pausal women with or without osteopenia/osteoporosis, several well-controlled studies have reported the effect of physical activity on bone markers. Dalsky et al. [17] demonstrated that weight-bearing exercise increased lumbar BMD as a result of decreased serum OC levels. Hatori et al. [16] also showed that walking exercise at an anaerobic threshold level increased lumbar BMD as a result of decreased urinary calcium excretion. Danz et al. [18] showed that combined aerobic and anaerobic exercise decreased radial BMD as a result of increased serum OC levels. Brooke-Wavell et al. [19] reported that 20 min walking a day for 2 years increased calcaneal broadband ultrasonic attenuation without any significant changes in bone markers in contrast to increased urinary DPD in the sedentary group. In these studies, the assessment of bone markers has not always been adequate. However, despite inconsistency in the results of exercise on bone metabolism, it may be possible that the positive effect of exercise on bone mass in postmenopausal women with or without osteopenia/osteoporosis might result from a suppression of bone turnover, bone resorption, or urinary calcium excretion. We also found using the measurements of bone markers including urinary NTX (more specific for bone than urinary DPD) and serum BAP that walking exercise, like bisphosphonates, decreased bone turnover in postmenopausal women with osteoporosis, although its efficacy was apparently less than that of bisphosphonates. This antiresorptive effect of exercise can be confirmed by a couple of experimental studies using ovariectomized rats [48,49].

The normal range of the urinary NTX level in Japanese women (30–44 years of age) is 9.3–54.3 nmol BCE/mmol Cr [50]. Thus, the subjects of this study did not always show extremely high bone turnover. In this population, the more the urinary NTX level decreased, the more lumbar BMD increased. It is also known that the more the bisphosphonate alendronate decreases the urinary NTX level, the more lumbar BMD increases in postmenopausal women with osteoporosis [20,51]. The change in the urinary NTX level at month 6 correlated with the long-term lumbar BMD change ($r = -0.41$) in treatment with alendronate in elderly women [20]. The correlation coefficient of the percent change in the urinary NTX level at month 3 and that in lumbar BMD at month 12 in our study ($r = -0.409$) was similar to the effect of alendronate in elderly women. Thus, the decrease in the urinary NTX level might play an important role for the maintenance or increase of lumbar BMD in intervention with exercise as well as bisphosphonates. To date, very few studies have demonstrated a significant association between physical activity and urinary NTX level in postmenopausal women with osteopenia/osteoporosis. Urinary NTX appeared to be a more responsive and useful bone marker than serum BAP and OC in an intervention consisting of moderate walking exercise in postmenopausal women with osteopenia/osteoporosis, and an early change in the urinary NTX level may be useful to predict the long-term response of lumbar BMD to exercise, although its efficacy for increasing lumbar BMD may be quite modest.

We could demonstrate a significant antiresorptive effect of walking exercise on bone in postmenopausal women with osteopenia/osteoporosis in a prospective study. However, there are limitations that we have to discuss before we conclude that walking exercise is efficacious. First, even though we performed a prospective observation, the subjects were not randomly divided into the exercise and nonexercise (control) groups. Because exercise training requires effort, the subjects who hoped to perform walking exercise were assigned to the exercise group. Further randomized controlled studies are needed to confirm our results. Second, one can say that the duration of the study might be too short to evaluate the effect of exercise on lumbar BMD as well as bone metabolism. In fact, lumbar BMD increased in the exercise group as compared with the control group, but the increase from the baseline was modest. However, the main purpose of this study was to examine the effect of exercise on bone formation and resorption markers, especially on the urinary NTX level. We could obtain a rapid exercise-related reduction in urinary NTX level, followed by its steady state. Therefore, we believe that the duration of this study might be sufficient, if we focus on the effect of exercise on bone resorption markers such as urinary NTX.

In conclusion, this study clearly demonstrates that the mechanism for the positive response of lumbar BMD to moderate walking exercise in postmenopausal women with osteopenia/osteoporosis appears to be the suppression of bone turnover, and that an early change in the urinary NTX level may be useful to predict the long-term response of lumbar BMD to exercise, although its efficacy for increasing lumbar BMD may be quite modest. Further studies are needed to elucidate the efficacy of walking exercise on the bone quality and the risk of falls as an exercise-related effect.

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ウォーキング大会参加者の随時血糖値

Non-fasting blood glucose level of the participant in a walking rally

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キーワード：ウォーキング、随時血糖値、糖尿病
Keywords：Walking, non-fasting blood glucose concentration, diabetes

1．はじめに

わが国では糖尿病罹患者の増加が著しく、この30～40年間に30倍近く増加していると考えられている（日本医学会、2004）。また最近の厚生労働省の調査によると糖尿病が強く疑われている人が平成９年には690万人であったが、平成14年には740万人に増加しており、糖尿病の可能性を否定できない人を含めると、その数は1370万人から1620万人と、この５年間に250万人も増加していることも明らかになっている（厚生労働省健康局、2004）。

糖尿病は、放置すると網膜症・腎症・神経障害などの合併症を引き起こし、さらに失明や透析治療が必要となる。また、脳卒中・虚血性心疾患などの心血管疾患の発症を促進することも知られている。このような糖尿病の合併症は、患者のQOLを著しく低下させるのみでなく、医療経済的にも大きな負担を社会に強いている。

糖尿病の発症予防には、一次予防として身体活動の増加や食事の改善などの生活習慣が第一であり、身体活動量の増加や食事の改善により糖尿病の予防が可能である。(The Diabetes Prevention Program (DPP) research group, 2002)

一方、糖尿病の前段階である耐糖能異常の状態あるいは糖尿病に罹患しても高血糖状態は自覚症状のないことから、高血糖及び初期糖尿病状態が見逃され、生活習慣改善の取り組みの開始が遅れることがある。したがって、生活習慣の改善により糖尿病状態の改善が可能である高血糖者や初期糖尿病罹患者を早期に発見することは糖尿病悪化の抑制に重要である。また、生活習慣の改善は健康に対する高い関心をもつことが、大きな動機付けとなる。特にウォーキングを習慣的に行っている人々は健康に関心が高く、さらに、糖尿病の一次予防として有効である身体活動量が多いと推測される。そこで、本研究ではウォーキング大会参加者の随時血糖値を測定し、糖尿病の可能性が高い人の割合を観察し、厚生労働省が行った平成14年度糖尿病実態調査の対象者と比較することを目的とした。

2．方　法

2005年５月３日（火）の午前７時30分から午後１時30分にかけて、第10回東京国際スリーデーマーチの中央会場（東京都武蔵野市・武蔵野中央公園）において、ウォーキング大会に参加した男女計202人（男性105人 平均年齢67歳、 女性97人 平均年齢60歳）を対象に随時血糖値の測定を行った。随時血糖値とは、空腹や空腹時間を考慮せず随時に測定した血糖値である。

性別、年齢、食事摂取時間を記入してもらった後の対象者の指尖から血液を微量採取し、その血糖値を血糖測定機器　アセンシアブリーズ(Bayer社) を用いて測定した。

3. 結　果

本研究の対象者数は全体では、10歳代１人（男性０人、女性１人）、20歳代５人（男性１人、女性４人）、30歳代４人（男性１人、女性３人）、40歳代９人（男性２人、女性７人）、50歳代35人（男性18人、女性17人）、60歳代84人（男性42人、女性42人）、70歳以上　64人（男性42人、女性22人）であった。

血糖値をウォーキング前に測定したのは149人（男性76人、女性73人）、ウォーキング後に測定したのは、53人（男性29人、女性24人）であった。ウォーキングを行った前に測定した人の随時血糖値（平均±標準偏差:120±38mg/dl）と、ウォーキングを行った後に測定した人の随時血糖値（131±38mg/dl）に有意差は見られなかった（P＞0.06）。また、糖尿病治療のための薬を服用している人は、３人（男性２人、女性１人）であった。

随時血糖値が200mg/dl以上の人は、全体で10人（男性９人（全男性中８％）、女性１人（全女性中１％））であった。随時血糖値が200mg/dl以上の人を年代別に見ると、男性では50歳代で18人中３人（50歳代男性中14%）、60歳代で42人中５人（60歳代男性中11%）、70歳代で38人中１人（70歳以上男性中３％）であった。随時血糖値が200mg/dl以上の女性は60歳代の42人中１人（60歳代女性中２％）のみであった（表１）。糖尿病治療のための薬を服用している人３人のうち１人が随時血糖値200mg/dl以上であった。

