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Resistance training and arterial compliance: keeping the benefits while minimizing the stiffening

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Objectives **This study aimed to determine the effects of moderate resistance training as well as the combined resistance and aerobic training intervention on carotid arterial compliance.**

Background **Resistance training has become a popular mode of exercise, but intense weight training is shown to stiffen carotid arteries.**

Methods **Thirty-nine young healthy men were assigned either to the moderate-intensity resistance training (MODE), the combined resistance training and endurance training (COMBO) or the sedentary control (CONTROL) groups. Participants in the training groups underwent three training sessions per week for 4 months followed by four additional months of detraining.**

Results **All training groups increased maximal strength in all the muscle groups tested ($P < 0.05$). Carotid arterial compliance (via simultaneous carotid ultrasound and applanation tonometry) decreased approximately 20% after MODE training (from 0.20 ± 0.01 to 0.16 ± 0.01 mm^2/mmHg, $P < 0.01$). No significant changes in carotid arterial compliance were observed in the COMBO (0.20 ± 0.01 to 0.23 ± 0.01 mm^2/mmHg) and CONTROL (0.20 ± 0.01 to 0.20 ± 0.01 mm^2/mmHg) groups. Following the detraining period, carotid arterial compliance returned to the baseline level. Peripheral (femoral) artery compliance did not change in any groups.**

Conclusions **We concluded that simultaneously performed aerobic exercise training could prevent the stiffening of carotid arteries caused by resistance training in young healthy men. *J Hypertens* 24:1753–1759 © 2006 Lippincott Williams & Wilkins.**

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Keywords: arterial structure and compliance, exercise, imaging, cross-training, ultrasonics

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Introduction

The aorta and large arteries play an important role in the cardiovascular system not only as blood conduits to the peripheral tissues, but also as a buffer for pressure changes resulting from intermittent ventricular ejection of blood. By absorbing a proportion of the energy in systole and releasing it in diastole, they maintain coronary blood flow and avoid an increase in left ventricular afterload. Through the impairment of this buffering function, reductions in arterial compliance or increases in arterial stiffness contribute to elevations in systolic blood pressure, left ventricular hypertrophy, and coronary ischemia [1,2]. Indeed, higher arterial stiffness is associated with a greater rate of mortality in patients with end-stage renal failure and essential hypertension [3,4]. Accordingly, any interventions that could act to decrease arterial compliance should be cautiously performed or even avoided.

Resistance training has become a popular modality of exercise performed by most populations, and has become an integral component of exercise recommendations endorsed by a number of national health organizations [5–7]. Resistance training has profound effects on the musculoskeletal system, thereby contributing to the maintenance of functional capacity and the prevention of sarcopenia and osteoporosis [7]. The effects of resistance training on the cardiovascular system, however, are not well understood. We have recently demonstrated that high-intensity resistance training is associated with reduced arterial compliance [8,9]. This finding was initially observed in cross-sectional studies comparing strength-trained individuals and sedentary controls [8] and later confirmed by interventional studies involving several months of resistance training interventions [9]. Considering a number of functional and physiological benefits that resistance training induces, practice of resistance training should not be discouraged. A remaining critical question is whether any type of resistance training could be performed regularly without inducing arterial stiffening. In this context, two strategies appear plausible. First, the intensity and volume of the resistance training used in the previous studies [8,9] were more strenuous and vigorous than those recommended for the comprehensive health programs [5–7]. It is not currently

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known whether moderate resistance training would induce similar arterial stiffening. Second, because regular aerobic exercise has been shown to increase arterial compliance [10,11], simultaneously performed endurance training may negate the effects of resistance training, thereby attenuating or preventing arterial stiffening. Neither of these possibilities has been tested, however.

Accordingly, the primary aim of the present study was to determine the effects of moderate-intensity resistance training as well as the combined strength and endurance training intervention on carotid arterial compliance. We hypothesized that the compliance of carotid arteries would not change following moderate-intensity resistance training as well as combined resistance and aerobic training. At the completion of the exercise intervention period, we implemented a period of detraining. We reasoned that if the observed changes in arterial compliance were induced by the prescribed exercise training, values should return to the baseline levels when the stimuli of exercise training were removed.

Methods

Participants

A total of 39 young healthy men were studied. None of the men had participated in any resistance or endurance training on the regular basis. All subjects were normotensive (< 140/90 mm Hg), non-obese (body mass index < 30 kg/m^2), and free of overt chronic diseases as assessed by medical history, physical examination, and a complete blood chemistry and hematological evaluation. Candidates who smoked in the past 4 years were taking cardiovascular-acting medications or anabolic steroids, or had significant intima–media thickening, plaque formation, and/or other characteristics of atherosclerosis (e.g. ankle–brachial index < 0.9) were excluded. All subjects gave their written informed consent to participate, and all procedures were reviewed and approved by the Institutional Review Board. Subjects were randomly assigned into either the moderate-intensity resistance training group (MODE, $n = 12$), the combined high-intensity resistance training and moderate-intensity aerobic exercise training group (COMBO, $n = 11$), or sedentary control group (CONTROL, $n = 16$). No endurance-training group was included because the primary focus of the present study was on resistance training. Before the intervention period, there were no significant differences in any of the variables between the groups (Table 1).

Table 1 Selected subject characteristics at baseline

Variable	CONTROL group	MODE group	COMBO group
N	16	12	11
Age (years)	22 ± 1	20 ± 1	21 ± 1
Height (cm)	172 ± 1	169 ± 2	171 ± 2
Body weight (kg)	68 ± 2	65 ± 2	66 ± 2
Body mass index (kg/m^2)	22 ± 1	23 ± 1	23 ± 1
Body fat (%)	21 ± 1	18 ± 2	21 ± 1
Lean body mass (kg)	55 ± 2	51 ± 1	53 ± 1
Peak oxygen consumption (ml/kg per min)	49 ± 3	52 ± 2	49 ± 2

Data presented as the mean ± SEM. CONTROL, sedentary control group; MODE, moderate-intensity resistance training group; COMBO, combined high-intensity resistance training and moderate-intensity aerobic exercise training group.

Measurements

The exercise intervention groups were studied five times: before training (baseline), at 2 months (midpoint of exercise training), at 4 months (completion of exercise training), at 6 months (midpoint of detraining), and at 8 months (completion of detraining). The non-exercising control group was studied three times: baseline, at 4 months, and at 8 months. In order to avoid potential diurnal variations, subjects were tested at the same time of day throughout the study period [9,10]. Furthermore, prior to each testing, subjects abstained from caffeine and fasted for at least 4 h; most subjects were studied after overnight fast. Subjects in the intervention groups were studied 20–24 h after their last exercise training session to avoid the acute effects of exercise [12], but while they were still considered to be in their normal (i.e. habitually exercising) physiological state.

Incremental exercise

To demonstrate that the participants had been sedentary, we measured the maximal oxygen consumption during an incremental cycle ergometer exercise [13]. The oxygen consumption, heart rate, and ratings of perceived exertion were measured throughout the protocol.

Strength testing

Maximal muscular strength in the intervention groups was assessed before and after resistance training using the following exercises: half squat, bench press, leg extension, leg curls, lat row, and abdominal bend. After 10 warm-up repetitions, one-repetition maximum (1 RM) values were obtained according to established guidelines. The day-to-day coefficient of variation for 1 RM strength in our laboratory is 4 ± 2%. The 1 RM test was not performed in the control group due to the potential risks involved in the testing.

Body composition

The body composition was determined using the bioelectric impedance method (coefficient of variance, 4 ± 2%) [14].

Arterial blood pressure at rest

Chronic levels of arterial blood pressure at rest were measured with a semi-automated oscillometric device (Form PWV/ABI; Colin Medical, Komaki, Aichi, Japan) over the brachial and dorsalis pedis artery. Recordings were made in triplicate with participants in the supine position.

Carotid artery intima–media thickness

The carotid artery intima–media thickness (IMT) was measured from the images derived from an ultrasound machine equipped with a high-resolution linear-array broad-band transducer as previously described [8]. Ultrasound images were analyzed by use of computerized image analysis software. At least 10 measurements of IMT were taken at each segment, and the mean values were used for analysis. This technique has excellent day-to-day reproducibility (coefficient of variance, 3 ± 1%) for the carotid IMT.

Carotid artery stiffness and compliance

A combination of ultrasound imaging of the pulsatile common carotid artery with simultaneous applanation of tonometrically obtained arterial pressure from the contralateral carotid artery permits non-invasive determination of arterial compliance [10,15]. The carotid artery diameter was measured from images derived from an ultrasound machine equipped with a high-resolution linear-array transducer. A longitudinal image of the cephalic portion of the common carotid artery was acquired 1–2 cm distal to the carotid bulb. To assess the effects of peripheral artery compliance, the same procedure was repeated on the common femoral artery. All image analyses were performed by the same investigator who was blinded to the group assignments.

Pressure waveforms and amplitudes were obtained from the common carotid artery with a pencil-type probe incorporating a high-fidelity strain-gauge transducer (SPT-301; Millar Instruments, Houston, Texas, USA) [10,16]. Because baseline levels of blood pressure are subjected to hold-down force, the pressure signal obtained by tonometry was calibrated by equating the carotid mean arterial and diastolic blood pressure to the brachial artery value [9,10]. In addition to arterial compliance [17], we also calculated the β-stiffness index, which provides an index of arterial compliance adjusted for distending pressure [18]. Arterial compliance and the β-stiffness index were calculated using the equations $[(D_1-D_0)/D_0]/[2(P_1-P_0)] \times \pi \times (D_0)^2$ and $[\ln(P_1/P_0)]/[(D_1-D_0)/D_0]$, where D_1 and D_0 are the maximal and minimum diameters, and P_1 and P_0 are the highest and lowest blood pressures. The blood pressure obtained at the ankle (Form PWV/ABI; Colin Medical) was used to calculate the femoral artery compliance. The day-to-day coefficients of variation were 2 ± 1, 7 ± 3, and 5 ± 2 for the carotid artery diameter, pulse pressure, and arterial compliance, respectively. The coefficient of variance for femoral arterial compliance was 7 ± 4%.

