

SHORT-TERM EFFECTS OF A CHICKEN EGG SHELL POWDER ENRICHED DAIRY-BASED PRODUCTS ON BONE MINERAL DENSITY IN PERSONS WITH OSTEOPOROSIS OR OSTEOPENIA

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EFEKT PRI KRÁTKODOBOM UŽÍVANÍ VÝROBKOV OBSAHUJÚCICH PRÁŠOK ZO ŠKRUPÍN SLEPAČÍCH VAJEC NA MINERÁLOVÚ DENZITU KOSTÍ U OSÔB S OSTEOPORÓZOU ALEBO OSTEOPATIOU

Abstract

Schaafsma A, Pakan I:

Short-term effects of a chicken egg shell powder enriched dairy-based products on bone mineral density in persons with osteoporosis or osteopenia

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Based on the high calcium content, chicken egg shells are an interesting source of calcium. We studied the short-term effects on bone mineral density (BMD) of the lumbar spine and hip in 9 women and one man (mean age \pm SD, 63.9 \pm 8.1 years) with osteoporosis or osteopenia. Also the effects on pain and general well-being were monitored. Ten women (62.5 \pm 5.0 years) from a population study on BMD served as a control group. During a study period of 4–8 months, the intervention group consumed twice daily a dairy-based supplement which resulted in a daily intake of, among others, 3.0 g of egg shell powder, 400 IU of vitamin D3 and 400 mg of magnesium. BMD of the lumbar spine (anteroposterior (AP) and lateral (LA) position) and hip were measured by dual-energy X-ray absorptiometry. After the intervention period, BMDs of the lumbar spine, total proximal femur and trochanter were significantly ($p < 0.05$) increased with (median) 4.4 %: (range) 1.7 to 10.4 % (lumbar spine AP), 5.7 %: -1.3 to 15.9 % (lumbar spine LA), 2.2 %: -1.9 to 9.4 % (total proximal femur), 1.8 %: -1.8 to 9.0 % (trochanter). Within a period of 4 months, an important reduction in pain was reported and as a consequence an improvement in general well-being. In the control group, BMDs of the lumbar spine AP and of the femoral neck significantly decreased over a period of 8 months with -0.7 % (-1.3 to 0.2 %) and -0.9 % (-2.4 to -0.1 %) respectively. Six women of the intervention group continued to use the supplement on their own free will and without any

Abstrakt

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Efekt pri krátkodobom užívaní výrobkov obsahujúcich prášok zo škrupín slepačích vajec na minerálovú denzitu kostí u osôb s osteoporózou alebo osteopatiou

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Z hľadiska vysokého obsahu kalcia sú pre nás zaujímavé skupiny slepačích vajec. Pozorovali sme krátkodobé efekty na kostnú hmotu (BMD) lumbálnej chrbtice a kĺbu u 9 žien a 1 muža (s vekovým priemerom 63,9 \pm 8,1 roka) s osteopéniou a osteoporózou. Sledovali sme aj účinok na bolesť a celkový zdravotný stav. Kontrolnú skupinu štúdie BMD tvorilo 10 žien (62,5 \pm 5,0 roka). Počas 4–8 mesiacov intervenčná skupina užívala dvakrát denne mliečne produkty s obsahom 3 g prášku zo škrupín slepačích vajec, 400 IU vitamínu D3, 400 mg magnézia. BMD lumbálnej chrbtice (anteroposterior (AP) a lateral (LA) position) a kĺbu sa merali pomocou dvojitého X-lúčov absorpčnej metódy (dual-energy X-ray absorptiometry). Po ukončení štúdie BMD lumbálnej chrbtice, proximálneho femuru a veľkého hrboľa sa zaznamenal významný ($p < 0,05$) rast priemerne 4,4 %: 1,7–10,4 % (lumbar spine AP), 5,7 %: -1,3–15,9 % (lumbar spine LA), 2,2 %, -1,9–9,4 % (total proximal femur), 1,8 %: -1,8–9,0 % (trochanter). Počas 4 mesiacov sme zaznamenali redukciu bolesti, čo malo vplyv na celkový zdravotný stav. V kontrolnej skupine BMD lumbálnej chrbtice AP a krčka femuru sa zaznamenal významný pokles počas 8 mesiacov: -0,7 % (-1,3–0,2 %), resp. -0,9 % (-2,4–(-0,1 %)). 6 žien v intervenčnej skupine užívalo produkt z vlastnej vôle bez akejkoľvek kontroly počas 24 mesiacov. Okrem posledných 3 mesiacov, keď užívali dvojité dávku, užívali produkt iba denne. Táto

