Journal of Advances in Medical and Pharmaceutical Sciences
8(4): 1-13, 2016, Article no.JAMPS.24382
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

A Comparative Study of the Ameliorative Effects of Skimmed Milk and Tuna Fish in the Dietary Management of Dexamethasone Sodium Phosphate Induced Osteoporotic Female Wistar Rats

H. K. Okafor[1*], A. I. Odugbemi[1] and S. E. Omogo[1]

[1]*Department of Biochemistry, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author HKO designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors AIO and SEO collected all data and also wrote part of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/24382
Editor(s):
(1) Hamdy A. Sliem, Internal Medicine, Suez Canal University, Egypt and College of Dentistry, Qassim University and EL-Jouf University, Saudi Arabia.
Reviewers:
(1) Anonymous, University of Modena and Reggio Emilia, Modena, Italy.
(2) Daniele Lazzarotto Harter, Federal University of Health Sciences of Porto Alegre, Brazil.
(3) Anonymous, McGill University, Canada.
(4) Vittorio Emanuele Bianchi, Clinical Center of Nutrirtion and Mretabolism, Stella Maris, San Marino.
Complete Peer review History: http://sciencedomain.org/review-history/15055

Original Research Article

Received 18th January 2016
Accepted 30th May 2016
Published 17th June 2016

ABSTRACT

Objective: This investigation was carried out to compare the moderating effects of skimmed milk and whole dried Tuna fish in osteoporotic female rats.

Methods: Sixty female albino Wistar rats were divided into two main groups. The first group (ten rats) fed basal diet and was maintained as negative control group. The second main group (fifty rats) was injected with 2 mg dexamethasone sodium phosphate daily (a synthetic corticosteroid) for 5 weeks after which the second main group was divided into five groups. Group 1 osteoporotic group fed basal diet, while groups from 2 to 5 osteoporotic rats fed basal diet supplemented with 5% and 10% skimmed milk, 5% and 10% Tuna fish powder respectively.

Results: The results indicated that the injection with cortisone caused osteoporosis of rats. Induced osteoporosis caused decreases in body weight gain (BWG), serum osteocalcin, Estradiol (E2),

*Corresponding author: E-mail: kekule080@yahoo.com;

calcium (Ca) and phosphorous (P) concentration in serum and femur bone, Bone mineral density (BMD), Bone mineral concentration (BMC) and increases in weight of liver and Parathyroid hormone (PTH) compared to negative control group (healthy rat). However, supplementation diet of osteoporotic rat with 5% and 10% skimmed milk and 5% and 10% dried whole dried Tuna fish respectively led to increases in body weight gain, serum osteocalcin, E2, Ca, P in serum and femur, BMD, BMC and decreases in weight of liver and PTH. Positive control group (osteoporotic rats) resulted to changes in the histological structure of bone compared to negative control group. The best results were observed in rats fed diet supplemented with 10% skimmed milk.

Conclusion: Administration of diet supplemented with 10% skimmed milk is more effective in the dietary management of dexamethasone induced osteoporosis in female rats than when supplemented with 5% or 10% powdered Tuna fish as a supplemental source of calcium.

Keywords: Osteoporosis; skimmed milk; tuna fish; osteocalcin; estradiol; calcium; bone mineral density; bone mineral concentration.

1. INTRODUCTION

Osteoporosis is considered a public health problem of major concern and it is characterized by decreased bone density which results in skeletal fragility and fractures [1]. In the United State, it is estimated that about 44 million Americans or 55% of people 50 years and older are both osteoporotic with low bone mass [2]. It is the most common articular disease in the elderly and has variable clinical presentations which often carry significant disability [3]. Osteoporosis is a major complication in patients who are usually treated with chronic glucocorticoid treatment [4]. Osteoporosis is more common in women than in men, partly because of the hormonal changes that occur at the menopause [5]. Osteoporosis increases gradually by age to reach 21.9% age groups 40-<50 years old. Relative osteoporosis among male adolescents is 16.7% and among female adolescent is 0.9% [6].

It is a disease predominantly involving deterioration of cartilage and bone; the loss of articular cartilage is marked clinically by a gradual onset of pain, stiffness and loss of mobility in synovial (weight-bearing) joints. Osteoporosis also negatively affects the quality of life of elderly patients. This disease is characterized by a marked reduction in bone mass and deterioration of bone tissue which results in skeletal fragility and susceptibility to fractures [7].

Osteoporosis has been reported to be influenced by diet, adequate nutrition, especially calcium intake, plays a major role in the prevention and treatment of osteoporosis [8]. The therapeutic approach is targeted mainly at the relief of symptoms [9]. Calcium intake influences peak bone mass achieved in early adulthood by influencing skeletal role in preventing bone loss and osteoporotic fractures in later life. Low calcium intake is widespread problem across countries [10]. Nutrition is among the modifiable factors that influence the risk of osteoporosis and fracture. Calcium and vitamin D play important roles in improving bone mineral density and reducing the risk of fracture [8,10].

Numerous studies have indicated that intake of calcium is associated with bone mineral density [11]. Dairy products are good sources of calcium but plant calcium may be also important in populations that do not consume a large amount of milk [12]. Dried skimmed milk is made by removing fat and almost all moisture from milk followed by pulverization. Dried skimmed milk constitutes more than 96% milk solid, 4% or less moisture and little fat (about 0.6%). It also contains about 36% good quality protein and about 51% lactose and is high in vitamin B complex, especially riboflavin. Diet is an equally important factor in bone health. Fish are very beneficial for bone health due to their roles in improving bone mineral density [13]. Anchovies, caviar, herring, salmon, sardine, tuna fish with bone are high in usable calcium. Small fish with bone may be an important source of calcium in human diet [14]. Fish such as salmon and tuna fish that include bones is considered non-dairy good sources of calcium [14,15].

