	61±1	60±1	55±1*†	54±1*†
SBP, mmHg	110±1	109±1	116±1*	121±1*‡	124±2*†	129±2*†‡
DBP, mmHg	62±1	62±1	70±1*	72±1*	72±1*	74±1*
PP, mmHg	48±1	48±1	45±1	48±1‡	52±1*†	55±2*†
Hypertension, %	0	0	8	11	20*†	30*†
Heart rate, beats/min	57±1	56±1	61±1*	62±1*	59±1	57±1
Total cholesterol, mmol/l	4.48±0.07	4.52±0.07	5.13±0.05*	5.19±0.05*	5.35±0.06*†	5.27±0.07*
HDL cholesterol, mmol/l	1.61±0.04	1.57±0.03	1.72±0.04	1.71±0.04*	1.68±0.04	1.68±0.05
Plasma glucose, mmol/l	4.90±0.04	4.88±0.04	5.14±0.05*	5.21±0.04*	5.22±0.05*	5.24±0.08*
Sit-and-reach, cm	47±1	32±1‡	46±1	31±1‡	41±1*†	26±1*†‡
Leg extension power, W/kg	23±1	22±1	18±1*	17±1*	14±1*†	13±1*†
N	62	62	82	57	27	26
Peak oxygen uptake, $ml \cdot min^{-1} \cdot kg^{-1}$	37±1	36±1	32±1*	29±1*‡	28±1*†	25±1*†

Values are means ± SE; *N*, number of subjects; high and poor, high-flexibility and poor-flexibility groups, respectively; SBP and DBP, brachial systolic blood pressure and diastolic blood pressure, respectively; PP, brachial pulse pressure. Hypertension ≥ 140/90 mmHg. The criterion for division between 2 groups was the mean value of the sit-and-reach test every 10 yr of age in each sex in this population. *$P < 0.05$ vs. young within same flexibility group; †$P < 0.05$ vs. middle-aged within same flexibility group; ‡$P < 0.05$ vs. high-flexibility within same age category.

Table 2. *Arterial stiffness in high- or poor-flexibility groups*

	Young		Middle-Aged		Older	
	High	Poor	High	Poor	High	Poor
N	98	92	104	100	71	61
baPWV, cm/s	1,080±12	1,085±11	1,200±12*	1,260±14*‡	1,384±24*†	1,485±29*†‡
N	38	37	82	84	37	31
Aortic PWV, cm/s	732±18	731±17	788±9*	825±12*‡	902±24*†	1,004±29*†‡
faPWV, cm/s	871±15	849±14	916±10*	970±26*	984±14*†	1,016±23*

Values are means ± SE; *N*, number of subjects. baPWV, brachial-ankle pulse wave velocity (PWV); aortic PWV, carotid-femoral PWV; faPWV, femoral-ankle PWV. The criterion for division between 2 groups was the mean value of the sit-and-reach test every 10 yr of age in each sex in this population. *$P < 0.05$ vs. young within same flexibility group; †$P < 0.05$ vs. middle-aged within same flexibility group; ‡$P < 0.05$ vs. high-flexibility within same age category.

baPWV and aortic PWV correlated with sit-and-reach in middle-aged (Fig. 1, *middle*) and older (Fig. 1, *right*) subjects. In young subjects (Fig. 1, *left*), there were no relationships. The slope of the relationship was steeper in older subjects than in middle-aged subjects in both baPWV and aortic PWV ($P < 0.001$).

A univariate regression analysis indicated that sit-and-reach positively correlated with peak oxygen uptake ($r = 0.20$, $P < 0.001$) and leg power ($r = 0.13$, $P < 0.05$). The analysis also indicated that baPWV negatively correlated with peak oxygen uptake ($r = -0.37$, $P < 0.001$) and leg power ($r = -0.32$, $P < 0.001$). A stepwise multiple-regression analysis revealed that among the components of fitness and age, sit-and-reach ($\beta = -0.14$), peak oxygen uptake ($\beta = -0.12$), leg power ($\beta = 0.17$), and age ($\beta = 0.61$) were independent correlates of baPWV.

DISCUSSION

The key new findings of the present study are as follows. First, in middle-aged and older subjects, arterial stiffness deteriorated in the poor-flexibility groups compared with the high-flexibility groups. Second, a negative relationship between flexibility and arterial stiffness was observed in middle-aged and older subjects, but there were no relationships in young subjects. These results support our hypothesis that a less flexible body indicates arterial stiffening, especially in middle-aged and older adults. Furthermore, age-related arterial stiffening was greater (~30% in baPWV) in the poor-flexibility than in the high-flexibility groups, which suggests that poor flexibility is associated with greater age-related arterial stiffening.

In general, because habitual exercise includes flexibility exercise (e.g., stretching during warming up or cooling down), an active person may tend to be more flexible than an inactive one (11). In fact, a positive relationship between cardiorespiratory fitness and flexibility was observed in the present study. It is well known that cardiorespiratory fitness was inversely related to arterial stiffness (25). The present study also showed that both peak oxygen uptake and leg power are inversely related to baPWV. Stepwise multiple-regression analysis re-

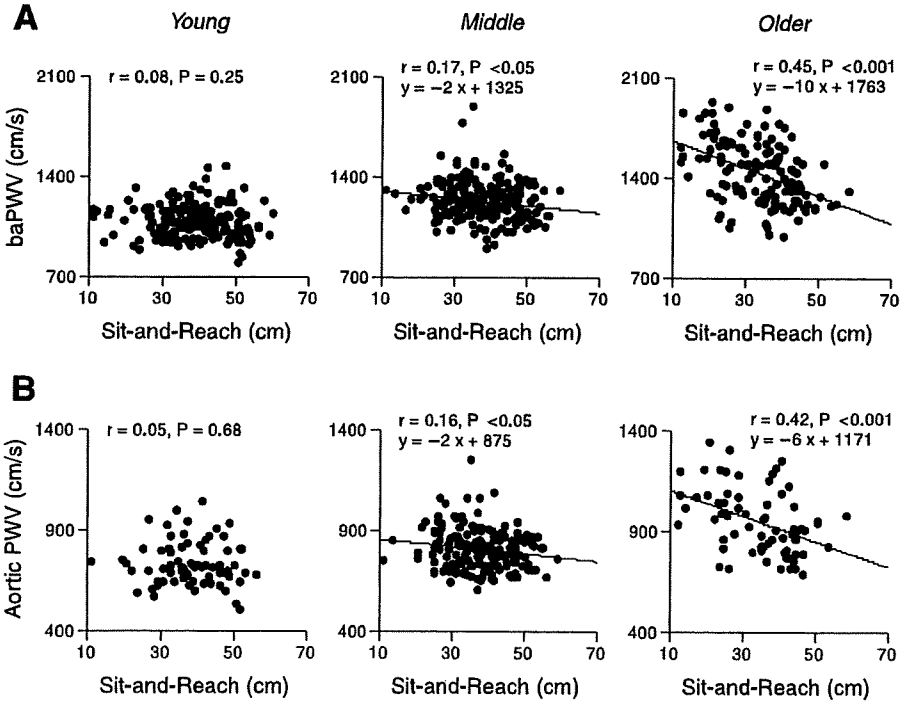

Fig. 1. Relationships between sit-and-reach and brachial-ankle pulse wave velocity (baPWV; *A*) or aortic PWV (*B*) in each age category. The baPWV and aortic PWV correlated with sit-and-reach in middle-aged (*middle*) and older (*right*) subjects. In both baPWV and aortic PWV, slope of the relationship was steeper in older subjects than in middle-aged subjects ($P < 0.001$).

vealed that among the components of fitness and age, sit-and-reach was an independent correlate of baPWV. These findings statistically support the idea that flexibility is identified as a determinant or predictor of arterial stiffness, independent of other components of fitness. On the other hand, the peak oxygen uptake was also an independent correlate of baPWV. This result may indicate that subjects who have low cardiorespiratory fitness have higher arterial stiffness than high cardiorespiratory fitness subjects in high-flexibility groups. The same might apply for physical activity. The interaction among flexibility and other components of fitness or physical activity in determining the arterial stiffness awaits further studies.

Recently, Cortez-Cooper et al. (7) examined the effects of strength training on central arterial compliance in middle-aged and older adults. In this previous study, a stretching exercise group was included as a control group. An unexpected finding of the study was that a stretching program significantly increased carotid arterial compliance. Together with our results, these findings suggest a possibility that improving flexibility induced by the stretching exercise may be capable of modifying age-related arterial stiffening in middle-aged and older adults.

Ehlers-Danlos syndrome is a rare connective tissue disorder inherited as an autosomal-dominant trait. As a result, the patients are pathologically hyperflexible. A previous study showed abnormally low values of aortic PWV in the ecchymotic Ehlers-Danlos syndrome (10). Furthermore, Boutouyrie et al. (5) reported that carotid distensibility was 27% higher in the vascular type Ehlers-Danlos syndrome than in control subjects. Thus patients with Ehlers-Danlos syndrome are less likely to have stiff arteries. In contrast, people with spinal cord injury seem to be immobile subjects, indicating the loss of ligamentous laxity. A recent study showed that the aortic PWV among people with spinal cord injuries was higher than that in control subjects (18). These pathological observations are in line with the present results.