Left ventricular dimensions, mass and function

Echocardiography was used to measure the left ventricular dimensions, wall thickness, and stroke volume according to established guidelines [19] as previously described [8]. The left ventricular mass and stroke volume were normalized for the body surface area. The ratio of the average left ventricular wall thickness to the left ventricular internal end-diastolic diameter was used as an index of relative wall thickness [8].

Exercise training intervention

In the first 4 months of study period, participants in all training groups underwent three supervised resistance training sessions per week. During each training session, participants in the COMBO group completed three sets of 8–12 exercises at 80% of 1 RM and subjects in the MODE group completed three sets of 14–16 exercises at 50% of 1 RM, in the following order: leg extension, seated chest press, leg curls, lateral row, squat, and sit-ups. The resistance of each exercise was increased progressively throughout the resistance training period. The recovery time between exercise bouts was controlled at 2-min intervals. Each resistance training session lasted approximately 45 min. Subjects in the COMBO group performed a cycle exercise at 60% of the maximal heart rate for 30 min immediately after each resistance training session. Training assistants verbally encouraged the subjects and ensured proper form and technique at each exercise session. Participants were instructed to refrain from any other regular exercise during the entire study period. Participants in the sedentary control group were instructed not to alter their normal activity levels throughout the study period.

Statistical analyses

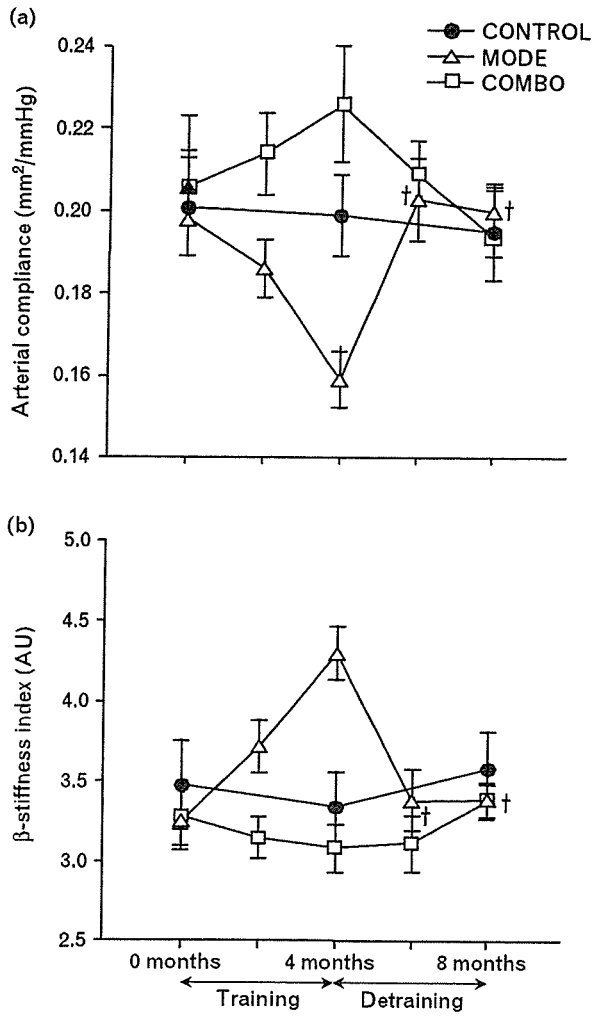
Changes were assessed by two-way analysis of variance (group × time) with repeated measures. In the case of significant F-values, a post-hoc test (Newman–Keuls method) was used to identify significant differences among mean values. To determine whether the changes in arterial compliance and the β-stiffness index were independent of changes in stroke volume, analysis of covariance was performed with stroke volume as the covariate. Pearson's correlation and regression analyses were performed to determine the relation between variables of interest.

Results

Before the intervention period, there were no significant differences in any of the variables between the groups (Table 1). In all groups, there were no changes in height, weight, body mass index, and body surface area throughout the intervention periods.

All the exercise intervention groups increased 1 RM strength significantly in all muscle groups tested ($P < 0.05$ to $P < 0.0001$). Percentage increases in 1 RM strength for the MODE and COMBO groups were 6 and 25% for leg extension, 13 and 14% for leg curl, 10 and 25% for squat, 8 and 17% for lateral row, 6 and 21% for bench press, and 12 and 21% for abdominal bend, respectively.

Fig. 1



Changes in (a) carotid arterial compliance and (b) β-stiffness index for the sedentary control group (CONTROL), the moderate-intensity strength training group (MODE), and the combined aerobic and strength training group (COMBO). Data presented as the mean ± SEM. *$P < 0.05$ versus baseline; †$P < 0.05$ versus 4 months.

Table 2 Hemodynamic and vascular indices

Variable	Baseline	After training	After detraining	Interaction
Brachial systolic blood pressure (mmHg)				
CONTROL group	118 ± 2	119 ± 1	120 ± 2	$F = 2.130$
MODE group	120 ± 3	117 ± 3	115 ± 2	$P = 0.086$
COMBO group	115 ± 2	116 ± 2	115 ± 2	
Brachial diastolic blood pressure (mmHg)				
CONTROL group	68 ± 2	73 ± 2*	73 ± 1	$F = 5.475$
MODE group	71 ± 2	66 ± 2*	68 ± 2	$P > 0.001$
COMBO group	67 ± 1	67 ± 2	67 ± 2	
Brachial pulse pressure (mmHg)				
CONTROL group	49 ± 2	47 ± 1	47 ± 1	$F = 2.407$
MODE group	49 ± 2	52 ± 2	47 ± 2	$P = 0.057$
COMBO group	48 ± 2	49 ± 1	48 ± 1	
Carotid systolic blood pressure (mmHg)				
CONTROL group	101 ± 2	104 ± 2	104 ± 1	$F = 1.653$
MODE group	105 ± 3	105 ± 4	104 ± 3	$P = 0.170$
COMBO group	99 ± 2	97 ± 2	98 ± 2	
Carotid pulse pressure (mmHg)				
CONTROL group	33 ± 2	32 ± 1	32 ± 1	$F = 2.383$
MODE group	36 ± 2	39 ± 3	36 ± 2†	$P = 0.059$
COMBO group	31 ± 1	30 ± 1	32 ± 1	
Carotid lumen diameter (mm)				
CONTROL group	5.91 ± 0.11	5.94 ± 0.14	6.06 ± 0.11	$F = 1.839$
MODE group	6.03 ± 0.13	6.02 ± 0.10	6.02 ± 0.11	$P = 0.131$
COMBO group	5.79 ± 0.09	5.91 ± 0.07	5.81 ± 0.09	
Δ Carotid lumen diameter (mm)				
CONTROL group	0.66 ± 0.03	0.66 ± 0.04	0.63 ± 0.03	$F = 3.460$
MODE group	0.74 ± 0.02	0.66 ± 0.04*	0.76 ± 0.04†	$P = 0.012$
COMBO group	0.71 ± 0.04	0.72 ± 0.03	0.69 ± 0.04	
Carotid intima–media thickness (mm)				
CONTROL group	0.50 ± 0.01	0.52 ± 0.02	0.50 ± 0.02	$F = 1.803$
MODE group	0.46 ± 0.01	0.45 ± 0.02	0.46 ± 0.01	$P = 0.138$
COMBO group	0.47 ± 0.01	0.52 ± 0.01	0.51 ± 0.02	
Femoral compliance (mm^2/mmHg)				
CONTROL group	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	$F = 0.950$
MODE group	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	$P = 0.441$
COMBO group	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	

Data presented as the mean ± SEM. CONTROL, sedentary control group; MODE, moderate-intensity resistance training group; COMBO, combined high-intensity resistance training and moderate-intensity aerobic exercise training group. *$P < 0.05$ versus baseline. †$P < 0.05$ versus after the training period.

The magnitude of increases was larger ($P < 0.05$) in the COMBO group than in the MODE group in all exercises except for the leg curl.

There were no significant differences in baseline arterial compliance and β-stiffness index between all four groups (Fig. 1). Carotid arterial compliance decreased after 4 months of MODE interventions ($P < 0.01$). In contrast, arterial compliance did not decrease, but rather tended to increase ($P = 0.06$), after 4 months of the COMBO intervention. Following the detraining period, arterial compliance values returned to the baseline level. Alterations in arterial compliance were primarily due to changes in arterial distension as the carotid pulse pressure remained unchanged (Table 2). In general, qualitatively similar results (although inverse in direction) were obtained by use of the β-stiffness index ($P < 0.01$). The femoral arterial compliance, an index of the compliance of peripheral (muscular) artery, did not change in any groups. In all groups, there were no significant changes in brachial and carotid systolic blood pressures, carotid IMT, and carotid lumen diameter (Table 2).

In all groups, there were no significant changes in heart rate at rest throughout the study period (Table 3). All the resistance training interventions increased the left ventricular mass index and the relative wall thickness ($P < 0.001$). In the COMBO group, the stroke volume index tended to increase during the training period ($P = 0.07$). There were no significant changes in the stroke volume index in any other groups. Following the detraining period, left ventricular structural and functional indices returned to baseline and were no longer significantly different from baseline. There were no such changes in the sedentary control group throughout the study period. To determine whether changes in stroke volume, a determinant of arterial compliance, could confound the interpretation of the present results, we performed several different analyses. When we performed a

Table 3 Cardiac indices

Variable	Baseline	4 months	8 months	Interaction
Heart rate at rest (beats/min)				
CONTROL group	58 ± 3	56 ± 2	57 ± 2	$F = 0.254$
MODE group	55 ± 3	54 ± 2	53 ± 2	$P = 0.906$
COMBO group	52 ± 3	48 ± 1	50 ± 1	
Left ventricular mass index (g/m^2)				
CONTROL group	131 ± 7	132 ± 7	131 ± 7	$F = 11.940$
MODE group	139 ± 4	151 ± 4*	137 ± 4†	$P < 0.001$
COMBO group	125 ± 5	143 ± 6*	127 ± 6†	
Relative wall thickness (%)				
CONTROL group	19.5 ± 0.4	19.7 ± 0.4	19.8 ± 0.4	$F = 15.793$
MODE group	19.0 ± 0.5	20.7 ± 0.5*	19.3 ± 0.5†	$P < 0.001$
COMBO group	19.0 ± 1.0	20.2 ± 0.9*	18.9 ± 0.9†	
Stroke volume index (ml/m^2)				
CONTROL group	47 ± 2	47 ± 2	46 ± 2	$F = 1.861$
MODE group	51 ± 1	50 ± 1	50 ± 1	$P = 0.130$
COMBO group	48 ± 2	50 ± 2	48 ± 2	

Data presented as the mean ± SEM. CONTROL, sedentary control group; MODE, moderate-intensity resistance training group; COMBO, combined high-intensity resistance training and moderate-intensity aerobic exercise training group. *$P < 0.05$ versus baseline. $^\dagger P < 0.05$ versus 4 months.

univariate correlation analysis between the stroke volume index and carotid arterial compliance in a pooled population, these two functions were not correlated ($r = 0.05$, $P = 0.93$). Additionally, changes in carotid arterial compliance were not associated with changes in stroke volume index in the combined exercise group ($r = 0.19$, $P = 0.26$). Moreover, when analysis of covariance was performed with the stroke volume as the covariate, the overall results on carotid arterial compliance were essentially the same.

