Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Framroze et al., J Nutr Food Sci 2015, 5:2
DOI: 10.4172/2155-9600.1000348

Research Article Open Access

A Comparative Study of the Impact of Dietary Calcium Sources on Serum Calcium and Bone Reformation Using an Ovariectomized Sprague-Dawley Rat Model

Bomi Framroze[1]*, Sunil Godase[2] and Sagar Sawant[2]

[1]*Hofseth Biocare AS, Molovegen 6, 6004, Alesund, Norway*
[2]*SA-FORD, V-10, MIDC Taloja, 410208, India*

Abstract

Osteoporosis is a multifactorial skeletal disease that is characterized by compromised bone strength predisposing a person to an increased risk of fracture. This ovariectomized (OVX) Sprague-Dawley rat model study measured the changes in serum calcium, as well as femur dimensions and mineral density, when consuming calcium carbonate, calcium gluconate and salmon bone calcium. Our results show that salmon bone calcium and to a lesser extent calcium gluconate showed a statistically significant increase in femur dimension and mineral density while calcium carbonate showed no impact on either bone parameter. Our results also show that none of the three calcium sources affected serum calcium levels after 8 weeks of treatment.

Keywords: Calcium; Carbonate; Gluconate; Salmon; Bone; OVX; Rat

Introduction

Osteoporosis is a multifactorial skeletal disease that is characterized by compromised bone strength predisposing a person to an increased risk of fracture [1]. Osteoporosis that is associated with ovarian hormone deficiency following menopause is the most common cause of age-related bone loss that is potentiated by a decrease in ovarian estrogen usually during the first decade after menopause onset [2]. Current therapies for postmenopausal osteoporosis still rely on drugs that inhibit bone reabsorption such as estrogen [3] and bisphosphonates [4] Long-term administration of these currently prevalent medications have shown an increased risk of serious side effects [5] and some agents, such as calcitonin [6,7], have been highly restricted due to their cancer promoting effects. Thus such therapies are increasingly only recommended for high risk women who have no contraindications [8].

Recent advances in bone studies have begun to indicate the use of a combination of anti-resorptive agents, such as estrogen with bone formation-stimulating agents like growth hormones [9]. However, many potential bone forming agents available today either have serious side effects, such as the anabolic steroids, or may not improve bone quality or decrease susceptibility to fractures, such as sodium fluoride.

We believe that discovering a naturally occurring substance that helps increase bone formation in postmenopausal women will be a useful tool in the orthopedists' treatment arsenal, helping to decrease the necessity and frequency of much harsher drug therapies.

A few reports have indicated that natural supplements may be effective in preserving bone mass using different models for osteoporosis [10,11]. In particular, studies using fish bone supplements have shown significant impact on bone density and growth [12-14]. These and other studies regarding the beneficial effects of natural foods have led us to hypothesize that salmon bone specially extracted to contain a high concentration of protein containing natural growth hormones as well as modest quantities of prebiotic fibers might be more effective in modulating bone mass due to ovarian hormone deficiency. The ovariectomized (OVX) Sprague-Dawley rat exhibits most of the characteristics of human postmenopausal osteoporosis and has been extensively used to model human osteoporosis [15]. These rodents are often the starting point for preliminary screenings, efficacy and toxicity studies of new pharmacological agents or therapeutic models, prior to clinical trials in humans.

To test our hypothesis we used OVX rats and added calcium from three different sources, namely, calcium carbonate, calcium gluconate and two doses of salmon bone calcium into their normal diets. Minimally processed salmon bone calcium has good, sustainable availability should this dietary source of calcium eventually show enhanced effectiveness in the prevention or treatment of osteoporosis in humans.

Materials and Methods

Study design and subjects

The objective of this study was to determine the dietary effects of minimally processed salmon bone calcium (SBC), calcium carbonate and calcium gluconate versus placebo on serum calcium levels, femur bone mineral density and bone regrowth in ovariectomized female Sprague-Dawley rats over 60 treatment days. This study was conducted according to GCP guidelines issued by the ICH and in accordance with the laws and regulations of India where the trial was performed. The final approved protocol and all the study related documents were reviewed and approved by an Institutional Animal Ethics Committee before the start of the study.