Consumption of a diet adequate with respect to the supply of calcium and bone mineralization, principally in infancy and adolescence, is the most efficient way of preventing osteoporosis [16]. Calcium absorption is restricted to only 30% of the amount in the diet and its absorption depends on factors such as gastric acidity which has been known to be as an indispensable factor in the liberation of the mineral in the soluble form, when bound to other food constituents [17]. In situations of inadequate ingestion for prolonged periods, the ion is removed from the bone matrix to maintain the biological functions.

The objective of this study is to investigate the comparative efficacies of skimmed milk and Tuna fish powder in the dietary management of osteoporosis in female Wistar Rats.

2. MATERIALS AND METHODS

2.1 Materials

Dexamethasone Sodium Phosphate (synthetic corticosteroid) Injections were purchased from Maruti Futuristic Pharma Pvt. Ltd, India. Skimmed milk powder (Chellarams brand) and Fresh Tuna fish were purchased from Oshodi local market, Lagos, Nigeria. The kit for bone marker was purchased from Sigma Aldrich Chemise GmbH, Germany. The experimental animals were procured from the animal house, College of Medicine, University of Lagos, Nigeria.

2.2 Methods

2.2.1 Preparation of tuna fish powder

Fresh Tuna fish (without heads) was thoroughly cleaned and washed. After that, the Tuna fish including bone was cooked with pressure for 40 minutes, then dried in oven at 60°C for 3 hours and subsequently milled to obtain the powder.

2.3 Experimental Design

Sixty female albino rats weighting 150±15 g were housed individually in ventilated cages under controlled condition at constant temperature (25°C) and lighting (12 hrs light - dark cycle) and given free access to food and water ad libitum. The rats were divided into two main groups. The first group (10 rats) fed on basal diet and served as a control negative group. Basal diet preparation was formulated as shown Table 1.

The second group (fifty rats) fed on basal diet and injected with 2 mg dexamethasone sodium phosphate (synthetic corticosteroid) to induce osteoporosis for 5 weeks. Thereafter 5 rats were taken from each group to test for the onset of osteoporosis, the second control group (forty five rats) divided into five groups containing nine rats each and fed on different diets for eight weeks as described below:

- Group 1: Osteoporotic rats fed on basal diet preserved as positive control group.
- Group 2: Osteoporotic rats fed on basal diet containing 5% skimmed milk.
- Group 3: Osteoporotic rats fed on basal diet containing 10% skimmed milk.
- Group 4: Osteoporotic rats fed on basal diet containing 5% Tuna fish powder.
- Group 5: Osteoporotic rats fed on basal diet containing 10% Tuna fish powder.

Each rat was weighed at the beginning, weekly and the end of the experiment while a daily record of food intake was made. At the end of experimental period (eight weeks), rats were euthanized after overnight fasting. The blood of each rat was collected and centrifuged at 250 rpm for 25 minutes to obtain the serum, which was kept at -18°C until analysis. The right femurs were harvested. Each right femur bone was carefully cleaned and the weight was recorded and then stored in formalin buffer (10% formalin) until analysis.

Table 1. Composition (g/kg) of the basal diet (BD)

Ingredients	Diet groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Casein (g)	200	200	200	200	200
Skimmed milk (g)	0	15	30	0	0
Tuna fish powder (g)	0	0	0	15	30
Vitamin mixture[1] (g)	20	20	20	20	20
Mineral mixture[2] (g)	80	80	80	80	80

[1]Vitamins (per kg of diet): thiamin, 15 mg; riboflavin, 10 mg; pyridoxin, 5 mg; nicotinamide, 95 mg; calcium panthotenate, 65 mg; folic acid, 2 mg; biotin, 0.1 mg; cyanocobalamin, 0.03 mg; retinyl palmitate, 1.5 mg; dl-a-tocopheryl acetate, 115 mg; cholecalciferol, 0.15 mg; menadione, 1.2 mg; ascorbic acid, 40 mg; myo-inositol, 90 mg; carrier wheat starch, 1.24 g

[2]Minerals (per kg of diet): $CaHPO_4$, 12 g; K_2HPO_4, 2.1 g; KCl, 3 g; NaCl, 3g; $MgCl_2$, 2.5 g; Fe_2O_3, 1.9 mg; $MnSO_4$, 115 mg; $CuSO_4$ $7H_2O$, 0.2 mg; $ZnSO_4$ $7H_2O$, 90 mg; KIO_3, 0.2 mg

Table 1: Composition (g/kg) of the basal diet (BD). Note: Group 2 and Group 3 had skimmed milk, Group 4 and Group 5 had 15g/kg and 30g/kg of Tuna fish respectively while all groups had vitamin mixture and mineral mixture

2.4 Biochemical Analysis

2.4.1 Bone marker

The serum estradiol E2 was evaluated according to the method described by [20]. Bone Marker: Serum osteocalcin concentrations [18], parathyroid hormone (PTH) levels [19], and estradiol E2 titres [19] were determined according previously described methods [18-20].

2.4.2 Measurement of bone mineral density and bone mineral concentration

The bone mineral concentration (BMC) and bone mineral density (BMD) of each femur were measured using the method of [21], by Dual Energy X-ray Absorption (DEXA) using Norland X-R46, version 3.9.6, Peachtree City, GA, USA) equipped with dedicated software for small animal measurements. This technique provided integrated measurements. These measurements by DEXA bone scanner yielded total, cortical and trabecular BMD and BMC [22].

2.4.3 Determination of calcium and phosphorus content in serum and femur

Serum calcium and phosphorus were estimated to using the method described by [23] while calcium and phosphorus in bone were determined in right femur by colorimetric method according to the method described by [24] and [25].

