

Title

Effects of additional resistance training during diet-induced weight loss on bone mineral density in overweight premenopausal women

Short running head

Effect of resistance training plus diet on bone

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Abstract

Bone loss accompanies a diet-induced weight loss and could be prevented with a combination of exercises. This study was conducted in order to examine the effects of additional resistance training during diet-induced weight loss on whole-body and selected regional bone mineral density (BMD). The participants for a 14-week weight-loss study were 42 overweight premenopausal Japanese women. They were randomly placed in either a diet-only group (D; $n = 21$) or a diet plus resistance training group (DR; $n = 21$). Whole-body BMD and body composition, lumbar-spine BMD, and 1/3 radial BMD were measured by dual-energy X-ray absorptiometry before and after the intervention. Bone formation and resorption markers were also measured. Thirty-five participants (83%) completed the study. Groups D ($n = 17$) and DR ($n = 18$) individuals lost 6.2 ± 3.5 kg and 8.6 ± 3.6 kg body weight, respectively. Reductions in percentage fat mass and fat mass in Group DR were significantly greater than in Group D, while lean mass decreased significantly in both groups. The effect of time on whole-body BMD was significant (-0.3%); however, whole-body bone mineral content, lumbar-spine BMD, and 1/3 radial BMD remained unchanged. There were no significant time-by-group interactions in the whole-body and regional BMD and bone markers. These results suggest that additional resistance training during weight loss has no effect on BMD in overweight premenopausal Japanese women. Further long-term studies with large numbers of subjects are needed.

Keywords: body composition; bone mass; resistance training; weight loss

Introduction

Obesity is closely associated with major health risk factors [1,2], and the prevalence of obesity continues to increase in developed and developing countries [3,4]. Because weight-loss treatment benefits the health of obese individuals [5], such individuals should be treated using an appropriate program. However, epidemiological studies have reported that people who lose weight also experience a reduction in their bone mass [6–9]. Therefore, weight loss is regarded as one of the risk factors for osteoporosis [10].

The fact that bone loss accompanies weight loss has been demonstrated in numerous intervention studies [11–18]. In addition, an observational study on elderly women revealed that the effect of weight loss on bone mass is mostly apparent in comparatively thinner women (most pronounced in those who weighed 60 kg or less, followed by those who weighed between 61 and 70 kg; whereas, among those who weighed more than 70 kg, no significant bone loss was observed) [9]. Since the criterion defining obesity in Japan is more severe than in the Western countries, due to a greater susceptibility to obesity-related complications [19], many comparatively thinner Japanese women are advised to reduce their body weight. Thus, it is very important to examine the preventive treatments designed to counter diet-induced bone loss in Japanese women.

Exercise has a positive influence on the skeleton [20,21], and the peak load is more important than the number of loading cycles in increasing bone mass [22]. Therefore, in clinical settings, resistance training is believed to have a greater effect on bone structure. However, there are a limited number of previous studies on the combination of resistance training and diet-induced weight loss, particularly in premenopausal women. A recent study

[23] revealed the significance of resistance training during diet-induced weight loss in older overweight adults with type 2 diabetes. The present study examined the hypothesis that additional resistance training during diet-induced weight loss would be more beneficial for bone mineral density (BMD) in overweight premenopausal Japanese women.

Methods

Participants

The participants for a 14-week weight-loss study were recruited through advertisements in local newspapers. Forty-two Japanese women who met the following criteria were selected: (1) a history of regular menstrual cycles, (2) good general health, and (3) not taking any medications known to interfere with bone metabolism. They were randomly placed in one of the following weight-loss groups: diet only (D; $n = 21$), or diet plus resistance training (DR; $n = 21$). The aim and design of the study were explained to each participant before they gave their written informed consent, and the study was approved by the Human Investigation Review Committee of the University of Tsukuba.

Dietary protocol

All the participants were instructed to restrict energy intake to 1200 kcal/d and to consume at least 800 mg of calcium per day. In order to ensure proper daily nutrition, participants consumed a well-balanced supplemental food product (MicroDiet; Sunny Health, Nagano, Japan) as one of their daily meals for the first month. This product was developed for very low-energy diets (173 kcal per pack) and comprises protein, carbohydrates, fat, various amino acids, vitamins and minerals [24]. Each of the other meals consisted of 80 kcal of eggs and/or dairy products; 80 kcal of meat, fish, and/or soybean products; 80 kcal of vegetables and fruits; and 160 kcal of carbohydrates and oils. In order to ensure the daily consumption of sufficient amounts of daily protein and calcium, participants were instructed to consume a protein supplement (FeedBack WheyProtein; Meiji Dairies, Tokyo,