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check on compliance, up to 24 months. They consumed the supplement only once daily except for the last three months when they were asked to take the double dosage again. After 24 months BMDs did not differ from baseline. This study shows that egg shell powder is a source of bioavailable calcium. Furthermore, this pilot study indicates that the chicken egg shell powder enriched dairy-based supplement increases BMD of subjects with a low bone mass in the short term and as a consequence delays bone demineralisation for a longer period. (Tab. 3, Fig. 1, Ref. 23.)

Key words: calcium, bone mineral density, egg shell powder, magnesium, osteoporosis, pain, vitamin D3.

Calcium is a very important building block of bone and often seen as key element in bone mineralisation and anti-demineralisation strategies (4, 5, 9). Dairy products which are known as the major source of calcium are only 'recently', about 10,000 years ago, introduced in the human diet (17). Before that time, the stone-age adult consumed a high amount of calcium from other sources (2, 11), probably including egg shell.

To support increased requirements or to overcome low intakes, several calcium sources are available for food enrichment. In postmenopausal women and elderly, a number of calcium sources, such as purified calcium carbonate (3, 6, 21), calcium citrate (7), osseino-mineral complex (3), oyster shell electrolysate (12), calcium lactate gluconate (19, 21, 22, 23), and milk-calcium (18, 22) have been studied for their effects on BMD. In general the effects were comparable, causing a small, sometimes transient increase in BMD or an inhibition of bone loss. Chicken egg shell powder, not commonly used as calcium source for humans, might have a higher impact on BMD as has been suggested by a Slovakian study with osteoporotic patients (14). The major component of egg shell powder is calcium carbonate (about 98 % of weight) whereas other minerals which are of interest in bone metabolism such as strontium and fluoride, are present in small amounts. Furthermore, it has been suggested by Slovakian researchers that egg shell powder contains bioactive substances (not published). Combinations of calcium with vitamin D (3, 7, 9, 15, 18, 21) and magnesium (1) enhance calcium effects on BMD of postmenopausal women in case their intakes are limited.

This pilot study aimed to study the short-term effects of an egg shell powder enriched dairy-based supplement also containing other minerals and vitamins including vitamin D3 and magnesium on BMD of the lumbar spine and hip, and on pain and general well-being.

Subjects, material and methods

Subjects

Ten persons (1 man and 9 postmenopausal women, 51–82 years of age) with radiological and dual-energy X-ray absorptiometric (DEXA) confirmed osteoporosis (T-score <-2.5 SD) or osteopenia (-1 < T-score > -2.5) entered the study. Characteristics of the subjects are given in Table 1. Beside the low bone mass they were apparently healthy based on a medical check. Ten apparently healthy women (55–73 years of age) from a former BMD population study, served as a control group. These controls were not studied at the same time as the intervention group, but they came

štúdia ukázala, že prášok zo škrupín slepačích vajec je prirodzeným zdrojom kalcia. Z tejto práce ďalej vyplýva, že prášok dokáže po krátkodobom užívaní spôsobiť u subjektov zvyšovanie BMD a má aj dlhodobý účinok pri demineralizácii kostí. (Tab. 3, obr. 1, lit. 23.)

Kľúčové slová: kalcium, minerálová denzita kostí, prášok zo škrupín slepačích vajec, magnézium, osteoporóza, bolesť, vitamín D3.

Tab. 1. Baseline characteristics.

	Intervention group	Control group
Number of participants	10	10
– women	9	10
– men	1	
Age (years±SD)	63.9±8.1	62.5±5.0
Body weight (kg)	74.1±10.1	73.0±9.7
Body height (cm)	167.4±8.4	166.5±5.5
BMI (kg/m ²)	26.4±2.7	26.4±4.0
Diagnose osteoporosis	10	10
– anteroposterior	5	5
– lateral	5	5

Data are presented as mean±SD. The intervention group received an egg shell powder enriched dairy-based supplement in combination with minerals and vitamins. The control group did not consume any supplement. BMI: body mass index.

from the same region and were measured with the same equipment at the same hospital. No one of both groups used additional calcium or vitamin D supplements or were on medication which are known to influence bone metabolism. All persons gave their written consent. The study was approved by the Medical Ethical Commission of the Medical Centre Leeuwarden.

