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ORIGINAL ARTICLE

Effect of antioxidants combined to resistance training on BMD in elderly women: a pilot study

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Abstract
Summary We determined the effect of antioxidants and resistance training on bone mineral density of postmenopausal women. After 6 months, we observed a significant decrease in the lumbar spine BMD of the placebo group while other groups remained stable. Antioxidants may offer protection against bone loss such as resistance training.
Introduction The purpose of this pilot study was to determine the effects of antioxidant supplements combined to resistance training on bone mineral density (BMD) in healthy elderly women.
Methods Thirty-four postmenopausal women (66.1± 3.3 years) were randomized in four groups (placebo, n=7; antioxidants, n=8; exercise and placebo, n=11; and exercise and antioxidants, n=8). The 6-month intervention consisted in antioxidant supplements (600 mg vitamin E and 1,000 mg vitamin C daily) or resistance exercise (3×/week). Femoral neck and lumbar spine BMD (DXA) and dietary intakes (3-day food record) were measured before and after the intervention. A repeated measure ANOVA and non-parametric Mann–Whitney U tests were used.
Results We observed a significant decrease in the placebo group for lumbar spine BMD (pre, 1.01±0.17 g/cm^2; post, 1.00±0.16 g/cm^2; $P<0.05$ respectively) while it remained stable in all other groups. No changes were observed for femoral neck BMD.
Conclusions Antioxidant vitamins may offer some protection against bone loss in the same extent as resistance exercise although combining both does not seem to produce additional effects. Our results suggest to further investigate the impact of antioxidant supplements on the prevention of osteoporosis.

Keywords Antioxidants · BMD · Bone · Bone densitometry · Exercise · Postmenopausal · Vitamin C · Vitamin E

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Introduction

Osteoporosis is a major public health problem that is characterized by a loss of bone mass associated with aging [1]. This process contributes to functional impairment since it increases susceptibility to fractures, primarily of the hip, spine, and wrist [2]. Hip fractures are a major problem as they lead to complication-associated death in more than 20% of cases and about 50% of individuals who survive remain disable [3]. Women are particularly affected by osteoporosis owing to the important role that estrogens play on bone turnover as they stimulate bone formation and inhibit bone resorption [4].

Resistance training has been associated with osteoporosis and the maintenance of bone mass [5] and to positively impact on multiple risk factors for osteoporotic fractures in previously sedentary postmenopausal women [6]. In this sense, exercise favors bone formation and contributes to increase bone density [7, 8]. However, the positive effects of resistance training could be limited by exercise-induced oxidative stress observed in older individuals.

In fact, moderate to high-intensity exercise has been shown to increase reactive oxygen species (ROS) and nitric oxide derivatives (NO) production in contracting skeletal muscle [9, 10]. The extent of oxidative stress is dependent on several factors, including age, oxygen consumption, antioxidant levels, and the presence of adequate repair systems [11]. Although regular exercise training is effective to enhance adaptive responses thereby diminishing oxidative stress in younger individuals [12–14], it appears that aging may partly attenuate these adaptations [15, 16]. It thus has been proposed that elders who exercise have greater requirements for dietary antioxidant [17] such as vitamins C and E [18].

Antioxidants have also been associated with bone health. When compared to matched controls, elderly osteoporotic women presented lower levels of dietary and endogenous antioxidants [19]. In accordance, epidemiological studies have shown low vitamin C intakes to be associated with a low bone mineral density (BMD) [20, 21] and an increased bone loss in postmenopausal women [22]. Moreover, low intakes of vitamin C and E have been related to an increased risk of hip fractures in smokers [23].

Up until now, no studies have examined changes in BMD in response to high intensity resistance exercise combined with vitamins C and E, specifically in postmenopausal women. Actually, it remains unclear whether exogenous antioxidants supplements are beneficial for BMD gains in exercising older individuals. Therefore, the purpose of this pilot study was to investigate if 6 months of resistance training combined with antioxidant treatment could counteract the loss of bone mass as compared to exercise alone.