2.4.4 Determination of protein

This was done using the A.O.A.C (Association of Official Agricultural Chemists) method [26].

2.5 Biological Evaluations

2.5.1 Rat growth assay

Total feed efficiency ratio (FER) was obtained by total increased weight divided by total consumed food of each rat during test period in each group. FER is equal with food conversion ratio (FCR). Generally, the amount of every other day consumed food and body weight gain by each rat was noted and was calculated by the following equation:

FER = body weights gain (g)/consumed food (g) [27].

2.5.2 Bone histology

Right femurs were immersed in 10% formalin till when ready for analytical purposes. The bone was then decalcified using a mixture of 10% of formic acid and hydrochloric acid solution for a period of 45 days. The processing of the rotten tissue for light microscopy was carried out and tissues were bedded in paraffin.

2.6 Statistical Analysis

The results obtained were analyzed using SPSS program (version 18.0) and expressed as mean and standard error of measurements (SEM). Statistical significance ($p<0.05$) among the groups were determined by one-way ANOVA followed by Duncan's multiple range test.

3. RESULTS AND DISCUSSION

Table 2 shows calcium, phosphorus and protein concentrations in skimmed milk and dried Tuna fish. The mineral calcium plays a major role in bone strength and is of prime nutritional importance in osteoporosis, being essential for bone health throughout life [28]. The primary role of calcium in the body is structural, providing the rigidity necessary for the skeleton and teeth to function mechanically. Bone contains about 99% of the body's calcium. Calcium in body fluids also exerts critical metabolic functions, binding to proteins, and operating as a signal transmitter and protein activator within cells. Muscle contraction and nerve transmission are two of the many body functions that rely on calcium for activation. Additionally, calcium is also involved in blood clotting [29]. Calcium is required for normal growth and development of the skeleton [30]. Adequate calcium intake is critical for achieving optimal peak bone mass and modifies the rate of bone loss associated with aging [30]. In the present study, there was a significant decrease ($P<0.05$) in the concentrations of calcium of Tuna fish powder compared to skimmed milk. The majority of the phosphorus in the body is found as phosphate (PO_4).Approximately 85% of the body's phosphorus is found in bone. Phosphorus is found in most foods because it is a critical component of all living organisms. Dairy products, meat, and fish are particularly rich sources of phosphorus. In the current study, there was a significant increase ($P<0.05$) in the concentrations of phosphorus and protein of Tuna fish powder compared to skimmed milk. From the present studies, it was revealed that skimmed milk contains a higher calcium concentration (2100 mg/100 g) as against Tuna fish with a calcium concentration of 1990 mg/kg. These results are in agreement with those obtained by [31], who mentioned that Tuna are high in major minerals such phosphorus, calcium and potassium. Protein is known to be a key component of bone tissue hence an adequate dietary supply is needed [32]. The majority of the research studies state that there is a positive association between protein intake and bone health. There are several epidemiological studies, both cross-sectional and longitudinal that has reported an association between dietary protein and bone [33,34]. These studies show that individuals who eat the most dietary protein have the highest BMD. Furthermore, prospective studies have observed that individuals with the highest protein intake have the slowest risk of bone loss [33,35]. One of the possible mechanisms by which dietary protein may contribute to the improvement of bone mass can be explained by the fact that increasing dietary protein is also known to increase levels of circulating insulin-like growth factor 1 (IGF-1), and conversely, a low-protein diet decreases IGF-1 [36]. IGF-1 is known to be a key mediator of bone growth but also has also been implicated in its role in the skeletal response to anabolic Parathyroid Hormone (PTH) therapy [37].

The results presented in Table 3 are in agreement with the findings of [38], who reported that glucocorticoids induced a significant lower weight gain (P<0.05) compared to control group. Meanwhile, rat's administration of diet supplemented with 10% skimmed milk and 10% Tuna fish recorded significant increase (P<0.05) in final body weight and body weight gain compared to positive control group. These results are accordance with the results obtained by [39], who reported that control positive (osteoporotic rats) showed significant decrease (P<0.05) in body weight gain. The decrease in body weight gain may be due to high dose from cortisone and long-term.

Effect of skimmed milk and Tuna fish on weight organs are shown in Table 4. Results revealed that there were no significant differences (P>0.05) in weight of kidney, heart and spleen between all groups. Meanwhile, positive control showed significant increase (P<0.05) in weight of liver and decreases in weight of femur bone compared to the diet supplemented groups with skimmed milk and Tuna fish, injection with cortisone for 5 week led to increases in liver weight and decrease in weight of femur bone. These results have been corroborated by other researchers who reported that cortisone caused an increase in size and weight of cells of regenerated liver through the increased infiltration of lipid and a decrease in femur bone weight [40,41].

In addition, [40] reported that mean value±SD of liver weight/body weight of positive control (rats administration cortisone) increased as compared to negative control group. However, [42] reported that administration of cortisone to rats increased the protein content and relative weight of the liver of animal. Group of rats fed with diet supplemented with the 5% skimmed milk powder, 10% skimmed milk powder and 10% Tuna fish powder respectively showed a significant increase (p<0.05) in their final weights compared with the positive control group (non-supplemented). Tuna fish at high concentration (10%) led to decrease in weight of liver. The decrease in liver weight of rats fed diet supplemented tuna fish may be due to omega-3 present in tuna fish. Also, [43], mentioned that rats administration omega-3 fatty acids decrease weight of liver (5.76 g) which increased (6.43 g) by treated with paracetamol. [44] reported that omega-3fatty acid have beneficial effect on liver disease.