食事摂取後時間（血糖値測定と直近食事の間の

表１　随時血糖値が200mg/dl以上の人数（人）

	総数	10~19歳	20~29歳	30~39歳	40~49歳	50~59歳	60~69歳	70歳~
男女計	10	0	0	0	0	3	5	1
男性	9	0	0	0	0	3	4	1
(%)	(8)					(14)	(11)	(3)
女性	1	0	0	0	0	0	1	0
(%)	(1)						(2)	

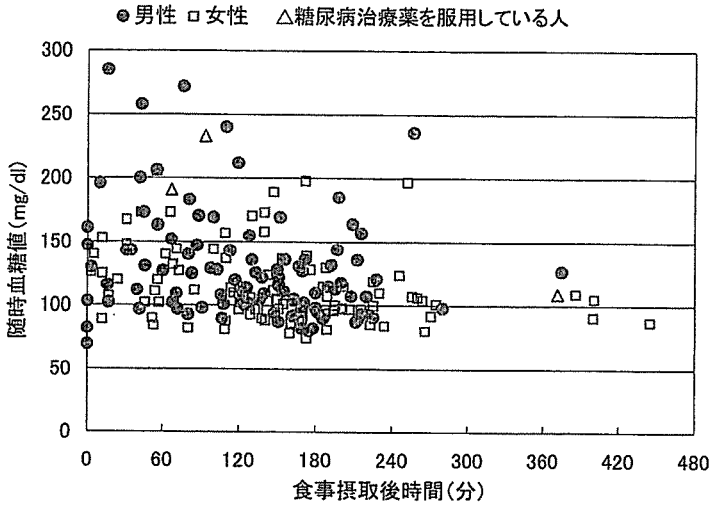

図１　食事摂取後時間と随時血糖値

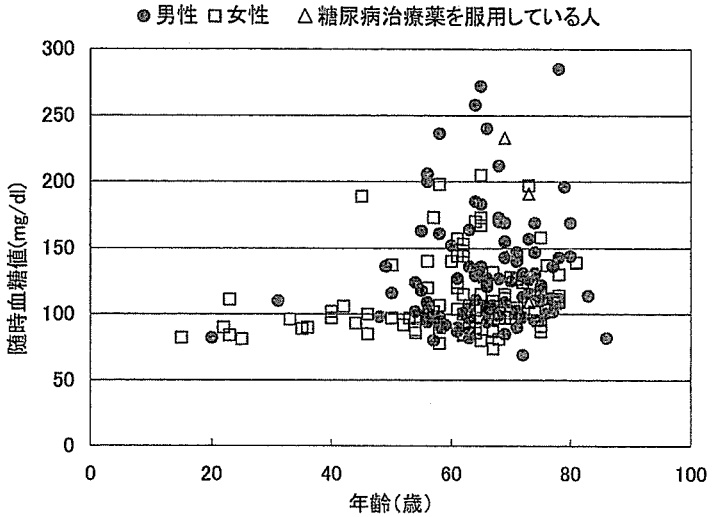

図２　年齢と随時血糖値

時間）と随時血糖値の関係をみると、食事摂取から時間が経つほど、血糖値が低くなっていることが観察された。（図１）

年齢と随時血糖値の関係をみると、年齢が若い人では高血糖者は見られず、年齢が上がるにつれ、男女とも50及び60歳代から高血糖値を示す人数が増加している傾向にあった。（図２）

4. 考　察

本研究により、糖尿病の可能性が高い随時血糖値が200mg/dl以上の対象者は、全体で男性８％、女性１％であり、平成14年度糖尿病実態調査報告（厚生労働省健康局）と比較すると（糖尿病が強く疑われている人：男性12.8%、女性6.5%）少ない傾向にあった（図３）。

本研究と平成14年度糖尿病実態調査を年齢別に比較すると、50歳代の男性では糖尿病が強く疑われる人の割合は14%と同じ値であった。また60歳代の男性では本研究の割合（11%）の方が平成14年度糖尿病実態調査の割合（17.9%）よりも低い値を示し、70歳以上男性においても本研究の割合（３%）の方が平成14年度糖尿病実態調査の割合（21.3%）よりも低い値を示した。今回、ウォーキング歴について調査を行っていないが、60歳代と70歳以上の男性で糖尿病が強く疑われる人の割合

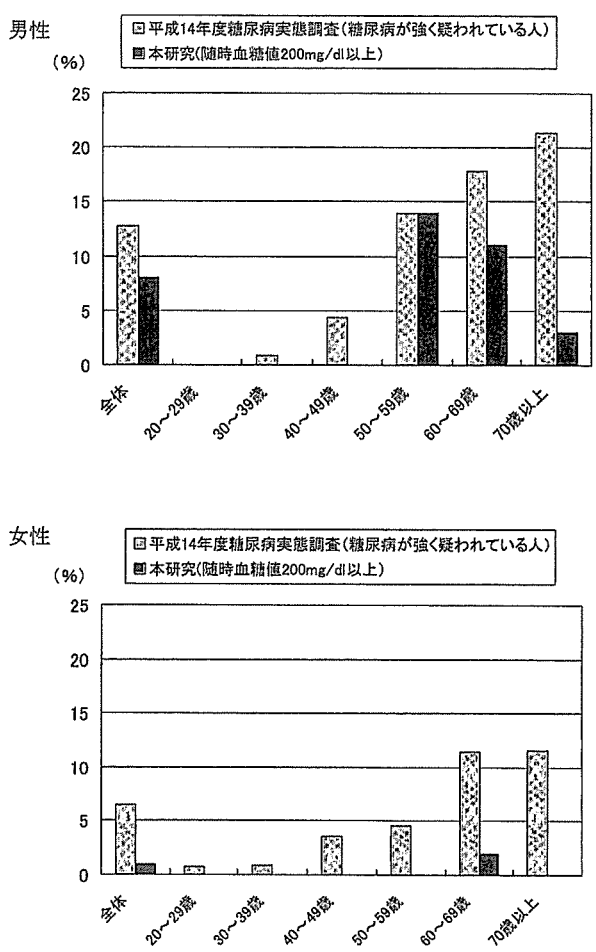

図３　本研究と平成14年度糖尿病実態調査との比較

が低い理由は、男性の場合は会社を退職した60歳代から糖尿病予防に有効なウォーキングを始め、年齢が高くなり、ウォーキング歴が長い人ほど糖尿病罹患者の割合が低くなるからであるということが推測される。一方、平成14年度糖尿病実態調査の値と同程度であった50歳代男性においては、ウォーキング歴が浅いか、もしくは単発的に今回のウォーキング大会に参加したためウォーキングによる糖尿病予防効果が現れていないのではないかと考えられる。

女性においては、随時血糖値が200mg/dl以上の人が60歳代の1人のみ（60歳代女性中2％）であり、平成14年度糖尿病実態調査の糖尿病が強く疑われる人の割合（11.5％）と比較すると低い値を示し、70歳以上女性においても本研究の割合（0％）の方が平成14年度糖尿病実態調査の割合（11.6％）よりも明らかに低い値を示した。この理由として、女性のウォーキング大会参加者は男性よりも早い年代からウォーキングを始めるひとが多いと推測されるため、ウォーキング歴が長く、ウォーキングの糖尿病予防効果が男性よりも早い年代から現れるからではないかと考えられる。また、50歳代の女性において本研究の割合（0％）は、平成14年度糖尿病実態調査の糖尿病が強く疑われる人の割合（5％）よりも低かったが、本研究の50歳代女性の対象者は17人と少なかったため平成14度糖尿病実態調査の値と比較することが不可能であり、この差が有意なものであるか不明である。