We can only speculate on the mechanisms responsible for the greater age-related arterial stiffening in the poor-flexibility groups. First, both arterial stiffness and flexibility may be structurally determined by similar compositions such as the muscles or connective tissues (e.g., elastin-collagen composition) (19). Thus age-related alterations in arterial stiffness may correspond to age-related alterations in flexibility within the same individual. Second, arterial stiffness is functionally determined by the vascular tone of the artery (19). Vascular tone is partially regulated by sympathetic nerve activity. Stretching of skeletal muscle causes an increase in sympathetic nerve activity via the central nervous system (26). Repetitive stimulation of transient sympathoexcitation induced by habitual stretching exercises, which improve flexibility, may chronically reduce resting sympathetic nerve activity. This reduction in sympathetic nerve activity may result in a decrease in arterial stiffness. On the other hand, the higher sympathetic nerve activity elevates blood pressure. In middle-aged and older subjects, systolic blood pressure in the poor-flexibility group was higher than in the high-flexibility group (Table 1). Elevated blood pressure can increase arterial stiffness (19). In this regard, heart rate (HR) appears to be low for young and older subjects, suggesting a well-conditioned population. If sympathetic nerve activity in poor-flexibility groups is higher than that in high-flexibility groups, then HR might be higher in poor-flexibility groups. Although sympathetic nerve activity increases with age, HR appears unchanged because of age-related decrease in intrinsic HR (6, 15). Further experimental studies are needed to verify the proposed mechanisms related to the present findings.

Our findings have potentially important clinical implications. Trunk flexibility can be easily evaluated over all ages and in any practical fields. Thus a measurement of flexibility as a physical fitness might contribute to assist in the prevention of age-related arterial stiffening. Stretching is widely recommended for injury prevention despite the limited evidence (9). In addition to the recommendation, we believe that flexibility exercise such as stretching, yoga, and pilates would be integrated as a new recommendation into the known cardiovascular benefit of regular exercise. However, although the present results are the first to provide evidence demonstrating that poor flexibility is associated with greater age-related arterial stiffening, the present cross-sectional study provided only associations among age, flexibility, and arterial stiffness. An intervention study is also needed to determine the cause-and-effect relationship between flexibility and arterial stiffness.

We used baPWV for an estimation of arterial stiffness. A major advantage of baPWV is its simple way of measurement by only wrapping the four extremities with blood pressure cuffs. This technique does not need the refined technique of applanation tonometry that is required for the measurements of aortic PWV. Although the value of baPWV mainly reflects stiffness in the central arteries (21, 28), baPWV includes stiffness from the brachial part, the ascending and descending aorta, and the abdominal aorta and leg part. When compared with central elastic arteries, peripheral arteries are generally considered to be of less clinical significance (20). Aortic PWV has been directly linked with cardiovascular mortality and morbidity (2, 16, 17, 22). In the present study, the same results as the baPWV were obtained by the use of the aortic PWV. (Table 2, and Fig. 1). In contrast, faPWV did not differ between the poor-flexibility and high-flexibility groups (Table 2). Therefore, we believe that baPWV provides qualitatively similar information as that derived from aortic PWV in this cross-sectional study and that the faPWV may be less sensitive to physical fitness or daily activity compared with the baPWV and aortic PWV (13, 23).

The present study has several limitations. First, we used the sit-and-reach test as an indicator of flexibility. The sit-and-reach test may be differentially influenced by arm and leg length or sex. In the present study, we set an individual zero point for each subject (see flexibility in METHODS for details). Thus the effects of arm and leg length were few. Furthermore, the differences between the two flexibility groups remained significant after normalizing baPWV and aortic PWV for sex when analyzed by ANCOVA. Although the sit-and-reach test has been commonly used to assess flexibility as health-related fitness, the test reflects trunk flexibility. We did not examine the flexibility of other regions such as neck, shoulder, and/or lower extremity. Further investigations are required to improve our understanding of the relationship between flexibility and arterial stiffness. Second, subjects in the present study included premenopausal women. The elastic properties of central arteries fluctuate with the phases of the menstrual cycle (12). However, we did not monitor the menstrual phase in the present study. Thus, if premenopausal women in this popula-

tion are tested during the early follicular phase, the relationship between flexibility and arterial stiffness could be analyzed more accurately.

In conclusion, the present results indicate that poor flexibility is associated with greater age-related arterial stiffening. The association was independent of cardiorespiratory fitness and muscular strength. These findings suggest the possibility that flexibility may be a predictor of arterial stiffening, independent of other components of fitness.

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Predicting $\dot{V}O_{2max}$ with an Objectively Measured Physical Activity in Japanese Women

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[1]*Health Promotion and Exercise Program, National Institute of Health and Nutrition, Tokyo, JAPAN;* [2]*Okayama Southern Institute of Health, Okayama Health Foundation, Okayama, JAPAN;* and [3]*Faculty of Sport Sciences, Waseda University, Saitama, JAPAN*

ABSTRACT

CAO, Z., N. MIYATAKE, M. HIGUCHI, M. MIYACHI, K. ISHIKAWA-TAKATA, and I. TABATA. Predicting $\dot{V}O_{2max}$ with an Objectively Measured Physical Activity in Japanese Women. *Med. Sci. Sports Exerc.*, Vol. 42, No. 1, pp. 179–186, 2010. **Purpose**: To investigate the use of the accelerometer-determined physical activity (PA) intensity variables as the objective PA variables for estimating $\dot{V}O_{2max}$ in Japanese adult women. **Methods**: The subjects of this study were 148 Japanese women aged 20 to 69 yr. 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 for 7 consecutive days. Using data of age, SC, MVPA, or VPA, and either body mass index (BMI) or waist circumference (WC), the nonexercise $\dot{V}O_{2max}$ prediction models were derived as BMI models^{MVPA}, WC models^{MVPA}, BMI models^{VPA}, and WC models^{VPA}, and cross-validated by using two separate cross-validation procedures. **Results**: SC, MVPA, and VPA were significantly related to $\dot{V}O_{2max}$ (r = 0.43, r = 0.52, and r = 0.58, respectively). The multiple correlation coefficients for the BMI and WC models^{MVPA} were 0.83 and 0.85, respectively, and for the BMI and WC models^{VPA}, they were 0.85 and 0.86, respectively. The SEE was 3.3 and 3.1 mL·kg^{-1}·min^{-1} for the BMI and WC models^{MVPA}, respectively, and it was 3.1 and 3.0 mL·kg^{-1}·min^{-1} for the BMI and WC models^{VPA}, respectively. All regression models demonstrated a high level of cross-validity supported by the minor shrinkage of the coefficient of determination and the increment of SEE in the predicted residual sum of squares procedure, and by small constant errors for the subgroups of age, SC, and $\dot{V}O_{2max}$ between 25 and 35 mL·kg^{-1}·min^{-1}. **Conclusions**: This study demonstrated that multiple regression models using data of MVPA or VPA were useful in predicting $\dot{V}O_{2max}$ for Japanese adult women.
Key Words: CARDIORESPIRATORY FITNESS, MAXIMAL OXYGEN UPTAKE, ACCELEROMETER, PREDICTION MODELS, INTENSITY

Cardiorespiratory fitness is known to be an objective, reproducible measure that reflects the functional consequences of physical training (36) and recent physical activity (PA) habits, and is a powerful predictor of chronic disease morbidity and mortality. Prospective observational studies have shown that low cardiorespiratory fitness is strongly associated with the risk for developing coronary heart disease (13), hypertension (1), type 2 diabetes mellitus (32), and metabolic syndrome (20) as well as mortality from cardiovascular disease (13,17), cancer (33), and all causes of mortality (17). Hence, cardiorespiratory fitness has been suggested to be included in the European Health Monitoring System for the adult population (35), National Health and Nutrition Examination Survey in the United States (4), and the Exercise and Physical Activity Reference for Health Promotion 2006 in Japan (26) in which the recommended reference value for maximal oxygen uptake ($\dot{V}O_{2max}$) to prevent lifestyle-related disease was reported. Although cardiorespiratory fitness is an important health indicator, cardiorespiratory fitness assessment is usually not performed in many health care settings because of the absence of feasible and practical assessment methods.

The impracticality of maximal and submaximal exercise tests for the assessment of cardiorespiratory fitness in the general public is well recognized. Therefore, a variety of nonexercise prediction models (2,7,12,15,21,22,30,38) have been developed as alternative approaches to fitness assessment and the estimation of $\dot{V}O_{2max}$. Those nonexercise models are effective for use in large epidemiological cohorts in which exercise tests to predict or measure

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$\dot{V}O_{2max}$ would be impractical. Those previous reports, however, have relied on a subjective self-reported 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 (31). 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. To overcome the deficiencies of existing nonexercise prediction models, Cao et al. (3) developed a nonexercise prediction model for estimating $\dot{V}O_{2max}$ using an objectively measured PA variable, pedometer-determined daily step counts (SC). They demonstrated that SC was useful in predicting $\dot{V}O_{2max}$ variance and helped their nonexercise $\dot{V}O_{2max}$ prediction model generate relatively accurate estimations of $\dot{V}O_{2max}$ in Japanese women. However, SC alone does not discriminate the intensity of movement or reflect the amount of time spent in specific intensity categories of PA, which may weaken the accuracy of that prediction model. The need for further study to investigate the ability of PA intensity variables to improve their prediction accuracy has been emphasized.