Discussion

The major findings of the present study are as follows. First, resistance training performed at a moderate intensity produced a magnitude of arterial stiffening similar to high-intensity resistance training previously reported [9]. Second, concurrently performed endurance training minimized arterial stiffening that was accompanied by high-intensity resistance training. These results suggest that a simultaneously performed aerobic training could negate and prevent the stiffening of carotid arteries caused by resistance training.

Historically, resistance training had been regarded as unsafe for individuals at high risk for future cardiac events because of the abrupt increases in blood pressure and myocardial oxygen demand during high-intensity resistance training [20]. These marked increases in arterial blood pressure during resistance exercise were thought to be initiating factors for arterial stiffening [8]. The majority of recent studies, however, have documented that low to moderate resistance training is a safe and viable form of exercise training as blood pressure increases are within the clinically acceptable range during moderate-intensity resistance training [21]. For these reasons, we hypothesized that resistance training performed at a moderate intensity would not result in a decrease in arterial compliance. In contrast to our working hypothesis, moderate resistance training significantly decreased arterial compliance (from 0.20 ± 0.01 to 0.16 ± 0.01 mm^2/mmHg), and the magnitude of the reduction in arterial compliance was similar to that we previously observed in high-intensity resistance training (from 0.20 ± 0.02 to 0.16 ± 0.01 mm^2/mmHg) [9]. Moreover, these changes in arterial compliance returned to the baseline levels a few months after the cessation of training, confirming that the change in carotid arterial compliance was indeed due to the effect of the moderate resistance training intervention. Furthermore, reductions in arterial compliance were accompanied by significant increases in left ventricular mass index and relative wall thickness, important clinical correlates of arterial stiffening. Even moderate-intensity resistance training therefore appears to stiffen or harden the large elastic arteries. Our present study provides a warning that even moderate resistance training, which is typically recommended to the general public, should be prescribed cautiously, especially for high-risk populations. However, one important consideration that should be emphasized is that the volume (i.e. three sets) of moderate-intensity resistance training used in the present study was still greater than that typically recommended for comprehensive health programs, where only one set of resistance exercises is recommended [6,7]. We therefore cannot exclude the possibility that moderate-intensity resistance training performed with fewer sets may not result in a reduction in arterial compliance.

In contrast to resistance training, regular aerobic exercise is shown to be efficacious in preventing and reversing arterial stiffening in healthy adults [10,11]. We hypothesized that by combining the stiffening effects of resistance training and the destiffening effects of endurance training, both interventions would negate each other and would cause no changes in arterial compliance. In the present study, we demonstrated that simultaneously performed endurance training prevented the reduction in arterial compliance that was accompanied by high-intensity resistance training. Additionally, there was a tendency for arterial compliance to increase with combined endurance and resistance training. From the standpoint of exercise adherence and compliance, this type of 'cross-training' is highly beneficial as it is more enjoyable and breaks the boredom that often results from long-term participation in a single exercise mode [22,23]. Taken together, these findings suggest that combined resistance and aerobic training may be an effective countermeasure for the unfavorable effects of strenuous resistance training.

It is not clear what physiological mechanisms explain the effects of combined training on arterial compliance. Chronic or repeated increases in flow exert their effects on endothelial vasodilatation by modulating the expression of nitric oxide synthase [24]. Carotid arteries

experience increases in blood flow and shear stress during aerobic exercise bouts [25,26], whereas carotid blood flow does not appear to change during resistance exercises [27,28]. Consistent with this, endothelial function is improved with regular aerobic exercise [29,30] as well as with combined resistance and aerobic training [31,32]. Resistance training alone, however, appears to have no effects on flow-mediated vasodilation [33]. One possibility is therefore that the combined aerobic and resistance training may have increased nitric oxide bioavailability, which in turn may have negated the opposing effects of resistance training on the arterial wall. Future studies will be needed to determine the physiological mechanisms underlying the influence of resistance and aerobic training on carotid arterial compliance.

Although endurance training performed concurrently with resistance training prevented the stiffening of carotid arteries, the magnitude of increases was larger in the combined training group than in the moderate-intensity training group in all exercises except for the leg curl. The strength gains were consistently smaller in the combined training group compared with the previously studied high-intensity resistance training alone [9], especially in the lower limbs. This occurred despite the fact that the same training intensity and volume were prescribed to both groups. These results are consistent with a number of previous studies demonstrating that subjects who perform a combination of endurance and strength training achieve lower strength gains than subjects performing weight training alone [34–36]. It should therefore be noted that simultaneous endurance and resistance training may prevent arterial stiffening, but could attenuate optimum gains in muscular strength. In order to minimize the antagonistic effects of endurance training on strength gains, it is recommended that strength and endurance training be performed on alternate days [36]. A smaller strength gain in the combined training group might confound the interpretation of our findings. The moderate-intensity resistance training that achieved much smaller strength gains, however, experienced a similar magnitude of arterial stiffening to the high-intensity training group. The effect of resistance training on arterial compliance therefore does not appear to be dependent upon the training intensity or strength gains.

There are several limitations of the present study that should be emphasized. First, the combined training group that performed moderate-intensity resistance training was not included in the present study. Because simultaneously performed endurance training negated the effects of 'high-intensity' resistance training, however, it is fairly reasonable to assume that it would negate the effects of 'moderate-intensity' (i.e. lesser stimuli) resistance training as well. Second, although arterial compliance and blood pressure often change simultaneously with interventions, changes in arterial compliance were not associated with the corresponding changes in blood pressure in the present study. Because changes in the elastic property of arteries appear to precede changes in blood pressure [37], it is possible that a longer duration of resistance training may have increased blood pressure. Third, we studied relatively small numbers of subjects in each group ($n = 11–16$), and included only young healthy men. Future studies targeting high-risk populations (e.g. the elderly) are needed.

In light of the current recommendation that resistance training should be incorporated into exercise prescription [5–7], the effects of resistance training to stiffen large elastic arteries are of particular concern. We examined two strategies that potentially prevent arterial stiffening associated with resistance training. We demonstrated that moderate-intensity resistance training produced significant reductions in arterial compliance. In contrast, combined resistance and aerobic training did not result in decreases in carotid arterial compliance. These results suggest that in order to negate and prevent the stiffening of carotid arteries caused by resistance training, aerobic training should be performed simultaneously with resistance training.

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Effects of Isoflavone and Exercise on BMD and Fat Mass in Postmenopausal Japanese Women: A 1-Year Randomized Placebo-Controlled Trial

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ABSTRACT: The combined intervention of isoflavone intake and walking exercise over 1 year in postmenopausal Japanese women exhibited a trend for a greater effect on prevention of bone loss at the total hip and Ward's triangle regions.

Introduction: The additive effects of isoflavones and exercise on bone and lipid metabolism have been shown in estrogen-deficient animals. In this study, we determined the effects of isoflavone intake, walking exercise, and their interaction on bone, fat mass, and lipid metabolism over 1 year in postmenopausal Japanese women.
Materials and Methods: A total of 136 postmenopausal women at <5 years after the onset of menopause were randomly assigned to four groups: (1) placebo, (2) walking (45 minutes/day, 3 days/week) with placebo, (3) isoflavone intake (75 mg of isoflavone conjugates/day), and (4) combination of isoflavone plus walking. BMD, fat mass, serum lipid, and serum and urinary isoflavone concentrations were assessed.
Results: A significant main effect of isoflavone on the reduction in trunk fat mass was obtained at 12 months. Significant main effects of walking on the reduction in fat mass in the whole body and the trunk were observed at 3, 6, and 12 months and that in the legs and arms at 6 and 12 months. Serum high-density lipoprotein (HDL)-cholesterol concentration significantly increased by 12 months after the walking and the combined intervention. After 12 months, a significant main effect of isoflavone on BMD was observed only at Ward's triangle. Walking prevented bone loss at the total hip and the Ward's triangle to significant degrees. The effect of the combined intervention on BMD at total hip and Ward's triangle regions was greater than that of either alone. No significant interaction was observed between isoflavone and walking in any measurements recorded during the study.
Conclusions: Our study suggest that combined intervention of 75 mg/day of isoflavone intake and walking exercise 3 times/week for 1 year showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either alone. Intervention with isoflavone in postmenopausal Japanese women showed a modest effect on BMD compared with those in Westerners. Further studies over longer treatment duration that include assessment of BMD at various regions are necessary to ascertain the clinical significance of the combined intervention of isoflavone plus walking in postmenopausal women.
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Key words: isoflavones, walking exercise, BMD, fat mass, postmenopausal women

INTRODUCTION

ESTROGEN DEFICIENCY OFTEN increases the risk of several chronic diseases such as osteoporosis, cardiovascular disease, and obesity.[1–4] Hormone replacement therapy (HRT) is an effective regimen that prevents these diseases in postmenopausal women.[5,6] However, the HRT trial carried out by the Women's Health Initiative (WHI) found that long-term use of HRT (follow-up, 5.2 years) poses serious risks and may increase the risk of heart attack and stroke.[7] Follow-up studies with the WHI will likely provide insight as to whether the increased risks and benefits of HRT therapy decline after the end of the therapy and the time at which this decline occurs as well as evaluation of the dose.[8]

Recently, there has been an increased interest in the role of phytoestrogens in preventing osteoporosis and hypercholesterolemia.[9] Soybean is a rich source of the isoflavones, genistein, and daidzein, which shows weak estrogenic effects in several organs.[10] A number of studies have

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previously reported that soybean isoflavones dose-dependently inhibited bone loss in both female and male osteoporotic animal models without causing notable effects on the reproductive organs.[11–13] However, the results obtained from several observational clinical studies are still conflicting. The discrepancies between the results may be caused by the variation in factors such as the duration of intervention, the bone sites measured, dietary intake, isoflavone intake among subjects and controls, and its metabolism.