Japan) containing 14.0 g of protein, 1.6 g of carbohydrate, and 0.18 g of fat (64 kcal per pack). When mixed with 200 ml of low-fat milk, the drink supplied 21.6 g of protein and 490 mg of calcium (171 kcal). The participants also kept daily food diaries during the 14-week intervention period, and met weekly as a group with a dietitian who encouraged weight loss through nutrition education and dietary behavior modification. In order to determine the dietary intake, 3-day food records were completed at baseline and at week 10. Experienced dietitians analyzed the data (Eiyoukun, version 4.0; Kenpakusya, Tokyo, Japan).

Resistance training

In addition to restricting energy intake, the participants in Group DR completed three 90-minute resistance training sessions per week on alternate days. Each session comprised warm-up, free-weight resistance training, and cool-down. The resistance training program designed for myopachynsis entailed bench presses, squats, leg curls, leg extensions, and sit-ups. Since participants had no prior experience of free-weight resistance training, each training session began with the use of a very light load (*e.g.*, only bar in bench presses) in order to learn performance safety and precision. From week 2 to 4, their training program was scheduled as follows: 1 set of warm-ups and 3 sets of intensive training with 12–15 repetitions in each set. Load intensity was decided based on the rating of perceived exertion (RPE) scale; the criterion being what the participants considered to be “somewhat hard”. At the beginning of week 5, 8–10-repetition maximum tests were performed for bench presses, leg curls, and leg extensions, and maximal lifts were estimated using generalized charts

[25]. Based on these results, the participants were instructed to increase their load intensity to 50% maximal lifts with 10–15 repetitions. The loads were gradually increased when they were able to complete 3 sets with 15 repetitions. During the last 4 weeks, they performed 10–15 repetitions with approximately 60% maximal lifts. At the end of week 14, the 8–10-repetition maximum tests were performed again for the 3 training events.

Body composition and bone mineral density

The participants were scanned using a Lunar DPX-L densitometer (Lunar Corporation, Madison, WI) with manufacturer-supplied software (version 1.35). Each scan was analyzed using the extended research analysis mode [26]. Whole-body BMD, bone mineral content (BMC), percentage fat mass, fat mass, and bone- and fat-free lean tissue mass (lean mass) were determined. Pixels of soft tissue were used to calculate the ratio of mass attenuation coefficients (R-value) at 40 to 50 keV (low energy) and 80 to 100 keV (high energy). Lumbar spine (L2–4) and 1/3 radial BMD were also measured using the same instrument. The within-participants coefficients of variation in this instrument were 0.3–1.1% for BMD (whole body, 0.3%; L2–4, 0.4%; 1/3 radial, 1.1%), 0.8% for whole-body BMC, 2.4% for percentage fat mass, 2.5% for fat mass, and 0.5% for lean mass in another population ($n = 17$ for L2–4 and 1/3 radial BMD or $n = 34$ for the other variables). Body weight was calculated as the sum of BMC, fat mass, and lean mass. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2).

Bone markers

Bone formation was estimated from the serum concentrations of bone-specific alkaline phosphatase (BAP) and osteocalcin (OC). BAP was determined by an enzyme immunoassay (SRL, Tokyo, Japan), and OC was determined by an immunoradiometric assay (Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan). All blood samples were collected in the morning after an overnight fast. Bone resorption was estimated from urinary deoxypyridinoline (DPD) cross-links and N-telopeptide of type I collagen (NTX). All urine samples were collected from the second urine voiding of the morning. DPD was measured by an enzyme immunoassay (Kotobiken Medical Laboratories, Tokyo, Japan) and expressed in terms of nanomoles per millimoles of urinary creatinine. NTX was measured by an enzyme immunoassay (SRL, Tokyo, Japan) and expressed as nanomoles of bone collagen equivalent (BCE) per millimoles of urinary creatinine. The within-participants coefficients of variation were 3.7% for BAP, 3.8% for OC, 4.3% for DPD, and 5.8% for NTX in another population where $n = 10$.

Physical activity

Participants were instructed to maintain their daily physical activity. The total number of steps of the participants in each group was calculated using a pedometer (Lifecorder; Suzuken, Nagoya, Japan). The accuracy and reliability of this pedometer was reported by Crouter et al. [27]. In order to determine the participants' physical activity throughout the study, they wore the pedometer for 7 days at baseline and at week 10 of the weight-loss program.