Accelerometers are widely accepted as valid objective measurement tools that allow researchers to estimate how much energy individuals are expending, as well as to quantify the SC and the amount of time spent in light, moderate, and vigorous PA (VPA), which correspond to <3 metabolic equivalents (METs), 3 to 6 METs and >6 METs, respectively. Recently, accelerometry has been used in the monitoring of the levels of PA in populations (9,25). In 2005, Plasqui and Westerterp (27,28) developed a nonexercise model for estimating $\dot{V}O_{2max}$ using a fitness index based on accelerometer counts and HR, and they cross-validated this model in 2006. They reported that this fitness index contributed significantly to the explained variation in $\dot{V}O_{2max}$, and the total explained variation of their nonexercise prediction model was 71%, with a SEE of 409 $mL \cdot min^{-1}$, or 13.7% of the average $\dot{V}O_{2max}$. However, this prediction model may be less feasible for use in certain clinical applications because of the cost and technical requirements for its use and because it uses a noncommercially available accelerometer. There are several accelerometry-based PA monitors that are commercially available. The Kenz Lifecorder (LC; SUZUKEN Co Ltd, Nagoya, Japan) 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 (19), over 24 h of typical daily activities undertaken in a respiratory chamber (19) and in a free-living environment using doubly labeled water as the criterion method (39). Furthermore, 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 (24). However, to our knowledge, there is no information on the prediction of $\dot{V}O_{2max}$ using the accelerometer-determined time spent in moderate to vigorous PA (MVPA) or VPA as the objective PA variables.

In the present study, we hypothesized that the accelerometer-determined PA intensity variables including the time spent in MVPA and VPA are potential predictors of cardiorespiratory fitness in Japanese women. To verify this hypothesis, the relationships between PA intensity variables and $\dot{V}O_{2max}$ were investigated. More specifically, the purpose of this study was to develop new nonexercise $\dot{V}O_{2max}$ prediction models using SC and the time spent in MVPA or VPA as the objective PA variables as well as additional covariates including age and body composition in Japanese women.

METHODS

The present study consists of three parts. First, the relationships between PA intensity variables and $\dot{V}O_{2max}$ were investigated. Second, validation procedures were used to develop new nonexercise prediction models that included body composition and objectively measured PA variables as predictor variables. Finally, the accuracy of the new nonexercise prediction models was assessed using two separate cross-validation procedures.

Subjects. One hundred and forty-eight Japanese women aged 20 to 69 yr participated in the present study. None of the subjects had any chronic diseases or were taking any medications that could affect the study variables. Seventy-six healthy women were tested in two independent institutions supervised by two of the coauthors N.M. and M.H., and 72 healthy women were tested in another institution by C.Z., M.M., and I.T. All subjects provided written informed consent according to local institute policy before the measurement of physical fitness. The research project was approved by the Ethical Committee of the National Institute of Health and Nutrition. The subjects' characteristics are described in Table 1.

Anthropometrics. Body mass was measured using an electronic scale (Inner Scan BC-600; Tanita Co., Tokyo, Japan) and was determined to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a stadiometer (YL-65; Yagami Inc., Nagoya, Japan). Body mass and height were measured with the subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated by dividing the body mass in kilograms by the square of height in meters ($kg \cdot m^{-2}$). Waist circumference (WC) was measured at the umbilical level with an inelastic measuring tape at the end of normal expiration to the nearest 0.1 cm.

Maximal aerobic power. $\dot{V}O_{2max}$ was measured using a maximal graded exercise test (GXT) with bicycle ergometers [Lode Excalibur (N.M.); Lode BV, Groningen, The Netherlands; Monark Ergomedic 828E (M.H., C.Z.), Varberg, Sweden]. The initial workload was 30–60 W, and

TABLE 1. Physical characteristics of the study subjects.

Variable	Mean ± SD *N* = 148	Range *N* = 148
Age (yr)	47.0 ± 12.5	20–69
Height (cm)	157.1 ± 5.6	141.9–172.4
Body mass (kg)	54.2 ± 8.4	34.6–91.0
BMI ($kg \cdot m^{-2}$)	22.0 ± 3.3	16.6–35.2
WC (cm)	77.8 ± 10.1	58.6–107.1
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	30.8 ± 5.9	19.5–51.0
SC ($steps \cdot d^{-1}$)	11,075 ± 4474	2996–31,048
MVPA (min)	26.6 ± 21.7	1.9–139.1
VPA (min)	2.5 ± 5.9	0.0–43.4

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

the work rate was increased thereafter by 15 $W \cdot min^{-1}$ until the subject could not maintain the required pedaling frequency (60 rpm). HR (WEP-7404; NIHON KOHDEN Corp., Tokyo, Japan) and a rating of perceived exertion were monitored throughout the exercise. During the progressive exercise test, the expired gas of subjects who were tested by two of the coauthors, NM and MH, was collected, and the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured and averaged over 30-s intervals using an automated breath-by-breath gas analyzing system [Aeromonitor AE-280S (M.H.); Minato Medical Science, Tokyo, Japan; Oxycon Alpha (N.M.), Mijnhardt b.v., The Netherlands]. The Aeromonitor AE-280S consists of a microcomputer, a hot-wire flow meter, and oxygen and carbon dioxide gas analyzers (a zirconium element-based oxygen analyzer and an infrared carbon dioxide analyzer). Gas was sampled at the rate of 220 $mL \cdot min^{-1}$ through a filter by a suction pump through the analyzers. The Oxycon Alpha consists of a microcomputer, a capillaryline, and oxygen and carbon dioxide gas analyzers (O_2, differential paramagnetic; CO_2, infrared absorption). Expiratory volumes were determined using a Triple V turbine volume sensor which was calibrated before each test according to the manufacturer's instructions. The systems were calibrated before each test with gases of known concentration. The expired air of subjects who were tested in the institution by C.Z., M.M., and I.T. (CZ) was collected over 30-s intervals in Douglas bags. An oxygen and carbon dioxide mass spectrometer (Arco-1000; Arco System, Ogaki, Japan) was used to analyze oxygen and carbon dioxide concentrations. The volume of expired air was determined using a dry gas volume meter (DC-5; Shinagawa Seisakusho, Tokyo, Japan) and converted to STPD. During the latter stages of the test, each subject was verbally encouraged by the test operators to give a maximal effort. Achievement of $\dot{V}O_{2max}$ was accepted if two of the following conditions were met: subject's maximal HR was > 95% the age-predicted maximal HR (220 − age), and the $\dot{V}O_2$ curve showed a leveling off.

Physical activity. PA was measured by activity monitors using a uniaxial acceleration sensor (LC) and a triaxial acceleration sensor (AM; Panasonic Electric Work Co., Ltd, Osaka, Japan). Subjects were instructed in 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. The activity monitor was firmly attached to their clothes at the waist with the aid of a clip. The technical and estimation equation details of the LC and AM have been described in previous studies (10,19,39). LC and AM have the same measurement range of acceleration, are similar in size, use simple regression models to convert acceleration into PA intensity, do not need any individual calibrations, and easily export the data from the software into an Excel spreadsheet. Previous studies have shown that both LC and AM have displayed reasonable estimates of energy expenditures under controlled conditions and in free-living conditions (19,39). Yamada et al. (39) found that the correlation between the total energy expenditure (TEE) measured by LC and the TEE measured by AM was high ($r = 0.94$, $P < 0.001$). They also found that no significant differences between the two activity monitors were observed in the time spent in MVPA and VPA. Furthermore, unpublished data from our institute showed that no significant differences between the two activity monitors were observed in SC ($N = 39$, average age 40.4 ± 9.6 yr, LC: 8557 ± 2573, AM: 8690 ± 2859, $P = 0.69$). The accelerometer-determined PA variables used in the present study for analyses included SC, MVPA, and VPA, but not TEE. Thus, it was reasonable to use two activity monitors in the present study, although Yamada et al. reported that the LC did not correlate with doubly labeled water (DLW)-derived TEE, and the AM did, after statistically controlling for the influence of age, weight, height, and %fat.

Statistical analyses. Measured and calculated values are presented as means ± 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), and SC into the first block and PA intensity variables into the second block. Because the outcome measurements were performed at different institutions and different activity monitors were used, the effects of institution and activity monitors were assessed by adding a dummy-coded institution variable and an activity monitor variable and then applying a multiple regression to determine whether the institution variable and activity monitor 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 SEE and relative SEE (%SEE). The new nonexercise prediction models were assessed using two separate cross-validation procedures, using the predicted residual sum of squares (PRESS) method (14) and various

TABLE 2. Correlations matrix of $\dot{V}O_{2max}$ and independent variables.

