Effects of increasing fruit and vegetable intake on bone turnover in postmenopausal osteopenic women

[1]Ebrahimof S., [1]Hoshiarrad A., [2]Hossein-Nezhad A., *[2]Larijani B., [1]Kimiagar SM.

[1]*School of Food Sciences and Food Technology, Shaheed Beheshti University of Medical Sciences,*
[2]*Endocrinology & Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran*

Received 26 May 2009; Revised 31 Oct 2009; Accepted 4 Nov 2009

ABSTRACT

Background and the purpose of the study: Adequate intake of fruits and vegetables could be helpful to prevent major non-communicable diseases**.** Some nutrients abundant in fruits and vegetables have been shown to affect bone health. In the present study effects of increasing fruit and vegetable intake on bone metabolism in postmenopausal women with osteopenia was evaluated.

Methods: In the present clinical trial 45 postmenopausal osteopenic women, between 50-60 years of age participated. Subjects were randomly assigned to intervention or control groups. Subjects in the control group were asked to continue their own dietary patterns and make no changes in their life style. To increase fruit and vegetable intake in the intervention group, they were given 6 extra servings of fruits and vegetables daily. Serum osteocalcin and crosslaps were measured at baseline and after 12 weeks of intervention. Twenty four hrs food recalls were used to assess dietary intake at baseline, during and at the end of the study.

Results: Increasing fruit and vegetable intake for 12 weeks reduced serum osteocalcin by 15% and crosslaps by 4%. The reduction was not statistically significant after adjustment for confounding factors. In 9 subjects of the intervention group, both markers of bone metabolism were reduced by 24 %. Baseline serum levels of both bone markers were significantly higher in these subjects.

Conclusion: Increasing fruit and vegetable intake may not reduce bone turnover in postmenopausal osteopenic women, but it may be effective for those who are at higher risk of bone fracture because of higher bone turnover.

Keywords: Fruit, Vegetable, Osteopenic, Postmenopause, Bone turnover

INTRODUCTION

Osteoporosis is a common chronic disease that affects many people around the world, especially the aging population. Osteoporotic fractures are a well known health burden to health care resources because they are a major cause of mortality and disability in the elderly (1). Bone loss begins at the age of 35- 45 in both sexes (2) but in women deficiency of esterogen at menopause accelerates its rate (3). According to global speculations prevalence of this disease will dramatically increase by the year of 2050 (4), most likely not only due to the aging population but also because of adverse changes in life style and diet (5). Peak bone mass (PBM) of Iranian population is similar to some other countries such as Lebanon, but the rate of bone loss in Iranian elderly is 1.5 times higher (6). A study on 40- 60 years old women in Tehran shows that 4.6% of postmenopausal women have osteoporosis in femur and 23.1% have osteoporosis in spine. Postmenopausal women compared to others had lower bone mineral density (BMD) at all bone sites (7). Currently higher intake of calcium and vitamin D (8) as well as some drugs such as hormones and bisphosphonates (9) are prescribed widely to slow the rate of bone loss and prevention of osteoporosis. Low BMD or Osteopenia if left untreated, may progress to osteoporosis and bone fractures.

Nutritional factors have an important role on the bone health. Research on diet and bone metabolism has focused primarily on calcium and vitamin D. Less is known about other components of diet. Cross sectional studies have shown that higher intakes of fruits and vegetables and some micronutrients such as potassium, magnesium, vitamin C and carotenoids have significant correlation with higher BMD and lower risk of osteoporosis (10). In clinical trials urinary calcium excretion and bone resorption markers were lower in those consuming more fruits and vegetables (11). A plant based diet has a lower acid load, whereas animal foods increase acidity (12). Dietary acid load may have negative effect on bone health through increasing urinary calcium excretion and bone resorption. Urinary calcium excretion is lower when urine is more alkaline

Correspondence: emrc@sina.tums.ac.ir

and higher when urine is more acidic (13). pH reduction even in normal limit inhibits osteoblasts activity but activates osteoclasts and increases bone resorption (14). Animal studies has revealed that onion or mixture of vegetables can significantly inhibit bone resorption and thus increase bone mass in rats (15). Benefits of increasing calcium through consuming more milk and dairy products is only obvious in those with low dietary calcium intake (16), and high intake of calcium supplements is associated with increased risk of renal stones (17). On the other hand there are many well known negative side effects for hormone replacement therapy such as increase in the risk of heart disease, stroke and breast cancer (18). These findings prompted us to perform the present study to evaluate the effect of fruit and vegetables intake on bone metabolism in postmenopausal osteopenic women.

