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EFFECTS OF PHYSICAL TRAINING ON BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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Abstract

Background and aims:.We hypothesized that low physical activity status (PAS) and increased Hcy concentrations stimulate osteoclastic activity, leading to increased collagen I breakdown in postmenopausal women.

Methods: Osteoporotic postmenopausal women (OP) and healthy postmenopausal women (NOP), age matched was included in the study.

Results: In patients with OP; plasma t-Hcy, urine Deoxypyridinoline were significantly higher than those found in NOP controls. In addition patients with OP also had lower PAS than the controls, which represent the insufficient physical activity. Pearson correlation analysis revealed negative correlation between t-Hcy and PAS, r=-0.42, p<0.03. Furthermore significant negative correlation was found between the levels of PAS and the BMD values for the spine in OP patients (r = -0.45, p<0.03).In contrast, was demonstrated significantly positive correlation between t-Hcy and cigarette smoking in OP patients, r =0.57, p<0.02.

Conclusions: We were able to show that OP group had a lower PAS, whereas the elevated t-Hcy was different from that of the NOP group. Slightly elevated homocysteinemia may contribute to increase osteoclastic activity and reduced osteoblastic activity in OP group. However; our results suggested a weak but negative relationship between PAS and BMD. Further investigation is needed to examine the relationship of PAS as an endogenous bioactive molecule to bone loss in postmenopausal women. Further studies are needed to understand the underlying mechanisms of these findings.

Key words: *osteoporosis, homocysteine, physical activity, deoxypyridinoline, DEXA*

Introduction

Osteoporosis is an important health problem in aging population, showing itself as fractures even after minor trauma (1). Falls are recognized as the most important risk factor that increases the tendency to osteoporotic fractures that have a high rate of morbidity and mortality (2). The underlying pathobiochemical mechanism remains unclear; disturbed cross-linking of collagen has been suggested (3). Elevated homocysteine is a strong risk factor for osteoporotic fractures among elders, yet it may be a marker for low B-vitamin status. Experimental animal studies have demonstrated that exogenous homocysteine addition alters the matrix organization, limits the formation of pyridinium cross-links (pyridinoline and deoxypyridinoline) and decreases the proteoglycan content. Total plasma homocysteine (t-Hcy) levels are lower in premenopausal and in pregnant women than in postmenopausal women. Sex steroid hormones may modulate plasma homocysteine levels (4,5).

In the laboratory, regular exercise positively impacts mechanical bone strength by creating small gains of bone mass at areas of mechanical stress. Animal experiments demonstrate that very small increases in bone density created by repeated mechanical loading can result in disproportionate increases in bone strength by inducing beneficial geometric changes of the bone structure . Of particular interest, recent lines of evidence have suggested that mechanical loading may decrease secretion of sclerostin by the osteocytes. This in turn leads to loss of inhibition of the *wnt* canonical signaling pathway, thereby increasing bone formation by nearby osteoblasts (6).

Although the mechanisms involved in the pathogenesis of osteoporosis are not fully understood, there is evidence to suggest that oxidative stress caused by reactive oxygen species (ROS) is associated with osteoporosis (7). The organism has enzymatic and non-enzymatic antioxidant systems neutralizing the harmful effects of the endogenous ROS products. Physical training is known to increase the antioxidant defence system and reduce exercise-induced oxidative stres (8). The non-invasive assessment of bone turnover has markedly improved previously few years with the development of specific and sensitive markers of bone resorption such as type I collagen degradation product: pyridinium cross-links associated peptides (9). Advantages of using collagen crosslink fragments as markers of bone resorption include: 1) crosslinks are formed only in mature collagen, and thus their release

reflects breakdown of mature collagen; 2) crosslink fragments are not absorbed from the gut, and thus dietary habits does not confound results; and 3) crosslink fragments are extremely stable in frozen urine samples for long periods (9,10). The total number of fractures, and hence the cost to society, will increase dramatically over the next 50 years as a result of demographic changes in the number of elderly people. Thus, prevention of osteoporosis by identifying risk factors or risk indicators, as well as the development of new treatment strategies, is major issues. A comparable situation can be found in developing and other industrialized countries. We examined the association between t-Hcy, DPD, BMD and the physical activity status of postmenopausal women.