	$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	Age (yr)	BMI (kg·m^{-2})	WC (cm)	SC (steps·d^{-1})	MVPA (min)
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})						
Age (yr)	−0.55**	–				
BMI (kg·m^{-2})	−0.46**	0.17*	–			
WC (cm)	−0.62**	0.34**	0.85**	–		
SC (steps·d^{-1})	0.43**	0.05	−0.01	−0.06	–	
MVPA (min)	0.52**	−0.14	−0.13	−0.26**	0.48**	–
VPA (min)	0.58**	−0.15	−0.19*	−0.30**	0.39**	0.67**

N = 148; * $P < 0.05$. ** $P < 0.01$.
BMI, body mass index; SC, daily step counts; MVPA, moderate-to-vigorous physical activity; VPA, vigorous physical activity; WC, waist circumference.

subsamples of the entire sample. The PRESS method of cross-validation 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^2p) can be calculated as 1 − (PRESS/SStotal). The PRESS SEE (SEEp) can be calculated using the following equation: SEEp = $\sqrt{\text{PRESS}/n}$. 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 among these subgroupings (CE). All analyses were done with SPSS Advanced Models 16.0J for Windows (SPSS Japan Inc., Tokyo, Japan). The statistical significance level was set at $P < 0.05$.

RESULTS

Results from cardiorespiratory fitness 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 0.43 for SC to a high of −0.62 for WC, indicating that each independent variable was related to $\dot{V}O_{2max}$. The correlation coefficients between $\dot{V}O_{2max}$ and MVPA or VPA were significantly higher than the correlation coefficient between $\dot{V}O_{2max}$ and SC. 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 0.55, 0.54, and 0.60, 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, body composition, and SC, the addition of MVPA raised the R^2 from 0.648 to 0.694 for the BMI model^{MVPA} and from 0.681 to 0.716 for the WC model^{MVPA}, representing increases of 7.1% for the BMI model^{MVPA} and 5.1% for the WC model^{MVPA} in the explained variance of $\dot{V}O_{2max}$. VPA significantly increased the explained variance in $\dot{V}O_{2max}$ by an additional 12.3% in the BMI model^{VPA} and 9.4% in the WC model^{VPA}, and decreased the SEE by 0.408 mL·kg^{-1}·min^{-1} in the BMI model^{VPA} and 0.334 mL·kg^{-1}·min^{-1} in the WC model^{VPA}. When the institution variable or activity monitor variable as an independent variable was added to the multiple regressions, we found that the institution variable and activity monitor variable were not statistically significant ($P > 0.05$) and produced no appreciable difference in the accuracy of the models ($R^2\Delta < 0.009$, 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, particularly in model^{VPA}: R^2 obtained from the BMI model^{VPA} and WC model^{VPA}, respectively, decreased by 0.014 and 0.011,

TABLE 3. Multiple regression non-exercise models estimating $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) in the entire sample.

	BMI Model (kg·m^{-2})		WC Model (cm)	
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	Coefficients	β	Coefficients	β
Model^{SC}				
Constant	50.327*		54.526*	
Age (yr)	−0.241*	−0.515	−0.196*	−0.402
Body composition	−0.667*	−0.371	−0.266*	−0.462
SC (10^3 steps·d^{-1})	0.587*	0.448	0.555*	0.431
R	0.805*		0.825*	
SEE (mL·kg^{-1}·min^{-1})	3.517		3.315	
SEE%	12.431		11.724	
Rp	0.788*		0.812*	
SEEp (mL·kg^{-1}·min^{-1})	3.628		3.406	
Model^{MVPA}				
Constant	48.543*		51.853*	
Age (yr)	−0.224*	−0.480	−0.175*	−0.360
Body composition	−0.623*	−0.346	−0.244*	−0.424
SC (10^3 steps·d^{-1})	0.427*	0.326	0.408*	0.317
MVPA (min)	0.067*	0.249	0.060*	0.226
R	0.833*		0.846*	
SEE (mL·kg^{-1}·min^{-1})	3.293		3.144	
SEE%	10.677		10.194	
Rp	0.818		0.833	
SEEp (mL·kg^{-1}·min^{-1})	3.389		3.222	
Model^{VPA}				
Constant	48.288*		51.466*	
Age (yr)	−0.219*	−0.469	−0.177*	−0.363
Body composition	−0.574*	−0.319	−0.226*	−0.393
SC (10^3 steps·d^{-1})	0.423*	0.322	0.408*	0.316
VPA (min)	0.316*	0.316	0.284*	0.290
R	0.853*		0.863*	
SEE (mL·kg^{-1}·min^{-1})	3.109		2.981	
SEE%	10.081		9.666	
Rp	0.839*		0.852*	
SEEp (mL·kg^{-1}·min^{-1})	3.201		3.054	

* $P < 0.0001$.
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.
SEEp, PRESS SEE; Rp, PRESS multiple correlation coefficients. SEE% calculated as (SEE/mean of measured $\dot{V}O_{2max}$ ×100).

TABLE 4. Constant error (CE) and SD for subgroups of the entire sample.

Subgroup	N (%)	BMI Model^{MVPA} CE	BMI Model^{MVPA} SD	WC Model^{MVPA} CE	WC Model^{MVPA} SD
Age					
<35 yr	26 (17.6%)	0.59	3.69	0.85	3.75
35–50 yr	58 (39.2%)	−0.76	2.98	−0.66	2.82
>50 yr	64 (43.2%)	0.03	3.08	0.18	2.90
SC					
<7500 steps·d^{-1}	27 (18.2%)	−0.67	3.01	−0.73	2.74
7500–9999 steps·d^{-1}	37 (25.0%)	0.36	2.77	0.61	2.66
≥10,000 steps·d^{-1}	84 (56.8%)	0.00	3.57	−0.03	3.36
$\dot{V}O_{2max}$					
<25 mL·kg^{-1}·min^{-1}	26 (17.6%)	−2.40	2.56	−2.01	2.69
25–35 mL·kg^{-1}·min^{-1}	91 (61.5%)	−0.16	2.88	−0.21	2.64
>35 mL·kg^{-1}·min^{-1}	31 (20.9%)	2.47	3.16	2.63	3.22

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

and the SEE obtained from the BMI model^{VPA} and WC model^{VPA} increased by 0.092 mL·kg^{-1}·min^{-1} and 0.073 mL·kg^{-1}·min^{-1}, respectively.

The second stage of the cross-validation analysis was to examine the accuracy of the models by analyzing and comparing the CE and the SD of the CE for various subsamples of the sample. These results are provided in Tables 4 and 5. Table 4 suggests that model^{MVPA} is most accurate in predicting $\dot{V}O_{2max}$ for individuals who are older (>50 yr), more active (SC ≥ 10,000 steps·d^{-1}), and with average fitness (25–35 mL·kg^{-1}·min^{-1}). Subgroups of $\dot{V}O_{2max}$ showed high absolute CE values (>2.6 mL·kg^{-1}·min^{-1}) in the high fitness subgroup. When prediction model^{VPA} was applied to the subgroups (Table 5), the CE values (<0.8 mL·kg^{-1}·min^{-1}) for all ages and SC groupings except for individuals who were less active (CE < 1.11 mL·kg^{-1}·min^{-1}) and for the average fitness subgroup (25–35 mL·kg^{-1}·min^{-1}) were small. At the extremes of fitness, the model^{VPA} systematically overestimated or underestimated $\dot{V}O_{2max}$ by about 2.9 mL·kg^{-1}·min^{-1}. Figures 1 and 2 show the tendency for model^{MVPA} and model^{VPA} to consistently underestimate for individuals with high fitness. For most of those subjects whose $\dot{V}O_{2max}$ was found to be ≥ 37 mL·kg^{-1}·min^{-1}, the prediction models systematically underestimated $\dot{V}O_{2max}$, and their average measured $\dot{V}O_{2max}$ value was 40.3 ± 4.0 mL·kg^{-1}·min^{-1} (N = 25). The average $\dot{V}O_{2max}$ estimated by the models for these subjects, however, were, for the BMI and WC models^{MVPA}, 37.3 ± 3.5 and 37.4 ± 3.7 mL·kg^{-1}·min^{-1}, respectively, and for the BMI and WC models^{VPA}, 37.9 ± 4.8 and 38.0 ± 5.1 mL·kg^{-1}·min^{-1}, respectively.

TABLE 5. Constant error (CE) and SD for subgroups of the entire sample.