MATERIALS AND METHODS

Study design and subject selection

The present study was a sequential matched randomized clinical trial. Subjects in the present study were 1-10 year post-menopause women aged 45- 60 years who had referred to bone mineral densitometry center of Shariati hospital in Tehran. Non smoking osteopenic women (according to WHO criteria (19)) who had not taken any medications or did not suffer from any condition that likely to affect their bone metabolism were chosen.

Goals of the study were explained for all subjects and each participant provided informed written consent. Study protocol was approved and sponsored by Endocrinology and Metabolism Research Center of Tehran University of Medical Science.

Subjects were randomly assigned to intervention and control groups. Age, menopausal age, history of dietary supplements use and type of vitamins and minerals supplements were asked at baseline. Physical activity level was determined by asking daily activities and according to physical activity guideline it was grouped as very low, low, medium and very high (20). Participants were asked to maintain their usual activity levels and also supplement intakes during the entire study.

Dietary intervention

Subjects in both groups were instructed not to modify their regular diet under any condition. Intervention group were asked to consume six extra servings of fruits and vegetables daily. The extra servings were about 400 gr of fruits and vegetables including 100 gr of cucumber, 100 gr of tomato, 70 gr of red cabbage, 50 gr of lettuce, 17 gr of prunes, 14 gr of dried apricots, 17 gr of dried figs and 22 gr of raisins, providing 249 kcal energy, 5.64 gr protein, 82.7 mg calcium, 1242.5 mg potassium, 70.6 mg magnesium and 57.7 mg vitamin C. The gram of servings of fruits and vegetables was determined according to USDA data bank (21). Fresh vegetables in the amount of 4- 5 kilograms (considering the amount that other members of family may eat) were given to intervention group subjects weekly. The definition of vegetables serving sizes was provided for each of them using a colored food photo album (22). The exact amount of fruits that each participant had to eat daily was carefully weighed by a digital balance and packed in plastic bags. The weekly amount considering possible use of other members of family was given to them at the beginning of each week. Subject of the intervention group were instructed how to add these extra fruits and vegetable to their regular diet. In order to avoid putting on weight, they were recommended to reduce their simple sugar and fat intake.

Measurements

BMD was assessed by dual-energy X-ray absorptiometry (DEXA) at the lumbar spine (lumbar vertebrae 2-4) and total hip (23). Each woman's weight (while wearing light clothing and no shoes) was recorded by using a balance-beam scale to the nearest 0.5 kg. Height was measured with a stadiometer to the nearest of 0.5 cm (24). Body mass index (BMI) was calculated by the following formula: [(weight (kg) / height (m^2))].

At the baseline and after 12 weeks intervention a blood sample was collected after an overnight fasting for 12-14 hrs and bone metabolism was assessed using two markers, i.e., the serum marker of bone formation, osteocalcin (OC), and serum marker of bone resorption, crosslaps (CL). Both bone markers were measured using ELISA method and Nordic Bioscience Diagnostic (Denmark) kits.

Dietary assessment

Dietary intake was assessed using 7 days of 24 hrs food recalls for each subject (2 recalls at baseline, 5 recalls during the study and 2 recalls at the end). A trained nutritionist asked each subjects to name all foods and drinks who consumed during the previous 24 hrs and assisted the respondent in estimating portion sizes. By using the manual for household measures, cooking yield factors and edible portion of foods, the amount of consumed foods and drinks was converted to grams (25). Food servings were computed according to USDA data bank (23). The composition of dietary intakes was analyzed using Iranian food composition table (26) which is modified and complemented by valid reference food composition tables (27).

Statistical analyses

Data were analyzed using Statistical Program for Social Sciences (SPSS) (version 14). Student *t*-test was used to compare means between two study groups and Paired test was used to detect changes within each group. Changes in dietary intake over time were evaluated by repeated measure ANOVA after adjustment for confounding factors. Statistical significance was defined as P-value <0.05.