Material and Methods

Subjects

Forty-nine postmenopausal women were recruited and divided in two subgroups; postmenopausal osteoporotic women (OP-34 women) and postmenopausal non-osteoporotic women (NOP-15) group. All Caucasian women were recruited from those of center of University of Gaziantep (GAZÜ) between September 2006 and January 2007. All of the 34 osteoporotic women had a natural menopause and their mean age (±SD) was 55.9±6.5 years. In addition all of the NOP women had a natural menopause and their mean age (±SD) was 54±4.7. Dietary habit of each woman was evaluated by an interview with a dietitian, and the following risk factors were measured: present and previous use of cigarettes, milk and milk products. We also excluded women who had taken any other bone-active agents (bisphosphonates, calcitonin or fluoride within the last 12 months, raloxifene or estrogen therapy within the last 3 months). In addition, we carried out routine serum biochemical screening (i.e., calcium, phosphate, thyroid stimulating hormone (TSH), transaminases, creatinine and hematocrit) to reveal the presence of asymptomatic forms of metabolic diseases. The duration of menopause (mean age±SD) was 119±52.7, 117±32.9 months for the OP women and NOP women, respectively. The participants included patients who reported, diabetes mellitus 2 (5.8%), use of antihypertensive medication 8 (16%) and use of cholesterol-lowering drugs 4 (8.1%), and current smoking of five or more cigarettes per day are given

The study's questionnaire also included demographic characteristics like age, gender, financial status and education level. Information about smoking habits was collected using a standardized questionnaire. Physical Activity Status (PAS) was defined as leisure-time activity of certain intensity and duration, at least once/week during the past year and was graded in qualitative terms such as: light (expended calories <4 kcal/ min), moderate (expended calories 4–7 kcal/ min) and vigorous (expended calories >7 kcal/ min). The rest of the subjects were defined as physically inactive. We also classified the physically active participants into those who adopted an endurance-type physical activity (activity involving repeated use of large muscles, such as in walking or bicycling) and a resistance exercise (to increase muscle strength, such as by lifting weights). Body mass index was (BMI) calculated as weight (in kg) divided by standing height (in m^2). Obesity was defined as body mass

Table 1. *Characteristics of the postmenopausal women (means ± SD)*

	OP	NOP
Number of subjects	34	15
Age (years)	55.9±6.5	54.1±4.7
BMI, (kg/m^2)	21.4±3.1*	26.1±2.6
Duration of menopause (mouths)	119±52.7	117±32.9
Number of deliveries (ranges)	1.8±0.8 (1-4)	1.6±0.3 (1-4)
Physical Activity Status	Sedentary: 78%* Light or moderate: 21%** Vigorous: <1%	Sedentary: 63% Light or moderate: 36% Vigorous: <1%
Smoking, %	7%	5%
Drinking>2 glasses of milk (%)	31%	32%
BMD (g/cm^2)	OP	NOP
AP lumbar spine (L1-L4)	0.63 ± 0.06*	0.83 ± 0.09
Z - score	-2.76 ± 0.12	-0.77 ± 0.13

* OP vs.NOP, $p<0.05$
** OP vs.NOP, $p<0.01$

index >29.9 kg/m^2. Waist and hip circumferences (in cm) were also measured. Participants whose average blood pressure levels were greater or equal to 140/90 mm Hg or were under antihypertensive medication were classified as hypertensives.

Postmenopausal women with liver or renal disease, endocrine or metabolic abnormalities, and/or receiving medicine known to influence bone mineralization or homocysteine metabolism were excluded. All women gave their written informed consent, and the study was approved by the medical Local Ethics Committee. Demographic, clinical, BMD and lifestyle characteristics are given in Table 1.

