Evaluation of food products fortified with oyster shell for the prevention and treatment of osteoporosis

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Revised: 25 December 2014 / Accepted: 7 January 2015 / Published online: 21 January 2015
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Abstract Production and evaluation of different diet formulas fortified with oyster shell for the prevention and treatment of osteoporosis. Eighty-eight female albino rats were recruited and divided into 11 groups (8 rats each). Group 1 represented negative control while the remaining groups were ovariectomized. Group 2 acted as positive control. Groups 3–5 were fed on basal diet. Groups 6–8 were fed on lentil soup while groups 9–11 were fed on vegetable soup. Group 4, 7, 10 were fed on diets fortified with oyster shell. Groups 5, 8 and 11 were fed on diet formulas fortified with calcium citrate. All calcium fortified diet formulas, especially lentil soup, have minimized risk factors associated with osteoporosis as indicated from the significant increase in tibial weight, total protein, total calcium and phosphorus with noticeable reduction in ALP activity compared to positive group. Maximum recovery was observed for

diet fortified with oyster shell. These data suggest that food products fortified with oyster shell as natural and inexpensive source could be beneficial for the prevention and treatment of osteoporosis.

Keywords Osteoporosis · Calcium · ALP · Oyster Shell · Nutraceuticals

Introduction

Calcium is the most abundant mineral in the body. More than 99 % of the body calcium is located in bones and teeth for structural purposes. The remaining fraction is present in tissues and plasma to regulate essential body functions such as muscle contraction and nerve impulses (Heaney 2009). It is reported that 45 % of blood calcium circulates freely while 45 % is bound to proteins for its metabolism. The remaining 10 % is complexed with either citrate, phosphate or bicarbonate (Gurr 2009). Calcium level in the body should be tightly maintained. Calcium intake should be sufficient to achieve maximum bone mass density (BMD) and to avoid bone fractures. In cases where there is insufficiency in the intake of calcium rich food, bones act as calcium reservoir to restore blood calcium level through resorption process (Gurr 2009; Rizzoli 2008) and this lead to osteoporosis.

Osteoporosis is a major health problem characterized by excessive loss of calcium from bones. This loss weakens bones architecture and increases the susceptibility for their fracture. Postmenopausal and ovariectomized women are at high risk for osteoporosis due to dramatic withdrawal of estrogen (Hidaka et al. 2006). It is reported that 55 % of the population around the world aged 50 and older are suffering from osteoporosis disorders. Osteoporosis and related

Electronic supplementary material The online version of this article (doi:10.1007/s13197-015-1725-3) contains supplementary material, which is available to authorized users.

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fractures are major causes of morbidity and mortality in the aging population (Prince 1997).

Increasing calcium intake can reduce fracture risks by as much as 60 % (Heaney 1992). Vitamin D increases absorption of calcium from intestine and decreases bone loss in elderly people. Several studies indicate that the minimum daily calcium intake required for bone protection is 1200–1500 mg (American Medical Association Council on Scientific Affairs 1997). Chapuy et al. (1992) reported that women who increased their calcium intake to 1700 mg/day and consumed additional vitamin D₃ for 18 months reduced the rate of hip fracture by 43 % and other vertebral fractures by 32 %. It has been reported that serum Alkaline phosphatase (ALP) increases in osteoporosis due to increased bone turnover rather than increased bone formation (Hulth et al. 1979). Serum calcium, phosphorus and protein decrease significantly in osteoporosis and are considered to important risk factor (Cheng et al. 2011; Thiebaud et al. 1997).

Nutrition plays a pivotal role in reducing osteoporosis associated risks and could be used in its prevention and treatment (Dawson et al. 1997). Milk, cheese and citrus contain the largest amount of calcium as each serving cup contains about 300 mg of calcium. Consuming half a cup of tofu, spinach, white beans, Chinese cabbage, red beans and brussel sprouts would provide 258, 122, 113, 79, 40.5 and 19 mg of calcium respectively (Weaver and Plawecki 1994). Bioavailability of calcium is another factor that should be taken into consideration as it varies significantly across various types of food regardless of their calcium content (Kärkkäinen et al. 1997).

Many individuals fail to achieve the recommended daily dose of calcium due to either insufficient intake of calcium rich food or decreased bioavailability (Cashman 2002). Therefore, it becomes imperative to increase the use of either calcium supplement or calcium fortified food that produce higher bioavailability rate to achieve optimal bone density. The main aim of this study was to produce and evaluate some calcium fortified food products using the natural source of oyster shells in various diet formulas such as basal diet, lentil and vegetable soups.