Study design

During the study period, varying from 4–8 months (mean 6.9 months) the intervention group consumed twice daily a dairy-based supplement (Table 2). BMD was measured at the start and at the end of this period. Blood and 24 h urine were collected at baseline, halfway and at the end of the study. Compliance to product intake was checked based on the request for new product. After this official study period, 6 women of the supplemented group used the supplement up to 24 months. During this additional period compliance to product intake was not recorded. Based on a questionnaire it can be assumed that they used only one supplement daily and not on a regular basis. From month 21–24 they consumed the supplement twice daily again. No restrictions were made for the daily diet. Based on a frequency list of dairy products at baseline and at the end of the study, total calcium intake during the intervention period was estimated at 2,200 mg per day. The calcium intake of the control group was calculated to be 1,150 mg per day. BMD of the control group was measured with an interval of 22 to 36 months. For a comparison with the results of the intervention group, data of the control group were interpolated to an interval of 8 months.

Tab. 2. Composition of the dairy-based supplement.

Nutrient	Unit	per 400 ml	US RDA ²
Milk protein	g	16.4	50
Milk fat	g	10.4	73 ³
Carbohydrates	g	42	238 ⁴
Energy	kJ (kcal)	1,428 (340)	7,980 (1,900)
Calcium total	mg	1800	800
from egg shell ¹	mg	1200	
from milk	mg	600	
Phosphorus	mg	520	800
Sodium	mg	236	500 ⁵
Potassium	mg	756	2,000 ⁵
Magnesium	mg	400	280
Iron	mg	3	10
Vitamin A	mmg-RE	600	800
Beta carotene	mg	2.0	
Vitamin D3	IU	400	200
Vitamin E	mg α -TE	20	8
Vitamin B6	mg	4.0	1.6
Vitamin B12	mmg	4.0	2.0
Vitamin C	mg	120	60

¹ Derived from Biomin a.s., Cifer, Slovak Republic. ² USA Recommended Dietary Allowances for women 51 years and older (16). ³ 35 % of total energy. ⁴ 50 % of total energy. ⁵ Estimated safe and adequate daily intakes.

The supplement (powder suspended in water) was taken at breakfast and with the evening meal in a total amount of 400 ml per day. Vitamin A is presented in retinol equivalents (g-RE), vitamin E in -tocopherol equivalents (mg -TE).

Material and methods

Body height and weight were measured by the same person with calibrated equipment during every visit. The outcomes were used to calculate the body mass index (BMI) (kg/m²).

BMD of the lumbar spine (L2-L4) in lateral (LA) and anteroposterior position (AP) and the hip (total proximal femur, femoral neck, trochanter, intertrochanter and Ward's triangle) were assessed by DEXA on a QDR 2000 (Hologic Inc., Waltman, MA, USA). The coefficients of variation for BMD measurements were 1.0 %. Non-fasting blood samples were analysed for calcium, phosphate and gamma glutamyl transferase (γ GT) (Hitachi 717 Chemistry Analyser, Boehringer Mannheim, Germany), and total alkaline phosphatase (colorimetric, according to the recommendations of the International Federation of Clinical Chemistry (IFCC)).

The volume of 24-h urine was measured and samples were analysed for creatinine and calcium (Hitachi 717 Chemistry Analyser) and for hydroxyproline (colorimetric, Hypronosticon, Organon Technica Nederland B.V., Boxtel, The Netherlands).

Statistical analysis

Data were analysed using SPSS 7.0 (SPSS Inc., Chicago, USA). Although BMDs were normally distributed according to the Shapiro-Wilk's test for normality, non-parametric tests (Mann-Whitney U-test and Wilcoxon signed-rank test) were preferred because of the small sample size. Although the intervention

group and the control group were not studied at the same time we did compare the changes (second measurement minus first measurement) in BMDs of both groups.

Results

Anthropometric measurements

In both groups no significant changes were noted in body height, body weight or BMI.