On the other hand, the effects of exercise on the prevention of bone loss, fat accumulation, and hypercholesterolemia in women around the menopausal period have been previously studied.[14–17] An exercise program designed for postmenopausal women should be safe and easy to perform and continue. Although high-intensity exercise could possibly increase the bone mass in pre/postmenopausal women, such exercise is also often associated with stress fractures, particularly in individuals with fragile skeletons.[18] Walking is a relatively safe exercise that is commonly performed by elderly people. Thompson et al.[14] indicated an inverse association between body composition variables and daily walking steps in middle-aged women. However, walking exercise has a relatively low impact on bones, and therefore, cannot be used exclusively for preventing bone loss in postmenopausal women.[15] Thus, a few studies have examined the effects of a combination of exercise and HRT on bone mass and body composition in postmenopausal women. Kohrt et al.[16] and Cheng et al.[17] assessed the independent and combined effects of exercise and HRT on the increase in BMD and the reduction in fat mass in postmenopausal women. Because the risk to benefit ratio of HRT continues to be debated,[19] other interventions that combine therapies using phytoestrogens with exercise training may be preferred. Therefore, we hypothesized that isoflavone intake combined with walking exercise would have beneficial effects on the bone and fat mass and serum lipid concentration in postmenopausal women. We have previously reported that, with regard to the prevention of bone loss and fat gain in estrogen-deficient animals, the effects of moderate-intensity exercise combined with isoflavone administration were better than those observed when either was used exclusively.[20–22]

In this study, we report the effects of soy isoflavone intake and/or walking exercise for 1 year on bone mass, body composition, and serum lipid concentration in postmenopausal Japanese women.

MATERIALS AND METHODS

Subjects

Subjects were recruited for this study through advertisements in local newspapers, and those who fulfilled the following criteria were enrolled for the study. Healthy postmenopausal women 45–60 years of age who were within 5 years of natural onset of menopause (defined as at least 12 months since the last menstrual cycle occurred) were enrolled in the study. The subjects had no history of previous use of HRT, lipid-lowering medications, antibiotics, or any other medication known to affect the skeleton. They provided written informed consent to participate in the study. The protocol was approved by the institutional review board of the NIH and Nutrition of Japan, and the study was performed in accordance with the guidelines of the Declaration of Helsinki.

In this study, 145 women who fulfilled the required criteria were called for the screening examination. The criteria for the invitation were as follows: willingness to participate, clinically healthy (no cardiovascular, musculoskeletal, respiratory, or other chronic diseases that might limit walking exercise), sedentary life style (no regular sports activities for at least 2 years), nondieting, nonsmoking, and having no apparent occupational responsibilities or leisure time activities that might impede their participation. Nine participants were excluded after the medical screening because of high serum estradiol (E2) concentrations (>20 pg/ml). Thus, 136 women were randomly assigned to four groups: (1) placebo, (2) placebo plus walking, (3) isoflavone, and (4) isoflavone plus walking. However, 28 women withdrew from the study because of illness, family problems, or they thought that participating in the study was troublesome. The remaining 108 subjects completed the 12-month study, and their data were included in the per protocol analysis (Fig. 1).

Intervention

Placebo or isoflavone capsules were blindly allocated to researchers and subjects throughout the study. Participants from the two groups, isoflavone and isoflavone combined with walking, received two capsules that contained a total of 75 mg of isoflavone conjugate (47 mg as aglycone form; Fujiflavone P40; Fujicco Co., Kobe, Japan) with dextrin, daily in the morning. The isoflavone conjugate weighing 75 mg contained daidzin (38.3 mg), malonyldaidzin (0.2 mg), acetyldaidzin (2.1 mg), daidzein (0.6 mg), genistin (8.6 mg), acetylgenistin (0.6 mg), genistein (0.2 mg), and glycitin (23.4 mg) with glycitein (1.0 mg). The remaining subjects were assigned to receive two placebo capsules containing only dextrin, daily in the morning.

Participants who were randomized into the walking groups were expected to attend three 1-h-long exercise classes each week. The exercise program consisted of a 10-minute warm-up period, a 45-minute supervised walking exercise session, and a 5-minute cool down period. Participants were given instructions with regard to the proper manner of walking to eliminate possible injury. The participants were instructed to maintain the speed of walking at 5–6 km/h, and this was monitored by a pedometer.

The nonwalking group participants did not engage in sports training and were asked to continue their normal activity levels. All participants were instructed to record their daily number of steps walked that were continuously monitored by the pedometer, and their diaries were obtained and checked once a month to ensure that they were up-to-date.

Questionnaire interview

Individual information was collected by trained interviewers in face to face interviews based on a structured and

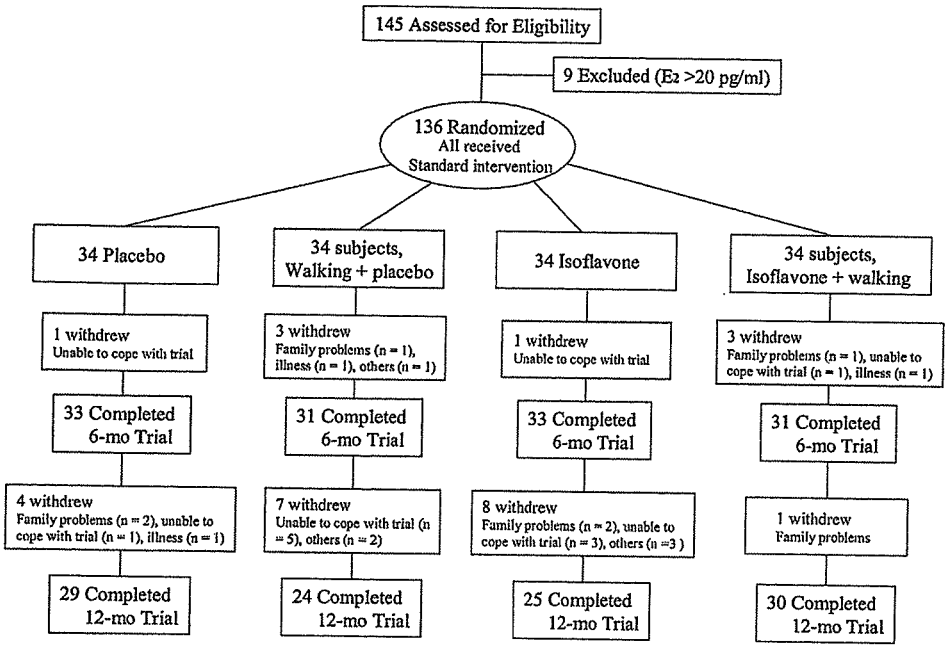

FIG. 1. Flow chart with details of the participants.

previously validated questionnaire that included the following: socio-demographic data; years since menopause; physical activities, including hours spent sitting, standing, walking, sports, and leisure activities; medications; smoking and drinking alcohol; and other factors that may have possible confounding effects on the relation between dietary isoflavone consumption and metabolism of bone and lipid. The participants were instructed to record the contents of daily meals, snacks, and beverages in the diet diary. The diary was collected continuously for 3 days to confirm the meal intake, and, if necessary, the participants were immediately instructed to adhere to the dietary regimen. Daily intakes of soy isoflavones, calcium, vitamin D, total energy, and protein were calculated from the daily record by the dietitian on the basis of the Fifth Revision of the Standard Tables of Food Composition in Japan.[23]

Blood and urine samples

Before BMD measurement, fasting (>12 h) blood samples were collected by venipuncture in EDTA-containing tubes, refrigerated immediately, and centrifuged at 1500 rpm for 30 minutes at 4°C within 2 h. Serum samples from each participant were stored frozen at –20°C. Serum concentrations of total cholesterol (TC) and triacylglycerol (TG) were determined using commercial kits (cholesterol C-test and triglyceride G-test, respectively; Wako Pure Chemical Industries, Osaka, Japan). Serum high-density lipoprotein (HDL)-cholesterol was measured by an enzymatic method (HDL-cholesterol test; Wako Pure Chemical Industries). Serum low-density lipoprotein (LDL)-cholesterol levels were calculated as follows: total cholesterol (mg/dl) – HDL-cholesterol (mg/dl) –TG (mg/dl) × 0.2. Estradiol (E2) was assessed by radioimmunoassay (Amersham Biosciences, Piscataway, NJ, USA). Serum bone-specific alkaline phosphatase (BSALP; Alkphase-B; Metra Biosystems, Mountain View, CA, USA) was measured by using a microplate coated with an anti-BSALP monoclonal antibody. Serum intact osteocalcin (OC) was measured by using a sandwich enzyme immunoassay (EIA) that uses polyclonal antibodies against 20 N-terminal residues (amino acids 1–20) and against 7 C-terminal residues (amino acids 43–49; Biomedical Technology, Stoughton, MA, USA). Urine samples were collected from a second voiding at the same time as blood collection, and they were stored at –20°C. Urinary deoxypyridinoline (DPD) was measured using a sandwich EIA (Pyrilinks-D Assay; Metra Biosystems).