本研究の結果より、ウォーキングを行っている中高年男女は、糖尿病罹患率が低い可能性が示唆された。糖尿病とは、身体の糖代謝能が低下している状態である。食事などで摂取した糖質の約90％は、骨格筋から取り込まれて処理されるので（DeFronzo et al, 1981）、身体全体の糖代謝能は骨格筋の糖代謝能により決まっていると考えられている。さらに、この骨格筋での糖取り込み速度は、糖輸送体である骨格筋のGLUT-4（glucose transporter 4）濃度と高い相関があることが報告されており（Goodyear et al, 1990、Henriksen et al, 1990）、身体全体の糖代謝能に骨格筋のGLUT-4濃度が大きな影響を与えると考えられる。身体トレーニングにより骨格筋のGLUT-4が増加する（Rodnick et al, 1990）。したがって、本研究で明らかとなったウォーキング実践者の糖尿病罹患率が低い理由として、①習慣的なウォーキング活動による糖取込み量増加という急性の効果と、②GLUT-4増加という慢性的なウォーキングの効果が関与していると推測される。最近、我々は、この骨格筋のGLUT-4発現の機序に関係していると考えられている転写活性化補助因子PGC-1α（peroxisome proliferator-activated receptor γ coactivator 1）の発現が身体運動により増加することを報告している（Terada et al, 2004）。このような糖取り込みに関するメカニズムの観点から見ても、ウォーキングを行うことが糖尿病予防に効果的であることが示唆された。

5. 結　論

本研究の結果から、男女とも60歳代、70歳以上のウォーキング大会参加者は糖尿病罹患率が低いことが明らかになった。これらのことから、ウォーキングを行う余暇時間がある年代で、ウォーキング歴がある程度長い人においては、ウォーキングが糖尿病になる可能性を低下させることが示唆された。

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Cooperative effects of isoflavones and exercise on bone and lipid metabolism in postmenopausal Japanese women: a randomized placebo-controlled trial

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Abstract

Cooperative effects of isoflavones and exercise on bone and lipid metabolism have been exhibited in estrogen-deficient animals; however, results from clinical trials have not been published. In this study, we determined the effects of isoflavone intake and walking and their interaction on bone and lipid metabolism in postmenopausal women over 24 weeks. The bioavailability and metabolism of isoflavones (daidzein in particular) were also examined to clarify the mechanism of their bone-protective effects in humans. One hundred twenty-eight subjects were randomly assigned to 4 groups: placebo; placebo combined with walking (3 times per week); isoflavone intake (75 mg of isoflavones conjugates per day); and isoflavone combined with walking. The subjects were classified by equol status (producers or nonproducers) as identified using production of equol from daidzein in fecal culture. Bone mineral density (BMD), body composition, and serum concentrations of isoflavones were assessed. Serum high-density lipoprotein cholesterol concentration significantly increased (6.1%, $P = .03$), and fat mass in the whole body significantly decreased (−4.3%, $P = .0003$) from the baseline in the combined intervention group. There were no significant differences in BMD between baseline and postintervention in any of the treatment groups. However, the percent changes in BMD in equol producers were −0.53% and +0.13% in the sub–whole body and total hip, respectively. This was significantly different compared with −1.35 and −1.77 for the sub–whole body and total hip, respectively, in nonproducers in the isoflavone group ($P = .049$ and .040, respectively). The mean serum equol concentration was significantly higher in equol producers than in nonproducers in the isoflavone groups, but not in the placebo group. The combination of isoflavones and exercise exhibited favorable effects on serum lipid and body composition of postmenopausal women. The findings of this study suggest that the preventive effects of isoflavones on bone loss depend on the individual's intestinal flora for equol production.

1. Introduction

Menopause is often associated with the incidence of several chronic diseases including osteoporosis, cardiovascular disease, and obesity [1-4]. Hormone replacement therapy (HRT) is the effective regimen to prevent these diseases in postmenopausal women [5,6]; however, it is accompanied by an increased risk of unfavorable outcomes [7].

Recently, phytoestrogens have received a great deal of attention for their potential role in preventive osteoporosis and hypercholesterolemia because they are not as likely as steroid hormones to cause undesirable side effects in estrogen-deficient animals and postmenopausal women [8-11]. The predominant phytoestrogens found in plants are soybean isoflavones, including genistin, daidzin, and glycitin, which have structures similar to that of estrogen [12]. We previously reported that genistein dose-dependently inhibited bone loss in both female and male osteoporotic animal models without any adverse effects [13-15]. However, conflicting results have been reported

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in several observational clinical studies, even among Asians who consume 10 to 100 times more isoflavones than Westerners [16]. Setchell et al [17] have recently suggested that equol, a specific intestinal bacterial metabolite of the isoflavone daidzein, is the single most important factor influencing the clinical efficacy of soy isoflavones in preventing bone loss, and individual variation in production capability may explain the mixed results in many studies.

On the other hand, it is well established that exercise is also effective in preventing bone loss and hypercholesterolemia resulting from estrogen deficiency in both animal and human studies [18-20]. Although high-intensity exercise can be expected to increase bone mass in pre/postmenopausal women, it is also often associated with stress fractures, especially in fragile skeletons. Walking is a relatively safe and common exercise among elderly people. However, it has a relatively low impact on bones and is, therefore, insufficient for the prevention of bone loss in postmenopausal women [21]. Thus, in clinical research, it has been shown that a combination of estrogen with exercise is more effective in increasing trabecular bone mineral density (BMD) in older women as compared with either treatment alone [22]. In this context, we have recently demonstrated that, in the prevention of bone loss and fat gain in estrogen-deficient animals, a combined intervention of moderate-intensity exercise and isoflavone administration was more advantageous than either treatment alone [23-25]. To assess this issue in humans, we examined the cooperative effects of soy isoflavone intake and walking on bone and lipid metabolism in postmenopausal Japanese women. Furthermore, we stratified the subjects by equol status, which is dependent on the individual's intestinal flora, to determine the actual effects of soy isoflavone on bone loss in early postmenopausal women.

The following questions were addressed in the present study:

1. Are there any cooperative effects of isoflavones and walking on bone and lipid metabolism and the body composition of humans?
2. Is there a positive association between soy isoflavone intake and the concentrations of serum isoflavones, including daidzein and equol, based on equol status?
3. Is there any difference in the effect of soy isoflavone intervention on the change in BMD between equol producers and nonproducers among postmenopausal Japanese women?

2. Materials and methods

2.1. Subjects

Subjects were recruited for this study through advertisements in local newspapers, and those who met the following criteria were enrolled in the study. Healthy postmenopausal women aged 45 to 60 years who were within 5 years of natural menopause defined as at least 12 months since last menstrual cycle were enrolled for the study. The subjects had not previously used hormone therapy, 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 National Institute of Health and Nutrition of Japan, and the study was carried out according to the guidelines of the Declaration of Helsinki.

One hundred forty-five potentially eligible women were invited to the screening examination. The criteria for the invitation were as follows: willingness to participate;

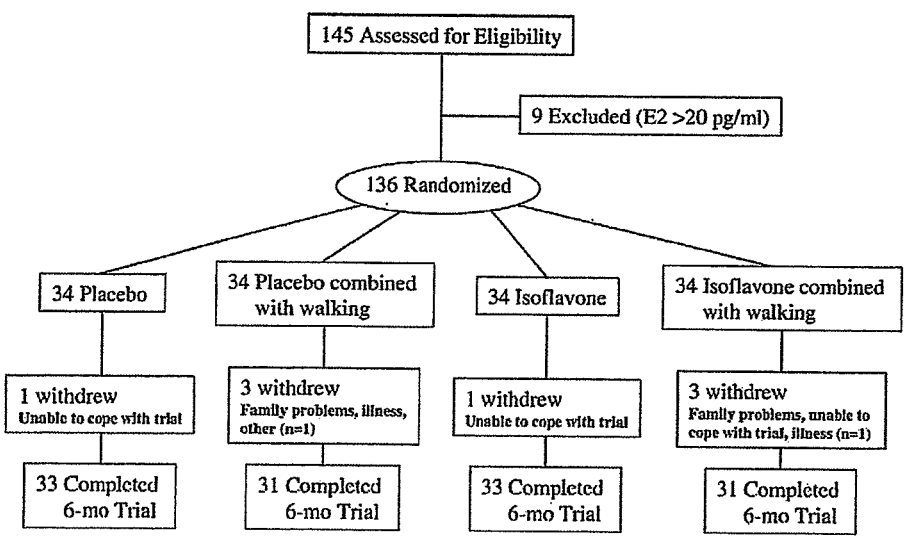

Fig. 1. Flow chart describing the progress of the participants during the trial.

clinically healthy (no cardiovascular, musculoskeletal, respiratory, or other chronic diseases that might limit walking exercise); sedentary (no regular sports activities for at least 2 years), nondieting, nonsmoking, and having no apparent occupational or leisure time responsibilities that might impede their participation. Nine participants were excluded at the medical screening because of their serum estradiol (E_2) concentrations (>20 pg/mL). Thus, 136 women were randomly assigned to 4 groups: (1) placebo; (2) placebo combined with walking; (3) isoflavone; and (4) isoflavone combined with walking. Eight women withdrew from the study because of illness, family problems, and feeling the intervention was a burden. The 128 subjects completed 6-month intervention and their data were included in the analysis (Fig. 1).