RESULTS

From 52 women enrolled the study, 45 (23 in intervention group and 22 in control group) completed the study. Table 1 summarizes baseline characteristics of subjects. No significant difference was observed between two groups at the beginning of the study. Sixty six percent of control group and fifty two percent of intervention group had very low level of the activity. Thirty four percent of controls and forty eight percent of intervention group reported low activity. No statistically significant difference was observed between two groups (p=0.21). Participants were asked not to change their habitual activity during the study. Forty six percent of controls and sixty five percent of intervention group didn't use calcium and vitamin D supplements but fifty four percent of controls and thirty five percent of intervention group reported the use of calcium and vitamin D supplements. There was no difference between two groups regarding this factor (p=0.21). All participants maintained their use of supplements during the study.

Reported intake of food groups over time and according to study groups are shown in Table 2. Dietary intakes of the study groups were similar at the baseline and no significant difference was observed. During the study, in addition to fruit and vegetables intake, two groups were different in meat and substitutes intake. At the end of the study only fruit and vegetables intake was different between two groups.

Evaluation of dietary changes by repeated measure ANOVA revealed that in the control group, the intake of grain products (p=0.0001) and fats (p=0.0001) changed during the study. In the intervention group the same change was observed for fruits and vegetables (both p=0.0001) as well as for grain products (p=0.0001), meat and alternatives (p=0.04) and fats (p=0.0001).

Table 3 summarizes energy and nutrients intakes of two study groups. As it shows energy and nutrients intake of study groups were the same at the baseline. During the study two groups were different in energy and protein intake as well as potassium, magnesium and vitamin C. At the end of the study, two groups were just different in potassium, magnesium and vitamin C intake.

Means±SD of weight in intervention and control group at baseline were 72.5±9.9 kg and 68.9±11.9 kg, respectively. At the end of the study it was 72.9±10.5 kg and 69.2±11.4 kg, respectively. No significant change in weight was observed during the study.

Tables 4 and 5 summarize baseline levels of bone metabolism markers and their changes during the study. After the intervention serum OC level in intervention group decreased about 15%. However following adjustment for confounding factors including unexpected changes in dietary intake, the observed decrease was not statistically significant.

Serum CL levels had 4% decrease in intervention group. In control group a small incensement was observed after intervention but none of the changes were statistically significant. After control for confounding factors the changes remained not significant.

Besides of the mentioned findings, we evaluated those participants in intervention group who had significant reduction in both bone turnover markers at the end of the study.

Nine subjects of the intervention group had significant reduction in both bone turnover markers (24% for each). We compared these subjects with 32 participants in two groups who did not have reduction in both bone turnover marker (4 participants had no change in bone turnover markers and were eliminated from further analysis). No significant difference in age and menopause age was observed. In addition, energy, food groups and nutrients intakes were not statistically different. The only observed difference was in their baseline serum levels of bone metabolism markers. Baseline serum levels of bone turnover markers was 25±8.9 ng/ml for OC and 0.8±0.3 ng/ml for CL. Figures 1 and 2 show the baseline serum level of bone metabolism markers in these 9 subjects in comparison with other subjects of intervention group. Baseline OC level in these 9 subjects was significantly higher than the rest (p=0.03). These subjects also had higher CL level at the baseline (p=0.05).

DISCUSSION

Results of this survey which was conducted on postmenopausal women with osteopenia show that increasing fruit and vegetables intake for 12 weeks can not reduce bone metabolism turnover in these subjects in comparison with controls.

There are not many clinical trials on the effects of fruit and vegetable intake on bone turnover but cross sectional and cohort studies have clearly shown the relationship between intake of fruit and vegetable and bone health. Magnesium, potassium, fruit and vegetables intakes are significantly associated with greater BMD in both men and women and among men, with lower bone loss over four years (13). Similar correlation has been reported for 40- 60 year old women. They showed that women who consume vegetables more than 9 times a week have higher BMD compared to others with lower intakes (7). In another study on rural population of Tehran higher BMD were found at heels in women consuming more than 1.5 servings of vegetables daily (28).