Measurement of serum and urinary isoflavones

Concentrations of isoflavones in serum and urine samples were determined for each subject by reverse-phase high-performance liquid chromatography (HPLC). Duplicate samples of serum and urine were incubated with sulfatase (EC 3.1.6.1; Sigma Chemical) and β-glucuronidase (EC 3.2.1.31; Wako Pure Chemical Industries) at 37°C for 2 h to release the aglycones of the isoflavones; this was followed by purification of reactants using a Sep-Pak C18 cartridge (Waters Co., Milford, MA, USA). Isoflavones were separated at 35°C by reverse-phase HPLC on a 4.6 × 250-mm Capcell Pak C18 column (Shiseido Co., Tokyo, Japan) using a Tosoh CCP and 8020 system with a diode array detector PD8020 (Tosoh Co., Tokyo, Japan). Elution was performed at a flow rate of 1 ml/minute with a linear gradient of acetonitrile solution (10–35%) containing a constant 0.1% concentration of acetic acid. Data were simultaneously acquired at 254 nm (daidzein, genistein, glycitein) and 280 nm (equol).

BMD and body composition

BMD of the lumbar spine (L_2–L_4), left hip, and subwhole body regions (excluding head region) and body composition were assessed by DXA at baseline and after 3, 6, and

TABLE 1. CHARACTERISTICS OF SUBJECTS OF DIFFERENT STUDY GROUPS AT BASELINE AND 12 MONTHS*

		Placebo (n = 33)	*Walking (n = 31)*	*Isoflavone (n = 33)*	*Isoflavone + walking (n = 31)*
Age (years)		54.9 (2.9)	55.2 (2.8)	53.8 (2.9)	54.4 (2.9)
Years since menopause		3.7 (2.1)	3.6 (1.8)	2.7 (1.4)	3.2 (1.4)
Height (cm)	Baseline	156.7 (6.3)	155.3 (6.3)	155.8 (4.3)	154.8 (5.5)
	After 12 months	156.3 (6.3)	154.8 (6.0)	155.3 (4.4)	154.7 (5.3)
Weight (kg)	Baseline	51.4 (7.1)	54.1 (7.3)	51.5 (5.4)	52.9 (5.3)
	After 12 months	51.7 (6.9)	52.6 (7.3)	51.2 (5.7)	51.8 (5.0)
BMI (kg/m^2)	Baseline	20.9 (2.2)	22.4 (2.9)	21.3 (2.5)	22.1 (2.0)
	After 12 months	21.2 (2.5)	21.9 (2.8)	21.3 (2.6)	21.7 (2.0)
Daily intake					
Isoflavone (mg)†	Baseline	48.1 (30.6)	47.7 (25.0)	44.4 (26.9)	49.4 (25.0)
	After 12 months	49.4 (35.0)	48.1 (24.1)	37.4 (26.9)	48.3 (26.7)
Calcium (mg)	Baseline	671.5 (190.9)	723.8 (221.5)	695.8 (253.5)	691.5 (213.3)
	After 12 months	620.3 (176.6)	687.9 (191.0)	597.1 (209.0)	709.6 (232.2)
Vitamin D (μg)	Baseline	9.2 (5.9)	12.3 (13.1)	9.8 (5.9)	9.2 (5.0)
	After 12 months	8.4 (5.1)	9.5 (6.9)	7.6 (4.9)	8.6 (5.8)
Vitamin K (μg)	Baseline	429.8 (172.2)	463.6 (207.0)	376.3 (211.0)	438.3 (181.9)
	After 12 months	378.2 (177.9)	504.1 (260.8)	365.2 (209.0)	439.8 (200.6)
Protein (g)	Baseline	75.0 (13.5)	79.5 (17.4)	72.4 (14.3)	73.5 (13.5)
	After 12 months	69.7 (14.3)	74.8 (14.2)	67.3 (16.0)	74.8 (14.3)
Total energy (kcal)	Baseline	1980.8 (327.5)	1992.8 (327.0)	1923.0 (361.0)	1995.9 (339.7)
	After 12 months	1861.2 (379.2)	2015.6 (305.2)	1811.4 (371.2)	2020.3 (285.4)
Average numbers of steps/day‡	During 12 months	6397 (2166)	8047 (2169)§	6111 (2395)	9024 (3113)§

* Mean (SD). There were no significant differences among the four groups for any of these characteristics at baseline and 12 months, except the number of steps walked. There were no significant differences between the baseline and after 12 months in each group.

† Excluding the isoflavone capsules used in the study.

‡ Significant main effect of walking ($p = 0.0004$) on the number of steps recorded by pedometer monitoring was analyzed using the two-factor ANCOVA model.

§ The walking and isoflavone intake plus walking groups were significantly different from the placebo and isoflavone intake alone groups ($p < 0.05$).

12 months using a Hologic QDR-4500A scanner (Hologic, Waltham, MA, USA). To minimize interobserver variation, all the scans and analysis were carried out by the same investigator, and the day-to-day CVs of his observations were <0.5, 1.9, 0.7, 0.3, 1.9, and 0.8% for BMD in the spine, femoral neck, trochanter, total hip, Ward's triangle, and whole body, respectively. Long-term precision was 0.35% by testing the spine phantom daily over the previous 1 year.

The whole body scans were divided into several regions such as arms, legs, trunk (pelvis, spine, and ribs), and head. The body compositions were analyzed by using manual DXA analysis software (version 11.2:3). The arm region was defined as the region extending from the head of the humerus to the distal tip of the fingers. The reference point between the head of the humerus and the scapula was positioned at the glenoid fossa. The leg region was defined as the region extending from the inferior border of the ischial tuberosity to the distal tip of the toes. The subwhole body was defined as the region extending from the shoulders to the distal tip of the toes. We selected a reference point that could be clearly visualized on the DXA system terminal.

Statistical analysis

All values are expressed as mean ± SD. Differences in baseline characteristics between the different groups were tested by one-factor analysis of covariance (ANCOVA). To determine whether the change over the course of the study was significantly different from the baseline in each group, paired *t*-test with Bonferroni adjustment was performed. The percent change in BMD, body composition, serum lipid, and biomarkers of bone turnover were calculated {[(post intervention − baseline values)/baseline values] × 100} for each group. Two-factor ANCOVA was performed to determine the main effects of isoflavone intake, walking exercise, and their interactions at 3, 6, and 12 months. Significant differences of the percent change in BMD and fat mass among the different groups were determined by ANCOVA with time as the repeated measure, without the use of the intention-to-treat principle. To adjust for the possible confounding, the body weight, height, and daily intake of calcium, vitamin D, protein, and total energy were used as covariates in the analyses of body composition, BMD, and serum biomarkers. Statistical analyses were performed using SPSS for Windows (version 13.0J), and a p value was set at <0.05.

RESULTS

General

There were no significant differences among the groups at baseline with regard to the subject characteristics (Table 1). The 1-year intervention of isoflavone intake and walking did not affect these parameters. At the beginning of the study, the average dietary isoflavone (glycoside form) intake was 48.1, 47.7, 44.4, and 49.4 mg/day in the placebo, walking, isoflavone, and isoflavone plus walking groups, re-

TABLE 2. SERUM ESTRADIOL, BIOMARKERS OF BONE TURNOVER, AND LIPID CONCENTRATIONS OF DIFFERENT STUDY GROUPS AT BASELINE AND 12 MONTHS*

		Placebo	*Walking*	*Isoflavone*	*Isoflavone + walking*
Estradiol (pg/ml)	Baseline	11.78 (2.64)	13.75 (6.55)	11.71 (3.48)	11.99 (3.08)
	After 12 months	12.17 (3.12)	12.63 (4.06)	12.43 (3.12)	12.40 (3.41)
	Percent change	4.83 (26.60)	3.07 (25.91)	9.73 (23.15)	7.88 (34.17)
Osteocalcin (ng/ml)	Baseline	10.51 (2.40)	10.47 (2.88)	9.23 (2.09)	9.50 (2.42)
	After 12 months	9.86 (2.44)	9.60 (2.15)	8.80 (2.15)	9.14 (2.27)
	Percent change	−7.78 (15.63)	−3.52 (20.77)	−2.87 (12.04)	−4.19 (12.29)
BSALP (U/liter)	Baseline	30.37 (11.61)	29.03 (6.65)	27.77 (8.63)	29.26 (8.26)
	After 12 months	29.19 (8.58)	30.38 (6.94)	27.27 (8.19)	28.01 (6.80)
	Percent change	−4.81 (12.40)	10.16 (32.36)	0.89 (18.93)	−3.68 (15.84)
DPD (nM/mM creatinine)	Baseline	7.76 (1.83)	7.65 (1.68)	7.30 (2.37)	6.88 (1.63)
	After 12 months	6.25 (1.47)	6.71 (1.76)	6.28 (1.60)	6.22 (2.17)
	Percent change	−14.02 (24.53)	−10.60 (25.17)	−8.33 (28.19)	−9.82 (23.11)
Total cholesterol (mg/dl)	Baseline	227.4 (33.4)	232.8 (31.5)	227.9 (29.5)	230.7 (35.2)
	After 12 months	231.2 (28.6)	227.2 (26.9)	232.9 (35.8)	228.3 (34.4)
	Percent change	−0.48 (9.71)	0.22 (7.94)	0.58 (11.07)	−0.14 (10.08)
HDL-cholesterol (mg/dl)	Baseline	71.7 (14.9)	71.0 (18.6)	74.2 (18.3)	66.2 (13.5)
	After 12 months	76.8 (14.7)	72.0 (18.1)†	76.0 (17.5)	71.3 (12.5)†
	Percent change	2.38 (9.33)	7.78 (10.74)‡	2.39 (10.36)	8.78 (11.02)‡
LDL-cholesterol (mg/dl)	Baseline	138.7 (29.2)	133.8 (24.0)	136.2 (27.6)	143.6 (31.0)
	After 12 months	136.0 (27.2)	133.1 (21.4)	135.1 (27.2)	142.3 (31.8)
	Percent change	0.30 (15.2)	0.62 (14.4)	0.58 (17.5)	0.28 (16.0)
TG (mg/dl)	Baseline	102.5 (49.0)	114.2 (70.2)	83.9 (38.5)	106.7 (55.1)
	After 12 months	87.6 (33.0)	103.2 (44.1)	87.4 (48.1)	81.6 (31.8)
	Percent change	−2.13 (29.72)	−5.71 (45.16)	6.17 (34.98)	−12.16 (32.75)

* Mean (SD).