2.2. Intervention

Placebo or isoflavone capsules were blindly allocated to researchers and subjects throughout the study. Participants in the 2 groups, isoflavone and isoflavone combined with walking, received 2 capsules containing a total of 75 mg of isoflavone conjugates (47 mg as aglycone form, Fujiflavone P40, Fujicco, Kobe, Japan) with dextrin, daily in the morning. The 75 mg of isoflavone conjugate 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 2 placebo capsules containing only dextrin, daily in the morning.

Participants who were randomized into the walking groups were expected to attend three 1-hour 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 cooldown period. Participants were carefully instructed on the proper manner of walking to eliminate possible injury. The participants were instructed to maintain the speed of walking at 5 to 6 km/h, and this was monitored with a pedometer.

Nonwalking group participants did not engage in sports training and were asked to continue their customary activity levels. All participants were instructed to record their daily physical activity level that was continuously monitored by the pedometer, and their diaries were obtained and checked for completeness once a month.

2.3. Questionnaire interview

Individual information was collected by trained interviewers in face-to-face interviews based on a structured and previously validated questionnaire, and included sociodemographic data; years since menopause; physical activities, including hours spent sitting, standing, walking, sports, and leisure activities; medications; smoking and alcohol drinking; and other factors that may have possible confounding effects on the relation between dietary isoflavone consumption and metabolism of bone and lipid. The dietary assessment of intakes of soy isoflavones, calcium, vitamin D, total energy, and protein was based on 3-day diet records obtained at baseline and at 6 months.

2.4. Blood and urine samples

Fasting (>12 hours) blood samples were collected before BMD measurement by venipuncture in EDTA-containing tubes, refrigerated immediately, and within 2 hours centrifuged at 1500 rpm for 30 minutes at 4°C. Serum samples from each participant were stored frozen at −20°C. Serum concentrations of total cholesterol and triacylglycerol (TG) were determined using commercial kits (Cholesterol C-Test and Triglyceride G-Test, Wako Pure Chemical, Osaka, Japan). Serum high-density lipoprotein cholesterol (HDL-C) in the serum was measured by an enzymatic method (HDL-Cholesterol Test, Wako Pure Chemical). Estradiol was assessed by radioimmunoassay (Amersham Biosciences, Piscataway, NJ). A serum bone-specific alkaline phosphatase (BALP) (Alkphase-B; Metra Biosystems Ink, Mountain View, CA) was measured by using a microplate coated with an anti-BALP monoclonal antibody. Serum intact osteocalcin was measured using sandwich enzyme immunoassay 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). Urine samples were collected from a second voiding at the same time as serum extraction and they were stored at −20°C. Urinary deoxypyridinoline (DPD) was measured using sandwich enzyme immunoassay (PYRILINKS-D Assay, Metra Biosystems Ink).

2.5. Measurement of serum isoflavones

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

2.6. Identification of equol production in feces

Human feces collected from 122 subjects at baseline were stored at −80°C until use. Frozen fecal samples were thawed at room temperature, and 1 g was diluted in 9 mL Dulbecco phosphate-buffered saline (−) buffer (Nissui, Tokyo Japan)

and suspended. Fecal suspensions (0.5 mL) were incubated with 4.5-mL brain heart infusion medium (Difco Laboratories, Tokyo, Japan) containing 10 μg/mL daidzein at 37°C for 96 hours in anaerobic grove box (Hirasawa, Tokyo, Japan) of $CO_2/H_2/N_2$ (10:10:80, vol/vol). The fecal cultures (0.5 mL) were harvested and extracted with ethyl acetate, and then the ethyl acetate (isoflavone fraction) was evaporated. The residues were redissolved in 1 mL of HPLC solvent. Isoflavones were separated at 40°C by reversed-phase HPLC on a 4.5 × 250 mm Capcell Pak C18 column (Shiseido). The mobile phase consisted of 17% methanol and 3% ethyl acetate in 0.05% phosphate (A) and 2% ethyl acetate in methanol (B) with a linear gradient of 0% to 40% B. The flow rate was 1 mL/min, and data were acquired at 280 nm. Daidzein and equol were obtained from Extra Synthese, Genay, France. Equol producer status was determined by production of equol from daidzein in fecal culture after 96 hours' incubation. The average conversion rate from daidzein to equol in equol producers and non-producers was 87.4% and 0%, respectively.

2.7. Bone mineral density and body composition

Bone mineral density, including the lumbar spine (L2-L4), left hip, and sub–whole body (excluding head region), and body composition were assessed by dual energy x-ray absorptiometry at baseline and after 6 months with the use of Hologic QDR-4500A scanner (Hologic, Waltham, MA). The same staff conducted the scans and analysis. The short-term within-subject in vivo precision error in our laboratory for BMD was 0.5% for the spine, 1.5% for the total hip, and 0.8% for the whole body. Long-term precision was 0.35% by daily testing the spine phantom over the previous 1 year.

2.8. Statistical analysis

All values are expressed as means and SDs. Differences in baseline characteristics between the different groups were tested by 1-factor analysis of covariance (ANCOVA). Paired t test with Bonferroni adjustment was performed to determine whether change over the course of intervention

Table 1
Characteristics of subjects by study groups at baseline and at 6 months of intervention

	Placebo (n = 33)	Walking (n = 31)	Isoflavone (n = 33)	Isoflavone + walking (n = 31)
Age (y)	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 6 mo	156.4 (6.1)	155.1 (6.3)	155.6 (4.3)	154.6 (5.3)
Weight (kg/m^2)				
Baseline	51.4 (7.1)	54.1 (7.3)	51.5 (5.4)	52.9 (5.3)
After 6 mo	51.0 (7.3)	53.1 (7.3)	50.9 (5.7)	52.1 (5.5)
BMI (kg/m^2)				
Baseline	20.9 (2.2)	22.4 (2.9)	21.3 (2.5)	22.1 (2.0)
After 6 mo	20.8 (2.3)	22.1 (2.9)	21.1 (2.6)	21.8 (2.0)
Daily intake				
Isoflavone (mg)[a]				
Baseline	48.1 (30.6)	47.7 (25.0)	44.4 (26.9)	49.4 (25.0)
After 6 mo	45.6 (26.8)	44.1 (24.2)	40.8 (25.5)	36.9 (27.1)
Calcium (mg)				
Baseline	671.5 (190.9)	723.8 (221.5)	695.8 (253.5)	691.5 (213.3)
After 6 mo	625.4 (190.0)	693.0 (220.3)	621.7 (219.1)	722.9 (220.0)
Vitamin D (μg)				
Baseline	9.2 (5.9)	12.3 (13.1)	9.8 (5.9)	9.2 (5.0)
After 6 mo	7.0 (3.9)	6.3 (3.4)	6.8 (3.5)	7.2 (4.1)
Vitamin K (μg)				
Baseline	429.8 (172.2)	463.6 (207.0)	376.3 (211.0)	438.3 (181.9)
After 6 mo	389.5 (145.5)	471.5 (226.6)	383.0 (235.6)	434.6 (206.2)
Protein (g)				
Baseline	75.0 (13.5)	79.5 (17.4)	72.4 (14.3)	73.5 (13.5)
After 6 mo	73.3 (16.2)	69.4 (13.3)	72.5 (18.2)	74.0 (10.9)
Total energy (kJ)				
Baseline	8287.7 (1370.3)	8337.9 (1368.2)	8045.9 (1510.4)	8350.8 (1421.3)
After 6 mo	7951.7 (1743.9)	8044.2 (1440.0)	8049.2 (1638.9)	8155.0 (1271.5)
No. of walking (×10^4)				
During 6 mo	109.3 (40.3)	144.4 (61.9)*	108.8 (49.4)	159.1 (52.2)*

Values are expressed as mean (SD). There were no significant differences among the 4 groups for any of these characteristics at baseline and at 6 months. There were no significant differences between the baseline and after 6 months of intervention in each group.