In the present study we added 6 extra servings of fruits and vegetables to usual daily intake of intervention group so their daily intake of fruits and vegetables increased to 12 servings during the study. The extra servings were about 400 grams of fruits and vegetables. This amount was determined according to the previous studies. In a cross sectional assessment of the present study groups which has been published previously (29), fruit and vegetable intake was significantly correlated with OC ($r = -0.4$, $p < 0.001$). Serum OC level in those consuming more than 400 grams fruit and vegetables daily was significantly lower than others (18±6.5 ng/ml compared with 30±13.7 ng/ml, $p < 0.05$). Animal studies have reported that the minimum effective dosage of fruits and vegetables which inhibit bone resorption rate is about 6.2 g/ kg body weight (15).

Type of extra fruits and vegetables was chosen on

Table 1. Characteristics of subjects under study

	Control group (n=22)	Intervention group (n=23)	P-value
Age (y)	55±3.7	55±3.5	0.9
Duration of menopause (y)	5.6±3.7	6.7±3.2	0.2
Anthropometric Characteristics			
Weight (kg)	68.9±11.9	72.5±9.9	0.7
BMI (kg/m^2)	28.6±4.3	29.3±3.9	0.4
Bone Mineral Density and Bone Metabolism Markers			
Total hip BMD (g/cm^2)	0.88±0.09	0.91±0.08	0.1
Spine BMD (g/cm^2)	1.0±0.06	0.99±0.09	0.6
Osteocalcin (ng/ml)	21.2±10.8	19.4±8.2	0.9
Crosslaps (ng/ml)	0.72±0.31	0.62±0.29	0.4

*Means±SDs

Table 2. Food groups intakes of participants according to the study groups*

	Control group (n=22)	Intervention group (n=23)	P-value
Grain products (serving/day)			
At baseline	6.5±1.5	7.1±1.2	0.3
During the study	5±1.3	5.4±1.4	0.4
At the end	7.4±4.2	7.5±3.3	0.9
Milk products (serving/day)			
At baseline	2.1±1.6	2.1±1.3	0.9
During the study	2±0.9	2±0.1	0.9
At the end	1.7±0.9	1.9±1.0	0.6
Meat and alternatives (serving/day)			
At baseline	2.2±1.2	2.1±1.2	0.9
During the study	2.4±0.1	3.3±0.1	0.007
At the end	2.6±1.5	2.3±1	0.4
Fruit (serving/day)			
At baseline	2.6±1.8	2.3±1.6	0.4
During the study	3.4±1.2	6.4±1.4	0.00
At the end	3.2±2.3	6.4±1.9	0.00
Vegatbles (serving/day)			
At baseline	2.2±1.2	2.7±1.5	0.2
During the study	2.1±0.8	5.8±1.2	0.00
At the end	2±2.3	5.7±2.4	0.00
Fats (serving/day)			
At baseline	3.4±1.9	3.2±1.7	0.7
During the study	3.7±2.1	3±1.1	0.2
At the end	2.3±2	1.7±1.2	0.2
Sweets (serving/day)			
At baseline	1.7±0.7	1.4±1.1	0.3
During the study	1.5±0.9	1±0.6	0.3
At the end	1.5±1.6	1.2±1	0.4

*Means±SDs

Table 3. Energy and nutrients intakes of participants according to the study groups*

	Control group (n=22)	Intervention group (n=23)	P-value
Energy (kcal/day)			
At baseline	1472±306	1511±337	0.7
During the study	1529±344	1779±267	0.009
At the end	1472±486	1691±288	0.07
Protein (gram/day)			
At baseline	49±15	49±13	0.9
During the study	51±12	62±11	0.004
At the end	47±15	54±12	0.1
Calcium (milligram/day)			
At baseline	745±270	800±286	0.5
During the study	808±259	908±301	0.2
At the end	688±326	857±311	0.08
Potassium (milligram/day)			
At baseline	2090±700	2029±643	0.8
During the study	2241±719	3418±770	0.00
At the end	2183±1115	3274±739	0.00
Magnesium (milligram/day)			
At baseline	159±49	146±36	0.3
During the study	183±57	245±50	0.00
At the end	179±67	218±55	0.04
Vitamin C (milligram/day)			
At baseline	164±85	136±77	0.3
During the study	144±53	214±90	0.003
At the end	132±94	195±83	0.02

