Impact of Lifestyle and Nutritional Adaptation Strategies on Bone Health of Middle to Older Aged Women at Risk of Osteoporosis

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ABSTRACT
Osteoporosis is defined as a disorder or impairment in bone mass, strength, and microarchitectur, leading to increased risk for fracture, disability, loss of independence and even death. Optimum nutrition and diet rich in nutrients specific to bone health goes a long way in optimising the bone mass, as well as in prevention against this disorder. This study investigates the impact of nutritional and lifestyle changes on women in middle to older age group who are at risk of osteoporosis. Out of total (N=139 Females) screened participants to get females (n=60) for study in 40-60 years age groups, 43% were found at risk of osteoporosis. Reduced intake of proteins and minerals, ignorance of diversity in fruit and vegetable consumption was significantly (p≤0.05; 0.01) correlated with decreased BMD score, serum calcium and phosphorus levels. After several developmental trials; a formulation of mixture of cereals (20%), soy flour (20%), diverse fruit (30%), honey (20%), fat (6%), two herbs (2%) and flax seeds (2%) with overall acceptability score of 8.25 and significantly (p=0.05) high in essential nutrients was selected for intervention to 50 % of women (pre-15n, post-15n) participating in this study for a period of 90 days. The BMD scores, anthropometric and biochemical markers were assessed before and after the intervention period. After random supplementation of both the groups, improvement in biochemical markers specific to skeletal health was reported in test groups. The participants also mentioned decrease in frequent occurrence of pain and related symptoms. The observed effect of supplement has a potential to effectively reduce the risk of osteoporosis among females.

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Introduction
Osteoporosis is a bone disorder characteristic of signs including slight to chronic pain in back, legs or other body parts leading to lower bone mass and deteriorating bone tissue if left untreated thereby increasing fractures risk. Once it starts, there is a rapid and continuous loss of bone mass as compared to its formation, lowering skeletal strength and making it vulnerable to fractures or an unrepairable broken bone. This Silent disease is often asymptomatic until a fracture occurs [1]. Three stages of life are critical for its prevention. From the time of adolescence till end of third decade of life when peak bone mass is attained. It makes it an ideal time to strengthen our skeleton for later years through weight-bearing exercise, taking calcium and enough sunlight, and quit smoking. At second important stage of menopause, rapid bone loss over a five-to-six-year period makes it all the more necessary to get ourselves screened. But, unfortunately in majority of cases it is not followed unless a woman has symptoms of osteoporosis. And the time of the first fracture, which may be an early sign of osteoporosis. There is variety of diagnostic tests for determining bone density for osteoporosis diagnosis. Tests like single-energy X-ray absorptiometry (SXA) and dual-energy X-ray absorptiometry (DXA or DEXA) measures the mineral content of the entire skeleton as well as of specific sites [2].

The process of bone remodelling is a continuous metabolic process. The discovery of key pathways regulating bone resorption (osteoclasts) and formation (ossification by osteoblasts) has identified new approach to treatment with distinctive mechanism of action. This process secures the skeletal architecture throughout the life and any imbalance in this, is an indication of a bone disorder [3]. Studies have proven that irrespective of the stage of life, eating a diet rich in bone nutrients maintain bone strength and carrying out a physically active lifestyle are two essential components of having strong bones. Diet helps in getting the right nutrients for the bone strength and development right from the adolescence.

A bulk of studies is available about importance of calcium and vitamin D, but still women suffer from bone disorders, especially during menopause. Protein is an important macro nutrient for maintaining strong bones and muscles. Phosphorus and certain trace minerals (magnesium, manganese, iron, copper, and zinc) and vitamin C and K are also involved in bone health [4, 5]. There exists a greater scope for plant phytochemicals and phenolic acids present in various food ingredients like soy, brown rice, oats, alfalfa, flax, and cocoa, etc, in reducing the risk of bone density loss. Time to time studies have revealed the role of vitamins and minerals present in dried foods like figs, prunes, raisins and dates in combination with fructooligosaccharides (FOS) showing beneficial results in maintaining bone mineral density [6, 7].
Besides diet, there exist some more factors which if changed for better, further increase the scope of osteoporosis prevention at any age. The first of these factors is exercise. It has many potential health benefits like maintaining an ideal body weight and making bones and muscles strong. Following an active life style, consuming foods rich in calcium and ensuring supply of sufficient vitamin D to our bodies has a tremendous scope to improve our bone and muscle health and reduce the risk of osteoporosis. Keeping all these benefits in view a set of following objectives was framed for this study. 
1. To assess the nutritional status and bone health markers of participants before and after supplementation study.
2. To develop a ready-to-eat multinutrient supplement with optimum sensory and nutritional attributes.
3. To study the impact of supplementation on nutritional status and bone health markers of females at risk of osteoporosis.