***Corresponding author:** Bomi Framroze, Hofseth Biocare AS, Molovegen 6, 6004, Alesund, Norway, Tel: +4770102630; E-mail: bomi.framroze@gmail.com, bf@h-bc.no

Received January 26, 2015; **Accepted** February 28, 2015; **Published** March 03, 2015

Citation: Framroze B, Godase S, Sawant S (2015) A Comparative Study of the Impact of Dietary Calcium Sources on Serum Calcium and Bone Reformation Using an Ovariectomized Sprague-Dawley Rat Model. J Nutr Food Sci 5: 348. doi:10.4172/2155-9600.1000348

40 Sprague Dawley (*Rattus novergicus*) female, nulliparous and non-pregnant, healthy young rats were used in the study. The health status of the animals used in this study was examined on arrival (initial preliminary examination) and only apparently healthy animals were selected for acclimatization. After acclimatization in the laboratory for 7 days, the rats were ovariectomized under anesthesia using a mixture of Ketamine and Xylazine, administrated intraperitoneally and set aside for a 2 week healing period. These 250-300 g rats were further acclimatized to the test conditions for a period of 5 days prior to dosing and examined for health status by a veterinarian before assignment.

Prior to initiation of test item/placebo administration, the animals were randomly allocated to the different groups (G1-G5 with 8 rats in each group) using the `Group Allocation` function in the MS Excel Add-in "Daniel´s XL Toolbar," (http://xltoolbox.sourceforge.net/) in such a way that the weight variation of animals were within 20% of group mean. The animals were marked individually by temporary tail marking with indelible ink and cage cards during acclimatization, and by micro tattooing of toe pads, after randomization. Following allocation to the study, each animal was assigned with an individual cage card, labeled with the Project number, species, strain, sex and animal number, group number, dose level, experiment start and end dates.

During the 60 day study, the rats were housed individually in polycarbonate cages. The average room temperature and relative humidity of experimental room were maintained between 21°C and 23°C and 50 to 55%, respectively, during the entire study period. Artificial light was set to give a cycle of 12 hours light and 12 hours dark. The animals were offered a conventional laboratory rodent diet *ad libitum* supplied by M/s. Nutrivet Life Sciences and filtered water was provided *ad libitum.*

Experimental design and procedures

Each group, G1-G5, was fed a powdered feed with G1 fed the placebo control diet containing no additional calcium added, G2 fed a diet containing a low dose of salmon bone calcium (SBC), G3 fed a diet containing a high dose of salmon bone calcium (SBC), G4 fed a diet containing a low dose of calcium gluconate and G5 fed a diet containing a low dose of calcium carbonate, as shown in Table 1 below.

The rat doses were based on the commonly used human dose of 1000 mg/day of calcium carbonate for an average 60 kg human. Based on body surface area calculations, the subject rat dose equivalent was estimated to be 105 mg/kg/day and this was the dose used for the salmon bone calcium (SBC) and calcium carbonate reference standard. However, since the salmon bone calcium and calcium gluconate used in the study contained less than half the amount of elemental calcium as calcium carbonate we included a second salmon bone powder dose at 210 mg/kg/day, and the same higher dose for calcium gluconate, as shown in Table 1.

The body weight of each rat was measured weekly. The dosage of test item/reference item consumed was calculated based on daily measurements of feed consumed by each individual rat. At the end of the 8 weeks study, the experimental rats were fasted overnight. Blood was collected by retro-orbital sinus puncture, after being anaesthetized using isoflurane.

All animals were observed twice daily throughout the treatment period for morbidity and mortality and were observed for clinical signs once daily during the acclimatization period and twice daily during the treatment period.

The body weights were recorded weekly. Percent body weight change for the animals was calculated by comparing to the first day body weight. Food consumption was determined daily by re-weighing the non-consumed diet from each cage. Food consumption per animal was calculated as follows: Food consumption (g/ animal/ day) = (Food input-Food leftover)

Clinical pathology evaluation of all surviving animals was performed at the end of treatment period, prior to scheduled necropsies. The animals were fasted overnight prior to blood collection. Blood was collected by retro-orbital sinus puncture, under isoflurane anesthesia. The blood samples for hematological analysis were collected from all 40 animals. Approximately 0.5 ml of blood was collected in K_2EDTA vials, using non-heparinized micro capillary tubes. The serum was separated by centrifugation at 10000 rpm for approximately 5 min, at 4 ± 1°C, for evaluation of the serum calcium concentration in mg/dl.