Effects of skimmed milk and Tuna fish on bone marker of osteoporotic rats are represented in Table 5. The concentration mean of serum osteocalcin, a parameter of bone formation in positive control group decreased significantly (P<0.05) in comparison to all the group administered with skimmed milk or Tuna fish powder and negative control group. Osteocalcin is chiefly deposited in the extracellular matrix of bone, but a small amount enters the blood. Serum osteocalcin is sensitive and specific marker of osteoblastic activity and its serum level thus reflect the rate of bone formation [45]. The osteoporotic rats fed with diet supplemented with 10% skimmed milk showed no significant difference (p>0.05) compared with positive control group. The increase in osteocalcin may be due to skimmed milk and Tuna fish having higher calcium content. These results are in agreement with [46], who reported that supplementation of the diet with skimmed milk high calcium for 16 week led to increase in total osteocalcin compared to control group that received diet no supplementation.PTH recreation contributes to an increase in bone resorption and osteoporosis [47], over production of PTH led to an increase in bone resorption compared with bone formation and contributes to general skeletal demineralization. However, rats feeding on skimmed milk and Tuna fish revealed significant increase (P<0.05) in E2 and significant decrease (P<0.05) in PTH. The reduction in PTH in rats fed on diet supplemented with 10% skimmed milk was higher compared to other groups. These results are collaborated by [48], who reported that supplementation diet with calcium showed a decrease in PTH.

Data in Table 6 illustrated the effect of skimmed milk and dried Tuna fish powder on calcium and phosphorus levels in serum of osteoporotic female rats. There was a significant increase (p<0.05) in the concentration of calcium in the serum of all the experimental groups administered with skimmed milk or Tuna fish powder with respect to the positive control group. However, there was no significant difference (p>0.05) in the concentration of calcium in the serum of all the experimental groups administered with 5% skimmed milk, 5% Tuna fish powder and 10% Tuna fish powder. Furthermore, there was a significant increase (p<0.05) in the concentration of phosphorus in the serum of all the experimental groups administered with skimmed milk or Tuna fish powder with respect to the positive control group. The increase in calcium may be due to cortisone decrease leading to limited calcium absorption from gastrointestinal tract. Glucocorticoid affects mineral homeostasis by reducing calcium absorption and causing secondary hyper parathyrodism. Glucocorticoid directly inhibit osteoblastic bone formation, impair intestinal calcium excretion absorption and promote renal calcium excretion [49]. All treatment showed significant increase p<0.05 in serum calcium and phosphorus, the highest concentration of calcium was observed in serum rats fed on diet supplemented with 10% skimmed milk. These results may be due to skimmed milk a have higher calcium content and easy absorbed by body. Also, lactose present in skimmed milk increase calcium absorption. These results were collaborated by [50], who stated that the best dietary of calcium is dairy products because of the favorable element calcium content and absorption ability of calcium was higher. [51] reported calcium from fish would be absorbed by body and the intake of small fish with bones could increase calcium bioavailability.

Data in Table 7 also showed a decrease in calcium and phosphorus femur bone compared to negative control group. Also, it increased urinary excretion of calcium and decrease bone uptake of calcium. All treatment groups with two concentrations (5% and 10%) skimmed milk and dried tuna fish respectively showed significant increase in calcium and phosphorous femur bone as compared to positive control group. The best results of femur bone calcium and phosphorous recorded of osteoporotic rats fed diet supplemented with 10% skimmed milk. These results may be due to higher bioavailability of calcium and phosphorous in skimmed milk and deposition of them in bone.

Total BMD and BMC of femur bone in each group are summarized in Table 8. Results from the current study showed that osteoporosis caused significant decrease of BMD and BMC of femur bone. The Glucocorticoid has deleterious effects on bone density [52]. Glucocorticoid induced osteoporosis and led to a suppression of bone formation by decreasing the number and functioning of osteoblast and induced bone loss [53,54,38]. Other past studies reported decrease of BMD and BMC in osteoporosis disease [55,56]. The mean BMD and BMC of osteoporotic rats fed diet supplemented with 10% skimmed milk were higher than other treatment groups. The increases in BMD and BMC due to increased calcium and phosphorus levels in diet led to increased amount of osteoblast cells which led to increased rate of bone formation. These results are in agreement with the result of the studies of [9] and [57], who reported that increasing calcium intake or dairy products is associated with a greater gain in BMD and BMC. [10] reported those milk and calcium intakes are related to bone mineral accretion during growth. [58] also stated that women with high intake of dark fish (Salmon) have protective effect of bone loss because they increase the intake of calcium and vitamin D preventing bone loss, possibly due to the effect of calcium in suppressing PTH secretion. [59] also reported that combined diet supplementation with soybean and skimmed milk enhanced bone mass density in Wistar rats. Calcium supplementation has also been attributed to have a positive effect on bone mineral density in postmenopausal women [60].

From the histopathological results obtained, the femur of rat from negative control revealed no histopathology change as seen in Fig. 1. However, the section of femur of rat from positive control group (osteoporotic rats) showed thin bone cortex and dilatation of bone marrow cavity as indicated in Fig. 2; cracks and necrosis in bone cortex was shown in Fig. 3. These results are in agreement with [40], who reported that treatment of rats treated with cortisone revealed a decreased bone mass and also caused a reduction in thickness of cortical bone. As presented in Fig. 4, the femur bone of experimental rats from group supplemented with 5% skimmed milk revealed normal bone cortex while the femur of experimental rats from group supplemented 10% skimmed milk revealed no histopathological changes except thick cortical bone (Fig. 5) and proliferation of osteoblasts (Fig. 6). These results are collaborated by that obtained by [9], who reported that Chinese children receiving milk supplements increased cortical bone thickness. Thick cortical bone was noticed in bone rat from group supplemented with 5% tuna fish showed thick of cortical bone (Fig. 7), while the section of Wistar rat from group supplemented with 10% tuna fish showed very thick cortical bone (Fig. 8).