Methodology

1. Ethics
All the methods for the study were carried out after obtaining permissions from the institutional ethical committee and taking consent from all the participants (No. DR 12505-14/16-04-14). Females with co-morbidities were not included in the study.

2. Measures and impact evaluation
Basic anthropometric measurements like height, weight, hip and waist circumferences were measured. Body mass index (BMI) was calculated (kg/m2) [2] and Waist/ Hip (W/H) was also determined [8]. Bone health markers like serum calcium, serum phosphorus and serum alkaline phosphatase levels were also assessed through standard conventional assays [9]. Bone mass density was assessed through broadband ultrasound under the supervision of practicing physician at three different sites like wrist heel and leg. A total of 139 females were screened it was repeated for two more times after an interval of every three months to select 60 females at risk of osteoporosis for supplementation trial. The details regarding their food intake and selection was obtained using food frequency questionnaire and calculated though MSU Nutriguide Computer Software [10]. Anthropometric measurements like BMI, W/H ratio was taken before and after the supplementation trial to check the impact. Biochemical markers like total and ionised calcium and serum phosphorus and serum bone alkaline phosphatase (BAP) levels were assessed both before and after the trial to check the bone turnover during the study.

3. Developing a ready-to-eat nutrient bar
The ingredients selected to make a formulation include- a 2:1:2 ratio of cereals (brown rice, cornflakes and oats r), defatted soy flour, 2:1:2 ratio of dried fruit (figs, dates and seedless golden raisins), 1:1 ratio of herbs (Taraxacum officinale and Ocimum basilicum), flax seeds, honey and rice bran oil. Weighed amounts of all the dry ingredients were taken and mixed together, except dried fruit solids. It was followed by addition of honey and date pulp-which was prepared beforehand. The prepared mixture was filled in moulds (11x3x1.5cm) and baked at 125°C for 25 min, cooled, packaged in PP packing and stored at a cool dry place. Each bar weighed about 50 g±2g. Out of 24 formulations (of different percentages); four were at par to each other on nutritional and sensory attributes. Out of these four, the formulation which got highest score on sensory evaluation was selected for supplementation. The bar selected for supplementation trial provided a daily requirement of nutrients viz: protein, Calcium, Phosphorus and Magnesium at 17, 43, 59 and 61 percent respectively with appreciable amounts of omega 3, isoﬂavones, and vitamin K and total carotenoids.

4. Participants
Two groups of 30 participants each were made based on age; premenopausal (Group-I: 40-50 years) and post-menopausal (Group-II: 50-60 years). Fifty percent of each group of females were randomly selected and supplemented with developed Nutrient bar for a period of three months and another fifty percent were taken as control to and the impact of supplementation was recorded at the end. Randomization was performed by entering the described variables of a subject into a computer program that automatically allocates and generates a unique ID code. Participants were informed about their group after randomization and researcher was also well informed about the groups. However, the technicians involved in screening the participants were blinded to the randomization and complete care was taken to protect identity of the participants.

5. Supplementation
Participants assigned to the intervention group received the developed supplement together with individually tailored standardized dietary advice after initial personal interaction and filling of questionnaires, for a period of three consecutive months. The participants were fully aware about the composition of the bar and possible benefits of consuming the supplement. Their consent was taken before the supplementation trial and they were provided with a choice to discontinue the intake if they overcome any discomfort during the supplementation period, after informing the researcher before discontinuation. They were suggested to consume the bar at the breakfast with milk or during anytime of the day as convenient to them. Participants were asked to return any unused nutritional supplements at 15 days interval in order to evaluate follow-up, adherence and use of the provided nutritional supplement for nutritional and other analysis. However, the participants taken as control continued consumption of their usual daily diets for the time and received a leaflet with national dietary guidance.