Materials and methods

Subjects

Thirty-four healthy women aged between 61 and 73 years were recruited from local newspaper advertisements in Sherbrooke, Quebec (Canada) to participate in a large trial which objective was to evaluate the efficiency of a combination of antioxidant supplements and resistance training on sarcopenia and insulin sensitivity in elderly men and women. Subjects had to meet the following criteria: (1) healthy, caucasian, and without major physical incapacity; (2) taking no medication that could influence metabolism; (3) non-smoker and moderate drinker (15 g of alcohol/day maximum, the equivalent of one alcoholic beverage/day); (4) body mass index (BMI) between 18 and 30 kg/m^2; (5) no consumption of antioxidant supplements (i.e. vitamins C, E) during the last month; and (6) postmenopausal and not taking hormone replacement therapy. A phone interview was conducted to screen for the aforementioned inclusion criteria. In the present study, data from 34 women were used for analysis purposes. After the nature and goals of the study were thoroughly explained, subjects provided written informed consent. This study was approved by the Ethics Committee of the Sherbrooke Geriatric University Institute.

Overview of experimental protocol

After screening by telephone, volunteers were invited for a visit at the Research Centre on Aging (Sherbrooke Geriatric University Institute). Upon arrival, body weight, height, and body composition (DXA) were assessed and instructions were provided for the 3-day dietary record. Following the visit, subjects were randomized in one of four groups (placebo, $n=7$; antioxidants, $n=8$; exercise and placebo, $n=11$; and exercise and antioxidants, $n=8$) and exercise groups were enrolled in the 6-month resistance training program. Subjects were instructed to maintain usual food and physical activity habits. After 6 months of intervention, subjects were submitted to a post-test session identical to the one conducted at baseline.

Resistance training program

The 6-month resistance training program consisted in supervised 60-min exercise sessions performed three times per week on alternating days. Each session consisted of two components: 15 min of warming-up, treadmill or cycle ergometer followed by static stretching; and 45 min of resistance training comprising exercises targeting large and small muscle groups including abdominals: leg press, bench press, leg extension, shoulder press, sit up, seated row, triceps extension, and biceps curl. Subjects performed three sets (eight repetitions per set) at 80% of a one repetition maximum (1 RM) for each exercise. Resting periods of 90–120 s separated each set. The 1 RM was assessed every 4 weeks in order to adjust exercise intensity for strength gains throughout the training program. A maximum of one missed exercise session per month was accepted for compliance purposes.

Antioxidant and placebo supplements

Participants were randomly assigned to receive either the antioxidant supplement (600 mg/day of vitamin E (dl-αtocopherol) and 1,000 mg/day of vitamin C (ascorbic acid) or the placebo (lactose). Antioxidants and placebos were supplied for 50-day periods and new capsules were given at the end of each period to ensure compliance of subjects. Vitamin E and placebos were supplied by Arkopharma (Arkopharma, Carros, France) while vitamin C and placebos were purchased at the Sherbrooke Geriatric Institute hospital pharmacy (Wampole Brands, Toronto, ON, Canada).

Body composition and anthropometric measurements

Body weight was determined using an electronic scale (SECA, Hamburg, Germany). Height was measured using a tape measure fixed to the wall with the subject in stocking feet. Body mass index was calculated as weight (kg) relative to height (m^2). Determination of fat mass (FM) and fat-free mass (FFM) was assessed in a supine position, using the dual-energy X-ray absorptiometry method (GE Prodigy Lunar, Madison, WI, USA). In our laboratory, the coefficients of variations for repeated determinations of BMD, FM, and FFM in ten adults (measured 1 week apart) were 0.9%; 5.7%, and 1.1% respectively.

Dietary intake

Each subject was instructed to maintain normal dietary habits throughout the period of data collection as previously mentioned[24]. Subjects were provided with a 5 kg (11 lb) food scale and instructed on how to complete a 3-day dietary record. Diets were recorded on 2 weekdays and 1 weekend-day. It has been demonstrated that a 3-day dietary record is valid to estimate dietary intakes in older adults without cognitive impairments [24]. Dietary analyses were completed by using Candat system, version 6.0 software (Candat, London, ON, Canada) to determine daily energy, macronutrients, and micronutrients intakes.