[a] Except isoflavone capsules used for intervention.

* $P = .0004$; significant main effect of walking on the number of walking recorded by pedometer monitoring was analyzed using the 2-factor ANOVA model described in Materials and Methods.

Table 2
Serum E_2, lipid concentrations, and biomarkers of bone turnover, and their percent changes by study groups at baseline and at 6 months of intervention

	Placebo (n = 33)	Walking (n = 31)	Isoflavone (n = 33)	Isoflavone + walking (n = 31)	Main effects	
					Walking	Isoflavone
E_2 (pg/mL)						
Baseline	11.78 (2.64)	13.75 (6.55)	11.71 (3.48)	11.99 (3.08)		
After 6 mo	10.84 (2.00)	11.41 (4.02)	10.48 (1.19)	10.84 (1.67)	NS	NS
% Change	−5.77 (15.44)	−7.23 (41.88)	−6.08 (19.98)	−5.03 (23.51)	NS	NS
Osteocalcin (ng/mL)						
Baseline	10.51 (2.40)	10.47 (2.88)	9.23 (2.09)	9.50 (2.42)		
After 6 mo	9.89 (2.47)	9.21 (2.37)*	8.62 (2.20)	8.92 (2.19)*	NS	NS
% Change	−4.99 (15.92)	−9.91 (20.63)	−3.60 (19.14)	−5.85 (12.60)	NS	NS
BALP (U/L)						
Baseline	30.37 (11.61)	29.03 (6.65)	27.77 (8.63)	29.26 (8.26)		
After 6 mo	29.55 (9.59)	30.15 (6.43)	28.50 (6.08)**	29.47 (7.41)	NS	NS
% Change	−0.31 (15.40)	4.96 (15.55)	8.09 (19.24)	3.00 (13.94)	NS	NS
DPD (nmol/L per mmol/L creatinine)						
Baseline	7.76 (1.83)	7.65 (1.68)	7.30 (2.37)	6.88 (1.63)		
After 6 mo	7.35 (2.04)	7.21 (1.42)	6.98 (1.22)	6.89 (1.61)	NS	NS
% Change	−2.89 (27.47)	−1.06 (25.36)	−1.38 (23.87)	2.63 (20.81)	NS	NS
Total cholesterol (mg/dL)						
Baseline	227.4 (33.4)	232.8 (31.5)	227.9 (29.5)	230.7 (35.2)		
After 6 mo	223.0 (37.0)	236.2 (32.3)	230.3 (32.0)	232.0 (36.6)	NS	NS
% Change	−0.97 (15.19)	1.85 (10.29)	1.56 (12.65)	2.10 (13.13)	NS	NS
HDL-C (mg/dL)						
Baseline	71.7 (14.9)	71.0 (18.6)	74.2 (18.3)	66.2 (13.5)		
After 6 mo	71.5 (13.6)	71.7 (19.4)	73.5 (18.2)	69.0 (13.4)*	NS	NS
% Change	0.60 (10.85)	1.96 (10.61)	−0.97 (12.65)	6.14 (12.09)	$P = .04$†	NS
TG (mg/dL)						
Baseline	102.5 (49.0)	114.2 (70.2)	83.9 (38.5)	106.7 (55.1)		
After 6 mo	93.8 (41.8)	100.5 (32.4)	84.4 (34.9)	100.7 (61.7)	NS	NS
% Change	−3.66 (31.09)	−1.84 (30.64)	8.15 (35.96)	2.37 (62.04)	NS	NS

Values are expressed as mean (SD). NS indicates not significant.

* $P < .05$, significantly different from the baseline.

** $P < .01$, significantly different from the baseline.

† $P = .04$; significant main effect of walking on percent change of HDL-C was analyzed using the 2-factor ANCOVA model described in Materials and Methods.

was significantly different from baseline in each group. Percent change in BMD, body composition, serum lipid, and biomarkers of bone turnover was calculated {[(post-intervention − baseline values)/baseline values] ×100} for each group. Two-factor analysis of variance or ANCOVA was performed to determine the effect of isoflavone intake, walking, and their interactions after 6 months of intervention. When the subjects were stratified by equol status,

Table 3
Serum isoflavone concentrations by study groups at baseline and at 6 months of intervention

	Placebo (n = 33)	Walking (n = 31)	Isoflavone (n = 33)	Isoflavone + walking (n = 31)	Main effects	
					Walking	Isoflavone
Daidzein (nmol/L)						
Baseline	159.7 (143.0)	142.1 (146.0)	166.7 (128.7)	242.5 (360.0)		
After 6 mo	268.7 (276.2)	210.8 (199.9)	888.8 (841.7)**	899.7 (719.4)**	NS	$P < .0001$†
Genistein (nmol/L)						
Baseline	180.7 (136.1)	164.3 (183.8)	220.0 (199.9)	304.2 (371.9)		
After 6 mo	281.4 (457.0)	178.0 (178.6)	322.4 (275.7)	336.2 (301.0)	NS	NS
Glycitein (nmol/L)						
Baseline	63.8 (43.6)	54.3 (46.3)	66.8 (43.0)	65.5 (59.4)		
After 6 mo	65.3 (37.4)	71.7 (40.6)	194.0 (186.8)**	168.3 (155.2)**	NS	$P < .0001$†
Equol (nmol/L)						
Baseline	73.8 (201.7)	37.2 (79.4)	93.9 (196.1)	97.8 (232.3)		
After 6 mo	31.7 (131.9)	41.2 (131.1)	238.4 (348.6)*	228.4 (297.0)*	NS	$P < .0001$†

Values are expressed as mean (SD).

* $P < .05$, significantly different from the baseline.

** $P < .001$, significantly different from the baseline.

† Significant main effect of isoflavone ($P < .0001$) on serum daidzein, glycitein, and equol concentrations at 6 months was analyzed using the 2-factor ANCOVA model described in Materials and Methods.

3-factor ANCOVA was used to determine the effects of isoflavone, walking, equol status, and their interactions. Body weight, height, and daily intake including calcium, vitamin D, protein, and total energy were used as covariates in the analyses of body composition, BMD, and serum biomarkers to adjust for possible confounding. The significant differences in serum isoflavone concentrations and percent change in BMD between equol producers and nonproducers in each group were examined by using Student t test. Statistical analyses were performed using the PC SAS program, version 6.12 (SAS Institute, Cary, NC), and statistical significance was set at less than .05.

3. Results

3.1. General

The physical characteristics, daily intake of nutrients, and activity levels of the subjects at baseline and at 6 months of intervention are shown in Table 1. There were no significant differences in age, years since menopause, height, weight, BMI, and daily intake of isoflavones, calcium, vitamin D, and total protein among the different treatments groups at baseline. Average daily intake of isoflavone from soy foods (except isoflavone capsules) in each group at baseline was 44.4 to 49.4 mg. Six months of intervention of walking and isoflavones did not affect these parameters. The number of steps recorded by pedometer monitoring during the 6 months of intervention was significantly higher in the 2 walking groups as compared with the nonwalking groups.

3.2. Classification of equol producers and nonproducers

Fresh fecal samples were collected from 122 subjects to classify equol producers and nonproducers. Sixty-eight subjects (55.7%) were classified as equol producers because their fecal bacteria were able to convert daidzein to equol.