Table 2. Calcium and phosphorus concentration of skimmed milk and dried Tuna fish powder

Experimental samples	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Protein (%)
Skimmed milk	2100±0.50^{a}	1140±0.20^{a}	35±0.20^{a}
Tuna fish powder	1810±0.20^{b}	1660±0.30^{b}	71±0.30^{b}

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)

Table 3. Effect of skimmed milk and dried Tuna fish on body weight gain, feed intake and feed efficiency ratio of osteoporotic female rats

Experimental samples	IBW (g)	FBW (g)	BWG (g)	FI(g)	FER
Negative control group	143.20±2.72^{a}	302.40±17.12^{c}	159.20±6.76^{c}	14.58±0.59^{d}	0.178±0.63^{a}
Positive control group	142.20±2.72^{a}	180.40±11.01^{d}	38.20±6.76^{c}	8.58±0.59^{a}	0.048±0.63^{b}
5% Skimmed milk	143.80±5.20^{a}	193.20±4.02^{a}	49.4±1.27^{d}	10.20±0.14^{c}	0.052±0.43^{b}
10% Skimmed milk	145.60±1.06^{a}	202.10±2.12^{b}	56.5±10.26^{b}	10.94±0.94^{b}	0.054±0.76^{b}
5% Tuna fish powder	143.0±5.19^{a}	191.20±11.14^{d}	48.2±8.08^{d}	10.02±0.55^{c}	0.049±1.08^{b}
10% Tuna fish powder	141.80±1.12^{a}	197.0±12.21^{b}	55.2±14.16^{b}	10.88±0.54^{b}	0.057±1.16^{b}

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)
IBW: Initial Body Weight, FBW: Final Body Weight, BWI: Body weight Gain, FI: Feed Intake, FER: Feed Efficiency Ratio

Table 4. Effect of skimmed milk and dried Tuna fish on weight of organs and weight right femur of osteoporotic female rats

Experimental samples	Weight of liver (g)	Weight of kidney (g)	Weight of heart (g)	Weight of spleen (g)	Weight of right femur (g)
Negative control group	4.280±0.145^{b}	1.270±0.020^{a}	0.620±0.074^{a}	0.732±0.167^{a}	1.420±0.015^{f}
Positive control group	6.480±0.645^{a}	1.310±0.070^{a}	0.640±0.114^{a}	0.750±0.114^{a}	0.7696±0.034^{d}
5% Skimmed milk	5.760±1.12^{c}	1.340±0.161^{a}	0.650±0.061^{a}	0.760±0.151^{a}	0.9460±0.018^{c}
10% Skimmed milk	5.690±0.573^{c}	1.380±0.327^{a}	0.660±0.089^{a}	0.760±0.134^{a}	1.3293±0.106^{a}
5% Tuna fish powder	5.580±0.342^{c}	1.320±0.083^{a}	0.630±0.070^{a}	0.740±0.130^{a}	0.9273±0.013^{e}
10% Tuna fish powder	5.240±0.585^{c}	1.36±0.044^{a}	0.620±0.109^{a}	0.650±0.109^{b}	1.1643±0.061^{b}

Results represented as mean±S.E.M Values with the same superscripts across the same column are not significantly different (P>0.05) IBW: Initial Body Weight, FBW: Final Body Weight, BWI: Body weight Gain, FI: Feed Intake, FER: Feed Efficiency Ratio

Table 5. Effect of skimmed milk and dried Tuna fish on bone marker of osteoporotic female rats

Experimental samples	Osteocalcin (ng/ml)	Parathyroid hormone (PTH) (pg/ml)	Estradiol (E2) (pg/ml)
Negative control group	29.60±1.61[g]	34.10±1.179[b]	27.23±1.74[a]
Positive control group	10.33±0.67[f]	69.50±1.439[a]	15.0±0.09[e]
5% Skimmed milk	23.27±1.28[a]	55.46±1.90[c]	19.33±2.25[c]
10% Skimmed milk	25.60±1.61[a]	35.13±3.70[e]	28.33±3.14[a]
5% Tuna fish powder	16.70±0.36[e]	59.66±1.72[c]	18.33±2.25[c]
10% Tuna fish powder	21.30±0.80[a]	49.30±0.97[d]	22.33±1.37[b]

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)

Table 6. Effect of skimmed milk and dried Tuna fish on calcium and phosphorus levels in serum of osteoporotic female rats

Experimental samples	Calcium (mmol/l)	Phosphorus (mmol/l)
Negative control group	4.201±0.12[a]	3.953±0.165[a]
Positive control group	2.058±0.354[c]	2.083±0.085[e]
5% Skimmed milk	3.141±0.83[b]	2.687±0.248[d]
10% Skimmed milk	4.127±0.62[a]	3.034±0.107[c]
5% Tuna fish powder	3.023±0.309[b]	3.237±1.890[b]
10% Tuna fish powder	3.253±0.98[b]	3.963±0.135[a]

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)

Table 7. Effect of skimmed milk and dried Tuna fish on calcium and phosphorus in bone (femur) of osteoporotic female rats

Experimental samples	Calcium (mg/100 g)	Phosphorus (mg/100 g)
Positive control group	18.971±0.124[a]	12.166±0.242[a]
Positive control group	10.833±1.351[d]	6.816±0.473[d]
5% Skimmed milk	15.600±0.473[c]	9.70±1.236[c]
10% Skimmed milk	18.933±0.334[a]	12.133±0.895[a]
5% Tuna fish powder	15.166±0.900[c]	10.760±0.374[b]
10% Tuna fish powder	18.733±0.338[a]	11.366±0.232[f]

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)

Table 8. Effect of skimmed milk and dried Tuna fish on bone mass density (BMD) and bone mass concentration (BMC) of osteoporotic female rats