Bone Mineral Density

Bone mineral density, (BMD) was evaluated using dual energy X-ray absorptiometry (DEXA, Lunar®, Madison, WI, and U.S.A). BMD measurements were performed on one site of the body (AP lumbar spine). Osteopenia corresponded to BMD scores between 1.0 and 2.5 SD below the peak bone mass. Osteoporosis was defined as a BMD more than 2.5 SD below the peak bone mass according to AP lumbar spine.

Blood sampling

Fasting blood samples were collected by standard venipuncture technique between 8:30-11:30 a.m. Plasma was separated after centrifugation of blood at +4°C, 1500 g for 10 minutes and stored at -70°C until analysis. Urine samples for the determination of pyridinium cross-links and creatinine excretion were obtained from an overnight urine specimen, including the early morning portion. After division into aliquots, the urine samples were also frozen at -70°C before assay. Urinary concentrations were expressed relative to the urinary creatinine levels.

Pyridinium cross-links

Urine DPD assays were performed with a solid phase chemiluminescent enzyme-labeled immunoassay method according to the manufacturer's instructions and the results were corrected for urinary concentration by creatinine (Pyrilinks-D®, Immulite One ®, DPC, USA). Calibration range and analytical sensitivity were 7-300 nM and 4.4 nM, respectively. The intra-assay and inter-assay coefficients of variation were 4.0 % and 6.5 %, respectively.

Total Homocysteine

The term "total homocysteine" (t-Hcy) includes: homocysteine, cysteine-homocysteine mixed disulfide, and homocysteinyl moieties of homocysteine. Serum t-Hcy concentrations were determined with Immulite® One analyzer and Immulite® reagent, solid phase chemiluminescent enzyme labeled immunoassay (Pyriliks-D, DPC, L.A, USA) according to the manufacturer's instructions. The assays were linear from 2 to 50 μmol/L; calibrators and controls were supplied by the manufacturers. Specifications of intra-assay and inter-assay coefficients of variation (CVs) of t-Hcy assays were assessed from quality control data of the laboratory, which were 9.1% and 9.9 %, respectively.

Statistical analysis

Statistical analysis was carried out using a statistical package for social sciences (SPSS 9® for windows). All the data were tested for their normal distribution. The data obtained from the study and control groups were compared using Students t-test and chi-square test. Bivariate comparisons were examined using Pearson correlation coefficients (MedCalc®) and were corrected for ties. Differences were considered statistically significant at $P<0.05$.

Results

At the DEXA evaluation, 34 women had osteoporotic (T score below -2.5), and 15 had normal BMD values according to spine BMD. Osteoporotic women had lower values of weight and body mass index (BMI). The number of pregnancies was found to range from 1 to 4 in all of the 34 postmenopausal women. The demographic, PAS and clinical data of the subjects are summarized in Table 1.

The patients in the postmenopausal osteoporotic group exhibited significantly lower spine BMD values (0.63 ± 0.06 g/cm^2), than the controls (0.83 ± 0.09), $p<0.05$. Serum t-Hcy and urinary DPD levels were significantly higher in osteoporotic postmenopausal women (11.86±3.8 μmol/L, 15.4 ±7.0 nM/mM cr) than in non-osteoporotic postmenopausal women (10.93±3.6 μmol/L, 10.6± 9.1 nM/mM cr), $p<0.05$, $p<0.01$, respectively Table 2.

Association was found between homocysteine levels and PAS (physically active 11.5±3.5 vs. sedentary 12.6±2.3 μmol/L, p=0.09). When we focused our interest on specific type of exercise, we observed that endurance physical activity was associated with lower homocysteine levels as compared to resistance exercise or sedentary (11.0±2.6 vs. 12.4±2.5 vs. 12.5±2.3 μmol/L, p=0.04). The differences were significant (p=0.04) even after adjusting for age, body mass index, smoking and dietary habits of the participants.

Interestingly, in postmenopausal osteoporotic women the homocysteine (t-Hcy) levels were negatively associated with the PAS, and positively associated with the smoking, r=-0.401, $p<0.038$ and r =0.52, $p<0.029$, respectively. Furthermore significant negative correlations were only found between the levels of PAS and the BMD values for the lumbar spine (r = -0.492, $p<0.035$). Higher levels of PTH were found in OP and lower levels were found in NOP.