Statistical analysis

Paired *t*-tests were used to assess the changes in all the variables between groups. The difference between groups in the descriptive characteristics before the intervention and dietary intake during the intervention were examined using unpaired *t*-tests. Two-way analyses of variance (ANOVA) with repeated measurements were performed to assess the significance of the main effects of time and time-by-group interactions. Values are expressed as the mean \pm standard deviation. The data was analyzed using SPSS statistical software (version 15.0J; SPSS, Chicago, IL), with the level of statistical significance set at 5%.

Results

Participant characteristics

Thirty-five of the original 42 participants (83%) completed the study (D, $n = 17$; DR, $n = 18$). The descriptive characteristics of the 35 participants are summarized in [Table 1](#). There were no significant baseline differences in age, height, body weight, BMI, and percentage fat mass between the groups.

Adherence to the interventions

Adherence to the weight-loss program averaged 87.4% (range 64.3–100%) in Group D (14 sessions) and 93.6% (range 77.5–100%) in Group DR (40 sessions). There were no significant differences between the groups.

Changes in dietary intake

As shown in [Table 2](#), the mean total energy intake calculated from the available food records (D, $n = 16$; DR, $n = 17$) decreased significantly. Fat and carbohydrate intake also decreased significantly, while calcium intake exhibited a significant increase. The intake of other nutrients remained unchanged. Although significant interactions were observed in certain nutrient intakes, there were no significant differences between the groups in any nutrient intake during the intervention.

Changes in physical activity

The total number of steps calculated before and during the intervention were 8037 ± 2523

steps/d in D and 9006 ± 2696 steps/d in DR, and 9109 ± 2810 steps/d in D and 8831 ± 2339 steps/d in DR, respectively. No significant time effect or time-by-group interaction was observed.

Changes in estimated maximal lifts

Estimated maximal lifts (measured only in Group DR) at the beginning of week 5 were 35.3 ± 5.0 kg for bench presses, 21.6 ± 4.1 kg for leg curls, and 52.4 ± 10.2 kg for leg extensions; at the end of week 14, the values significantly increased to 37.4 ± 4.6 kg (+6.4%), 24.5 ± 4.1 kg (+14.0%), and 60.5 ± 10.4 kg (+17.2%), respectively.

Changes in body composition and bone mineral density

As shown in [Table 3](#), the participants in Groups D and DR lost 6.2 ± 3.5 kg and 8.6 ± 3.6 kg of their body weights, respectively, during the 14-week intervention. Reductions in percentage fat mass and fat mass in Group DR were significantly greater than in Group D. Lean mass decreased significantly in both groups. The main effect of time in whole-body BMD was significant (-0.3% , $n = 35$), while whole-body BMC, L2–4 BMD, and 1/3 radial BMD remained unchanged. There were no significant time-by-group interactions.

Changes in bone markers

As shown in [Table 3](#), a significant decrease in BAP and a significant increase in OC were observed in the serum bone-formation markers. In the urinary bone-resorption markers, DPD exhibited a significant increase, while NTX remained unchanged. There were no

significant time-by-group interactions.

Discussion

Although we hypothesized that additional resistance training during diet-induced weight loss would be more beneficial for BMD in overweight premenopausal Japanese women, the results of this study do not support the hypothesis. We have demonstrated that the additional resistance training increased percentage fat mass and fat mass loss, while no significant effects of resistance training on BMD were observed. Our 14-week weight-loss intervention caused a significant decrease in whole-body BMD, while the change was very small (-0.3%). Further, there were no significant changes in other regional BMD. Bone formation and resorption were each estimated by 2 markers; however, the directions of changes were not the same and did not explain the BMD changes. These results suggest that the 14-week diet-induced weight loss caused a small decrease in the whole-body BMD, but that additional resistance training has no effect on the whole-body and regional BMD.

There are limited and conflicting reports relating to whether the inclusion of resistance training into a diet-induced weight-loss program can offset the loss of BMD. Andersen et al. [28] compared bone mass changes between diet only and diet plus resistance training in obese women. The subjects in the diet plus resistance training group participated in 3 exercise sessions per week for 24 weeks. Their total and lower-body strength increased significantly by 30–40%, while no significant differences were observed between groups in any of the body composition measurements and whole-body BMD. Daly et al. [23] investigated whether the addition of supervised resistance training to a moderate diet induced weight-loss program could possibly maintain BMD compared to the moderate weight-loss program alone in older (>60 years) overweight men and women with type 2

diabetes. During the 6-month intervention, body weight decreased similarly in both groups, but whole-body BMD, BMC, and lean mass were maintained in the resistance-training group in contrast to their decrease in the weight-loss program alone. Significant group differences were observed. In this study, the apparent effects of resistance training were exhibited compared to that in the study of Andersen et al. [28] and the present study, which may be explained by the difference in either the characteristics of the participants or the exercise program.