6. Statistical Analysis
Mean and standard deviations for different parameters were computed, correlations were drawn to determine relationship of food and supplied nutrient to the risk of disease. Kruskal Wallis Test (SPSS17) was applied to select the best suited formulation of bar making for supplementation. Paired t-test was used to determine significant changes in various parameters of anthropometry and bone health markers both before and after the trial between test and control groups among pre (40-50 years) and post (50-60 years) menopausal women.

Results and Discussion
Participants who had repeated BMD scores less than -1 and without any co-morbidities were selected for the study. Normal score for BMD is above 1 SD or “T” score >-1 About half of the assessed population included university lecturers constituting 36% of those at risk of osteoporosis and majority of these were following a sedentary lifestyle pattern. While screening 139 participants in three screening tests to get 60 females for the study, 61 were not fulfilling the criteria for inclusion in the study, 11 were dropped because of other health related issues and 7 did not agree for the trail. Participants in the age group of 40-60 were assessed recording 43 percent of females at risk of osteoporosis and supporting the estimates of other studies [11]. The IOF One-Minute Osteoporosis Risk Test questionnaire provided information regarding genetic risk and secondary risk factors involved in osteoporosis. Of the total (60) at risk of disorder, genetic risk was found among 8% of participants,
7% were suffering from osteoarthritis and; only 3% were having thyroid problems.

It was also found that about 78% had onset of menopause between 47 to 55 years of age as supported by many other studies [12, 13]. The majority of women were either obese or overweight, with low BMD and high BMI (Table-1). This might support the fact that higher BMI is often related to increased fat deposition rather than more lean mass and may show an inverse relation between BMI and bone mass as revealed in some studies [14]. The calculated W/H ratio was low in pre-menopausal women and slightly higher for post-menopausal women in spite of low BMD scores. This increases the risk of osteoporosis for pre-menopausal women as low W/H ratio has been associated with osteoporosis [15]. For premenopausal group the average serum calcium, phosphorus and alkaline phosphate levels were in range of 8.44 to 8.82 mg/dl, 3.93 to 3.96 mg/dl and 205.25 to 201.00 IU/L, respectively. In post-menopausal group the average serum calcium, phosphorus and serum bone alkaline phosphate (BAP) levels were in range of 8.91 to 9.00 mg/dl, 3.82 to 4.08 mg/dl and 194.00 to 230.4 IU/L, respectively. These low values for serum calcium and serum phosphorus as seen in both the groups are related to postmenopausal osteoporosis and are considered as bio chemical markers of risk of osteoporosis directly related to BMD scores [15].

The correlation coefficients derived for women at risk of osteoporosis to their food intake revealed significantly (<.05) lower intakes of fruits, vegetables, and pulses relating to lower scores on BMD (r = 0.61, 0.69, and 0.52) and low serum calcium levels (r = 0.72, 0.63, and 0.49). Further, the intake of calories was slightly lower than the required and fats contributed more calories in both the groups. Protein intakes were lower than the required values for the age, gender, and BMI in both the groups of women which is often associated with reduced muscle mass with increased vulnerability to muscle weakness, sarcopenia and frailty, all contributing to an increased risk of falling [16,17]. Besides low intakes of calcium, the assessed participants were not getting enough sunlight which is negatively correlated with the 25(OH)D values. A three day 24 hour food recall questionnaire was prepared to collect dietary information. All these measurements were recorded before the supplementation trail.

The formulations prepared were analysed before they were used in development of bars to select most nutritionally and organoleptically suitable one. The nutritional scores of four formulations for the ready-to-eat Nutrient bar which were at par are presented in Table 2. Almost all formulations had similar nutritional profile however, highest incorporation of fruits (30%) and low percentage of cereal mix (20 %) resulted in better flavor and texture. So, the formulation with highest sensory score of 8.25 (Table 3) was selected for the preparation of nutrient bars for supplementation trial. The addition of herbs Taraxacum officinale and Ocimum basilicum enhanced the flavour and increased the nutritive value. All the ingredients had functional properties specific to bone health as shown by nutritive evaluation. The supplement was rich in nutrients like proteins, calcium phosphorus, magnesium, vitamin K, carotenoids and isoflavones when analysed. All these nutrients and active compounds had been proven to improve bone health in many studies [6, 18].