*Means±SDs

the base of studies on animals (15). These studies showed that 25 food items can inhibit bone resorption. Concerning Iranians' dietary habits and availability of fruits and vegetables throughout the year, 4 types of vegetables including lettuce, red cabbage, cucumber and tomato and also 4 types of fruits including dried apricot, dried fig, raisins and prune were chosen. According to these surveys, most foodstuffs which have been reported to have bone inhibitory effects are distributed among vegetables and the only fruits with significant effects were prune and orange. In this study in addition to prune which its effect on inhabitation of the bone loss inhibition has been shown in postmenopausal women (30), dried fig , dried apricot and raisins were chosen because of their high potassium content. Potassium has been shown to have an important role in bone health (10, 31).

In a similar study it was shown that the Dietary Approaches to Stop Hypertension (DASH) for 3 months reduces serum OC by 8-11% and C-teriminal telopeptide of type 1 collagen (CTX) by 16-18% (both P <0.001) in healthy men and women (18). Buclin et al evaluated the effect of alkaline diet on bone metabolism and urinary CTX marker in healthy young men and reported 19% reduction of the marker in comparison with acidic diet (17). In order to compare the results of the present study with similar reported surveys attention should be paid to two points, first the bone characteristics of subjects under study and second the characteristics of administered diet.

In both of the mentioned studies participants were a sample of the healthy population and thus had different bone metabolism compared with subjects of this study. Menopause and bone loss affect levels of the bone metabolism markers level. In postmenopausal women following reduction in estrogen level bone resorption and formation increases and higher levels of bone turnover markers in serum and urine are detectable (32). Some studies comparing bone turnover markers in women revealed that those with the highest level of bone metabolism markers have the least BMD (33). Regarding these facts it seems that the intervention period or the amount of effective fruits and vegetables for these subjects may be different from healthy and young subjects.

Potassium and magnesium intakes in two mentioned reports were higher than this study. In Buclin's study the administered diet had high potassium content (6396 mg/d) (13) while DASH diet contained 4700 mg/d potassium and 500 mg/d magnesium (11). Potassium and magnesium intakes of the present study were lower (3418 mg/d and 245 mg/d, respectively). However,

Table 4. Serum OC levels before and after intervention according to the study groups (n=45) *

(Serum OC level (µg/l	Before Intervention	After intervention	Changes	P-value
(Control group (n=22	19.7±7.4	19.3±7.5	-0.41±5.5	0.5
(Intervention group (n=22	19.9±8.4	16.9±7.7	-3.1±4.5	0.7
p-value	0.9	0.3	0.2	

*Means±SDs

Table 5. Serum CL levels before and after intervention according to the study groups (n=45) *

(Serum CL level (µg/l	Before Intervention	After intervention	Changes	P-value
(Control group (n=22	0.68±0.25	0.69±0.29	0.01±0.19	0.2
(Intervention group (n=23	0.61±0.3	0.56±0.23	-0.05±0.17	0.8
p-value	0.4	0.1	0.3	

* Means±SDs

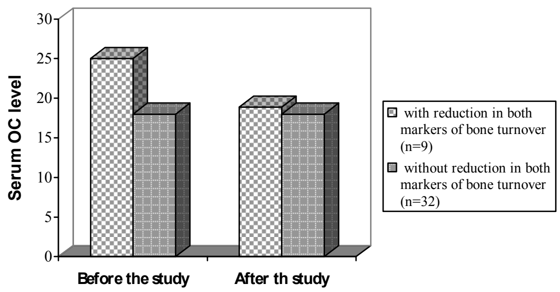

Figure1. OC serum levels in participants with reduction in both bone markers compared to others without reduction in both markers*
* Means±SDs