† Significantly different from the baseline values, $p < 0.05$.

‡ Significant main effect of walking exercise on percent change in HDL-cholesterol ($p = 0.003$) was analyzed using the two-factor ANCOVA model.

BSALP, bone-specific alkaline phosphatase; DPD, deoxypyridinoline; TG, triacylglycerols.

spectively. At the end of the study, there was no significant difference in the dietary isoflavone intake among the groups and when compared with the intake at baseline in each group. The number of steps recorded by the pedometer during the 1-year study was significantly higher in the two walking groups than in the nonwalking groups (Table 1).

Serum estradiol, lipids, and bone biomarkers

Serum concentrations of estradiol, lipids, and biomarkers of bone turnover at both baseline and 12 months of the study are shown in Table 2. Statistically significant differences were not observed with respect to serum concentrations of estradiol, lipids, and biomarkers of bone turnover at baseline among the groups. When these indices at 12 months were compared with those at baseline, serum E2, TC, and TG concentrations, urinary biomarker of bone resorption (DPD), and serum biomarker of bone formation (osteocalcin and BSALP) were observed to be unaffected by walking, isoflavone intake, and the combined intervention. At the end of the study, the HDL-cholesterol concentration significantly increased from baseline by 7.78% and 8.78% in the walking group and the isoflavone intake plus walking group, respectively. By using the two-factor ANCOVA analysis, we observed a significant main effect of walking ($p = 0.003$), but not of isoflavone intake ($p = 0.53$), on the percent change in HDL-cholesterol. On the other hand, there were no significant main effects on TC and LDL-cholesterol concentrations.

BMD and body composition

There was no significant difference among the four groups at baseline with respect to fat and lean mass in the whole body; fat mass in the trunk, arms, and legs regions; and BMD in subwhole body, lumbar spine, and hip regions (Table 3). Analysis by two-way ANCOVA model, walking exercise induced a significant reduction in the percent change in fat mass in the whole body and trunk region at 3 ($p = 0.001$ and $p = 0.005$, respectively), 6 ($p = 0.001$ and $p = 0.006$, respectively), and 12 months ($p = 0.0002$ and $p = 0.001$, respectively; Figs. 2A and 2B). The intervention with walking exercise also induced a significant reduction in the percent change in fat mass in the legs after 6 and 12 months ($p = 0.003$ and $p = 0.0001$, respectively; Fig. 2C). A significant main effect of walking exercise on suppression of fat mass gain in the arms was observed after 6 and 12 months ($p = 0.019$ and $p = 0.001$, respectively; Fig. 2D). Isoflavone intake significantly suppressed the percent change in fat mass gain in the trunk region after 12 months ($p = 0.027$); however, no interaction was observed between the effects of isoflavone intake and walking exercise (Fig. 2B). Analysis by ANCOVA with time as the repeated measure revealed that the percent change in fat mass in the whole body, trunk, and legs regions was significantly different in the walking alone and the isoflavone plus walking groups compared with that in the placebo group ($p < 0.05$). The percent change in fat mass in the arms in the isoflavone

TABLE 3. BMD AND BODY COMPOSITION OF DIFFERENT STUDY GROUPS AT BASELINE*

	Placebo (n = 33)	*Walking (n = 31)*	*Isoflavone (n = 33)*	*Isoflavone + walking (n = 31)*
Whole body fat mass (kg)	15.1 (4.5)	16.8 (4.3)	15.0 (4.1)	16.1 (3.5)
Trunk fat mass (kg)	6.5 (2.5)	7.9 (2.6)	6.8 (2.4)	7.7 (2.3)
Arms fat mass (kg)	0.9 (0.2)	1.0 (0.3)	0.9 (0.3)	1.0 (0.2)
Legs fat mass (kg)	2.9 (0.9)	2.9 (0.7)	2.7 (0.7)	2.7 (0.6)
Whole body lean mass (kg)	36.9 (3.7)	37.9 (4.2)	37.1 (3.2)	37.5 (3.2)
Subwhole body BMD (g/cm^2)†	1.002 (0.096)	0.979 (0.100)	1.003 (0.108)	1.008 (0.070)
Lumbar spine BMD (g/cm^2)	0.907 (0.130)	0.879 (0.122)	0.891 (0.123)	0.909 (0.097)
Total hip (g/cm^2)	0.787 (0.126)	0.780 (0.114)	0.777 (0.125)	0.807 (0.089)
Femoral neck BMD (g/cm^2)	0.676 (0.114)	0.671 (0.116)	0.668 (0.106)	0.699 (0.094)
Trochanter BMD (g/cm^2)	0.599 (0.122)	0.592 (0.097)	0.591 (0.089)	0.600 (0.078)
Ward's triangle (g/cm^2)	0.544 (0.153)	0.523 (0.151)	0.542 (0.151)	0.534 (0.134)

* Mean (SD). There were no significant differences among the four groups for any measurements at baseline.
† The subwhole body was defined as the region extending from the shoulders to the distal tip of the toes.

plus walking group was significantly different from that in the placebo group ($p < 0.05$).

On analyzing the percent change in BMD, walking showed significant main effects on the preservation of BMD in the total hip region after 12 months ($p = 0.039$; Fig. 3A). Interventions with isoflavone and walking showed significant main effects on the preservation of BMD at Ward's triangle after 12 months ($p = 0.044$ and $p = 0.0001$, respectively; Fig. 3B); however, no interaction effect was observed between the two interventions. There were no significant effects of either isoflavone or walking on the percent change in BMD in the femoral neck, trochanter, lumbar spine, and subwhole body regions (Figs. 3C–3F).

Serum and urinary isoflavones concentrations

Table 4 shows the serum and urinary concentrations of isoflavones at baseline and 12 months. At baseline, there were no significant differences in the concentrations of serum isoflavones among the four groups. Compared with baseline values, the administration of isoflavones showed a marked increase in the serum concentrations of daidzein, glycitein, and equol, but not in the concentration of genistein. In contrast, the placebo treatment did not modify the concentration of circulating isoflavones. Using the two-factor ANCOVA model, we found significant main effects of isoflavone intake on serum concentrations of daidzein ($p < 0.005$), glycitein ($p < 0.005$), and equol ($p < 0.001$) after 12 months. Similar patterns were observed in the concentrations of urine isoflavones among the four groups.

DISCUSSION

The major novel findings of this study are as follows: (1) walking exercise for 6 months can significantly decrease the fat mass in the whole body, trunk, legs, and arms regions; (2) isoflavone intake significantly reduced fat mass in the trunk region and decreased bone loss at Ward's triangle region after 1 year; and (3) the combined intervention of isoflavone and walking exercise for 1 year showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either alone in postmenopausal Japanese women.

Important changes in the body composition of a female occur around the menopausal period. These changes include a decrease in bone mass and an increase in fat mass that may be managed by either replacement of estrogen, exercise, or by their combined intervention.[24] Although several animal experiments have consistently indicated the positive effects of soy isoflavone in retarding bone loss caused by estrogen deficiency, the results obtained from studies on humans are still controversial.[9] The results of the clinical trials varied from an improvement of BMD in the lumbar and femoral neck after 1 year[25,26] to no effect.[27] In one study, the loss of BMD in the lumbar spine, but not in the hip, in women who consumed a red clover–derived isoflavone supplement, 45 mg of aglycone form daily, was significantly lower than in those consuming a placebo.[25] Furthermore, Morabito et al.[26] reported that 54 mg/day of genistein treatment for 1 year significantly increased the BMD of the lumbar spine and the femoral neck in Italian women. On the other hand, daily intake of soy extracts containing 80 mg of isoflavones, but not 40 mg, significantly increased the BMC, but not BMD, of the trochanter after 1 year in postmenopausal Chinese women; however, this effect was observed only in those women with low initial bone mass.[27]

To the best of our knowledge, this is the first observational study on the effects of isoflavone intervention on BMD in Japanese women who were followed up for 1 year. We found that soybean isoflavones prevented a decrease in BMD in Ward's triangle in postmenopausal women after 1 year. However, at other regions such as the lumbar spine, total hip, and subwhole body, the main effects of isoflavone intake were not observed despite the fact that the serum and urine concentrations of isoflavones were high compared with those in the previous study.[26] A putative explanation is that Ward's triangle is the area that is probably the most sensitive to estrogen because of its higher trabecular bone content compared with that of other regions.[28] Among the different regions of interest (ROIs), Ward's triangle in many studies has shown the largest age-related bone loss.[29,30] The ability to predict osteoporotic fractures for Ward's triangle has been at least as good as for the femoral neck ROI.[31,32] However, it is suggested that the