Experimental samples	BMD (g/cm^2)	BMC (g/cm^2)
Negative control group	0.16010±0.003[c]	0.11993±0.028[a]
Positive control group	0.0820±0.011[e]	0.10256±0.034[b]
5% Skimmed milk	0.12876±0.005[d]	0.10776±0.007[c]
10% Skimmed milk	0.15770±0.006[c]	0.10963±0.028[d]
5% Tuna fish powder	0.12733±0.002[d]	0.10431±0.004[e]
10% Tuna fish powder	0.14490±0.002[b]	0.10654±0.001a[f]

Results represented as mean±S.E.M, n=5
Values with the same superscripts across the same column are not significantly different (P>0.05)

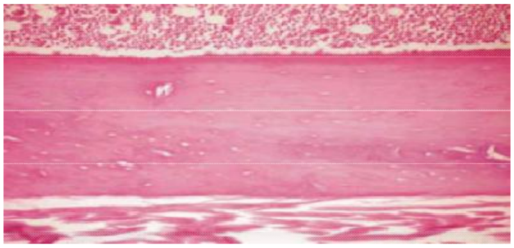

Fig. 1. Photomicrograph of femur of experimental rats from negative control group showing no histopathological changes. White horizontal lines of scale are discernible. (H and E x 200)

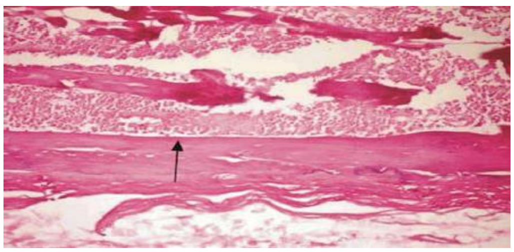

Fig. 2. Photomicrograph of femur of experimental rats from positive control group showing thin bone cortex (black arrow) and dilatation of bone marrow cavity (H and E x 200)

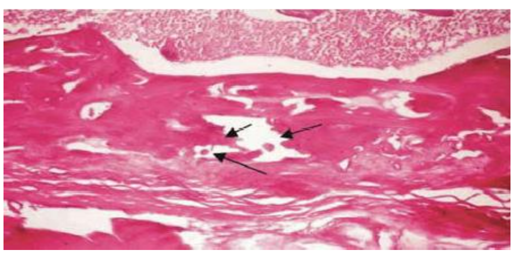

Fig. 3. Photomicrograph of femur of rats from positive control group showing cracks and necrosis (black arrows) in bony cortex (H and E x 200)

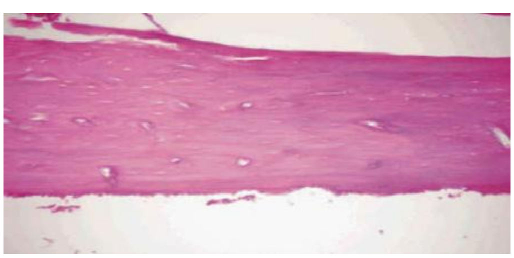

Fig. 4. Photomicrograph of femur of rats from group supplemented with 5% skimmed milk showing normal bone cortex (H and E x 200)

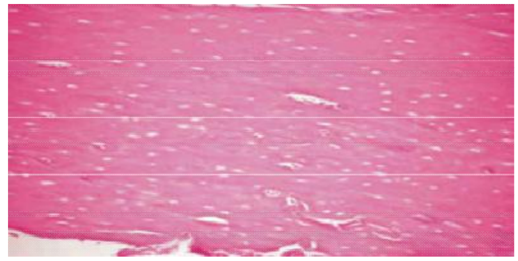

Fig. 5. Photomicrograph of femur of rats from group control supplemented with 10% skimmed milk showing thick cortical bone (H and E x 200)

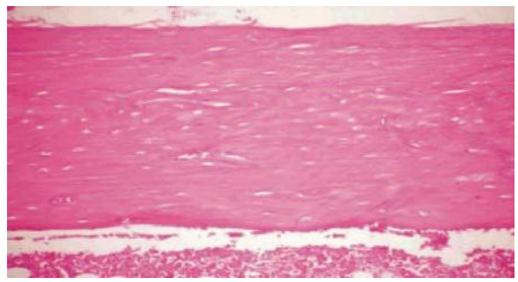

Fig. 6. Photomicrograph of femur of rats from group supplemented with 10% skimmed milk showing proliferation of osteoblasts (H and E x 200)

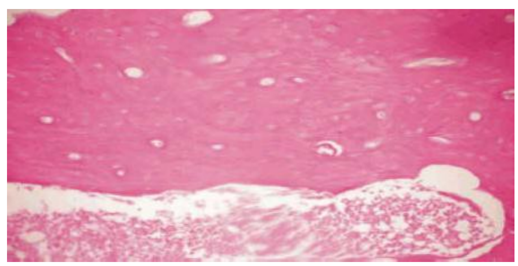

Fig. 7. Photomicrograph of femur of rats from group supplemented with 5% Tuna fish showing thick cortical bone (H and E 200)

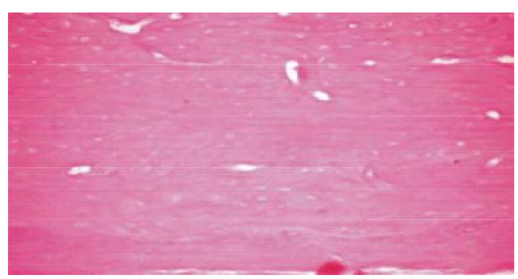

Fig. 8. Photomicrograph of femur of rats from group supplemented with 10% tuna fish showing very thick cortical bone (H and E x 200)