Materials and methods

Eighty eight female albino rats, ranging from 90 to 110 g, were randomly divided into 11 groups (8 rats each). The first group acted as negative control. The remaining rats were all ovariectomized and fed on low calcium diet for 30 days to act as osteoporotic rats. The

second group represented positive control. The remaining nine groups were divided as follows;

Group 3: Osteoporotic rats fed on basal diet only for 45 days.

Group 4: Rats were fed on basal diet fortified with oyster shell for 45 days.

Group 5: Rats were fed on basal diet fortified with calcium citrate for 45 days.

Group 6: Rats were fed on basal diet and lentil soup for 45 days.

Group 7: Rats were fed on basal diet and lentil soup fortified with oyster shell for 45 days.

Group 8: Rats were fed on basal diet and lentil soup fortified with calcium citrate for 45 days.

Group 9: Rats were fed on basal diet and vegetable soup for 45 days.

Group 10: Rats were fed on basal diet and vegetable soup fortified with oyster shell for 45 days.

Group 11: Rats were fed on basal diet and vegetable soup fortified with calcium citrate for 45 days.

The basal diet contained casein, corn oil, starch, sugar, cellulose, salt and vitamin mixture.

Lentil soup was made of lentil, carrot, tomatoes powder, onion, garlic and celery. The composition of vegetable soup was identical to that of lentil soup with the exception of replacing lentil by green bean and parsley. The two soups were heated at 60 °C for 12–20 h until they become dry powders. Each soup was then incorporated into the whole diet at 20 % level. Oyster shell and Ca citrate fortified diets contained 1.2 % crushed oyster shell and 2.3 % of calcium citrate respectively so that each fortified diet would contain 7.5 g calcium/kg. All vegetables used were washed properly and then dried under vacuum. This study was approved by the University Ethics Committee. Animals were treated according to ethical guidelines.

Serum was then prepared for further biochemical analyses. At the end of the experiment, animals were sacrificed and organs including liver, kidney, heart, lung and spleen were excised and weighed. Total calcium (Kessler and Wolfman 1964), phosphorus (Fiske and Subbarow 1925), total protein (Gornall et al. 1949) and ALP activity (Marsh et al. 1959) were determined in serum for all groups according to standard laboratory methods. Tibias were dissected out and weighed separately. All data are expressed as mean±SD for the eight rats of each group. The results are analyzed according to statistical analysis system (SAS 1999). Duncan's *s* at 5 % level of significance was used to compare between means according to Sendecor and Cochran (1980).

Results and discussion

The phytochemical composition for lentil and vegetable soups is shown in Tables S1 (Supplementary information). Lentil

Table 1 Changes in some biochemical parameters in female albino rats fed on low calcium diet for 30 days ($n=8$ rats)

Parameters	Initial group	Osteoporotic group
Total protein (g/100 ml)	6.59±0.08 ^d	5.66±0.07 ^c
Alkalinephosphatase activity (μ/L)	81.23±1.44 ^a	141.02±0.49 ^c
Total calcium (mg/100 ml)	14.63±0.24 ^a	10.71±0.18 ^c
Phosphorus (mg/100 ml)	7.94±0.10 ^a	4.47±0.13 ^c
Tibial bone weight (mg)	200±2.67 ^a	139.38±1.99 ^c

Data is expressed as mean ± SD for eight rats/each group.

Unshared superscript letters in the same row for different groups indicate significance values of $p < 0.05$

soup contains more protein and carbohydrate and less fiber than vegetable soup.

Ovariectomized female albino rats fed on low calcium diet showed a remarkable decrease in serum total protein, total calcium and phosphorus by 14.1, 26.8 and 43.7 % respectively when compared to the negative control group (Table 1). ALP has increased dramatically by 73.6 % (Table 1). Osteoporotic rats showed a remarkable reduction in tibial bone weight by 30.3 %. All these biochemical changes were significant and this indicates that ovariectomized rats could be used as positive control for osteoporotic animals. These data is consistent with previous studies (Hulth et al. 1979; Cheng et al. 2011; Thiebaud et al. 1997). There were no significant changes in relative organs weight of liver, kidney, heart, lung and spleen to the body weight (Supplementary information, Tables S2). These results were in agreement with Hamalainen (1994).

There was a significant increase in body weight for all osteoporotic rats that were fed with different calcium fortified formulas. The weight increase is relative to the type of the diet formula used in experiments (Supplementary information, Table S3). The diet formula containing lentil soup gave the highest body weight gain (43–46 %) among all treated groups. This might be attributed to the high content of protein and carbohydrate in lentil soup. There was no significant change in the relative organ weight to the body weight in all treated groups.