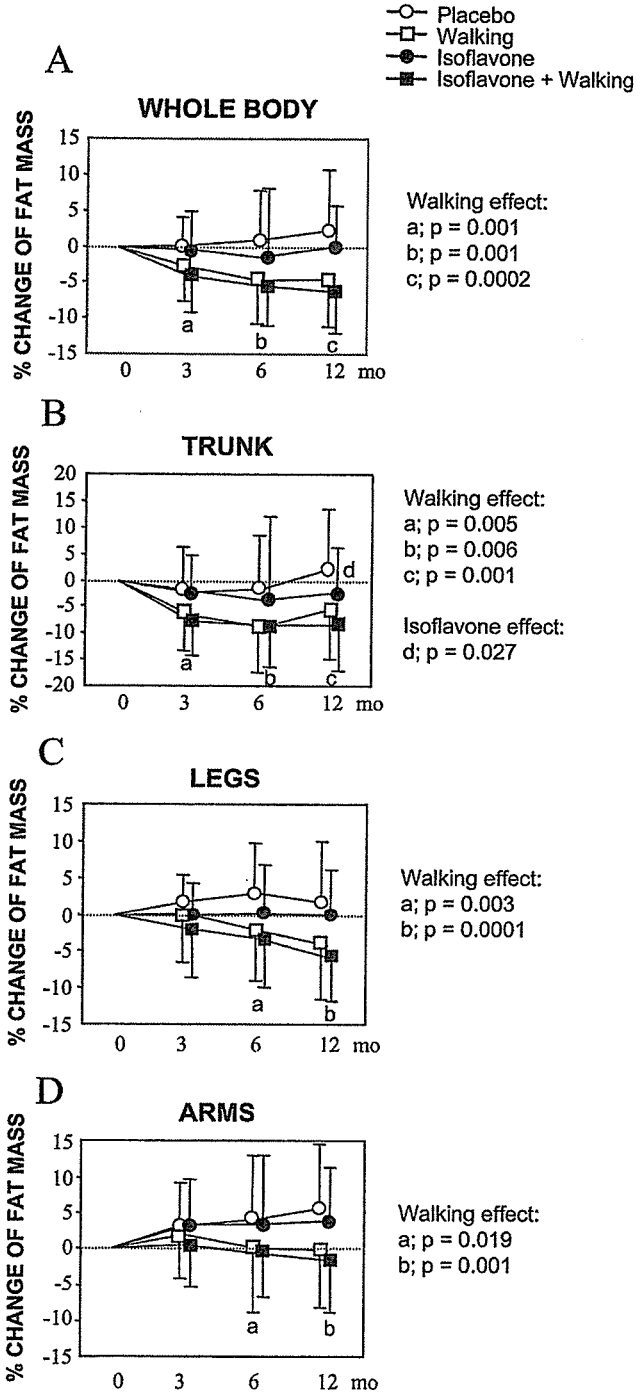

FIG. 2. The percent change in fat mass (±SD) from the baseline to 12 months of the study. The changes shown by the walking exercise and isoflavone intake plus walking exercise groups were significantly different from the placebo group ($p < 0.05$) with regard to (A) the whole body, (B) trunk, and (C) leg regions. (D) The isoflavone intake plus walking exercise group was significantly different from the placebo group ($p < 0.05$) with regard to the arm region. There was a significant main effect of walking exercise on (A) the whole body and (B) trunk region after 3 months, and on (C) the arm and (D) leg regions after 6 months. (B) There was significant main effect of isoflavone intake after 12 months on the trunk region. All p values were calculated using ANCOVA with time as the repeated measure. The main effects of isoflavone intake and walking exercise over 12 months were determined by two-factor ANCOVA.

measurement of BMD in the Ward's triangle is unreliable. In this study, the hip scan was analyzed carefully using the comparison software that enables adaptation to a position and an angle at the first measurement. Therefore, it is believed that the results of this study reflect the change in BMD in the Ward's triangle.

It is known that daidzein can be metabolized to equol, which is biologically more active than its precursor, and could ultimately influence the effect on the subject's health.[33] Although rodents are constitutive equol producers, equol production in humans depends on the intestinal microflora of an individual.[9] Thus, assessment of the correlation between equol status of the subjects and bone effects of isoflavone might be necessary to know the actual clinical effectiveness of isoflavone.

In this study, we expect that the combined intervention of isoflavone and walking exercise would provide a greater benefit than either intervention used exclusively. The combined intervention resulted in a positive change in BMD in the Ward's triangle after 1 year. By using the two-factor ANCOVA model, we found significant main effects of both interventions at this site, although the interaction was not observed. We also found that the combined intervention trend more effective than either intervention used exclusively for preventing bone loss in the total hip region. It is reported that a low estrogen level in females may decrease the sensitivity of bones for detecting mechanical loads.[34] Research on ovariectomized animals have shown that combining isoflavones with exercise training can be beneficial in increasing BMD to a greater extent than either intervention used exclusively.[20,22,35] In addition, several clinical trials have shown that a combination of exercise and HRT results in an increase in BMD that is more than either intervention used exclusively.[36] This study is the first to suggest that the combined intervention of isoflavone and walking exercise for 1 year may partly prevented bone loss in postmenopausal women. It is necessary to focus on the fact that BMD in Ward's triangle increased by 5% in case of the combined intervention, whereas it decreased by 2.5% in the case of placebo-administered controls. This benefit may show a clinically significant reduction in the number of fractures.[37]

In this study, we also indicated that any isoflavone intake, walking exercise, and combined intervention decreased body fat mass in postmenopausal women. We noticed that isoflavone intake significantly reduced fat mass in the trunk region but not in the other regions. There is evidence that estrogens modulate central body fat deposition in women. This evidence includes observations that menopause triggers an increase in central adiposity (i.e., trunk fat mass)[38] and that fat accumulation in postmenopausal women is attenuated by replacement of estrogens, exclusively or in combination with progestins.[15,39,40] Although isoflavones may play a role in the prevention of regional fat mass deposition as a natural alternative to estrogens or HRT, further studies are required to clarify dose–response relationships and the mechanisms leading to this effect. As expected, walking exercise and walking combined with isoflavone intake resulted in reductions in total and regional fat mass after 3, 6, and 12 months. These findings are con-

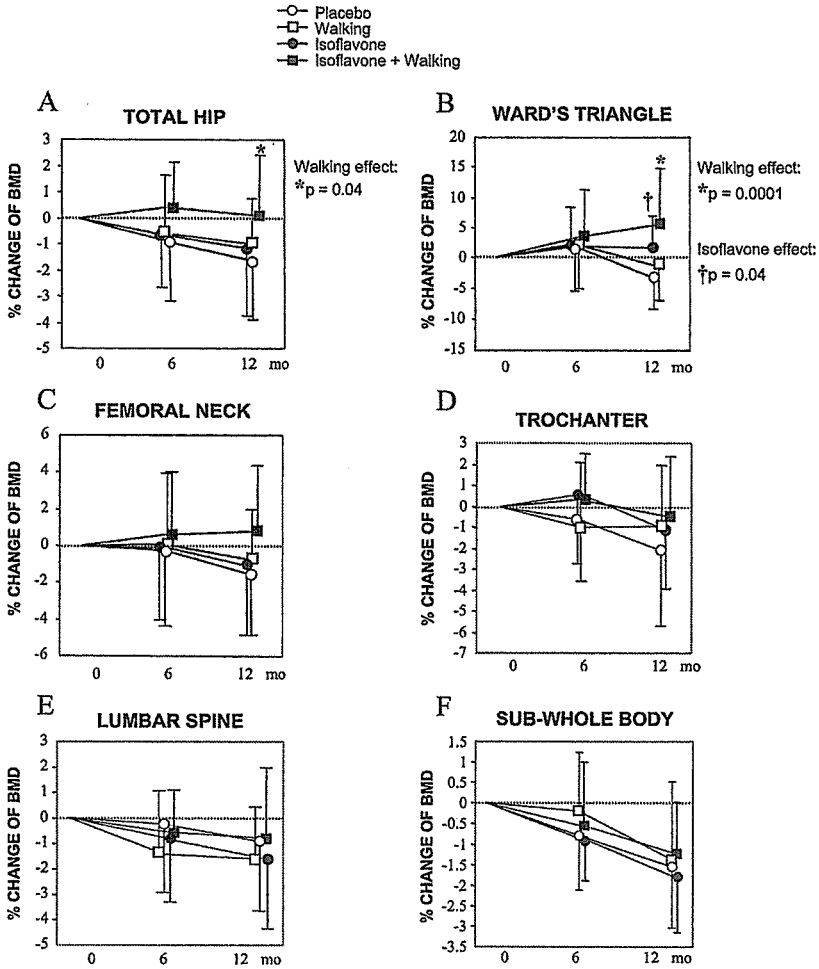

FIG. 3. The percent change in BMD (±SD) from baseline to 12 months of the study. (A) There was a significant main effect of walking exercise on the total hip region after 12 months. (B) There were significant main effects of walking exercise and isoflavone intake on the Ward's triangle after 12 months. There were no significant main effect on (C) the femoral neck, (D) trochanter, (E) lumbar spine, and (F) subwhole body. The subwhole body refers to the whole body scan excluding the head region. The main effects of isoflavone intake and walking exercise over 12 months were determined by two-factor ANCOVA.

TABLE 4. SERUM AND URINARY ISOFLAVONE CONCENTRATIONS OF DIFFERENT STUDY GROUPS AT BASELINE AND 12 MONTHS*

		Placebo	*Walking*	*Isoflavone*	*Isoflavone*	*Isoflavone + walking*	*Main effects‡* *Walking isoflavone*
Serum isoflavone							
Daidzein (nM)	Baseline	159.7 (143.0)	142.1 (146.0)	166.7 (128.7)	242.5 (360.0)		
	After 12 months	413.2 (611.4)	390.3 (564.6)	1270.7 (1698.4)†	903.3 (1097.9)†	NS	$p < 0.005$
Genistein (nM)	Baseline	180.7 (136.1)	164.3 (183.8)	220.0 (199.9)	304.2 (371.9)		
	After 12 months	443.6 (628.2)	454.7 (704.3)	633.9 (959.2)	470.7 (413.8)	NS	NS
Glycitein (nM)	Baseline	63.8 (43.6)	54.3 (46.3)	66.8 (43.0)	65.5 (59.4)		
	After 12 months	74.5 (111.4)	88.4 (109.7)	272.7 (408.8)†	178.2 (182.3)†	NS	$p < 0.005$
Equol (nM)	Baseline	73.8 (201.7)	37.2 (79.4)	93.9 (196.1)	97.8 (232.3)		
	After 12 months	143.4 (330.9)	275.3 (463.0)	515.8 (528.8)†	503.3 (507.8)†	NS	$p < 0.001$
Urinary isoflavones							
Daidzein (μM)	Baseline	8.38 (12.13)	8.53 (10.57)	6.73 (9.93)	7.29 (10.53)		
	After 12 months	7.30 (9.28)	8.17 (13.41)	16.97 (17.78)	22.22 (39.33)†	NS	$p < 0.01$
Genistein (μM)	Baseline	3.39 (5.17)	3.96 (7.40)	2.92 (3.32)	3.12 (5.12)		
	After 12 months	3.01 (3.49)	3.39 (7.09)	3.84 (3.58)	5.09 (6.42)†	NS	NS
Glycitein (μM)	Baseline	1.66 (2.38)	1.90 (4.86)	1.11 (1.32)	1.25 (1.62)		
	After 12 months	1.50 (2.67)	2.59 (4.25)	7.04 (6.92)†	8.32 (14.78)†	NS	$p < 0.0005$
Equol (μM)	Baseline	1.82 (4.06)	5.09 (10.86)	3.01 (5.71)	1.98 (3.59)		
	After 12 months	3.68 (9.20)	4.14 (8.45)	8.28 (10.01)†	8.52 (9.81)†	NS	$p < 0.05$

* Mean (SD).