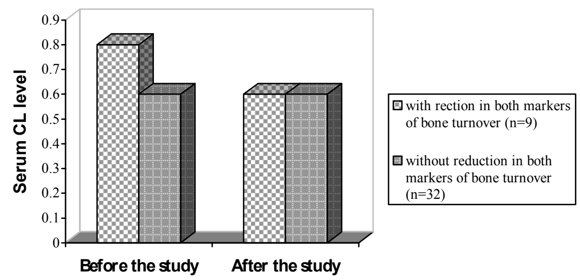

Figure2. CL serum levels in participants with reduction in both bone markers compared to others without reduction in both markers*
* Means±SDs

intervention group consumed 12 servings of fruits and vegetables daily which is higher than in DASH diet (11). Other than fruits and vegetables, whole grains and low fat dairy foods were sources of potassium and nuts, seeds and legumes were good sources of magnesium (11). Lower intakes of these nutrients can somewhat explain differences in results of this study. High intakes of potassium and magnesium can help acid-base balance by neutralizing metabolic acid produced by consumed proteins and amino acids especially methionin and systein (12). In human with normal kidney function, the acid-base balance depends to the ability of kidneys to excrete excess acid and the availability of a base for buffering (34). In the acute phase potassium and sodium contained in the blood fluid barrier are the first line of defense for buffering metabolic acidosis, and thus they spare the bone tissue (34). In the chronic state of metabolic acidosis, bone crystals are dissolved to provide calcium, carbonate and citrate for buffering (34). Much of the work to support these observations has been carried out in adults or animal models (35).

Dairy products and calcium intake in the intervention group was also lower than those of the similar studies. Lin et al reported intakes of 2.7 servings of dairy products and 1250 mg calcium daily (11). In Buclin's study in addition to 1888 mg daily calcium in experimental diet, subjects were given a single oral dose of 1 gram calcium on the last day of experiment. In this study intake of dairy products was about 2 servings daily which is the least amount recommended for people over 50 years old (36). However, our participants consumed more dairy products in comparison with subjects of other studies on Iranian population. Serving of 1±0.7 daily dairy products has been reported for 20-59 year old women (37). Calcium intake of participants in intervention group was 908 mg/d which is lower than 1200 mg/d that is recommended for this age group but is higher than 631±257 mg/d that was reported in 40-60 year old women (7). Calcium is a key nutrient in bone metabolism and studies have shown its short (38) as well as long term (39) effects on bone health in pre- and post-menopausal women. In these studies calcium supplements significantly reduced bone resorption or bone formation. It seems that adequate intake of calcium is necessary to enhance possible effects of fruits and vegetables. Low intake of calcium could weaken the impact of increase in fruits and vegetables.

As mentioned, the amount and type of fruits and vegetables were chosen according to the previous studies in rats (15) but adding extra servings of fruits and vegetables could not significantly reduce bone turnover markers in intervention group compared to the control group. After 12 weeks of intervention

OC level reduction was 15% which is higher than the levels report by Lin et al but adjustment for confounding factors made the reduction statistically not significant. At the end of the study 4% change was observed for CL in both groups. While the serum level of CL decreased in intervention group, it increased in the control group. In any event, although the changes showed positive effects of extra fruits and vegetables but statistical test could not confirm it.

In general clinical trials on humans have time limitation and as a result long range effects can not be detected. Studies on drugs such as bisphosphonates and hormone substitutes have shown that at least 3- 9 months is required to observe significant changes in bone metabolism so it is possible that a longer period was required for this nutritional intervention. Although basic results of the present study could not show the effect of increasing fruit and vegetables intake on bone turnover markers, subjects under study were those who had shown significant reduction in both turnover markers. After the intervention, in 9 subjects of the intervention group, both markers of bone metabolism were reduced by 24%. The reduction rate was higher than those of the similar studies (10, 11). Dietary intakes of these subjects were similar to others in intervention group but their baseline serum levels of both bone turnover markers were significantly higher than others. Studies have shown that such people with high bone turnover have better response to treatment with bisphosphonates, calcitonin and hormone replacement therapy (40,41). More research in this field is required to clarify whether baseline bone turnover should be considered as a confounding factor in bone metabolism studies.

In conclusion, consuming 12 servings of fruits and vegetables for 12 weeks did not reduce bone metabolism markers in all participants in intervention group but it had a significant effect on those with high bone turnover. High bone turnover was correlated with higher risk of bone fracture and as a result reducing bone turnover can be an effective approach in lowering the bone fragility. Increasing fruit and vegetable intake may be complementary to what is currently recommended to prevent bone fractures, especially for those who have high bone turnover rate.

ACKNOWLEDGEMENT

We extend our sincerest thanks to all women who served as samples of this study. We would also like to thank Ms Nahid Zandi for her supportive contribution.