Feeding osteoporotic rats with different diet formulas and different calcium sources exhibited remarkable changes in serum biochemical parameters. Ovariectomized rats fed on basal diet only showed 10.2 % increase in total protein (Table 2). Ovariectomized rats fed on oyster shell and calcium citrate fortified basal diets showed significant increase in total serum protein by 59 and 62.5 % respectively. Rats fed on lentil soup diet only showed 37.8 % increase in total protein. Feeding ovariectomized rats with oyster shell and calcium citrate fortified lentil soup increased total serum protein remarkably by 72.3 and 69.3 % respectively (Table 2). Feeding ovariectomized rats with vegetable soup resulted in 17.1 % increase in total serum protein. The total serum protein has increased significantly by 46.1 and 58.3 % following the feeding of ovariectomized rats with oyster shell and calcium citrate fortified vegetable soup respectively. The results shows

that not only did all the treated groups recovered their serum protein content but also exceeded the initial value (6.59 g/dl) for the negative control group. Rats fed on oyster shell fortified lentil soup recorded the highest amount of total serum protein followed by calcium citrate (Table 2).

Ovariectomized rats fed on basal diet, lentil and vegetable soups for 45 days showed a significant decrease in elevated ALP value (Table 2). Osteoporotic rats fed on basal diet only exhibited 25.8 % reduction in ALP value. Ovariectomized rats fed on oyster shell and calcium citrate fortified basal diets showed a reduction in ALP values by 35.5 and 30.2 % respectively. Rats fed on lentil soup diet only showed 29 % reduction in ALP value. Feeding osteoporotic rats with oyster shell and calcium citrate fortified lentil soup resulted in a significant decrease in ALP value by 42.1 and 40.5 % respectively. Feeding ovariectomized rats with vegetable soup resulted in 26.3 % decrease in ALP value. The latter has decreased remarkably by 38.4 and 32.3 % following the feeding of osteoporotic rats with oyster shell and calcium citrate fortified vegetable soup respectively. Most rats fed on calcium fortified diets have recovered normal ALP activity as in the negative control group. Rats fed on lentil soup fortified with oyster shell or calcium citrate were the closest group that mimicked the negative control group in ALP values (Table 2). Oyster shell fortified lentil soup recorded the highest recovery for ALP followed by calcium citrate.

The effect of feeding osteoporotic rats with different diet formulas and different calcium sources on serum total calcium is illustrated in Table 2. Osteoporotic rats fed on basal diet only showed 28 % increase in total serum calcium. Ovariectomized rats fed on oyster shell and calcium citrate fortified basal diets showed noticeable increase in total calcium by 30.7 and 40.8 % respectively. Rats fed on lentil soup diet only showed significant increase in total serum calcium by 37.9 %. Feeding ovariectomized rats with oyster shell and calcium citrate fortified lentil soup increased total serum calcium remarkably by 35.9 and 36.9 % respectively. Feeding ovariectomized rats with vegetable soup resulted in great increase in total serum calcium by 35.6 %. The total serum calcium has increased significantly by 53 and 46.9 % following the feeding of ovariectomized rats with oyster shell and calcium citrate fortified vegetable soup respectively. Most groups fed on calcium fortified diets have recovered, if not exceeded, the total serum calcium in the negative control group. Rats fed on vegetable soup fortified with oyster shell recorded the largest amount of total serum calcium when compared to other groups (Table 2). These results are in agreement with Hamalainen (1994) who reported that low serum calcium in calcium deficient rats is reversed by the administration of calcium salts.

There has been a dramatic change in the amount of serum phosphorus across all treated groups (Table 2). Osteoporotic rats fed on basal diet only showed a remarkable increase in serum phosphorus by 98.8 %. Ovariectomized rats fed on oyster shell and calcium citrate fortified basal diets showed

Table 2 Serum protein (g/100 ml), alkaline phosphatase activity (μ L), total calcium (mg/100 ml), phosphorus (mg/100 ml) and tibial bone weight (mg) in osteoporotic female Albino rats fed on calcium fortified diet formulas for 45 days ($n=8$ rats)