Subgroup	N (%)	BMI Model^{VPA} CE	BMI Model^{VPA} SD	WC Model^{VPA} CE	WC Model^{VPA} SD
Age					
<35 yr	26 (17.6%)	−0.13	2.93	0.36	3.15
35–50 yr	58 (39.2%)	−0.49	3.16	−0.45	2.98
>50 yr	64 (43.2%)	−0.80	2.61	−0.56	2.59
SC					
<7500 steps·d^{-1}	27 (18.2%)	−1.09	2.94	−1.11	2.64
7500–9999 steps·d^{-1}	37 (25.0%)	0.47	2.74	0.69	2.61
≥10,000 steps·d^{-1}	84 (56.8%)	0.08	3.26	0.03	3.10
$\dot{V}O_{2max}$					
<25 mL·kg^{-1}·min^{-1}	26 (17.6%)	−2.89	2.28	−2.48	2.48
25–35 mL·kg^{-1}·min^{-1}	91 (61.5%)	0.13	2.72	0.05	2.50
>35 mL·kg^{-1}·min^{-1}	31 (20.9%)	1.94	2.92	2.12	3.05

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

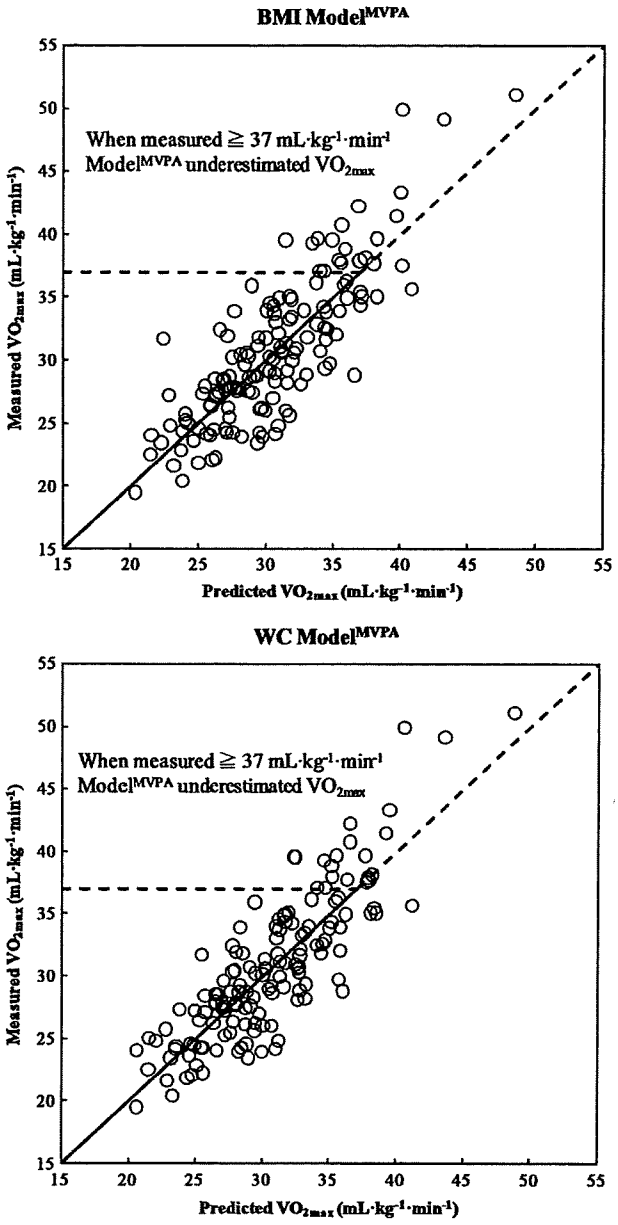

FIGURE 1—Relationships between the measured and predicted $\dot{V}O_{2max}$ values for the multiple regression model^{MVPA}. The *solid line* is the line of equality (measured $\dot{V}O_{2max}$ = predicted $\dot{V}O_{2max}$). The areas within the *dashed lines* show where the model^{MVPA} tends to underestimate $\dot{V}O_{2max}$. Data from the entire sample (N = 148) were used for the analysis.

DISCUSSION

The results of the present study showed that the PA variables of the time spent in MVPA and VPA were significantly related to $\dot{V}O_{2max}$, thus supporting our first hypothesis that the accelerometer-determined PA intensity variables

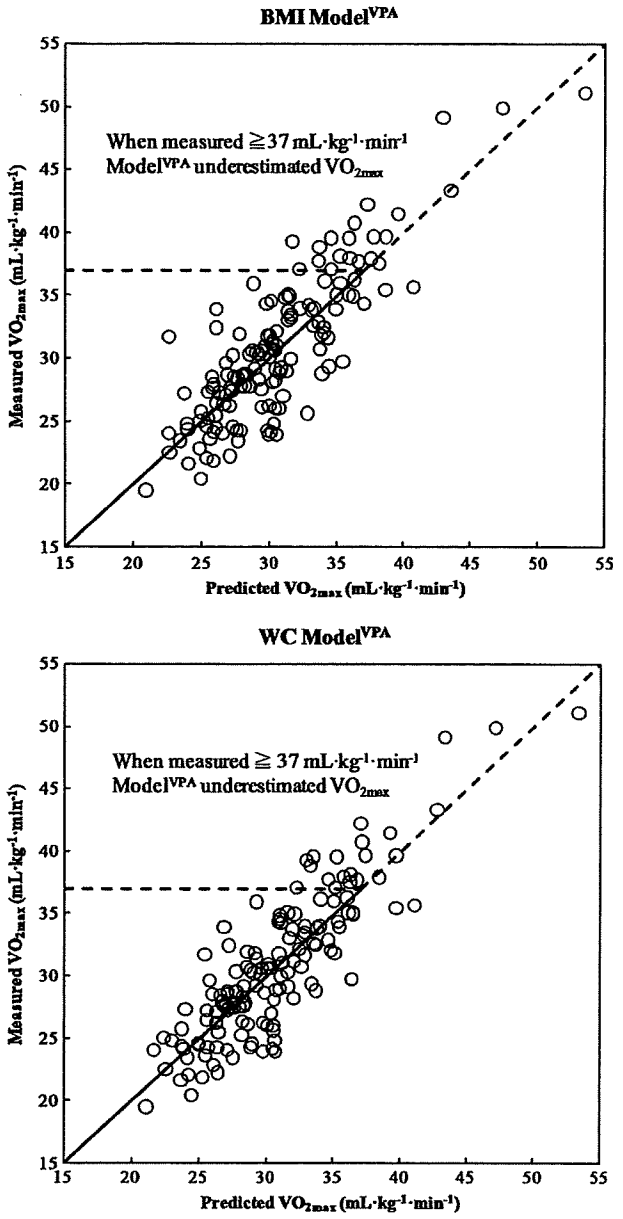

FIGURE 2—Relationships between the measured and predicted $\dot{V}O_{2max}$ values for the multiple regression model^{VPA}. The *solid line* is the line of equality (measured $\dot{V}O_{2max}$ = predicted $\dot{V}O_{2max}$). The areas within the *dashed lines* show where the model^{VPA} tends to underestimate $\dot{V}O_{2max}$. Data from the entire sample (N = 148) were used for the analysis.

including the time spent in MVPA and VPA are potential predictors of cardiorespiratory fitness in Japanese women. Furthermore, this study indicates that the nonexercise model for the prediction of $\dot{V}O_{2max}$ can be substantially improved by the inclusion of both of these objectively measured PA variables of the time spent in MVPA and VPA, which are easily and reliably measured using an accelerometer.

A positive relationship between the objectively measured intensity of PA and cardiorespiratory fitness has been established in youth (5,6,8). However, few studies have reported this relationship in adults. Hebestreit et al. (11) conducted a study of 71 patients with cystic fibrosis (aged 12–40 yr) and found MVPA, assessed by 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 $\dot{V}O_{2max}$. The present study is the first to examine the relationships between accelerometer-determined MVPA and VPA and $\dot{V}O_{2max}$ in healthy adults. The results of the present study demonstrated that each of the independent variables used in this study was independently related to $\dot{V}O_{2max}$. The correlation coefficient of 0.52 between accelerometer-determined MVPA and $\dot{V}O_{2max}$ found in this study was similar to that found in patients with cystic fibrosis (11) and was higher than the correlation coefficient (r = 0.25) in children reported by Dencker et al. (6). Furthermore, the results of the present study also showed stronger relationship (r = 0.58) between VPA and $\dot{V}O_{2max}$ compared with those in children (r = 0.30–0.38) (5,6) and adolescents (r = 0.45) (8). The relationships tended to be stronger for VPA than that for MVPA, which is consistent with other findings reported in children (6). These findings suggest that the proportion of the variance in cardiorespiratory fitness explained by VPA increases with aging and that VPA make a bigger contribution to the variance in cardiorespiratory fitness compared with MVPA. Those prior studies, in conjunction with the present study, document the value of using accelerometer-determined PA intensity variables including the time spent in MVPA and VPA when estimating $\dot{V}O_{2max}$.

In our previous study, an equation was developed to predict $\dot{V}O_{2max}$ from age, BMI, and SC in Japanese women. We found that SC was useful in predicting $\dot{V}O_{2max}$ variance and helped the nonexercise $\dot{V}O_{2max}$ prediction model generate relatively accurate estimations of $\dot{V}O_{2max}$ in Japanese women. However, SC alone does not discriminate the intensity of movement or reflect the amount of time spent in specific intensity categories of PA, which may weaken the accuracy of that prediction model. To further increase the accuracy of a nonexercise prediction model, a new equation using additional accelerometer-determined PA intensity variables including the time spent in MVPA and VPA was developed. The new nonexercise equations in the present study resulted in a validity coefficient of R ranging from 0.83 to 0.85 for model^{MVPA} and 0.85 to 0.86 for model^{VPA}, and a value of SEE ranging from 3.14 to 3.29 mL·kg^{-1}·min^{-1} for model^{MVPA} and 2.98 to 3.11 mL·kg^{-1}·min^{-1} for model^{VPA} (Table 3). Previously published nonexercise test prediction models reported varying success in predicting a measure of cardiorespiratory fitness, with SEE and R values ranging from 3.44 to 8.63 mL·kg^{-1}·min^{-1} and 0.46 to 0.88, respectively (2,7,12,15,16,18,21,22,27–30,34,37,38). In addition, the SEE for the nonexercise prediction model^{VPA}s in this study were lower than the 10–20% values reported for most

submaximal exercise methods used to estimate $\dot{V}O_{2max}$ (23). Therefore, the R values determined by the regression model in the present study were within the range of those associated with previous nonexercise methods for estimating $\dot{V}O_{2max}$, and the SEE values were lower than those previous values. 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 0.65 to 0.68 when using age, body composition (BMI or WC), and SC. The coefficient of determination increased to R^2 values ranging from 0.69 to 0.72 when MVPA was added to the equations. When VPA was added to the equations as a surrogate for MVPA, the regression model resulted in R^2 values ranging from 0.73 to 0.75. Those results indicated that the accelerometer-determined PA intensity variables, which have not been used in previously developed equations, substantially improved the accuracy of the estimation of $\dot{V}O_{2max}$ in adult women when compared with the use of age, BMI, and SC alone. Furthermore, the results of the present study also showed that model^{VPA} was more accurate than model^{MVPA}.