Statistical methods

Values are displayed as means±standard deviation (±SD). A one-way ANOVA was performed to assess differences between groups at baseline. A repeated measure ANOVA was used to examine differences between pre-test and post-test BMD. Because of the small sample size, changes between pre- and post-intervention values were also examined with the use of non-parametric Mann–Whitney *U* tests. We also compared the delta BMD (post-intervention BMD−pre-intervention BMD) between groups with the use of a one-way ANOVA. In all cases, a *P* value below 0.05 was considered significant. All analyses were performed with the SPSS software version 11.0.

Results

Mean age was 66.1±3.3 years. As shown in Table 1, at baseline, groups were similar for body composition and dietary intake variables, and no strength difference was found between both exercise groups for all 1 RM (results not shown). Body weight and BMI decreased significantly in placebo group but remained stable in other groups. Caloric intake in exercise and placebo group decreased significantly while it remained relatively stable in other

Table 1 Descriptive characteristics

	Placebo group		Antioxidants group		Exercise and placebo group		Exercise and antioxidants group	
	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
Age (years)	67.4±3.8		65.5±4.5		65.4±3.5		66.3±4.0	
Weight (kg)	64.20±7.56	63.43±7.57*	61.15±9.02	61.10±8.77	66.61±8.47	67.85±10.57	65.46±9.06	65.05±8.33
BMI (kg/m^2)	26.00±2.83	25.68±2.83*	25.12±12.89	25.11±2.78	26.54±2.74	27.01±3.44	26.25±2.89	26.09±2.56
Caloric intakes (Kcal/day)	1642±366	1735±213	2047±381	1736±449	2139±510	1877±343*	2018±530	1740±359
Vitamin D (mg/day)	748±441	785±524	648±512	625±567	173±132	283±234	342±395	624±485*
Calcium (mg/day)	1063±364	1089±323	1584±509	1349±426	888±396	917±278	1094±261	1109±383
Vitamin C (mg/day)	118±46	110±38	186±65	128±83*	135±43	154±118	119±78	81±26
Vitamin E (mg/day)	1.64±0.99	1.19±0.72	2.73±2.44	1.90±1.02	4.05±7.22	2.60±3.53	1.86±1.54	1.08±0.77

Mean±SD
*$P \leq 0.05$ significantly different from baseline values
**$P \leq 0.05$ significantly different between groups

Table 2 BMD variations in postmenopausal women

	Placebo group		Antioxidants group		Exercise and placebo group		Exercise and antioxidants group	
	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
Femoral neck BMD (g/cm^2)	0.90±0.06	0.90±0.07	0.85±0.10	0.86±0.12	0.91±0.08	0.91±0.08	0.94±0.14	0.93±0.14
Delta femoral neck BMD (g/cm^2)	0.0019		0.0077		−0.0023		−0.0129	
Lumbar spine BMD (g/cm^2)	1.01±0.17	1.00±0.16*	0.96±0.15	0.96±0.14	1.06±0.13	1.06±0.16	1.09±0.11	1.08±0.11
Delta lumbar spine BMD (g/cm^2)	−0.0148**		0.0013		−0.0014		−0.0035	

Mean±SD

*$P \leq 0.05$ significantly different from baseline values

**$P \leq 0.05$ significantly different between groups

groups. No significant difference was observed in baselines values for calcium and vitamin E intakes (excluding intervention). The daily consumption of vitamin D increased significantly in exercise and antioxidants group but remained similar in other groups. Finally, while daily intakes of vitamin C decreased significantly in antioxidants group, it remained stable for other groups.