Table 2. *Biochemical marker variables of postmenopausal women*

Biochemical markers	OP(n=34)	NOP(n=15)	Reference limits
DPD (nM/mM creatinine)	15.4 ±7.0 **	10.6± 9.1	3.0-7.4
Parathyroidhormone	65.17±33.1 **	45.07±24.4	10-73
Serum calcium (mg/dl)	9.4±0.35	9.36±0.44	8.8-10.2
Serum creatinine (mg/dl)	1.01±0.42	1.06±0.17	0.4-1.2
t-Hcy (μμmol/L)	Total:11.86±3.8 * Sedentary:12.6±2.3 Low-moderate:11.5±3.5	Total: 10.93±3.6 Sedentary:10.97±3.7 Low-Moderate:10.78±3	5-10

* OP vs.NOP, $p<0.05$
** OP vs. NOP, $p<0.01$

Discussion

In this study, we have found lower PAS and higher DPD, t-Hcy levels in postmenopausal osteoporotic women compared to postmenopausal non-osteoporotic controls. Although, our study indicates that there is a significant correlation between the plasma levels of PAS and lumbar spine BMD. Hyperhomocysteinemia-induced cell dysfunction can occur due to a variety of mechanisms (11-13). Among other biological effects, high blood Hcy levels increase oxidative stress. Hcy directly activates osteoclast formation and activity through increased generation of intracellular ROS (13,14). Hcy is susceptible to auto-oxidation with the secondary generation of reactive oxygen species; it is also can of inhibiting the activity of the antioxidant enzymes glutathione peroxidase and superoxide dismutase (15,16). In experimental studies, the induction of hyperhomocysteinemia by dietary folic acid restriction has been found to increase oxidative stress; the reduction of hyperhomocysteinemia by folate therapy has been shown to reduce oxidative stress in patients with hyperhomocysteinemia (17,18). These findings suggest that, in individuals with mild to moderate hyperhomocystinemia, increased bone resorption by osteoclasts may contribute to osteoporosis (13). A mechanistic role of Hcy in bone metabolism is known to occur in patients with homocystinuria, a genetic disorder characterized by severe hyperhomocysteinemia. It has been suggested that disturbed cross-linking of collagen fibrils and reduced fibrillin-1 and -2 deposition lead to a disturbed architecture of bone matrix in homocystinuric patients (19,20). Physiologic concentrations of Hcy directly activate osteoclast formation and activity through stimulation of p38 MAPK (p 38 Mitogen Activated Protein Kinase) and integrin $\alpha 3$. Furthermore Hcy upregulated Tartarate Resistant Acid Phospahatase (TRACP+) multinucleated cells and TRACP activity, stimulated actin ring formation, and increased the number of nuclei per cell. In vitro studies suggest that Hcy interferes with the formation of collagen cross-links, prevents insolubilization of fibrils, inhibits lysyl oxidase, and may delay the synthesis of more complex cross-links in collagen (10,21). Collagen crosslinking is a determinant of bone quality. Collagen crosslinking is affected by tissue maturation as well as the degree of mineralization.

Vitamin B_6 acts as a coenzyme of lysyl oxidase, whereas homocysteine is known as an inhibitor of lysyl oxidase. Cross-links can form enzymatically by the action of lysyl oxidase or non-enzymatically, resulting in advanced glycation end products. Thus, Homocysteine and vitamin B_6 (pyridoxal) are also regulatory factors of collagen crosslinking. So, high levels of plasma homocysteine may affect collagen cross-link formation in bone (22). Two clinically studies with more than 4000 patients reported an increased risk for osteoporotic fractures in elderly persons with moderate hyperhomocysteinemia (23,24). Folate and vitamin B_{12} deficiencies are the most common causes of increased Hcy concentrations in elderly individuals. Several studies found that vitamin B_{12} and folate status are related to BMD and fracture risk (25,26).