To estimate the prediction model's performance, we conducted two cross-validation analyses based on PRESS and various subsamples of the sample. For the PRESS procedure, the shrinkage of the coefficient of determination (< 0.014) and the increment of SEE (<0.092 $mL \cdot kg^{-1} \cdot min^{-1}$) for each prediction model were minor (Table 3). In the second stage of the cross-validation analysis, the CE values of both model^{MVPA} and model^{VPA} were small except for individuals at extremes of fitness. The results of two cross-validation analyses provide evidence for supporting the validity of the prediction model used in present study. Our finding of a significant underestimation of $\dot{V}O_{2max}$ among individuals with high fitness (Figs. 1 and 2) has been consistently observed in the previous studies (15,38). The present study drew on a smaller estimation bias (<3 $mL \cdot kg^{-1} \cdot min^{-1}$) compared with the study by Wier et al. (38) (<8 $mL \cdot kg^{-1} \cdot min^{-1}$). Wier et al. (38) 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 because of high fitness. Furthermore, they suggested that the estimation bias can be corrected by modifying the intercept using the CE value.

Compared with the Plasqui and Westerterp (28) study and our previous study (3), the present study drew on a similar or larger sample and achieved a better prediction accuracy and model stability, as evidenced by the larger R^2, smaller SEE values, absence of systematic bias, and minor shrinkage of the R^2 and increment of SEE in the PRESS procedure. Various tests should be evaluated not only for their accuracy and validity but also for their applicability in a varied study population, their cost, and the ease and convenience of the protocol. The wide age range of the highly varied women who obtained measurements of $\dot{V}O_{2max}$ in our study helps support the generalizability of the prediction model. In addition, because each of these predictor variables is easily obtained, it is believed that the nonexercise $\dot{V}O_{2max}$ prediction model using SC and MVPA or VPA as a surrogate for the PA variable can be a routine component of primary health care examinations for women in large epidemiological cohorts.

This study has several limitations. First, the prediction model developed in this study may have limited generalizability because it was developed in a group of relatively healthy Japanese women 20 yr and older. 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., men, children and adolescents, individuals with metabolic syndrome, and other racial groups) because the relationship between objectively measured PA and $\dot{V}O_{2max}$ in such groups may have different characteristics than that 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 investigate that possibility.

To our knowledge, this study marks the first attempt to develop new nonexercise $\dot{V}O_{2max}$ prediction models using accelerometer-determined PA intensity including the time spent in MVPA or VPA as the objective PA variables that can be used in large epidemiological cohorts. This study 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. The new nonexercise prediction equations derived in this study are applicable to estimating $\dot{V}O_{2max}$ in Japanese women.

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Original Article

Effects of Acetic Acid Bacteria Supplementation on Muscle Damage After Moderate-Intensity Exercise

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Abstract

Objective: Acetic acid bacteria were traditionally used to produce fermented food. Furthermore, acetic acid bacteria contain unique membrane lipids that would be expected to attenuate inflammation. This study examined the effects of oral intake of acetic acid bacteria isolated from fermented milk on muscle damage after moderate-intensity exercise.

Methods: In a double-blind placebo-controlled crossover trial, 40 untrained subjects (16 men and 24 women; age, 46.4 ± 4.3 yr; height, 162.8 ± 10.8 cm; weight, 60.2 ± 9.4 kg; body mass index, 22.6 ± 2.9 kg/m^2) took 111 mg of dried acetic acid bacteria per day (supplemented group) or 111 mg of cornstarch per day (placebo group) for 1 week and walked for 60 min on the last day of administration.

Results: Leukocyte, lymphocyte and neutrophil counts, IL-6 and creatine kinase (CK) activity, myoglobin (Mb) concentration and perceived pain in parts of the body were measured pre and post exercise. All values, except for IL-6, were significantly increased post-exercise compared with pre-exercise in both groups. However, neutrophil counts and ankle pain were significantly lower in the supplemented group. In addition, the increase of CK activity in the supplemented group was significantly attenuated at 24 h after exercise (supplemented group, 114 ± 54 U/l; placebo group, 126 ± 68 U/l). The supplemented group also demonstrated a trend toward a lower level of CK activity after exercise ($p = 0.06$). Other values did not differ between groups.

Conclusion: These results suggested that acetic acid bacteria supplementation was useful to attenuate muscle damage after moderate-intensity exercise.

KEY WORDS: acetic acid bacteria, dietary supplement, walking, neutrophil, creatine kinase

Introduction

Regular exercise prevents and ameliorates lifestyle-related diseases, such as type II diabetes, hypertension and ischemic heart disease [1-3]. Regular exercise of moderate intensity is sufficient to be of benefit with regard to those diseases [4]. On the other hand, exercise, especially unaccustomed exercise, induces muscle damage related to inflammation after exercise [5], and even moderate-intensity exercise is not an exception [6-8]. One factor in failure to continue regular exercise may be exercise-induced muscle damage.

Exercise-induced muscle damage is initiated by mechanical muscular contraction, which induces production and release of inflammatory mediators, such as cytokines and chemokines, resulting in neutrophil mobilization into the circulation. Circulating neutrophils infiltrate muscle tissue and cause muscle damage due to phagocytosis. Damaged muscle releases myocellular proteins such as creatine kinase (CK) and myoglobin (Mb) into the circulation [9,10].

Thus, exercise-induced muscle damage is caused by inflammation after exercise. Oral intake of α-tocopherol and allicine were reported to reduce exercise-induced muscle damage through attenuation of inflammation [11,12]. In addition to these compounds, some lipids, such as phosphatidylcholine (PC), terpenoids and sphingolipids, have been reported to inhibit production of inflammatory mediators [13-15] and would be expected to reduce muscle damage through attenuation of inflammation. In general, they are abundant in plants, but scarce in bacteria. However, acetic acid bacteria have unique membrane lipid components compared with other bacteria, and their membrane lipids consist of PC, terpenoids and sphingolipids [16]. Acetic acid bacteria are traditionally used to produce fermented food, such as vinegar, and can be obtained as viable cells, especially from fermented milk [17,18]. Although acetic acid bacteria contain unique membrane lipids and have a history of being ingested, the effects of ingestion of acetic acid bacteria on physiological function have not been studied.

With this background, in this study we focused on acetic acid bacteria isolated from fermented milk to clarify whether acetic acid bacteria supplementation attenuates inflammation and can reduce exercise-induced muscle damage after moderate-intensity exercise in untrained humans.

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Methods

Subjects

Forty healthy volunteers (16 men and 24 women; 46.4 ± 4.3 yr; height, 162.8 ± 10.8 cm; weight, 60.2 ± 9.4 kg; body mass index, 22.6 ± 2.9 kg/m^2) participated in this study. Subjects were recruited by an advertisement through a contract research organization, HUMA R&D Co., Ltd. (Minato-ku, Tokyo). None of the subjects had an exercise habit. Excluded from the study were current smokers; those who walked > 7000 steps per day; had a history of medical illness; took chronic medication or supplements, such as vitamin E and coenzyme Q10; had a food allergy; or had donated blood within 3 months prior to the study. Before obtaining written consent, we informed the subjects of the purpose of this study as well as possible risks and discomfort. The study protocol was approved by the ethics committee of two separate groups, the Mizkan Group Corporation and the HUMA R&D Co., Ltd., and was performed in accordance with the Declaration of Helsinki. The experiment was conducted under the management of medical doctors.

Acetic acid bacteria supplement

Acetic acid bacteria, *Acetobacter malorum* NCI 1683 (S24) [19], was isolated from fermented milk by the method described by Entani and Masai [20]. Similarity of the 16S rRNA sequence was 100% between the strain *Acetobacter malorum* NCI 1683 (S24) and *Acetobacter malorum* LMG 1746T (DSM 14337T). As the result of DNA-DNA hybridizations, DNA-DNA relatedness values between that strain and *Acetobacter malorum* LMG 1746T (DSM 14337T) was 76%. Thus, the strain was confirmed to be *Acetobacter malorum* NCI 1683 (S24). Bacteria were homogenized at high pressure and powdered by spray drying. This powder was enclosed in a capsule with soybean oil and beeswax. *Acetobacter malorum* NCI 1683 (S24) capsules were comprised of 7.4% dried acetic acid bacteria, 73% soybean oil and 17% beeswax. Placebo capsules contained these ingredients at the same ratios, with cornstarch replacing dried acetic acid bacteria.