REFERENCES

1. Hossein- Nezhad, A., Maghbooli, Z., Bandarian, F., Mortaz, S., Soltani, A., Larijani, B., Association of bone mineral density and lifestyle in men, Iranian Journal of Public Health, 2007; Suppl: 51-56
2. Larijani, B., Moayyeri, A., Keshtkar, A.A., Hossein-Nezhad, A., Soltani, A., Bahrami, A., Omrani, G.H., Rajabian, R., Nabipour, I., Peak bone mass of Iranian population: the Iranian Multicenter Osteoporosis Study, J Clin Densitom, 2006;9:367-374.
3. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev, 2000; 2:115-37.
4. Hossein-Nezhad, A., Maghbooli, Z., Bandarian, F., Mortaz, S., Soltani, A., Larijani, B., Association of bone mineral density and lifestyle in men, Iranian Journal of Public Health,2007; Suppl: 51-56
5. Bunker V. The role of nutrition in osteoporosis. Bri. J. Biomed. Sci, 1994; 51: 228-240.
6. Larijani B, Hossein-nezhad A, Mojtahedi A, Pajouhi M, Bastanhagh MH, Soltani A, Mirfezi SZ and Dashti R. Normative data of bone mineral density in healthy population of Tehran, Iran: A cross sectional study. BMC Musculoskelet Disord, 2005; 6: 38.
7. Moayyeri, A., Ahmadi-Abhari, S., Hossein-nezhad, A.,Larijani, B., Soltani, A. Bone mineral density and estimated height loss based on patients' recalls, Osteoporos Int, 2006;17:834-840.
8. Roudsari, A.H., Tahbaz, F., Hossein-Nezhad, A., Arjmandi, B., Larijani, B., Kimiagar, S.M., Assessment of soy phytoestrogens' effects on bone turnover indicators in menopausal women with osteopenia in Iran: a before and after clinical trial, Nutr J, 2005 ;4:30.
9. Manson JE, Martin KA. Clinical practice: Postmenopausal hormone replacement therapy. N Engl. J. Med, 2001; 345: 34-40.
10. Wattanapenpaiboon N, Lukito W, Wahlqvist ML, Strauss BJ. Dietary carotenoid intake as predictore of bone mineral density. Asia. Pac. J. Clin. Nutr, 2003; 12: 467-473.
11. Lin PH, Ginty F, Appel L J, Aickin M, Bohannon A, Garnero P, Barclay D, Svetkey LP. The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults. J. Nutr, 2003; 133: 3130-3136.
12. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. J. Am. Diet. Assoc, 1995; 95: 791-797.
13. Maurer M, Riesen W, Muser J, Hulter HN, Krapf R. Neutralization of western diet inhibits bone resorption independently of K intake and reduces cortisol secretio in humans. Am. J. Physiol, 2003; 284: F32-F40.
14. Arnett T. Regulation of bone cell function by acid-base balance. Pro. Nutr. Soc, 2003; 62: 511-520.
15. Mühlbauer RC, Lozano A, Palacio S, Reinli A, and Felix R. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone, 2003; 32: 372-380.