BMD

During the intervention period of 4—8 months, mean BMDs of the lumbar spine (AP and LA position), total proximal femur and trochanter increased significantly (Table 3). Individually changes in spinal BMD in AP and LA position are shown in Figure 1.

The control group showed a significant decrease in BMD of the lumbar spine in AP position and femoral neck over a period of 8 months. One woman had a marked increase in BMD of the total proximal femur. This increase of 1.7 % over 8 months coincided with a high calcium intake of 1,510 mg per day.

At baseline, mean BMDs of the total proximal femur, trochanter and intertrochanter were higher in the control group. After 8 months

Tab. 3. Bone mineral densities at baseline and their percental changes after 4—8 months intervention group) and 8 months (control group).

Measured site		Intervention group	Control group	p-value ³ intervention vs control
Lumbar spine AP ¹	Baseline	0.828 \pm 0.152 (0.518 - 1.082)	0.858 \pm 0.086 (0.707 - 0.977)	0.436
	Change (%)	4.1 (1.5-11.0)**	-0.8 (-1.3 - 0.1)**	0.000
Lumbar spine LA ²	Baseline	0.536 \pm 0.095 (0.373 - 0.652)	0.577 \pm 0.069 (0.504 - 0.703)	0.326
	Change (%)	6.5 (-1.3 - 15.9)**	-1.4 (-5.6 - 3.6)	0.001
Femoral neck	Baseline	0.612 \pm 0.125 (0.452 - 0.914)	0.696 \pm 0.110 (0.513 - 0.888)	0.082
	Change (%)	0.9 (-5.3 - 7.8)	-0.5 (-2.6 - -0.1)**	0.009
Total prox. femur	Baseline	0.720 \pm 0.139 (0.484 - 1.021)	0.829 \pm 0.125 (0.608 - 1.032)	0.031
	Change (%)	1.9 (-2.3 - 9.5)*	0.1 (-2.5 - 1.7)	0.017
Trochanter	Baseline	0.553 \pm 0.102 (0.400 - 0.750)	0.645 \pm 0.097 (0.472 - 0.781)	0.041
	Change (%)	1.8 (-1.8 - 9.4)*	-0.1 (-1.8 - 0.6)	0.086
Intertrochanter	Baseline	0.824 \pm 0.171 (0.550 - 1.180)	0.965 \pm 0.154 (0.717 - 1.231)	0.043
	Change (%)	4.9 (-4.9 - 12.1)	-0.2 (-2.4 - 4.0)	0.086
Ward's triangle	Baseline	0.452 \pm 0.150 (0.310 - 0.760)	0.551 \pm 0.190 (0.361 - 0.965)	0.288
	Change (%)	0.0 (-8.9 - 8.9)	-0.3 (-7.6 - 5.5)	0.683

Data are expressed as mean \pm SD (and range) in g/cm², and as median percental change (and range) from baseline. For group characteristics see Table 1, for supplement composition see Table 2.

¹ Lumbar spine in anteroposterior position.

² Lumbar spine in lateral position.

³ Mann-Whitney U-test based on absolute BMDs at baseline and the changes in BMDs after 4—8 months

* p<0.05, ** p<0.01: Wilcoxon signed rank-test compared with baseline and based on absolute BMDs.