All 40 animals were subjected to necropsy at the end of the treatment, after euthanasia (overdose of carbon dioxide) followed by exsanguinations. All animals were subjected to a full, detailed gross necropsy; which included examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

The femur bones were stored in normal saline and evaluated for femur length, femur cortical thickness and femur bone mineral density (BMD).

Results

All raw data from this study was analyzed using "Sigma Plot 11.0" statistical software (Supplied by Cranes Software International Ltd. Bangalore). The mean and standard deviation was calculated using Microsoft Excel Sheets and all data summarized in tabular form. All continuous data were checked for normality using Shapiro-Wilk test and for homogeneity of variance using Equal Variance test. All homogenous data were analyzed using ANOVA. Data showing significance in their variances were subjected to Dunnett's t-test.

No apparent treatment related clinical signs or symptoms were observed in any of the animals, throughout the study period as shown in Table 2 below.

The percentage body weight changes of treated animals were comparable with the controls, throughout the treatment period. No statistically significant difference was observed in the body weights, in any of the treatment groups, as compared to the control animals. Food consumption for all animals of the treatment groups was comparable and did not show any significant treatment related difference, as compared to the control group. The RBC of treated animals also showed no change, as compared to the control animals (Table 3).

As compared to some studies reporting increased serum calcium

Group	Treatment	Dose (mg/kg/day)	Number of animals
G1	Ovariectomized (control)	0	8
G2	Ovariectomized + Salmon bone calcium	105	8
G3	Ovariectomized + Salmon bone calcium	210	8
G4	Ovariectomized + Calcium Gluconate	210	8
G5	Ovariectomized + Calcium Carbonate	105	8

Table 1: Experimental design.

Group Number	Group	Dose (mg/kg)	No. of Animals/ Group	Day of observations	Clinical signs
G1	Placebo control	0	8	1–60	Normal
G2	Salmon bone calcium	105	8	1–60	Normal
G3	Salmon bone calcium	210	8	1–60	Normal
G4	Calcium gluconate	210	8	1–60	Normal
G5	Calcium carbonate	105	8	1 - 60	Normal

Table 2: Clinical Signs/Symptoms.

Group Number (N)	G1 (8)		G2 (8)		G3 (8)		G4 (8)		G5 (8)	
Dose (mg/kg)	**0**		**105**		**210**		**210**		**105**	
Parameter ↓	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calcium mg/dl	9.97	0.38	9.83	0.37	9.95	0.41	9.74	0.59	10.18	0.34

SD: Standard Deviation; N: Number of animals in group.

Table 3: Serum calcium levels.

Group Number (N)	G1 (8)		G2 (8)		G3 (8)		G4 (8)		G5 (8)	
Dose (mg/kg)	0		105		210		210		105	
Parameter ↓	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Femur length (mm)	36.10	0.61	37.17*↑	0.68	37.55*↑	0.55	37.26*↑	0.62	36.85	0.68
Femur Cortical Thickness (mm)	0.54	0.05	0.61	0.06	0.72*↑	0.06	0.64*↑	0.06	0.58	0.06
Femur Bone Mineral Density (mg/cm^2)	218.90	2.92	231.54*↑	2.93	239.31*↑	5.01	227.83*↑	5.12	224.35	4.52

Key: N: Number of animals in group; SD: Standard deviation; *↑: Value significantly differ at 95% level of significance (P<0.05)

Table 4: Femur measurement.

levels on calcium supplementation, [16,17] the serum calcium values of all treated groups in our study did not show any elevation from the calcium concentration in the placebo control and were all statistically comparable to each other. Serum calcium levels were analyzed using a Selectra E with a Dry ISE Clinical Chemistry Analyzer, on the same day of sample collection.

Bone development parameters as measured by femur bone changes were significantly different for the different groups as shown below in Table 4. The femur length was significantly increased in groups G2, G3 and G4; femur cortical thickness (bone regrowth) and femur bone mineral density (BMD) significantly increased only in groups G3 and G4 as compared to the G1 control group of animals.

At gross necropsy, none of the animals showed any external abnormal changes.

Discussion

Standard of care for osteoporosis treatment today includes bisphosphonates drugs [18] such as teraparatide and alendronate. These drugs are particularly useful in acute cases of glucocorticoid-induced osteoporosis but are known to have certain disadvantages for chornic long-term use, [19] such as accelerating the development of some forms of cancer.