	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Group 11
Total protein	6.24 $\pm$ 0.21 ^d	9.00 $\pm$ 0.25 ^b	9.20 $\pm$ 0.30 ^b	7.80 $\pm$ 0.21 ^c	9.75 $\pm$ 0.30 ^a	9.58 $\pm$ 0.23 ^a	6.63 $\pm$ 0.12 ^d	9.29 $\pm$ 0.22 ^b	8.96 $\pm$ 0.21 ^b
ALP	104.65 $\pm$ 2.35 ^a	90.9 $\pm$ 0.96 ^a	98.37 $\pm$ 2.90 ^a	100.13 $\pm$ 2.15 ^a	81.63 $\pm$ 2.67 ^{ab}	83.85 $\pm$ 0.63 ^a	103.99 $\pm$ 1.44 ^a	86.86 $\pm$ 1.51 ^a	95.49 $\pm$ 0.84 ^a
Total calcium	13.71 $\pm$ 0.20 ^a	14.00 $\pm$ 0.23 ^a	15.08 $\pm$ 0.31 ^a	14.77 $\pm$ 0.20 ^a	14.55 $\pm$ 0.39 ^a	14.66 $\pm$ 0.31 ^a	14.52 $\pm$ 0.25 ^a	16.28 $\pm$ 0.37 ^a	15.73 $\pm$ 0.55 ^a
Total phosphorus	8.89 $\pm$ 0.21 ^a	9.84 $\pm$ 0.15 ^a	8.63 $\pm$ 0.15 ^a	8.00 $\pm$ 0.21 ^a	9.44 $\pm$ 0.14 ^a	9.57 $\pm$ 0.16 ^a	8.13 $\pm$ 0.16 ^a	9.60 $\pm$ 0.25 ^a	8.47 $\pm$ 0.34 ^a
Tibial weight	190.00 $\pm$ 2.58 ^a	210.00 $\pm$ 2.67 ^a	200.00 $\pm$ 2.67 ^a	224.00 $\pm$ 4.22 ^a	226.00 $\pm$ 4.23 ^a	224.30 $\pm$ 2.58 ^a	200.00 $\pm$ 4.63 ^a	215.00 $\pm$ 2.67 ^a	210.00 $\pm$ 4.22 ^a

Data is expressed as mean $\pm$ SD for eight rats/each group.Unshared superscript letters in the same row for different groups indicate significance values of $p < 0.05$

significant increase in serum phosphorus by 120.1 and 93.1 % respectively. Rats fed on lentil soup diet only showed significant increase in phosphorus by 79 %. Feeding ovariectomized rats with oyster shell and calcium citrate fortified lentil soup increased serum phosphorus noticeably by 111.2 and 114.1 % respectively. Feeding ovariectomized rats with vegetable soup resulted in remarkable increase in serum phosphorus by 81.9 %. The serum phosphorus has increased significantly by 114.8 and 89.5 % following the feeding of ovariectomized rats with oyster shell and calcium citrate fortified vegetable soup respectively. The data shows that all groups recovered their serum phosphorus far behind normal values for the negative control group. Maximum recovery was observed in the group received basal diet especially that was fortified with oyster shell. The fore mentioned results are in agreement with Hamalainen (1994) who reported that calcium deficient rats excrete magnesium and phosphorus in urine in high concentration and most of these changes are reversed by the administration of calcium salts.

Feeding the osteoporotic rats with different diet formulas and different calcium sources exhibited remarkable changes in tibial bone weight (Table 2). Ovariectomized rats fed on basal diet only showed 36.3 % increase in tibial weight. Ovariectomized rats fed on oyster shell and calcium citrate fortified basal diets showed significant increase by 50.7 and 43.5 % respectively. Rats fed on lentil soup diet only showed 60.7 % increase in tibial weight. Feeding ovariectomized rats with oyster shell and calcium citrate fortified lentil soup increased tibial weight remarkably by 62.1 and 60.9 % respectively. Feeding ovariectomized rats with vegetable soup resulted in 43.5 % increase in tibial weight. The tibial weight has increased significantly by 54.2 and 50.7 % following the feeding of ovariectomized rats with oyster shell and calcium citrate fortified vegetable soup respectively. The results shows that not only did all the treated groups recovered the weight of tibial bones when compared to the positive control group but also exceeded the initial value (200 mg) for the negative control group. Rats fed on oyster shell fortified lentil soup recorded the maximum weight for tibial bones followed by calcium citrate.

Conclusion

Oyster shell fortified diet formulas have remarkably minimized risk factors associated with osteoporosis. They have remarkably decreased elevated levels of ALP. They have also significantly increased total calcium, phosphorus, total protein level and tibial bone weight. Almost absolute recovery for all these parameters was noticeable in the group received oyster shell fortified lentil soup. This study clearly indicates that consuming oyster shell in food as a calcium source does have a potential effect in restoring calcium level in the body. This could be used for prevention and treatment of osteoporosis

using natural and inexpensive sources. Its application in dairy and food industry could be very valuable and desirable. Further studies may be required to have clinical implications of these nutraceuticals.

Conflict of interest None of the authors have any personal, commercial, or financial conflicts of interest with respect to this work.

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