A difference between the studies conducted by Daly et al. [23] and Andersen et al. [28] was the mean age of their participants. The participants of the former study were older (66.9 yr in the weight-loss group and 67.6 yr in the weight-loss plus resistance training group) than the latter (38.1 yr in the diet-only group and 41.1 yr in the diet plus resistance training group). Moreover, Daly et al. [23] included men as participants (6 of the 13 participants in the weight-loss group and 10 of the 16 participants in the weight-loss plus resistance training group). There is no information regarding the influence of age or gender on the effect of resistance training on bone during weight loss. However, it may be inferred that older populations are more prone to bone loss with weight loss; in women, this is at least in part due to a reduced dietary calcium intake and/or the efficiency of absorption [29]. The potential hormonal mechanisms regulating bone loss during weight loss may also be related [29]. Comparatively younger women in the study by Andersen et al. [28] as well as in our study may face a lower risk of losing bone mass during weight loss; therefore, the effect of resistance training on bone may be masked in this population.

The difference between the study conducted by Daly et al. [23] and the present study

was the target intensity of the resistance training. The target intensity of the former was 75% to 85% of the one-repetition maximum strength, and the upper and lower body muscle strength increased by 43% and 33%, respectively. On the other hand, our target intensity was 50% to 60% of the maximal lift, and the maximal lifts of bench press, leg curls, and leg extensions increased by 6.4%, 14.0%, and 17.2%, respectively. The length of the training programs was also different; it was longer in the study by Daly et al. [23] (6 months) than in the present study (14 weeks). As the result, in the present study, lean mass decreased significantly in Group DR as well as in Group D. We set the exercise program considering the safety and feasibility for the participants; although, we should have set the target intensity higher and the duration longer to demonstrate a more apparent effect of exercise on myopachynsis and bone metabolism.

Our 14-week weight-loss intervention caused a slight decrease in the whole-body BMD; however, whole-body BMC, lumbar-spine BMD, and 1/3 radial BMD remained unchanged, while body weight was remarkably reduced. As previously described, comparatively younger premenopausal women may not be prone to losing BMD during weight loss. In addition, adequate protein and calcium intakes may be effective in preventing the loss of BMD. We provided a well-balanced supplemental food product and a protein supplement with low-fat milk to ensure proper daily nutrition. Supplemental protein [30,31] and calcium [14,18,31–33] have been reported to be effective in preventing bone loss during weight loss.

The limitations of this study are as follows: First, the relatively small number of participants may increase the probability of type II errors. Second, the study was 14 weeks

in duration, which was a rather short period for observing bone metabolism, although sufficient for conducting an energy-restriction intervention. With a longer observation period for the weight-loss program, we would be in a better position to comment on the long-term effects of combining exercise with energy restriction. Finally, the observed changes in BMD or BMC may actually have been artifacts caused by changes in the amount of soft-tissue mass producing errors in the bone densitometry measurements [34–36]. In the present study, the whole-body BMD declined significantly, whereas there was no significant change in BMC; this is because the bone area increased (+0.8%, not significant). The increase in bone area may imply an increase in bone diameter or may be the result of low instrument sensitivity.

In conclusion, the results of this study have demonstrated that the additional resistance training during diet-induced weight loss increased percentage fat mass and fat mass loss, while no significant effects on BMD were observed in overweight premenopausal Japanese women. Further long-term studies involving large numbers of subjects are required in order to demonstrate the effectiveness of exercise in preventing bone loss accompanying weight loss.

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Table 1 Descriptive characteristics of participants

	Diet only	Diet plus resistance training	<i>P</i> value for group difference†
Age (yr)	40.3 ± 6.5	42.3 ± 7.4	.409
Height (cm)	156.7 ± 6.2	157.7 ± 4.8	.596
Body weight (kg)	67.2 ± 7.0	68.5 ± 8.0	.605
Body mass index (kg/m ²)	27.4 ± 2.5	27.5 ± 2.5	.883
Percentage fat mass (%)	32.9 ± 6.2	35.2 ± 4.9	.226

Values are presented as mean ± standard deviation. †Group difference between diet only group and diet plus resistance training group analyzed by unpaired *t*-test.