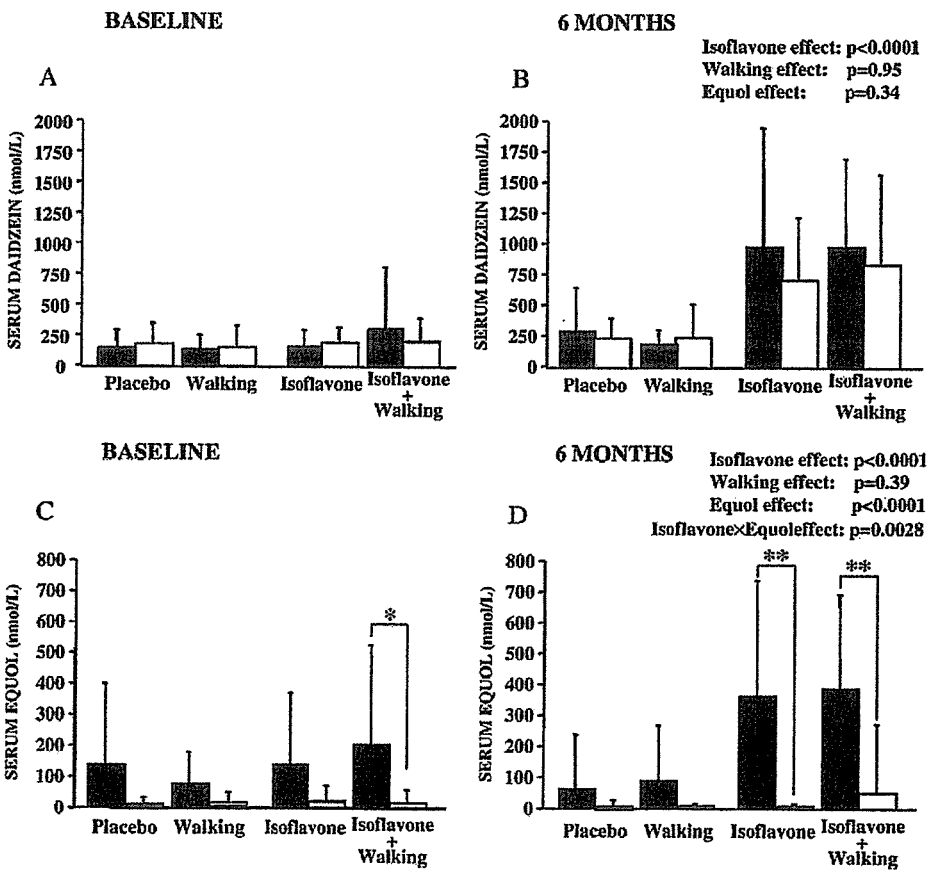

Fig. 2. Mean (SD) serum daidzein and equol concentrations in the study groups at baseline (A, C) and at 6 months of intervention (B, D). The subjects were stratified by equol status in each study group, and referred to as equol producers (■) and nonproducers (□). Differences in daidzein and equol concentrations among the study groups were nonsignificant at baseline (A, C). Differences in daidzein concentration between equol producers and nonproducers were not significant at baseline (A). Differences in equol concentration between equol producers and nonproducers in the isoflavone combined with walking group were significant (Student t test; $^*P < .05$) at baseline (C). The main effects of isoflavone, walking, and equol status and their interaction on serum daidzein and equol concentrations at 6 months were analyzed using 3-factor ANCOVA model described in Materials and Methods (B, D). Differences in daidzein concentration between the equol producers and nonproducers in each group were nonsignificant at 6 months (B). Differences in equol concentration between equol producers and nonproducers in the 2 isoflavone intervention groups (isoflavone and isoflavone combined with walking) were significant (Student t test; $^{**}P < .01$) at 6 months of intervention (D).

Fifty-two subjects (42.6%) were classified as nonproducers. Two subjects (1.6%) could not be classified as either equol producers or nonproducers. The number of equol producers and nonproducers in each group is as follows: the number of equol producers was 17, 15, 22, and 14 in the placebo, walking, isoflavone, and isoflavone combined with walking groups, respectively; the number of nonproducers was 11, 13, 11, and 17 in the placebo, walking, isoflavone, and isoflavone combined with walking groups, respectively. The equol producers defined from fecal analysis were almost the same as those who had high concentration of serum equol.

Serum concentrations of E_2, lipids, and biomarkers of bone turnover at both baseline and at 6 months of intervention are shown in Table 2. Statistically significant differences in serum concentrations of E_2, lipids, and biomarkers of bone turnover at baseline were not observed among the different groups. When these indices at 6 months were compared with those at baseline, the serum E_2, total cholesterol, TG concentrations, and the urinary biomarker of bone resorption (DPD) were not changed by walking, isoflavone, or their combination. High-density lipoprotein cholesterol concentration significantly increased from baseline by 6.1% ($P = .03$) in the combination of walking and isoflavone group, but did not significantly change in the other groups. Intact osteocalcin significantly decreased from baseline by 9.9% and 5.9% in walking and combined groups ($P = .01$ and .01, respectively). BALP significantly increased from baseline by 8.1% in the isoflavone group.

Table 4
Body composition, BMD, and their percent changes by study groups at baseline and at 6 months of intervention

	Placebo (n = 33)	Walking (n = 31)	Isoflavone (n = 33)	Isoflavone + walking (n = 31)	Main effects	
					Walking	Isoflavone
Sub–whole-body BMD (g/cm^2)						
Baseline	1.002 (0.096)	0.979 (0.100)	1.003 (0.108)	1.008 (0.070)		
After 6 mo	0.994 (0.089)	0.976 (0.095)	0.996 (0.105)	1.002 (0.069)	NS	NS
% Change	−0.74 (1.55)	−0.26 (1.58)	−0.69 (1.19)	−0.88 (1.57)	NS	NS
Whole-body lean mass (kg)						
Baseline	36.9 (3.7)	37.9 (4.2)	37.1 (3.2)	37.5 (3.2)		
After 6 mo	37.2 (3.9)	38.0 (4.3)	37.5 (3.5)	37.9 (3.2)	NS	NS
% Change	0.77 (2.39)	0.23 (2.27)	0.82 (1.75)	0.79 (1.84)	NS	NS
Whole-body fat mass (kg)						
Baseline	15.1 (4.5)	16.8 (4.3)	15.0 (4.1)	16.1 (3.5)		
After 6 mo	15.0 (4.5)	16.2 (4.2)*	14.5 (4.0)*	15.4 (3.7)*	NS	NS
% Change	0.17 (6.75)	−3.37 (6.35)	−2.92 (8.82)	−4.33 (6.03)	$P = .04$‡	NS
Lumbar spine BMD (g/cm^2)						
Baseline	0.907 (0.130)	0.879 (0.122)	0.891 (0.123)	0.909 (0.097)		
After 6 mo	0.904 (0.129)	0.866 (0.113)	0.884 (0.121)	0.901 (0.099)	NS	NS
% Change	−0.27 (2.64)	−1.30 (2.26)	−0.73 (2.44)	−0.90 (2.28)	NS	NS
Total hip (g/cm^2)						
Baseline	0.787 (0.126)	0.780 (0.114)	0.777 (0.125)	0.807 (0.089)		
After 6 mo	0.781 (0.121)	0.775 (0.109)	0.773 (0.126)	0.803 (0.086)	NS	NS
% Change	−0.66 (1.99)	−0.60 (2.31)	−0.50 (2.54)	−0.41 (1.86)	NS	NS
Femoral neck BMD (g/cm^2)						
Baseline	0.676 (0.114)	0.671 (0.116)	0.668 (0.106)	0.699 (0.094)		
After 6 mo	0.672 (0.104)	0.667 (0.110)	0.665 (0.094)	0.696 (0.093)	NS	NS
% Change	−0.25 (3.74)	−0.39 (4.09)	−0.04 (4.15)	−0.35 (3.68)	NS	NS
Trochanter BMD (g/cm^2)						
Baseline	0.599 (0.122)	0.592 (0.097)	0.591 (0.089)	0.600 (0.078)		
After 6 mo	0.594 (0.115)	0.589 (0.094)	0.593 (0.092)	0.597 (0.075)	NS	NS
% Change	−0.69 (2.80)	−0.31 (3.44)	0.29 (3.19)	−0.37 (2.49)	NS	NS
Trunk fat mass (kg)						
Baseline	6.5 (2.5)	7.9 (2.6)	6.8 (2.4)	7.7 (2.3)		
After 6 mo	6.1 (2.3)*	7.4 (2.5)*	6.4 (2.4)*	7.0 (2.2)*	NS	NS
% Change	−2.94 (9.84)	−6.62 (9.46)	−6.18 (14.23)	−7.56 (8.55)	NS	NS
Legs fat mass (kg)						
Baseline	2.9 (0.9)	2.9 (0.7)	2.7 (0.7)	2.7 (0.6)		
After 6 mo	3.0 (0.9)†	2.8 (0.7)*	2.7 (0.7)	2.6 (0.6)*	NS	NS
% Change	2.41 (7.22)	−1.45 (7.09)	0.91 (6.56)	−2.09 (6.86)	$P = .009$#	NS

Values are expressed as mean (SD).