Instead the use of functional foods to slow down the onset of osteoporosis has seen increased research attention. Several studies have shown the positive impact of eating fish bones on bone development and slowing down osteoporosis [20,21]. One aspect of our results shows that after 8 weeks of daily dose administration of salmon bone calcium in ovariectomized Sprague Dawley rats, it may be concluded that salmon bone calcium positively impacted bone development parameters. As can be seen in Figure 1, salmon bone calcium at a human equivalent dose of 2000 mg/day showed the highest increase in all three bone development parameters. Calcium gluconate, at the same high dose, also showed statistically significant improvement in all three bone development parameters, albeit at lower numerical values. While salmon bone calcium at the lower 1000 mg/day dose showed statistically significant improvements in only two of the three bone development parameters, namely femur length and femur BMD.

Another aspect of our results shows that minimally treated salmon bone calcium contains equal amounts of phosphate and carbonate anions as well as a mix of gluconate and glycinate amino acid salts, which may play a significant role in improved incorporation into bone tissue in osteoporosis treatment.

Another aspect of our results shows the presence of natural protein growth factors [22] and prebiotic non-digestible oligosaccharide fibers [23] in the salmon bone calcium which could be responsible for increased absorption of calcium, in spite of lower levels of elemental calcium intake, as compared to calcium carbonate. In particular, other researchers [24] have shown that the presence of oligosaccharides in the diet can lead to enhanced calcium absorption, particularly when present as part of a functional food.

We are planning further trials to investigate the role of the different bioactive factors present in minimally processed salmon bone fraction by selectively removing these components, such as the protein and oligosaccharide fractions, present in it. Assessing the different roles these components may play in improved bone development versus the simple presence of more effective counter-anions, remains a focus of research in our laboratory.

Our current results do show that consuming minimally processed food fractions may confer the same quantitative biological effect as consuming the natural whole food. This adds further evidence to the theory that eating whole foods for their nutritive and health benefiting effects May not be substitutable by highly-refined supplements derived from these foods [25,26] and should necessitate the development of a range of new minimally processed nutritional supplements.

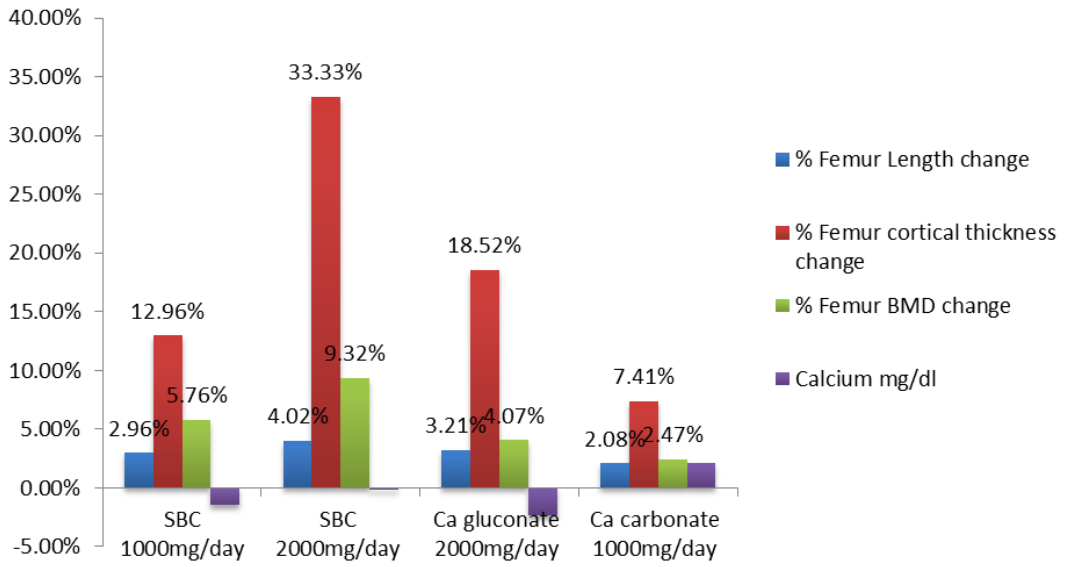

Figure 1: Percent change in serum calcium and bone parameters versus placebo control.