† Significantly different from the baseline values, $p < 0.05$.

‡ Significant main effect of isoflavone on serum and urinary daidzein, glycitein, and equol concentrations at 12 months were analyzed using the two-factor ANCOVA model.

NS, not significant.

sistent with the evidence reported by several clinical trials that assessed the combined effects of exercise and HRT on fat mass.[15,40,41]

In addition to bone and fat mass, walking exercise significantly increased HDL-cholesterol concentration. This finding is consistent with our previous study.[22] However, there was no significant main effect with respect to LDL-cholesterol concentrations. This lack of an effect is in agreement with other studies[42,43] examining the effects of soy isoflavones on LDL-cholesterol in postmenopausal women.

Our study had several limitations. First, with regard to our Japanese subjects, intake of soy products during this study was not controlled, because soy products are included in most Japanese foods. Compared with American and European women, Asian women consume a diet that is higher in isoflavones (average conjugated isoflavone intake of each group was 44.4–49.4 mg/day in our subjects). High dietary isoflavone intake might increase the threshold for the skeleton to respond to isoflavone supplementation. Thus, this might moderate the effect of isoflavone intervention in Japanese women. Second, a short-duration study cannot adequately assess the benefits related to bone quality, because bone is a slowly responding organ. A complete bone remodeling cycle takes ~180 days, and therefore, studying only one cycle with regard to the effect of any intervention on bone is not sufficient. Thus, the duration of our study (1 year) was not sufficient to test the clinically relevant changes, which require a longer trail that lasts for at least 2 years. Third, the time at which the biomarkers for bone metabolism are tested may differ from that of other studies. In this study, no significant differences were observed with regard to the serum and urinary biomarkers between the baseline and 1 year in each group. Although, there is a clinical study showing that the bone formation marker (BSALP) was not affected by isoflavone intervention,[38] other trials reported that the bone markers changed at 4–8 weeks after the treatment with isoflavone in the postmenopausal Japanese women.[44,45] Thus, these bone markers may have been assessed early during the study.

In conclusion, combined intervention of 75 mg/day of isoflavone intake and walking exercise 3 times/week for 1 year showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either alone. Intervention with isoflavone in postmenopausal Japanese women showed a modest effect on BMD compared with those in Westerners. Walking exercise prevented bone loss at total hip and Ward's triangle and also reduced the fat mass in the whole body. These results suggest that the walking exercise or combined with isoflavone intake over 1 year may offer a potential regimen for primary prevention of osteoporosis and lifestyle-related disease in postmenopausal women. Further studies over longer treatment duration that include the assessment of BMD at various regions are necessary to ascertain the clinical significance of the combined intervention of isoflavones plus walking in postmenopausal women.

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原著

生活習慣病の予防・改善のための運動療法 ーベンチステップ運動を用いた無作為化比較試験

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要約

目的：従業員の定期健康診断で生活習慣の改善を指示された人を対象に，ステップ台を用いた８週間の運動プログラムを実施し，その前後での運動能力や体重，腹囲，総コレステロール値，血圧などを測定することでベンチステップ運動の有効性を検討する．**方法**：対象者33名を無作為に２群に振り分け，運動群には運動負荷試験により決定した乳酸閾値に相当する台高と昇降頻度でのベンチステップ運動（踏み台昇降）を行った．運動は10分連続を１単位として１日３単位，週21単位の運動を指示し，試験期間中の食事指導や食事制限は行わなかった．**結果**：運動群では８週間の試験終了後に運動能力の有意な向上のほか，腹囲や総コレステロール値に有意な改善が認められた．予定されたプログラム（週21単位）の完全実施率は11.8%のみであり，週14単位以上実施した人を合わせても42.2%の実施率であった．しかし週14単位以上実施した人では有意な体重減少が認められ，高血圧の人も全例に血圧の低下が認められた．**結論**：乳酸閾値でのステップ運動を８週間継続することで腹囲や高コレステロール血症者の総コレステロール値の有意な低下が示された．またこの運動を週14単位以上行うことで体重減少や血圧低下も認められ，生活習慣病を予防・改善するための運動量としては乳酸閾値でのステップ運動を１日２単位，週14単位以上行うことが推奨される．

キーワード　生活習慣病，運動療法，ベンチステップ運動，無作為化比較試験〔人間ドック21(4)：18−23，2006〕

はじめに

近年の急速な人口高齢化の進展に伴い疾病構造も変化し，虚血性心疾患，脳血管疾患，糖尿病などの生活習慣病を持つ人の割合が増加し大きな問題となっている．これらの生活習慣病を予防・改善することが健康づくりのための重要課題と考えられており，厚生労働省の標語にも「１に運動，２に食事，しっかり禁煙，最後にクスリ」と示されている．しかし「平成15年国民・健康栄養調査」によると，わが国では運動習慣を持つ者の割合は男性28.3%，女性24.1%であり，国民の３分の２以上が運動習慣を身につけていない状態にある．この原因の一つに，最も効率的な運動の強度や量，頻度などに関して明確に示したものが少ないことが考えられる．今回我々は，乳酸閾値で決定された台高と頻度を用いた自宅で行うステップ運動プログラムの有用性を検討するため，無作為化比較試験を計画した．

対象と方法

平成17年秋に行われた飯塚病院の従業員定期健診で，肥満度（body mass index：BMI）25以上，血圧：収縮期血圧135mmHg 以上かつ/または拡張期血圧85mmHg 以上，総コレステロール220mg/dl 以上，中性脂肪150mg/dl 以上，HDL コレステロール39mg/dl 以下のうち１項目以上を満たし，産業医および保健師により生活習慣の改善が必要であると指示された67名を対象に，本試験の主旨を説明し文書による同意を得られた者について研究を行った．

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活動性の感染症合併例や狭心症などの心疾患の疑われる例，重症の糖尿病合併例，膝関節疾患など下肢の運動障害をある例は除外された．文書同意の得られた33名は年齢，性，BMI，総コレステロール値を層別化因子として運動群とコントロール群の２群に無作為に振り分けられた（表１）．

トレーニングを行う際の運動強度には乳酸閾値（lactate threshold：LT）を利用した．方法としては20cmのステップ台を用い，初回負荷40回/分で20回づつ漸増させる各負荷４分間の運動負荷試験を行った．各負荷終了30秒前に心拍数を記録し，負荷終了直後に耳朶採血を行い，ラクテート・プロ（アークレイ社製）を用いて血中乳酸濃度を測定した．LT 強度は Ayabe[1] らの開発した方法で求めた．対象者全員に運動負荷試験を行い，運動群に対しては個々人の乳酸閾値を基に調節した台高と昇降頻度でのステップ運動を１回に連続10分を１単位として，１日３単位以上を目標とした．運動は自宅での実施を基本としたが，週に１回職場内でインストラクターをつけた運動を全員で行うことを推奨した．また各自の運動実施状況を記録してもらい，週に一度回収した．尚，全試験期間を通じて食事制限や指導は行わないことにした．

トレーニングの前後において，運動負荷試験，脚伸展パワー測定，身体測定（身長，体重，腹囲，血圧，脈拍数），血液検査（血算，血液生化），血管内皮機能検査を行った．血管内皮機能は Peripheral arterial tonometry を用い，上腕血流を５分間駆血し駆血解放後１分間の血管の拡張反応を人差し指につけたプローブで細小動脈の血流拍動の高さを反対側の血流拍動をコントロールとしてコンピューター解析した[2]．統計学的解析には paired t-test を用いた．

なお，本研究は試験前に飯塚病院内で開催された倫理委員会の承認を得て実施された．

結　果

８週間の運動プログラム実施状況を表２に示す．指示通りの１日３単位，週21単位以上実施者は２名のみ（11.8%）で１日平均２回，週14単位以上実施者を合わせても42.2%の実施率であった．各々の測定値の変化を表３に示す．運動群においては乳酸閾値が5.5METs から6.2METs へと有意に（$p=0.001$）上昇し，脚伸展パワーも863Wから959Wへと上昇が認められた．腹囲異常者14

表１　プログラム参加者の背景

	運動群			コントロール群		
	男	女	合計	男	女	合計
参加人数	10	7	17	10	6	16
年齢	46.5	43.0	45.1	46.0	42.3	44.6
body mass index	27.7	29.1	28.2	27.2	29.0	27.9
総コレステロール値(mg/dl)	221	205	215	225	214	221

表２　プログラム実施状況

実施回数（単位/週）	実施人数（%）
0－7	5（29.4%）
7－14	5（29.4%）
14－21	5（29.4%）
21以上	2（11.8%）

表３　運動による各種パラメーターの変化

項　目	振り分け	参加者数	運動前値	運動後値	p値
乳酸閾値	T	17	5.5±0.3	6.2±0.3	0.001
(METs)	C	15	5.5±0.4	5.4±0.3	
脚進展力	T	17	863±111	959±113	0.011
(W)	C	15	1,110±123	1,087±119	
腹囲	T	14	98.2±2.2	95.9±2.0	0.007
(cm)	C	15	97.2±1.7	96.9±1.9	
body mass	T	14	28.8±0.7	28.8±4.9	
index	C	13	28.7±0.6	28.8±0.6	
総コレステロール値	T	9	237±5.9	228±4.9	0.019
(mg/dl)	C	9	250±9.1	248±9.0	
血管内皮機能	T	13	2.13±0.18	2.45±0.08	0.079
	C	12	1.89±0.13	1.90±0.17	

T；運動群

C；コントロール群