* $P < .05$, significantly decreased as compared with the baseline.

† $P < .05$, significantly increased as compared with the baseline.

‡ $P = .04$; significant main effect of walking on percent change of whole-body fat mass was analyzed using the 2-factor ANCOVA model described in Materials and Methods.

$P = .009$; significant main effect of walking on percent change of the leg fat mass at 6 months was analyzed using the 2-factor ANCOVA model described in Materials and Methods.

By the 2-factor ANCOVA analysis, there was a significant main effect of walking (P = .04), but not isoflavone (P = .53) on the percent change in HDL-C.

3.3. Serum isoflavone concentrations

Table 3 shows the serum concentration of isoflavones at baseline and at 6 months. At baseline, there were no significant differences in the concentrations of isoflavones, except genistein (P = .041), among the 4 groups. The administration of isoflavones resulted in a marked increase in the serum concentrations of daidzein (P < .001), glycitein (P < .001), and equol (P < .05), but not that of genistein from baseline. In contrast, the placebo treatment did not modify the circulating concentrations of isoflavones. When using the 2-factor ANCOVA model, we found a highly significant effect of isoflavone (P < .0001).

Since it has been reported that the production of equol depends on the individual's intestinal flora, the subjects were stratified into 2 subgroups, referred to as equol producers and nonproducers. The serum concentrations of daidzein and equol are shown in Fig. 2. The production of equol in individuals was confirmed by measuring their ability to produce equol in feces. Differences in daidzein concentration between equol producers and nonproducers in each group were nonsignificant at baseline (Fig. 2A). However, the serum equol concentration was significantly higher in equol producers than in nonproducers in the isoflavone combined with walking group at baseline (P < .05) (Fig. 2C). After 6 months of intervention, the serum daidzein concentration markedly increased in the 2 isoflavone-administered groups, but not in the 2 placebo groups, regardless of whether they were equol producers or nonproducers (Fig. 2B). When using the 3-factor ANCOVA model, we found a significant main effect of isoflavone (P < .0001). On the other hand, serum equol at 6 months significantly increased from baseline (P < .05) in equol producers in the 2 isoflavone groups (Fig. 2D). In contrast, serum equol remained at baseline levels in nonproducers, which were significantly lower than those of equol producers (P < .01). In placebo and walking groups, serum equol remained at baseline levels in both equol producers and nonproducers (Fig. 2D). Again, using the 3-factor ANCOVA model, we found 2 significant main effects of isoflavone and equol status (both were P < .0001) and their interaction (P = .0028) on equol concentrations at 6 months (Fig. 2D).

3.4. Body composition and BMD

There was no significant difference among the 4 groups at baseline with respect to body composition, fat and lean mass in the whole body, and fat mass of the trunk and legs (Table 4). Fat mass in the whole body significantly decreased from baseline in the isoflavone (−2.9%, P = .006), walking (−3.4%, P = .004), and combined intervention (−4.3%, P = .0003) groups, but slightly increased in the placebo group (0.2%, P = .56). Fat mass in the legs significantly decreased in walking (−1.45, P = .04) and combined interventions (−2.09%, P = .01), but was not significantly changed in the isoflavone group (0.91%, P = .41). On the contrary, the fat mass in the legs significantly increased in the placebo group (+2.41%, P = .04) compared with baseline. Using the 2-factor ANCOVA model, we found significant main effects of walking on whole-body fat mass (P = .04) and legs fat mass (P = .009), but a nonsignificant effect of isoflavone (P = .12 and .41, respectively). There were no significant differences in BMD among the different groups in the sub–whole body, lumbar spine, and hip regions at baseline (Table 4). The percent changes in BMD in all the regions after 6 months showed similar trends in each group, but statistically significant main effect and interactions were not detected by the 2-factor ANCOVA model. When the subjects were stratified according to their equol-producing status, no statistically significant main effects or interactions were observed by 3-factor ANCOVA. However, the percent changes in BMD in equol producers were −0.53% and +0.13% in the sub–whole body and total hip, respectively, which were significantly different as compared with the percent changes of −1.35% and −1.77% in nonproducers in the isoflavone group (P = .049 and .040, respectively) (Fig. 3). In contrast, there were no significant differences in the percent changes in BMD between the equol producers and nonproducers in the placebo, walking, and isoflavone combined with walking groups.

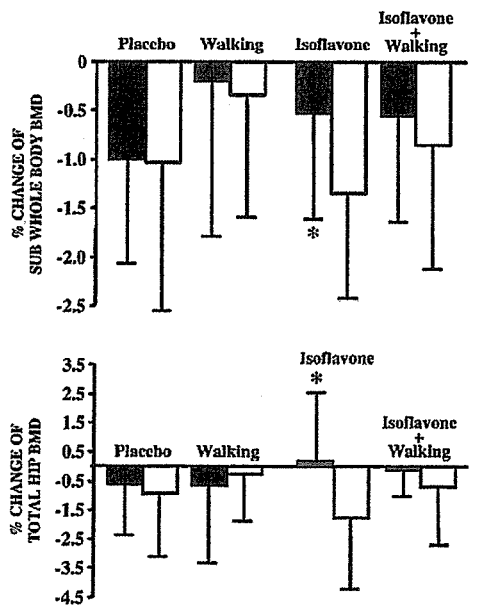

Fig. 3. Mean (SD) percent changes in BMD in the whole body and total hip at 6 months of intervention in the study groups. The subjects were stratified by equol status in both the study groups, referred to as equol producers (■) and nonproducers (□). Differences between equol producers and nonproducers in the isoflavone group were significant in the sub–whole body and total hip (Student t test; *P < .05).

4. Discussion

This randomized placebo-controlled study shows that the combined intervention of soy isoflavone intake and walking is most effective in decreasing the body fat and increasing the serum HDL-C concentration in early postmenopausal women, although the significant main effect was found for walking alone. Although isoflavone intervention for 6 months did not show a significant bone-protective effect, there was a significant difference in the percent change in BMD in the sub–whole body and hip regions between subjects stratified by their equol status.

It has been reported that soy isoflavone administration effectively lowered the serum cholesterol level in ovariectomized animals [25,26]. However, it is unclear whether isoflavones have clinically relevant and beneficial effects on lipid metabolism in humans [27]. In this study, it appeared that isoflavone intake for 6 months did not affect the blood lipid concentrations in healthy postmenopausal women. This result is similar to the reports from several clinical trials, which did not observe a hypocholesterolemic effect of isoflavones in postmenopausal women [27,28].

On the other hand, it has been reported that physical activity, such as brisk walking, significantly improved lipid metabolism and reduced body fat among overweight and obese postmenopausal women [29]. In a previous study, we reported that the combined intervention of exercise and isoflavone intake increased the HDL-C and decreased the body fat mass in ovariectomized mice [25]. In this study, we also observed an increase in HDL-C and a decrease in fat mass in postmenopausal women in the isoflavone combined with walking group. These results suggest that a combination of these 2 interventions may be a useful regimen for the management of serum lipids and body composition in postmenopausal women.