References

1. Dequeker J, Mundy GR (1998) Bone structure and function. In: Klippel J, Dieppe PA (eds) Mosby, London Rheumatology 8: 1-12.
2. Gruber HE, Ivey JL, Baylink DJ, Matthews M, Nelp WB, et al. (1984) Long-term calcitonin therapy in postmenopausal osteoporosis. Metabolism 33: 295-303.
3. Centrella M, Canalis E (1985) Local regulators of skeletal growth: a perspective. Endocr Rev 6: 544-551.
4. Bennett AE, Wahner HW, Riggs BL, Hintz RL (1984) Insulin-like growth factors I and II: aging and bone density in women. J Clin Endocrinol Metab 59: 701-704.
5. McHorney CA, Schousboe JT, Cline RR, Weiss TW (2007) The impact of osteoporosis medication beliefs and side-effect experiences on non-adherence to oral bisphosphonates. Curent Medical Research and Opinion 23: 3137-3152
6. Canalis E, McCarthy T, Centrella M (1988) Growth factors and the regulation of bone remodeling. J Clin Invest 81: 277-281.
7. Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA, et al. (1981) Impaired growth hormone secretion in the adult population. J Clin Invest 67: 1361-1369.
8. Genant HK, Baylink DJ, Gallagher JC (1989) Estrogens in the prevention of osteoporosis in postmenopausal women. Am J Obstet Gynecol 161: 1842-1846.
9. Turner CH (1991) Toward a cure for osteoporosis: reversal of excessive bone fragility. Osteoporos Int 2: 12-19.
10. Benvenuti S, Tanini A, Frediani U, Bianchi S, Masi L, et al. (1991) Effects of ipriflavone and its metabolites on a clonal osteoblastic cell line. J Bone Miner Res 6: 987-996.
11. Yamazaki I (1986) Effect of ipriflavone on the response of uterus and thyroid to estrogen. Life Sci 38: 757-764.
12. Malde MK, Bügel S, Kristensen M, Malde K, Graff IE, et al. (2010) Calcium from salmon and cod bone is well absorbed in young healthy men: a double-blinded randomised crossover design. Nutr Metab (Lond) 7: 61.
13. Marian KM, Susanne Bügel, Mette Kristensen, Ketil Malde, Ingvild EG, et al. (2010) Calcium from salmon and cod bone is well absorbed in young healthy men: a double blinded randomized crossover design. Nutrition and Metabolism 7: 61.
14. Huo J, Deng S, Xie C, Tong G (2010) Preparation and biological efficacy of haddock bone calcium tablets. Chinese Journal of Oceanology and Limnology 28: 371-378.
15. Yoon GA, Kim YM, Chi GY, Hwang HJ (2005) Effects of tuna bone and herbal extract on bone metabolism in ovariectomized rats. Nutrition Research 25: 1013-1019.
16. Wronski TJ, Yen CF (1991) The ovariectomized rat as animal model for post-menopausal bone loss. Cells Mater Suppl 1: 69-74.
17. Nicar MJ, Pak CY (1985) Calcium bioavailability from calcium carbonate and calcium citrate. J Clin Endocrinol Metab 61: 391-393.
18. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, et al. (2008) Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. BMJ 336: 262-270.
19. Saag KG, Shane E, Boonen S, Marín F, Donley D, et al. (2007) Teriparatide or Alendronate in Glucocorticoid-Induced Osteoporosis. N Engl J Med 357: 2028-2039.
20. Kennel KA, Drake MT (2009) Adverse effects of bisphosphonates: implications for osteoporosis management. Mayo Clin Proc 84: 632-637.
21. Kass-Wolff JH (2004) Calcium in women: healthy bones and much more. J Obstet Gynecol Neonatal Nurs 33: 21-33.
22. Kim S, Ravichandran YD, Kong C (2012) Applications of Calcium and its Supplement derived from Marine Organisms. Critical Reviews in Food Science and Nutrition 52: 469-474.
23. Dempster DW, Raisz LG (2015) Bone Physiology: Bone cells, modelling and remodelling. Nutrition and Bone Health 37-56.
24. Singh RD, Banerjee J, Arora A (2015) Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. Bioactive Carbohydrates and Dietary Fibre 5: 19-30.
25. Cashman K (2003) Prebiotics and calcium bioavailability. Curr Issues Intest Microbiol 4: 21-32.
26. Fardet AA (2015) Shift toward a new holistic paradigm will help to preserve and better process grain products' food structure for improving their health effects. Food and Function 6: 363-382.