16. Kanis JA and Johnell A. Long-term risk of osteoporotic fracture in Malm. Osteoporosis Int, 2000; 11: 669-674.
17. Curhan GC, Willett WC, Speizer FE, Spiegelman D, Stampfer MJ. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stone in women. Ann. Intern Med, 1997; 126: 497-504.
18. Shoemaker JR, Klemes AB. Alternate options to hormone replacement therapy for osteoporosis. J. Am. Osteopath. Assoc, 2003; 103: S1-S5.
19. World Health Organization. Assessment of fracture risk and its application to screening for osteoporosis. World Health Organization Technical report series 843. 1994; 1-129.
20. Carol D. Energy. In: Mahan LK & Escott-Stump S. Food, nutrition and diet Therapy.11th edition. Philadelphia. WB Saunders, 2004; 21-36.
21. www.nal.usda.gov/fnic/foodcomp/Data/SR15/sr15.html
22. Ghaffarpour M, Hoshiarrad A, Kianfar H, Banieghbali B. Food Photo Album. 1st ed. Tehran, Nutrition and Food science Research Institute Press, 1386.
23. Baran DT, Faulkner KG, Genant HK, Miller PD, Pacifici R. Diagnosis and management of osteoporosis: guidelines for utilization of bone densitometry. Calcif Tissue Int 1997; 61: 433-440.
24. DeHoog S. The assessment of nutritional status. In: Mahan LK & Escott-Stump S. Food, nutrition and diet therapy. 9th edition. Philadelphia. WB Saunders, 1996; 361-368
25. Ghaffarpour M, Hoshiarrad A, Kianfar H. The manual for household measures, cooking yield factors and edible portion of foods. 1st ed. Oloom Keshavarzi Press. Tehran, 1376.
26. Sarkissian T, Rahmanian N, Azar M. Food Composition table of Iran. Vol I: Raw Material. Tehran, Institute of Nutrition Sciences and Food Technology. No 131,1980.
27. USDA, National Nutrient Database for Standard Reference. US Development of Agriculture. Agriculture Research Service. Release 15, 2002. Available: Nutrient Data Laboratory Home Page, http:// www.nal.usda.gov.finc.food.com.
28. Ebrahimof S, Adibi H, Salehomom N, Hosseinni S, Larijani B. Fruit and vegetable intake and Bone Mineral Density in residents of villages surrounding Tehran. Iran J Pub Health 2004; suppl 1: S49-S56.
29. Ebrahimof S, Hossein-nezhad A, Hoshiarrd A, Rahmani M, Valaei N, Larijani B, Kimiagar M. Fruit and vegetable intake and bone turnover markers in postmenopausal osteopenic women referring to bone densitometry unit of Shariati Hospital during 2000-2004. Pejouhandeh, 2006; 11: 159-165.
30. Deyhim F, Stoecker BJ, Brusewitz GH, Devareddy L, Arjmandi BH. Dried plum reverses bone loss in an osteopenic rat model of osteoporosis. Menopause, 2005; 12: 755-62.
31. Tuker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP. Potassium, magnesium, and fruit and vegetables intake are associated with greater bone mineral density in elderly men and women. Am. J. Clin. Nutr, 1999; 69: 727-736.
32. Eshaghi, S.M., Hossein-nezhad, A., Maghbooli Z., Larijani,B., Relationship between Mandibular BMD and Bone Turnover Markers in Osteoporosis Diagnosis, Iranian Journal of Public Health, 2008; Suppl : 63-71.
33. Schneider DL and Barrett-Connor EL. Urinary N-telopeptide levels discriminate normal, osteopenic, osteoporotic bone mineral density. Arch. Intern. Med, 1997; 157: 1241-1251.
34. Green J, Kleeman CR. Role of bone in regulation of systemic acid-base balance. Kidney Int, 1991; 39: 9-26.
35. Mueller K, Trias R. Bone density and composition: age-related and pathological changes in water and mineral content. J Bone Joint Surg, 1996; 48A: 140-148. http://www.mypyramid.gov/pyramid/index.html.
36. Mirmiran P, Mohammadi F, Bayegi F, Kalantari N, Azizi F. Dietary intakes of Tehran's residents according to food guide pyramid. Iran Medical University magazine, 1381; 32: 767-777.
37. Blumsohn A, Herrington K, Hannon RA, Shao P, Eyre DR and Eastel R. The effect of calcium supplementation on the circadian rhythm of bone resorption. J. Clin. Endocrinol. Metab, 1994; 79: 730-735.
38. Kamel S, Fardellone P, Meddah B, Lorget- Gondelmann F, Sebert J L and Brazier M. Response of several markers of bone collagen degradation to calcium supplementation in postmenopausal women with low calcium intake. Clin. Chem, 1998; 44:1437-1442.
39. Chailurkit LO, Ongphiphadhanakul B, Piaseu N, Saetung S and Rajatanavin R. Biochemical markers of bone turnover and response of bone mineral density to intervention in early postmenopausal women: an experience in a clinical laboratory. Clin. Chem, 2001; 47: 1083-1088.
40. Gonelli S, Cepollaro C, Pondrelli C, Martini S, Monaco R and Gennari C. The usefulness of bone turnover in predicting the response to transdermal estrogen therapy in postmenopausal osteoporosis. J. Bone Miner. Res, 1997; 12: 624-631.