The effects of dietary soy isoflavones on biomarkers of bone turnover have been investigated in a few human trials. In this study, we found that serum BALP significantly increased from baseline in the isoflavone group. This result is similar to that of Morabito et al [30] as well as that of Arjmandi et al [31] who reported that the intervention of isoflavones increased serum BALP in postmenopausal women. The beneficial effect of soy isoflavones on BMD is still controversial, although impressive data from many studies on animal models of postmenopausal osteoporosis support a significant bone-protective effect of genistein and daidzein [32]. Nagata et al [33] reported that neither soy product and isoflavone intake nor serum isoflavone concentrations were associated with BMD. However, Somekawa et al [34] reported a significantly positive correlation between isoflavone intake and BMD at the lumbar spine in postmenopausal Japanese women. Several previous studies on dietary intervention have examined the effect of soy isoflavone on bone loss in postmenopausal women. In a study reported by Potter et al [35], diets containing 90, 56, and 0 mg of soy isoflavones per day over 6 months affected the BMD of the lumbar spine by 2.2%, −0.2%, and −0.6%, respectively. Chen et al [36] reported that soy isoflavone aglycone supplementation of 80 mg/d resulted in mild, but statistically significant and favorable percent changes in hip BMC (but not BMD) compared with placebo in Chinese postmenopausal women who had lower baseline BMC values. In Western women, the loss of BMD and BMC in the lumbar spine, but not in the hip, was significantly lower in women taking 45 mg/d of a red clover–derived phytoestrogen supplement for 1 year than in those taking a placebo [37]. Furthermore, Morabito et al [30] reported that genistein treatment (54 mg/d) for 1 year significantly increased the BMD of the lumbar spine and femoral neck in Italian women. This improvement in BMD was similar or slightly greater than that observed with HRT treatment. In this study, we did not find an effect of soy isoflavone on BMD even in the combined group after 24 weeks of intervention. The most possible explanation for the discrepancy between the promising findings and our results is that the duration of the intervention in our 6-month trial has been too short. Other considerable factors are the dose of isoflavones and daily intake of isoflavones from the diet of the subjects. However, the dose of isoflavones used in this study, 47 mg as aglycone form, was not low compared with those in the previous studies [30,36,37]; the Japanese diet contains higher soy products and more readily absorbed forms of isoflavones compared with Western and Chinese diets [38]. In our trial, the subjects were not restricted intake of soy products during the intervention (average isoflavone intake of each group was from 44.4 to 49.4 mg/d). The duration of the supplementation and these dietary differences in soy foods may be major reasons for the lack of isoflavone's effects on BMD in this study. These conditions need to be elucidated in further research.

Setchell et al [17] recently suggested that equol, a specific metabolite of daidzein produced by intestinal bacteria, may be the single most important factor that influences the clinical efficacy of soy isoflavone in preventing bone loss. This metabolite is not found in soy, but is formed by the intestinal flora in only 45% of the postmenopausal women studied. Setchell et al [17] reported that the lumbar spine BMD of equol producers increased by 2.4% ($P < .001$) as compared with the control group, whereas there was no significant change in BMD in the nonproducers after 2 years of intervention with isoflavones. In this study, we also stratified the subjects based on the equol-producing capacity of the individual's intestinal flora to investigate the actual effects of soy isoflavone on bone loss in early postmenopausal women. These results firstly showed that the loss of BMD in the sub–whole body and total hip were significantly lower in equol producers compared with nonproducers in Japanese treated with isoflavone. Furthermore, we demonstrated that the beneficial effect of isoflavones on bone could be attributed to the serum concentration of equol,

which was significantly higher in equol producers than in nonproducers in the isoflavone group. In contrast, because serum equol remained at baseline levels in both equol producers and nonproducers in the placebo groups, the loss of BMD did not differ between the producers and nonproducers. Our findings strongly support the hypothesis that the clinical effectiveness of soy products in bone health may be because of the ability of the subject to biotransform soy isoflavones to the more potent estrogenic metabolite, equol. Thus, the failure to distinguish subjects who are equol producers from those who are nonproducers in previous clinical studies could plausibly explain the variance in the reports on the benefits of soy intake [32]. Several specific intestinal bacteria capable of metabolizing soy isoflavone to equol have been identified from human feces [39,40]. Examination of equol production by the subjects who maintain the bacteria is now under investigation.

We are also interested in evaluating the effect of walking on the metabolism of isoflavones and bone loss in postmenopausal women because combined intervention of a submaximal dose of isoflavone and a moderate intensity of running exercise expressed more advantageous effects on the prevention of bone loss and fat gain in female and male osteoporotic model of mice than either treatment alone [23,24]. In this study, although the intervention of isoflavone combined with walking decreased fat mass in whole body and legs, we did not find any efficacy of the combined intervention on the change in BMD in postmenopausal women. These results suggest that the adjustment on bone metabolism needs longer term than lipid metabolism in humans. Bone remodeling is a relatively slow process, and the time required to complete a cycle may increase with age. Thus, longer-term trial would be required to evaluate the effects of isoflavone and isoflavone combined with walking on bone mass.

In conclusion, the combined intervention of soy isoflavone and walking for 6 months exhibited cooperative effects on modifying lipid metabolism and body composition in postmenopausal Japanese women. The beneficial bone effects of isoflavones depend on the equol-producing capability of an individual's intestinal flora in humans.

Acknowledgments

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Original article

Determination of reference intervals for vibratory perception thresholds of the lower extremities in normal subjects

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Abstract The assessment of vibratory perception thresholds (VPTs) is important for evaluating human somatosensory functions and provides new aspects in clinical practice and research. However, there has been little information concerning determinants of the VPT in normal individuals, and there has been no investigation to determine the reference intervals for the lower extremities by vibrometers using appropriate statistical analysis. We determined reference intervals for the VPT in lower extremities of 377 healthy subjects (241 males, 136 females; ages 11–74 years) using Suzuki-Matsuoka vibrometer-5 according to the National Committee for Clinical Laboratory Standards guidelines. The VPT was measured at 12 points: ulnar styloids, patellae, medial and lateral malleoli and the tip of the great toes, dorsal aspect of the distal phalanx of the fifth toes. The effects of age, sex, height, weight, alcohol consumption, and smoking on the VPTs were also investigated. The VPTs of normal subjects increased significantly with age. The difference between the two sides was negligible for determining reference intervals of the VPT. The average VPT was higher in men than in women at the lateral malleolus, the great toe, the patella, and the ulnar styloid but not significantly different at the fifth toe or the medial malleolus. There were no significant differences in the VPTs among the four sites of the foot. The reference interval for the VPT of the lower extremity is less than 13×10^{-2}G. The influence of body mass index, smoking, and alcohol consumption on the VPT was not significant. We provide the reference interval for the VPT of lower extremities in normal subjects. This information can serve as a basis for future clinical applications of VPT measurements.

Introduction

Quantitative assessment of somatosensory deficit and motor dysfunction is of considerable clinical significance for the diagnosis and treatment of spinal disorders. However, clinical evaluation for sensory perception has mostly been carried out qualitatively owing to technical limitations as well as time constraints.

In recent years, vibratory perception threshold (VPT) testing has been used to detect peripheral neuropathy in terms of amplitude.[1,2] It has been shown that the psychophysical responses to vibration are determined by activation of the Meissner and Pacinian corpuscles, which are rapidly adapting receptors.[3] It is, therefore, important to determine the VPT in terms of acceleration, as these mechanoreceptors respond to acceleration of stimulation.[4] The development of biothesiometers — simple, hand-held measuring devices for VPT — has enabled quantitative measurement of peripheral, sensory nerve function. The Suzuki-Matsuoka vibrometer-5 (SMV-5; Medience, Tokyo, Japan) (Fig. 1), which is one of such biothesiometers, was developed for the diagnosis of diabetic neuropathy. It is a fine instrument that can measure responses to acceleration of vibratory stimulation.

In Japan, the SMV-5 has gradually been used for several clinical studies.[5,6] Ohnishi et al. made a comparative study of the three vibrometers (SMV-5, Vibratron II, TM-31A) and reported that SMV-5 was the most reliable.[7] When diagnosing peripheral neuropathy based on measurement of the VPT, the determination of reference intervals for VPT data is epidemiologically and clinically important. However, there has been little information concerning determinants of the VPT in normal individuals, and there has been no investigation to determine the reference intervals for the lower extremities by the vibrometers using appropriate statistical analysis. In 1995 the National Committee for Clinical Laboratory Standards (NCCLS) proposed a guideline for terminology and procedures for determining reference intervals (C28-A).[8]

The purpose of the present study was to establish reference intervals for the VPT in the lower extremity of healthy Japanese subjects according to the NCCLS

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