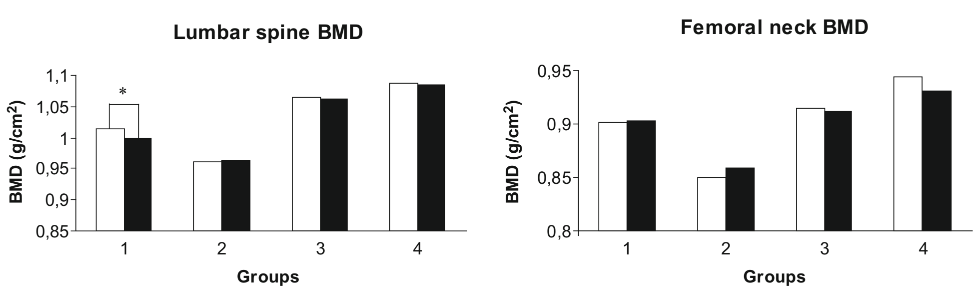
As shown in Table 2, we examined femoral neck and lumbar spine following 6 months of intervention. While the placebo group displayed a significant loss in lumbar spine BMD ($P<0.05$), all other groups remained stable (Fig. 1). Accordingly, delta lumbar spine BMD was significantly greater in the placebo group than the three other groups ($P<0.05$), indicating that lumbar spine BMD was maintained in all groups except for the placebo group. These results were confirmed with non-parametric analyses. However, no significant difference was observed between groups for changes in femoral neck BMD.

Conclusions

The aim of this pilot study was to investigate if the combination of resistance training and antioxidant supplements could impact on bone mass loss in postmenopausal women. We observed a bone loss in the placebo group corresponding to the typical average loss for postmenopausal women [25, 26]. Hence, it seems that antioxidants exerted a beneficial effect on BMD in postmenopausal women, to the same extent as resistance training. However, no interaction effect was observed when combining resistance training and antioxidant supplements. Groups were similar for calcium, vitamin D, vitamin E, and vitamin C intakes. Differences between groups in BMD could thus not be attributed to differences in dietary intakes.

The findings of this study support that, in postmenopausal women, high resistance training is effective to stop or limit the loss of lumbar spine BMD but possibly not femoral neck BMD. It may be in part attributable to the metabolic properties of these two different skeletal regions of interest. In fact, the lumbar spine comprises a greater proportion of cancellous (or trabecular) bone as compared to femoral neck [27].On the other hand, femoral neck comprises a greater proportion in cortical (or compact) bone [27]. Bone turnover is greater in cancellous bone than in cortical bone [27] which may explain why resistance exercise exerts a greater effect in this region. In support of that, a previous animal study showed that resistance exercise resulted in a significant increase in cancellous bone markers but not in cortical bone [28].

Fig. 1 BMD variation pre- and post-intervention between each experimental group



* $P \leq 0.05$ Significantly different from baseline values

Antioxidant supplements

Our results suggest that vitamins C and E may favorably impact on lumbar spine BMD. A previous study suggested that the use of vitamins C and E supplements is associated with decreased levels of serum CTx, a marker of bone resorption [29], bone loss occurring when resorption exceeds formation. In accordance, in vitro cell models demonstrate that ROS stimulate osteoclast differentiation [30, 31] and inhibit osteoblast differentiation [32]. Conversely, antioxidants may reduce the damaging effects of oxidative stress on bone mass by reducing the upregulated osteoclastic differentiation and enhancing the downregulated osteoblastic differentiation. Our results suggest to further study the role of antioxidants in the regulation of both osteoclastic bone resorption and formation.

Limitations

Our study had some limits that need to be mentioned. First, the small sample size may have reduced the statistical power. Nevertheless, we used non-parametric statistical tests to confirm the results. Secondly, our pilot study was of short duration but strongly supports to use a longer intervention period to (1) assure long-term effect and (2) examine if, on the long run, the combination of resistance exercise and antioxidants is beneficial as compared to exercise or antioxidants alone.

Recommendations

It is not appropriate to elaborate formal nutritional recommendations based on this pilot study, it provides some insights as to the nutritional parameters that may be beneficial for bone health. Further research is needed to determine appropriate recommendations for this population especially since nutrition and exercise are two effective and accessible strategies towards health maintenance in the aging population.

Our results demonstrated that 6 months of antioxidant supplementations may have the same positive effect on lumbar spine BMD than 6 months of resistance training although antioxidant supplementations combined with resistance training did not provide any additional effect. These results are interesting because this is the first study to examine the combination of these interventions in healthy elderly women suggesting another effective strategy to delay age-related BMD loss. Further investigations are necessary to confirm our findings.

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Conflicts of interest None.

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