In the current study the supplement was microbiologically analyzed before the trial and was found safe for consumption. BMD scores, anthropometric and bone health markers were analysed to record the changes if any. To study the impact of supplementation the parameters recorded before the supplementation were compared with those recorded after the trial among test and control groups for both premenopausal (Group I: 40-50 years; represented in Table 4) and post-menopausal (Group: 50-60 years; represented in Table 5) women participants. After three months of supplementation, no significant difference was observed in BMD scores on all the three sites of both the groups. However, in premenopausal group (40-50 years), the anthropometric values showed a significant (p<0.01, t=3.5) decrease of 3.8 percent in hip circumference with non-significant decline in waist circumference but a significant (p<0.05, t=1.215) increase of 3.4 percent in W/H ratio due to the benefits obtained through exercising. A significant (p<0.01, t=6.5) increase of 2.6 percent in weight among the females of control group resulted in 2.5 percent increase in their BMI which was significant (p<0.01, t=3.33). In the post-menopausal test group (50-60 years) significant (p<0.01, t=6.30, t=7.18) increase of 3.7 and 1.4 percent in weight and hip circumferences was recorded, whereas waist circumference has decreased significantly (p<0.05, t=3.32) by 0.68 percent resulting in net significant (p<0.01, t=3.73), increase of 3.82 percent in BMI with reduced abdominal fat. The W/H ratio decreased significantly (p<0.05) by 2.4 percent in the test group. While as, in control group, weight and waist measurements had significantly (p<0.01, t=3.07 t=2.35) increased by 2.66 and 0.60 percent respectively, thereby increasing BMI by 2.70 percent, where t=4.38, p<0.01. W/H ratio decreased significantly (p<0.05, 2.46) by 1.22 percent due to increase in waist circumference and changes in hip circumferences. It is also found that bone mineral density in the adult women to a large extent depends on the combined impact of dietary and some non-modifiable factors [19].

Decreased total and ionised calcium and increased serum bone alkaline phosphatase (BAP) levels are simple, easy, common biochemical markers which can still be used to assess the bone turnover and risk of developing osteoporosis and fractures [20, 21]. In the pre-menopausal (40-50 years) test group highly significant (p<0.01) increase in serum calcium (13.26 percent, t=18.52) and serum phosphorus (28 percent, t=14.00) levels was recorded. While as, in the control group the serum calcium and serum phosphorus levels had significantly (p<0.01 t=1.89; p<0.10 t=3.48) decreased by 0.95 and 4.83 percent respectively. Further, a significant (p<0.01 t=9.15) fall of 16.4 percent in the elevated alkaline phosphatase levels of test group and a non-significant increase of 2.3 percent in same levels of control group was observed. In post-menopausal test group (50-60 years), a highly significant (p<0.01) increase in serum calcium levels (14.6 percent, t=17.29) and serum phosphorus levels (31.15 percent, t=25.15) was recorded. But in the control group significant (p<0.05 t=2.6; p<0.01 t=3.0) reduction of 1.1 percent in calcium and 5.9 percent in phosphorus levels was observed. The elevated alkaline phosphatase levels of the test group has fallen significantly (p<0.01 t=6.45) by 20.96 percent while as a non-significant change in control group was recorded for same. The lower serum calcium, serum phosphorus and increased alkaline phosphate levels as bio chemical markers for risk of osteoporosis among post-menopausal women and their direct relation to skeletal health was evaluated before also [22]. These biochemical markers are non-invasive and inexpensive methods which can be repeated often and have a role in the diagnosis of postmenopausal osteoporosis [23, 25].

Conclusion

The results obtained showed no change in BMD scores but significantly positive changes in the anthropometric measurements and bone health markers after supplementation were recorded;
indicating that diet rich in vitamins, minerals, phyto-nutrients, including all food groups, together with lifestyle changes play an important role in improving our skeletal health. More such research work comparing nutrients to dietary sources to supplement use would advance our understanding of the underlying mechanisms and aid in creating recommendations for osteoporosis prevention especially among females. However, long-term prospective intervention studies are needed to further examine the dietary effects on bone loss and fracture.

References