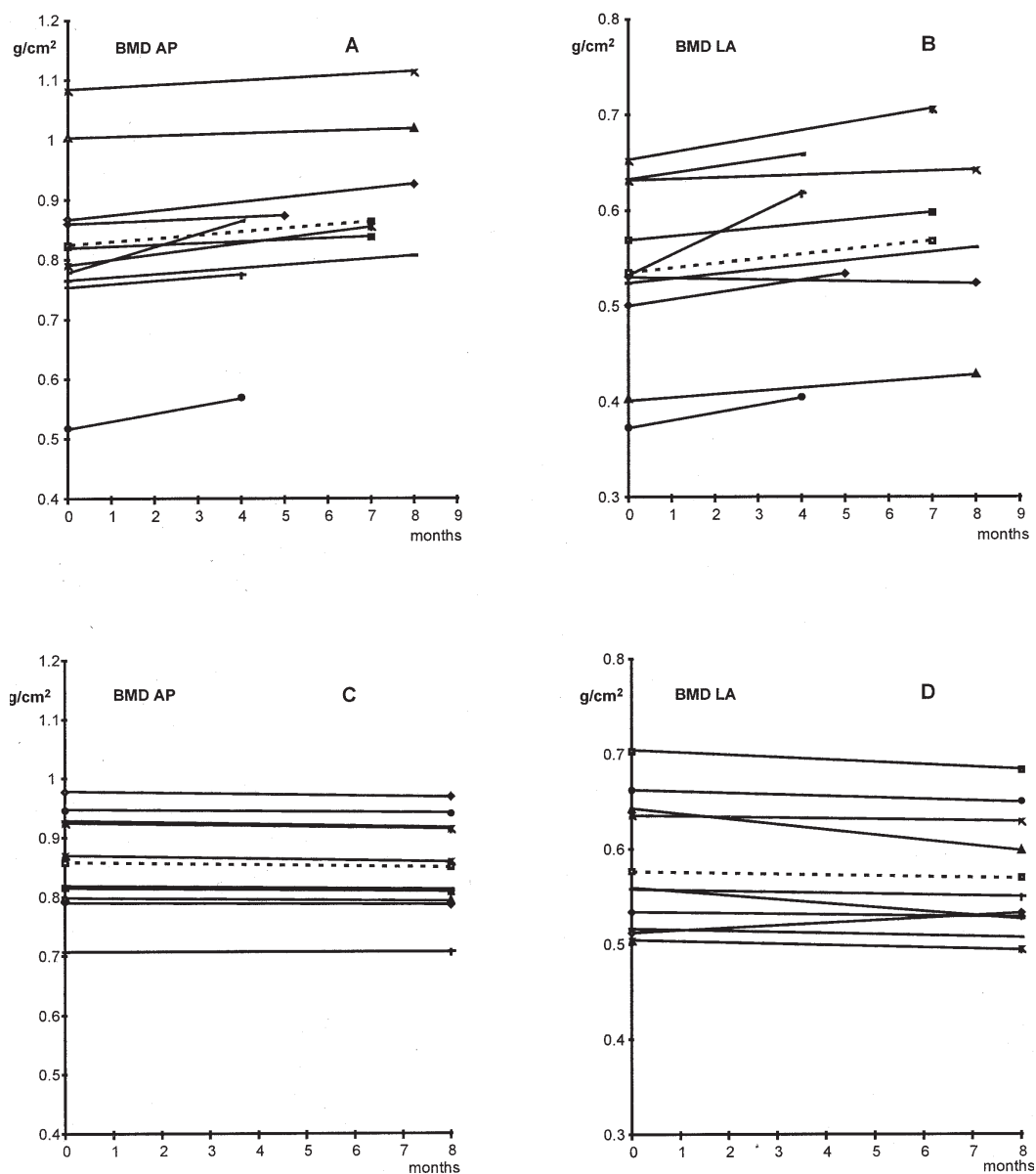


Fig. 1. Individual changes in lumbar spine BMD of the intervention group in AP (panel A) and LA position (panel B), after 4–8 months. Panel C (AP) and D (LA) show the results of the control group over an interpolated period of 8 months.

BMD is expressed as g/cm² as measured by DEXA. Mean BMDs presented as dashed lines. For a description of both groups and the supplement of the intervention group see Table 1 and 2. Mean spinal BMD of the intervention group increased significantly ($p < 0.05$) in AP and LA position. The control group showed a significant decrease of spinal BMD in AP position.

these differences disappeared. Comparing the changes in BMDs, the increases in the intervention group of the lumbar spine, femoral neck and total proximal femur were significantly different from the losses in the control group (Table 3).

In the small group of 6 women, BMDs after 24 months (data not shown) did not differ from baseline. The control groups lost bone at the lumbar spine AP ($p = 0.007$) and femoral neck ($p = 0.005$) over the same time interval. The increase in BMD of the lumbar spine AP at 24 months in the intervention group was still

significantly different from the decrease as found in the control group.

Biochemical measurements

Mean baseline concentrations (\pm SD) of serum calcium (2.36 ± 0.06 mmol/l) and total alkaline phosphatase (52.2 ± 11.3 U/l) did not change during the intervention period.