Supplementation

A double-blind placebo-controlled crossover trial with randomization was used. Subjects in the supplemented group received oral supplementation with dried acetic acid bacteria capsules for 1 week, while subjects in the placebo group received placebo capsules for 1 week. Both groups performed the exercise experiment on the last day of that week. Capsules were ingested 3 times a day and total intake of acetic acid bacteria or cornstarch was 111 mg per day. Subjects recorded the ingested capsule count every day, and compliance was assessed by capsule count. The interval between administration of the supplement and administration of the placebo was 2 weeks. No side effects were observed by oral administration of the supplement or the placebo in any subject.

Experimental protocol

A flow diagram of the experimental protocol and procedure is shown in *Figure 1*. Briefly, the exercise consisted of walking for 60 min with the aim of achieving a Heart Rate (HR) of 120 - 130 beats per min and a Rating of Perceived Exertion (RPE) of 12 - 13 [21]. Subjects performed exercise on a flat surface in a gymnasium. During exercise, subjects recorded their HR using the HR monitor, POLAR F11 (Polar Electro Oy, Kenpele, Finland), and the RPE every 15 min. They maintained their exercise intensity throughout the 60-min period. Only ingestion of water at room temperature was allowed during the experiment. The contents of dinner before the experimental day and the menu and portions for breakfast, lunch and dinner on the experimental day were the same among subjects. The day before each experimental day, subjects finished dinner before 2000 h, and then fasted overnight.

Visual analog scale (VAS)

Subjects were asked to record perceived pain on a 100 mm VAS from 0 (Most severe pain) to 100 (No pain) before (Pre), immediately after (Post), 7-h post (Post 7 h) and 24-h post exercise (Post 24 h). The VAS consisted of 6 scales, which evaluated body, leg, thigh, calf, hip and ankle pain.

Blood sampling

Peripheral venous blood samples were collected by antecubital venipuncture before (Pre), immediately after (Post), 2-h post (Post 2 h) and 24-h post exercise (Post 24 h).

Total and differential leukocyte counts

Total leukocyte count in EDTA-treated blood was measured by a Sysmex microcell counter K-4500 (TOA Medical Electronics, Kobe-city, Hyogo). The different leukocyte types were classified using the Olympus BH2 (Olympus Corporation, Shinjuku-ku, Tokyo). The absolute number of each cell type was calculated from results of the total leukocyte count based on the percentage of each cell type.

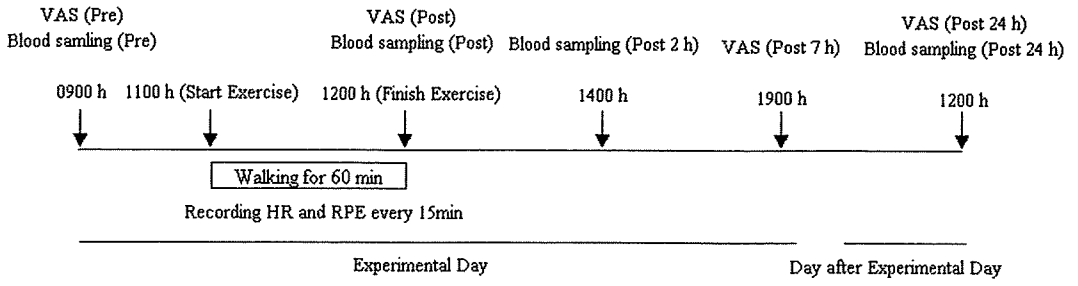

Fig. 1. Experimental protocol

Serum biochemistry

Serum samples were separated from whole blood in Vacutainer blood-collection tubes by centrifugation at 1,000 g for 10 min after blood was left to clot at room temperature for 30 min. These samples were stored frozen at −80 degrees until assayed. Serum CK activity was measured using a biochemical assay kit and Mb concentration was measured by a radioimmunoassay kit. IL-6 concentration was measured by a chemiluminescent enzyme immunoassay.

Statistical analysis

Data were presented as means (*M*) and standard deviation (*SD*). Mixed-model one-way repeated measures analysis of variance (ANOVA) was used to compare all values from the pre-exercise value to each time point within each group. Mixed-model two-way repeated measures analysis of covariance (ANCOVA) across groups and time points, with each pre-exercise value as a covariate, was used to detect the main effect for groups after exercise. Student's paired *t*-test was used for between group comparisons at each time point. Significance was evaluated for all statistics at $p < 0.05$. The statistical analysis was performed using a statistical package (SPSS 11.5 for Windows, Chicago, IL, USA).

Results

Exercise intensity

HR and RPE data during exercise are summarized in *Table 1*. These data show that the physical task level in this study, which was maintenance of an HR of 120 - 130 beats per min and RPE of 12 - 13 during moderate intensity exercise, was achieved. There were no significant differences between groups in HR and RPE at each time point.

Total and differential leukocyte counts

In the placebo group, the circulating neutrophil counts from Post to Post 24 h was significantly increased compared with the Pre value, while it was significantly increased from Post to Post 2 h in the supplemented group (*Figure 2*). A significant effect in both groups on circulating neutrophil counts was observed after exercise. However, the neutrophil counts were lower in the supplemented group than in the placebo group (*Figure 2*). Circulating leukocyte and lymphocyte counts increased after exercise in both groups, with no significant difference between groups (*Table 2*).

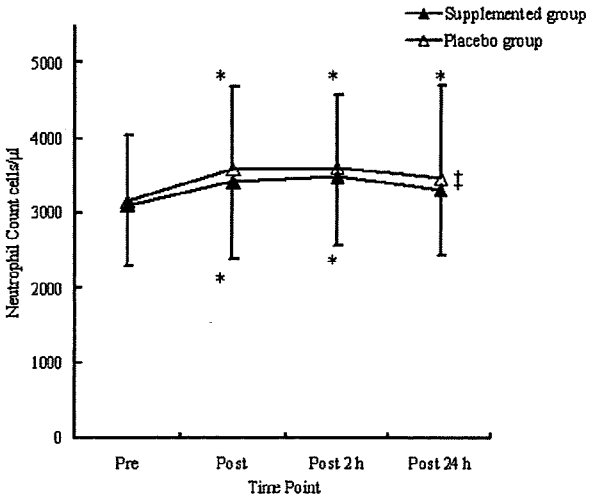

Fig. 2. Pattern of circulating neutrophil counts in supplemented and placebo groups after moderate-intensity exercise. Values are $M \pm SD$. Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.05$. ‡Significant effect for groups detected by mixed-model repeated measures ANCOVA with pre-exercise value as a covariate; $p < 0.05$.

Table 1 Heart rate and Rating of Perceived Exertion data during moderate-intensity exercise

Variable	Group	15 min	30 min	45 min	60 min	Average during exercise
HR	S	121 ± 5	122 ± 4	123 ± 4	123 ± 4	122 ± 3
(beats/min)	P	120 ± 6	122 ± 4	123 ± 3	123 ± 3	122 ± 4
RPE	S	11.9 ± 2.2	12.5 ± 2.2	12.9 ± 2.4	13.3 ± 2.6	12.7 ± 0.4
	P	11.7 ± 1.6	12.3 ± 2.1	12.6 ± 2.2	13.1 ± 2.6	12.4 ± 0.3

Note. Values are $M \pm SD$. S, supplemented group (n=40); P, placebo group (n=40).

Table 2 Effects of moderate-intensity exercise on total and differential leukocyte counts in supplemented and placebo groups

Parameter	Group	Pre	Post	Post 2 h	Post 24 h
Leukocytes	S	5300 ± 1109	5893 ± 1339**	6125 ± 1250**	5395 ± 1330
(cells/μl)	P	5464 ± 1391	5970 ± 1564**	6163 ± 1508**	5533 ± 1702
Lymphocytes	S	1841 ± 583	2054 ± 638	2188 ± 576**	1815 ± 447
(cells/μl)	P	1888 ± 628	1969 ± 652	2146 ± 697*	1766 ± 491

Note. Values are $M \pm SD$. Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.05$. **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.01$. S, supplemented group; P, placebo group.

Serum CK activity

In both groups, in comparison with the Pre value, serum CK activity was significantly increased at all time points from Post to Post 24 h. In the supplemented group, the increase in serum CK activity was significantly attenuated in comparison with that in the placebo group at Post 24 h (*Figure 3*). Although it was not significant, the effect in both groups on serum CK activity was observed after exercise, with the change less in the supplemented group than in the placebo group ($p = 0.06$) (*Figure 3*).

Mb concentration

Mb concentration was significantly increased only at Post exercise compared with the Pre value in both groups, but was significantly lower in the supplemented than in the placebo group (*Table 3*). However, the difference between groups was nearly the same both Pre and Post exercise, suggesting that the effect of acetic acid bacteria supplementation on Mb concentration was minor.

Serum IL-6 concentration

In comparison with the Pre value, the only significant increase in serum IL-6 was at Post 2 h in the supplemented group. No significant changes from the Pre value were observed at any time point in the placebo group (*Table 3*).

VAS

Perceived body and leg pain as well as perceived thigh, calf and hip pain were significantly increased after exercise in both groups, showing that moderate-intensity exercise induced perceived pain (*Table 4*). As shown in *Figure 4*, perceived ankle pain was also significantly increased after exercise in both groups, with the VAS score higher in the supplemented group than in the placebo group. This indicated that acetic acid bacteria supplementation reduced perceived ankle pain after exercise although pain in other parts of the body did not differ significantly between groups.