4. CONCLUDING REMARKS

In conclusion, significant improvements in osteocalcin, parathyroid hormone, estradiol, calcium and phosphorus levels in serum and bone (femur) including bone mass density (BMD) and bone mass concentration (BMC) were attained in all four diets supplemented groups. These findings show skimmed milk and Tuna fish are effective at positively moderating metabolic dysfunctions associated with dexamethasone sodium phosphate induced osteoporotic rats. 10% skimmed milk dietary supplementation is more therapeutically beneficial when compared with the other three methods. This may have implications for humans, which indicates for more research into this important field of medicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. McCarron DA, Heaney RP. Estimated health care saving associated with adequate dairy food intake. American Journal of Hypertension 2004;17:88-97.
2. National Osteoporosis Foundation (NOF). New release. Health forum to feature the national osteoporosis foundation. National Osteoporosis Foundation, Washington, United States; 2007.
3. Easton BT. Evaluation and treatment of the patient with osteoarthritis. J Fam Pract. 2001;50:791-7.
4. Aslan M, Simse KG, Yildirium U. Effect of short-term treatment with systemic prednisone on bone healing an experimental study in rats. Dent Traumatal. 2005;21(4):222-225.
5. World Health Organization (WHO). Prevention and management of osteoporosis. Report of a WHO study group. Geneva, WHO. Technical Report Series. 2003;No. 921.
6. National Nutrition Institute. National survey for the determination of bone mass density among adolescents and adults in Egypt. National Nutrition Institute, Cairo, Egypt; 2004.
7. Deal C. Potential new drug targets for osteoporosis. Nat. Clin. Pard. Theum. 2009;5:20-27.
8. Rizzoli R, Boonen S, Brand ML, et al. The role of calcium and vitamin D in the management of osteoporosis. Bone. 2008; 42:246- 249.
9. Hochberg MC. Nutritional supplements for knee osteoarthritis-still no resolution. N Engl J Med. 2006;354:858-60.
10. Zhu K, Prince R. Calcium and bone. Clinical Biochemistry. 2012;45(12): 936-942.
11. McLeod K, McCann SF, Horvath PJ, et al. Predictors of change in calcium intake in postmenopausal women after osteoporosis screening. Journal of Nutrition. 2007;137: 1968-1973.
12. Park HM, Hea JH, Park Y. Calcium from plant sources is beneficial to lowering the risk of osteoporosis in postmenopausal Korean women. Nutrition Research. 2011; 3:27-32.
13. Katja K, Shakuntala H, Mohammed A. Effect of consumption of the nutrient-dense, freshwater small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: A randomised, controlled study of efficacy. British Journal of Nutrition. 2008;83:581–597.
14. Tichenal CA, Dobbs J. A system to assess the quality of food source of calcium. Journal of Food Composition and Analysis. 2007;20(8):717-724.
15. Food and Nutrition Board. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. National Academy of Sciences, National Research Council, Washington D.C; 1997.

16. Prince R, Devine A, Dick I, et al. The effects of calcium supplementation (milk powder or tablets) and exercise on bone density in postmenopausal women. J Bone Miner Res. 1995;10:1068-75.
17. New SA. Intake of fruit and vegetables: Implications for bone health. Proc Nutr Soc. 2003;62(4):889-99.
18. Coleman RE, Mashiter Fogelman I, et al. Osteocalcin: A potential marker of metastatic bone disease and response to treatment. European Journal of Cancer and Clinical Oncology. 1988;24: 1211-1217.
19. Goltman D, Henderson B, Loveridge N. Cytochemical bioassay of PTH: Characteristics of the assay and analysis of circulating hormonal forms. Journal of Clinical Investigation. 1980;65:1309-1317.
20. Ratcliffe WA, Carter GD, Dowsett MJ. Estradiol assay: Application and guidelines for the provision of clinical biochemistry service. Annals of Clinical Biochemistry. 1988;25:466- 483.
21. Marie-Pierre S, Wang J, Shen WZ, et al. Dual-energy X-ray absorptiometry-measures lean soft tissue mass: Differing relation to body cell mass across the adult life span. Journal of Gerontology. 2004; 59:B796-B800.
22. Baginsk ES, Marie SS, Clark WL, et al. Direct micro determination of serum calcium. Clinica Chemica Acta. 1973; 46(1):46-54.
23. Patricia AD. Direct colorimetric determination of phosphorus in serum and urine. Clinica Chemica Acta. 1972;39(1):81-88.
24. Ginder EM, King JD. Rapid colorimetric free determination of calcium with methyl thymol blue. Clinical Chemistry. 1972;58: 379-382.
25. Goldenberg H. Rapid colorimetric determination of phosphorus. Clinical Chemistry. 1966;12:871-875.
26. A.O.A.C. Official Methods of Analysis. 18th Ed. Analytical chemists, Washington DC. No. 935.13 and 965.17. 2006;Chapter 4: 57-61.
27. Swenson WA, Smith Jr LL. Gastric digestion, food consumption, feeding periodicity, and food Conversion efficiency in walleye (Stizostedion vitreum vitreum). Journal of the Fisheries Research Board of Canada. 1973;30(9):1327-1336.
28. North American Menopause Society. The role of calcium in pre and postmenopausal women: 2006 position statement of the North American Menopause Society. Menopause. 2006;13:862-77.
29. Heaney RP. Dairy and bone health. J Am Coll Nutr. 2009;28 (Suppl1):82S-90S.
30. National Osteoporosis Foundation; 2008. Available:www.nof.org/osteoporosis/diseasefacts.html
31. Kris-Etherton PM, Harris WS, Apple LJ. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Circulation. 2002;106(21):2747-2757.
32. International Osteoporosis Foundation. Invest in your bones. Bone Appétit − The role of food and nutrition in building and maintaining strong bones; 2006.
Available:http://www.iofbonehealth/org/publications/bone-appetit.html
33. Promislow JH, Goodman-Gruen D, Slymen DJ, et al. Protein consumption and bone mineral density in the elderly: The Rancho Bernardo study. Am J Epidemiol. 2002; 155:636-44.
34. Hannan MT, Tucker KL, Dawson-Hughes B, et al. Effect of dietary protein on bone loss in elderly men and women: The Framingham Osteoporosis study. J Bone Miner Res. 2000;15:2504-12.
35. Rapuri PB, Gallagher JC, Haynatzka V. Protein intake: Effects on bone mineral density and the rate of bone loss in elderly women. Am J Clin Nutr. 2003;77:1517-25.
36. Bonjour JP, Schurch MA, Chevalley T, et al. Protein intake, IGF-1 and osteoporosis. Osteoporos Int. 1997;7(Suppl3):S36-42.
37. Bikle DD, Sakata T, Leary C, et al. Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. J Bone Miner Res. 2002; 17:1570-8.
38. DiMunno D, Delle Seide A. Glucocorticoids induced osteoporosis and rheumatic disease. Pathogenesis, prevention and treatment. Rheumatisms. 2005;58(1): 11-21.
39. Oliveira ML, Bergamaschi CT, Silva OL, et al. Mechanical vibration preserves bone structure in rats treated with glucocorticoids. Bone. 2010;46(6):1516-1521.
40. Gouda SMH. Effect of flaxseeds, pumpkin seeds and sesame seed on bone of rats suffering from osteoporosis. PhD Thesis,