In 24-h urine no significant differences could be detected between start and end excretions of hydroxyproline (150 ± 67.5 μ mol/

24 h, median and range: 156: 61-272) and calcium (5.5±3.6 mmol/24 h, median and range: 5.6: 1.2-12.0), or their ratios with creatinine.

Discussion

In this pilot study the effects of an egg shell powder, magnesium and vitamin D3 enriched dairy-based product were studied on BMD of the lumbar spine and hip in 10 subjects with spinal osteoporosis or osteopenia. Ten postmenopausal women on a normal diet who participated in a population study in the same region served as a control group. After a study period of 4–8 months, the intervention group significantly increased BMD of the lumbar spine in AP (+4.4 %) and LA position (+5.7 %), total proximal femur (2.2 %) and trochanter (1.8 %). The control group showed a decreased BMD of the lumbar spine in AP position (-0.7 %) and femoral neck (-0.9 %) over a comparable, interpolated period.

The total daily intake of 2,200 mg of calcium by the intervention group is comparable with the amount of about 2,000 mg of calcium per day in the study of Prince et al (19). In our study the increases in BMD of the lumbar spine are higher compared with the increases over the first 6 months in the study of Prince et al. The difference might be explained by a lower bone mass of our subjects and/or the supplementation of magnesium (preservation of calcium in bone, calcitriol synthesis) (1, 10), vitamin D3 (calcium absorption) (3, 7, 18) and/or chicken egg shell powder (14). Beside being a source of calcium, chicken egg shell powder contains small amounts of micro-elements, such as strontium and fluoride which may have an additional effect on bone metabolism over purified calcium (20). Although this study lasted for only 4–8 months, calcium supplementation studies up to 4 years indicate that the first year, with an important contribution during the first 6 months, is indicative for longer term effects (9, 22). Our BMD measurements in 6 women after 2 years suggest a strongly delayed bone loss despite the reduced intake of supplement and suggested lower compliance to product intake.

The intervention and control group were not studied at the same time, but we found it acceptable to compare both groups as the women were recruited from the same region just two years before our study started, and were measured with the same equipment in the same hospital. The control group intended to have higher BMDs at all measured sites (significant for total proximal femur, trochanter and intertrochanter) at baseline. Therefore no statistical differences were found between both groups after 4–8 months despite significant improvements of BMDs in the intervention group. When comparing the changes in BMDs, the intervention group showed a significant increase at the lumbar spine, femoral neck and total proximal femur (Table 3).

The high calcium intake by the intervention group did not disturb serum calcium levels nor did it cause an increase in urinary calcium. In theory, an increased calcium excretion of 2.4 mmol per 24 h might have been expected because of the extra calcium intake and the sodium content of the test product (Table 2) (8, 13). Perhaps

the rather high, mean calcium excretion of 5.5 mmol/24 h at baseline masked the expected increase. In other words, a decreased calcium excretion because of an inhibited bone resorption was compensated by an increased excretion as a result of a higher calcium intake. Another study with postmenopausal women (18) also reported no increase in urinary calcium after an additional daily calcium intake of 800 mg (total daily calcium intake of 1,500 mg).

A positive side effect of the test product, probably an effect of egg shell powder, was a reduction in pain and improvement of general well-being as reported by the participants. Although these effects are based on a non-validated questionnaire, they confirm what has been reported by others (14).

In conclusion, this study shows that calcium from egg shell powder was available to the body and, in combination with other minerals and vitamins, including vitamin D3 and magnesium, increases BMD in the short-term. Therefore, egg shell powder might be an interesting source of calcium for men and women with involutional osteoporosis or osteopenia. The markedly higher gain in BMD of the lumbar spine in particular, compared with other calcium studies over the same length of study period (9, 12, 19, 23), might point to an effect over the remodelling transient. A placebo or reference product controlled study is warranted to confirm the reported results and to substantiate the role of egg shell powder in healthy postmenopausal women.*