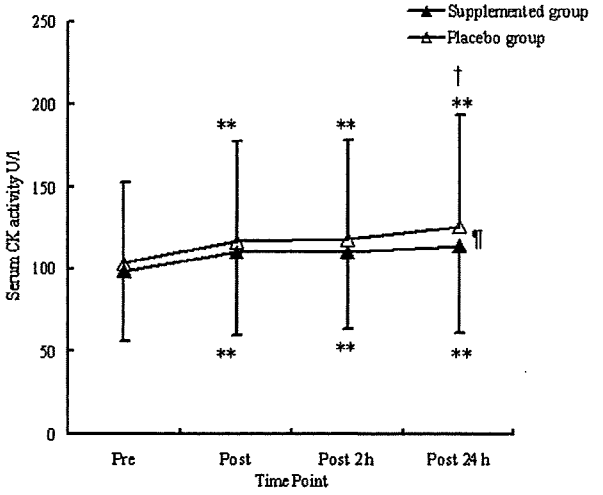

Fig. 3. Pattern of serum creatine kinase activity in supplemented and placebo groups after moderate-intensity exercise. Values are $M \pm SD$. Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by the mixed-model repeated measures ANOVA; $p < 0.01$. †Significant difference in comparison of the mean difference between groups detected by paired Student's *t*-test; $p < 0.05$. ¶Trend towards effect for groups detected by mixed-model repeated measures ANCOVA with pre-exercise value as a covariate; $p < 0.1$.

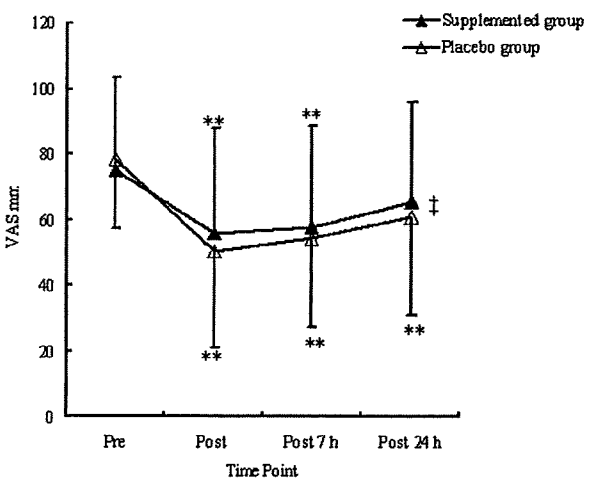

Fig. 4. Perceived ankle pain in supplemented and placebo groups after moderate-intensity exercise. Values are $M \pm SD$. Subjects were to rate pain by a 100 mm visual analog scale from 0 (Most severe pain) to 100 (No pain) before (Pre), immediately after (Post), 7-h post exercise (Post 7 h) and 24-h post exercise (Post 24 h). **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.01$. ‡Significant effect for groups detected by mixed-model repeated measures ANCOVA with pre-exercise value as a covariate; $p < 0.05$.

Table 3 *Effects of moderate-intensity exercise on biochemical markers in supplemented and placebo groups*

Parameter	Group	Pre	Post	Post 2 h	Post 24 h
Mb	S	35 ± 11	45 ± 3**†	36 ± 22	36 ± 12
(ng/ml)	P	38 ± 18	48 ± 3**	40 ± 20	34 ± 11
IL-6	S	1.3 ± 2.0	1.5 ± 1.9	1.7 ± 2.5**	1.4 ± 2.3
(pg/ml)	P	1.7 ± 4.5	1.4 ± 1.6	1.4 ± 1.5	1.7 ± 3.1

Note. Values are $M \pm SD$. Blood samples were collected before (Pre), immediately after (Post), 2-h post exercise (Post 2 h) and 24-h post exercise (Post 24 h). *Significant difference in comparisons of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.05$. **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.01$. †Significant difference in comparison of the mean difference between groups detected by paired Student's *t*-test; $p < 0.05$. S, supplemented group; P, placebo group.

Table 4 Effect of moderate-intensity exercise on perceived pain as evaluated by a visual analog scale (VAS) in supplemented and placebo groups

Variable	Group	Pre	Post	Post 7 h	Post 24 h
VAS for body pain	S	62 ± 25	52 ± 27*	47 ± 25**	60 ± 28
(mm)	P	67 ± 22	50 ± 22**	47 ± 25**	62 ± 27
VAS for leg pain	S	69 ± 25	47 ± 29**	46 ± 28**	58 ± 28*
(mm)	P	74 ± 18	42 ± 24**	47 ± 25**	59 ± 27**
VAS for thigh pain	S	78 ± 21	57 ± 29**	55 ± 27**	63 ± 26**
(mm)	P	80 ± 19	54 ± 29**	56 ± 27**	63 ± 27**
VAS for calf pain	S	74 ± 28	52 ± 28**	51 ± 29**	61 ± 29**
(mm)	P	80 ± 19	48 ± 30**	50 ± 27**	61 ± 30**
VAS for hip pain	S	79 ± 22	62 ± 26**	59 ± 28**	68 ± 27*
(mm)	P	80 ± 22	61 ± 28**	60 ± 27**	69 ± 26**

Note. Values are $M \pm SD$. Subjects were asked to evaluate pain on a 100 mm VAS from 0 (Most severe pain) to 100 (No pain) before (Pre), immediately after (Post), 7-h post exercise (Post 7 h) and 24-h post exercise (Post 24 h). *Significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.05$. **Highly significant difference in comparison of the mean difference between pre-exercise values and values at each time point detected by mixed-model repeated measures ANOVA; $p < 0.01$. S, supplemented group; P, placebo group.

Discussion

In this study, 40 untrained subjects performed a 1-h walking experiment to examine the effects of acetic acid bacteria supplementation on muscle damage after moderate-intensity exercise in a double-blind placebo-controlled crossover trial. The results indicated that acetic acid bacteria supplementation attenuated circulating neutrophil counts, serum CK activity, and ankle pain that were continuously induced after moderate-intensity exercise.

Walking is a popular form of exercise in daily life [22], and brisk walking for 1 h has been recommended in general as moderate-intensity exercise [4]. Therefore, we used walking for a 1-h period as a model for moderate-intensity exercise. Perceived muscle pain after exercise consisted of acute muscle pain immediately after exercise and delayed onset muscle soreness caused by inflammation. In this study, estimations by VAS could not distinguish between acute and delayed muscle pain. Perceived muscle pain persisted continuously until 24 h after exercise, and was most severe immediately after exercise in all parts of the body. In addition, neutrophils, which are induced by inflammatory mediators, and serum CK activity, which is a marker of muscle damage, were also increased up until 24 h after exercise. These results suggested that the exercise model used in this study induced muscle damage by inflammation and might be suitable to assess the effect of acetic acid bacteria supplementation.

The disruption of muscle cells due to mechanical muscular contraction and phagocytosis of neutrophils results in CK and Mb efflux into the circulation. Therefore, serum CK activity and Mb concentration are generally used markers of muscle damage [23,24]. However, our results showed that time-dependent changes differed in these muscle damage markers. Mb concentration increased only immediately after exercise before returning to the pre-exercise value, while serum CK activity continued to increase until 24 h after exercise. This difference in patterns has been reported previously, with the explanation that the molecular weight of Mb was smaller than that of CK and that Mb is quickly removed from the circulation after exercise [24]. Therefore, one possible explanation of the differences in the pattern between serum CK activity and Mb concentration after exercise in this study was that the elimination rate of Mb was higher than the accumulation rate in the circulation, resulting in the disappearance of Mb in blood soon after exercise.

We found that circulating neutrophil counts, which kept increasing up to 24 h after exercise, were attenuated by acetic acid bacteria supplementation. The result for neutrophils was similar to that for serum CK activity and suggested that acetic acid bacteria supplementation attenuated serum CK activity through the effect of neutrophil migration after exercise. Neutrophil migration was reported to be activated by cytokines and chemokines [25-27]. Therefore, we measured IL-6, one of the major cytokines, but found no significant increase in the placebo group after exercise nor an attenuation in the supplemented group. This result suggested that inflammatory mediators other than IL-6 participate in neutrophil migration. This result suggested that inflammatory mediators other than IL-6 participate in neutrophil migration. The membrane lipid of acetic acid bacteria contains PC, terpenoids and sphingolipids. These components could be expected to attenuate inflammatory mediators through regulation of NF-kappaB activation [14,15,28]. This suggested that one possible explanation of the anti-inflammatory effect of acetic acid bacteria supplementation was due to the regulation of NF-kappaB activation. The mechanism of the anti-inflammatory effect of acetic acid bacteria supplementation and the component responsible for this effect must be clarified in the future.

Perceived ankle pain in the supplemented group was reduced in comparison with the placebo group, although perceived pain in the body, leg, thigh, calf and hip did not differ significantly between the two groups. Because the ankle supports the entire body weight during exercise, the ankle might have been more affected by the exercise load compared than the other parts examined. Therefore acetic acid bacteria supplementation might have had a marked affect on perceived ankle pain.

In conclusion, results of this study suggested that oral intake of acetic acid bacteria was useful in attenuating muscle damage by inflammation after moderate-intensity exercise.