Nutrition & Food Science, Home Economy Department, Faculty of Education, Ain Shams University, Egypt; 2012.

41. Stanley LE, Erich H, Alfred GC. Effects of cortisone on regenerating rat liver. J. Gen Physiol., 1954;37(4):559-574.
42. Howard Clark J, Leroy AJV. Effect of cortisone upon liver enzymes and protein synthesis. JPET. 1957;117(2):202-207.
43. Meganathan M, Gopal KM, Sasikal KP, et al. Evaluation of antioxidant effects of omega-3 fatty acid against paracetamol induced liver injury in albino rats. Research Journal of Pharmaceutical Biological and Chemical Sciences. 2011; 2(1):426-433.
44. Andrea J, Gerald FW, Trevor AM, et al. Omega-3 fatty acid supplementation decrease liver fat content in polycystic ovary syndrome: A Randomized controlled trial employing protein magnetic resonance spectroscopy. Journal of Clinical Endocrinal Metabolism. 2009;94(10): 3842-3848.
45. Weaver CM, Peacock M, Martine BR, et al. Quantification of biochemical markers of bone turnover by kinetic measures of bone formation and resorption in young health females. Journal Bone Mineral Research. 1997;12(10):1714-1720.
46. Kruger MC, Booth CL, Coad, et al. Effect of calcium fortified milk supplementation with or without vitamin K on biochemical markers of bone turnover in premeno-pausal women. Journal of Nutrition. 2006; 22(11-12):1120-1122.
47. MuCkane WR, Khosla S, Risteli SJ, et al. Role of estrogen deficiency in pathogenesis of secondary hyperparathyr-oidism and increased bone resorption in elderly women. Proceeding of the Association of American Physicians. 1977; 109:174-180.
48. Micheal P, Bettina B, Helmut WM, et al. Effect of short-term vitamin D3 and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. The Journal of Clinical Endocrinology & Metabolism. 2011;86(4): 1633-1637.
49. Goodman SB, Jiranck W, Petrow E, et al. The effects of medication on bone. Journal of American Academy Orthopedic Surgeons. 2007;15:450-460.
50. Napoli N, Thompson J, Civitell RM, et al. Effects of dietary calcium compared with calcium supplements on estrogen metabolism and bone mineral density. American Journal of Clinica Nutrition. 2007;85:1428-1433.
51. Larson T, Thilsted SH, Kong-Sback K, et al. Whole small fish as a rich calcium source. British Journal of Nutrition. 2000; 83(2):191-196.
52. Sedo Sarkis K, Medeiros Pinheiro M, Luaa Szejnfeld V, et al. High bone density and bone health. Endocrinology Nutrition. 2012;59(3):207-214.
53. Canalis E. Effect of glucocorticoids on type 1 collagen synthesis, alkaline phosphates activity and deoxy ribonucleic acid content in cultured rat calvarias. Endocrinology. 1983;112:931-939.
54. Canalis E. Effect of cortisol on periosteal and non-periosteal collagen and DNA synthesis in cultured rat calvarias. Calcifical Tissue International. 1984;36: 158-166.
55. Wu J, Wang XX, Takasaki MA. Cooperative effects of exercise training and genistein administration on bone mass in overiectomized mice. Journal Bone of mineral Research. 2001;16:1829-1836.
56. Matsumoto H, Jieng GZ, Hashimoto T, et al. Effect of organic germanium compound (Ge-132) on experimental osteoporosis in rats; the relationship between transverse strength of bone mineral density (BMD) on bone mineral content (BMC). International Journal Oral Medical Sciences. 2002; 1:10-17.
57. Bakary D, Ann P, Mustapha C, et al. Effect of calcium supplementation on bone mineral accretion in Cambian children accustomed to a low-calcium diet. The American Journal of Clinical Nutrition. 2000;71(2):544-549.
58. Farina EK, Kiricad DP, Roubeneff R, et al. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: The Framingham osteoporosis study. The American Journal of Clinical Nutrition. 2011;93:1142-1151.
59. Muguruma M, Ahmed AM, Kawahara SA. Combination of soybean and skimmed milk reduces osteoporosis in rats. Journal of Functional Food. 2012;4(4):810-818.

60. Shea B, Wells G, Cranney A, et al. Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. Endocr. Rev. 2002;23:552-59.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15055