References

1. **Abraham G.A.:** The importance of magnesium in the management of primary postmenopausal osteoporosis. *J. Nutr. Med.* 1991; 2: 165–178.
2. **Barger-Lux M.J., Heaney R.P.:** The role of calcium intake in preventing bone fragility, hypertension, and certain cancers. *J. Nutr.* 1994; 124: 1406–1411.
3. **Chevalley T., Rizzoli R., Nydegger V. et al.:** Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin D replete elderly patients. *Osteoporosis Int.* 1994; 4: 245–252.
4. **Compston J.E.:** The role of vitamin D and calcium supplementation in the prevention of osteoporotic fractures in the elderly. *Clin. Endocrinol.* 1995; 43: 393–405.
5. **Dawson-Hughes B.:** The role of calcium in the treatment of osteoporosis. S. 1159–1168. In: Marcus R., Feldman D., Kelsey J. (Eds.): *Osteoporosis*. San Diego, Academic Press 1996.
6. **Dawson-Hughes B., Dallal G.E., Krall E.A., Sadowski L., Sahyoun N., Tannenbaum S.:** A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *New Engl. J. Med.* 1990; 323: 878–883.
7. **Dawson-Hughes B., Harris S.S., Krall E.A., Dallal G.E., Falconer G., Green C.:** Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. *Amer. J. Clin. Nutr.* 1995; 61: 1140–1145.

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- 8. Devine A., Criddle R.A., Dick I.M., Kerr D.A., Prince R.L.:** A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. *Amer. J. Clin. Nutr.* 1995; 62: 740–745.
- 9. Devine A., Dick I.M., Heal S.J., Criddle R.A., Prince R.L.:** A 4-year follow-up study of the effects of calcium supplementation on bone density in elderly postmenopausal women. *Osteoporosis Int.* 1997; 7: 23–28.
- 10. Driessens F.C.M., Verbeeck R.M.H.:** On the prevention and treatment of calcification disorders of old age. *Med. Hyp.* 1988; 25: 131–137.
- 11. Eaton S.B., Eaton III S.B., Konner M.J., Shostak M.:** An evolutionary perspective enhances understanding of human nutritional requirements. *J. Nutr.* 1996; 126: 1732–1740.
- 12. Fujita T., Fukase M., Miyamoto H., Matsumoto T., Ohue T.:** Increase of bone mineral density by calcium supplement with oyster shell electrolyte. *Bone Mineral* 1990; 11: 85–91.
- 13. Lemann J.:** Urinary excretion of calcium, magnesium, and phosphorus. S. 50–54. In: Favus M.J. (Ed.): *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. New York, Raven Press 1993.
- 14. Makai F., Chudacek J.:** The treatment of osteoporosis with Biomin-H. *Arch. Gerontol. Geriatr.* 1991; 2: 487–490.
- 15. Meunier P.J., Chapuy M.C., Arlot M.E., Delmas P.D., Duboeuf F.:** Can we stop bone loss and prevent hip fractures in the elderly? *Osteoporosis Int.* 1994; 4: 71–76.
- 16. National Research Council.** Recommended Dietary Allowances. 10th Edition. Washington, D.C., National Academy Press 1989.
- 17. Nelson D.A.:** An anthropological perspective on optimizing calcium consumption for the prevention of osteoporosis. *Osteoporosis Int.* 1996; 6: 325–328.
- 18. Nelson M.E., Fisher E.C., Dilmanian F.A., Dallal G.E., Evans W.J.:** A 1-y walking program and increased dietary calcium in postmenopausal women: effects on bone. *Amer. J. Clin. Nutr.* 1991; 53: 1304–1311.
- 19. 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: 1068–1075.
- 20. Reginster J.Y., Halkin V., Henrotin Y., Gosset C.:** Treatment of osteoporosis: role of bone-forming agents. *Osteoporosis Int.* 1999; 3: 91–96.
- 21. Reid I.R., Ames R.W., Evans M.C., Gamble G.D., Sharpe S.J.:** Effects of calcium supplementation on bone loss in postmenopausal women. *New Engl. J. Med.* 1993; 328: 460–464.
- 22. Reid I.R., Ames R.W., Evans M.C., Gamble G.D., Sharpe S.J.:** Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomized controlled trial. *Amer. J. Med.* 1995; 98: 331–335.
- 23. Thamsborg G., Jensen J.E.B., Kollerup G., Hauge E.M., Melsen F., Sorensen O.H.:** Effect of nasal salmon calcitonin on bone remodeling and bone mass in postmenopausal osteoporosis. *Bone* 1996; 18: 207–212.

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