Table 2 Comparisons of baseline data and changes in dietary intake between groups

	Diet only			Diet plus resistance training			P value for		
	Baseline	During	Change	Baseline	During	Change	Group dif.†	Time effect‡	Interaction‡
Total energy (kcal)	2046 ± 314	1302 ± 288	-743 ± 298*	2292 ± 391	1272 ± 204	-1020 ± 373*	.725	.000§	.026§
Protein (g)	68.5 ± 11.8	74.7 ± 12.9	6.2 ± 14.9	80.3 ± 16.5	74.0 ± 11.1	-6.2 ± 12.1	.870	.995	.013§
Fat (g)	66.4 ± 17.1	35.9 ± 10.3	-30.5 ± 17.7*	72.2 ± 17.8	36.0 ± 9.4	-36.2 ± 16.2*	.977	.000§	.342
Carbohydrate (g)	281 ± 46	169 ± 38	-112 ± 37*	313 ± 66	160 ± 32	-153 ± 67*	.431	.000§	.037§
Calcium (mg)	463 ± 128	1044 ± 213	581 ± 228*	599 ± 216	969 ± 182	370 ± 121*	.286	.000§	.002§
Magnesium (mg)	177 ± 54	194 ± 48	17 ± 73	200 ± 62	180 ± 46	-20 ± 65	.425	.920	.137
Phosphorus (mg)	947 ± 161	1072 ± 232	125 ± 252	1148 ± 261	1012 ± 173	-136 ± 210	.401	.893	.003§

Values are presented as mean ± standard deviation. *Significant intra-group difference using paired *t*-test. †Group difference during intervention between diet only group and diet plus resistance training group analyzed by unpaired *t*-test. ‡Significance of time effect and interaction analyzed by repeated two-way (time by group) analysis of variance. §*P* < 0.05.

Table 3 Comparisons of baseline data and changes in body composition and bone markers between groups

	Diet only		Diet plus resistance training		<i>P</i> value for	
	Baseline	Change	Baseline	Change	Time effect	Interaction
Body weight (kg)	67.2 ± 7.0	-6.2 ± 3.5*	68.5 ± 8.0	-8.6 ± 3.6*	.000§	.053
Percentage fat mass (%)	32.9 ± 6.2	-4.0 ± 2.2*	35.2 ± 4.9	-6.4 ± 2.9*	.000§	.008§
FM (kg)	22.3 ± 5.6	-4.5 ± 2.6*	24.3 ± 5.3	-6.9 ± 3.0*	.000§	.019§
LM (kg)	42.5 ± 4.5	-1.7 ± 1.5*	41.7 ± 4.4	-1.8 ± 1.2*	.000§	.867
WB BMC (kg)	2.46 ± 0.29	0.00 ± 0.06	2.47 ± 0.26	0.02 ± 0.08	.282	.358
WB BMD (g/cm ²)	1.20 ± 0.06	-0.01 ± 0.01*	1.20 ± 0.08	0.00 ± 0.01	.042§	.393
L2-4 BMD (g/cm ²)	1.26 ± 0.11	0.00 ± 0.02	1.25 ± 0.10	0.00 ± 0.03	.549	.788
1/3R BMD (g/cm ²)	0.69 ± 0.06	0.00 ± 0.01	0.68 ± 0.06	0.00 ± 0.02	.780	.691
BAP (U/l)	23.9 ± 8.3	-3.2 ± 4.9*	20.3 ± 5.0	-1.7 ± 3.1	.001§	.289
OC (ng/ml)	1.8 ± 0.9	0.9 ± 1.5	1.8 ± 0.7	1.1 ± 1.1*	.000§	.643
DPD (nmol/mmolCRE)	7.3 ± 2.4	0.6 ± 2.1	6.2 ± 1.8	1.7 ± 2.2*	.004§	.161
NTX (nmolBCE/mmolCRE)	35.2 ± 13.7	4.2 ± 10.6	31.1 ± 12.4	-0.5 ± 10.2	.300	.198

Values are presented as mean ± standard deviation. FM, fat mass; LM, bone- and fat-free lean tissue mass; WB, whole body; BMC, bone mineral content; BMD, bone mineral density; L2-4, lumbar spine; 1/3R, radius; BAP, bone specific alkaline phosphatase; OC, osteocalcin; DPD, deoxypyridinoline cross-links; CRE, creatinine; NTX, type I collagen cross-linked N-telopeptides; BCE, bone collagen equivalent. *Significant intra-group difference using paired *t*-test. §*P* < 0.05.