In particular, although total Hcy levels were unchanged, as described also by Wright et al. and De Cree et al, we found a lower level of reduced Hcy in physically active patients than sedentaries (12,13). Since Hcy is the only analyte imbalanced, its alteration is not likely to be the result of oxidative stress induced by physical exercise but rather it depends on a change in the metabolic pathways in which it is involved. Hence, it can be supposed that physical activity influences Hcy synthesis and/or Hcy removal processes, but for the complexity of the Hcy/methionine cycle further studies are necessary to characterize the mechanisms involved in Hcy lowering after physical exercise.

Some in vitro and animal studies have suggested that, oxidative stress decreases bone formation by modulating the differentiation and survival of osteoblasts and stimulating bone resorption. (27). However, only a few of the clinical studies has showed ROS an important role of oxidative stress in the development of osteoporosis (16,21). Oxidative stress is defined as a disturbance in the balance between the production of ROS and antioxidant defenses, including enzymatic

and non-enzymatic systems (28). Also, the bone resorbing osteoclasts generate a high level of superoxide anion ($O_2^{\bullet -}$) (28). The results predict that not only the bone loss of estrogen deficiency but also that seen in other situations in which ROS have been implicated, such as in aging, obesity and inflammation, might be caused by a prolonged augmentation of ROS signaling in bone cells (29,30). Recent reports implicated that osteoclast differentiation might require ROS as a signaling intermediate and in vivo bone resorption could be controlled by antioxidants (15) and Yilmaz et.al. that there was relation between serum antioxidant and antioxidant vitamin levels in sportsmen (8). In addition, a reduction of SOD activity with aging has been already reported (30,31) The results predict that not only the bone loss of estrogen deficiency but also that seen in other situations in which ROS have been implicated, such as in aging, obesity and inflammation, might be caused by a prolonged augmentation of ROS signaling in bone cells (30). Whichever mechanism underlies the bone loss; previous studies results predict that osteoporosis should be prevented by antioxidant therapies that increase the degradation of hydrogen peroxide in bone (32,33). Therefore, supplementation of antioxidant-vitamin -enriched diet to the therapy might shed light on the development of novel therapeutic strategies for osteoporosis (34,35).

Aerobics, weight-bearing, and resistance exercises have all been shown to be beneficial for maintaining bone density in postmenopausal women. Given concerns of sports-related injuries and degenerative joint disease associated with aging, many studies have examined the effect of walking, a fairly safe intervention, on BMD (6). Walking for 1 h, four times a week, appears to decrease bone turnover as evidenced by a decline in urinary N-telopeptide excretion (a marker of bone turnover) and modest increases in lumbar spine density (6,13). Recent interest on the effect of whole body vibration on musculoskeletal health has led to studies exploring the viability of such an approach on bone density. In a small 8-month study, whole body vibration on a reciprocating plate was more effective than daily walking in improving balance and BMD in physically untrained, otherwise healthy postmenopausal women. These results need to be reproduced in larger randomized controlled trials but may offer an interesting alternative in the future for those unable to perform daily exercise (17).

Our study had several potential limitations. Firstly, the small study population comprised of women who visited a clinic, and may not have been representative of the general population residing in a community, thus possibly resulting in selection bias. Secondly, we cannot insist that the genotypes are functionally relevant. Thirdly, it may be argued that the Pearson correlation coefficients should be applied to the p values obtained in our study. If, Pearson correlation coefficients were strictly adopted, associated p values could not retain all significances. However, although there is a chance of type I error owing to association, when considering the facts that. The posible associations between vitamin D and PTH levels were not definition in this study. Although only 2% of these older women had vitamin D insufficiency according to the classic definition, serum levels of PTH indicative of hyperparathyroidism were present in about 18%. This suggests that the existing criterion for vitamin D insufficiency may be too low (36).

In conclusion, we thought increased levels of t-Hcy, DPD and decreased values of BMD may be associated with a PAS in postmenopausal osteoporosis. Further studies are needed to understand the underlying mechanisms of these findings.

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