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Positive effects of a chicken eggshell powder-enriched vitamin–mineral supplement on femoral neck bone mineral density in healthy late post-menopausal Dutch women

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Although bone metabolism is largely under genetic control, the role of nutrition is considerable. The present study evaluates the effects of chicken eggshell powder, a new source of dietary Ca, and purified CaCO₃ on bone mineral density (BMD) of the lumbar spine and hip. Besides BMD we also looked at biochemical markers of bone and Ca metabolism. Both Ca sources were provided in combination with minerals and vitamins including Mg, cholecalciferol and phylloquinone. We designed a randomised, double-blind, placebo-controlled study to take place over 12 months. Healthy Caucasian women (*n* 85), selected by age (≥ 50 and < 70 years), from the databases of general practitioners were recruited by telephone calls. They had to be at least 5 years post-menopausal, with lumbar spine T-score being > -2.5 . At baseline, their mean habitual daily Ca intake was adequate. The women were randomly allocated to: eggshell powder-enriched (group A; *n* 24), purified CaCO₃-enriched (group B; *n* 22), or a placebo product (group C; *n* 27). BMD was measured at baseline and then after 6 and 12 months of supplementation as were the biochemical markers bone-specific alkaline phosphatase, amino-terminal propeptide extension of type I collagen, deoxypyridinoline, calcitonin, intact parathyroid hormone, calcidiol, and urinary Ca. After 12 months of supplementation, only mean BMD of the femoral neck in group A was significantly increased ($P=0.014$) by 1.75% (95% CI 0.18, 3.32) compared with a decrease of -0.60% (95% CI $-1.92, 0.72$) in group C. This increase coincided with significant decreases in markers of bone resorption and formation. No significant changes were seen in BMD at other sites, including lumbar spine, nor in groups B and C. No differences were found between groups A and B, or B and C.

The present study indicates that healthy late post-menopausal women with an adequate Ca intake at baseline may increase BMD of the hip within 12 months following supplementation with the chicken eggshell powder-enriched supplement.

Eggshell powder: Calcium carbonate: Bone mineral density: Postmenopausal women

Age-related decrease in bone mass and loss of bone architecture contribute to bone fragility. Nutritional prevention strategies, aiming to reduce the risk of fragility fractures, should therefore start as soon as possible. For women, bone loss accelerates at the onset of menopause as a result of a decreased production of oestrogen. This loss is often not due to nutrient deficiency and cannot substantially be influenced by the diet. Before bone has come into a new steady state, 5 to 10 years have passed. Nutritional

interventions therefore seem to be most effective following that period (Heaney, 1996).

At least Ca and vitamin D are recognised as important in primary prevention strategies and should be present in nutritional supplements. However, also for Mg and vitamin K, evidence for positive effects on bone is compelling (Schaafsma *et al.* 2001). Mg is thought to prevent Ca leakage from bone by regulating the pH of extracellular fluid. Furthermore, it is necessary for the synthesis of calcitriol

Abbreviations: BMD, bone mineral density; D-Pyr, deoxypyridinoline; 25(OH) D, calcidiol; PINP, amino-terminal extension of type I collagen; PTH, parathyroid hormone.

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(Driessens & Verbeeck, 1988). Two studies with post-menopausal women suggest an effect of Mg on bone mineral density (BMD) of hip (Rude & Olerich, 1996) and forearm (Stendig-Lindberg *et al.* 1993) after 2 years of supplementation with 250 and 500 mg daily. Vitamin K is an essential co-factor in post-translation carboxylation of osteocalcin, which is important for proper mineralization of new bone (Sokoll *et al.* 1997). Post-menopausal women with fractures of the distal radius were found to have a low percentage of carboxylated osteocalcin. These levels increased considerably following supplementation with 1 mg phylloquinone/d for only 2 weeks (Douglas *et al.* 1995). Recently we showed that also relatively small daily doses of phylloquinone (80 µg) can increase plasma carboxylated osteocalcin levels in post-menopausal women (Schaafsma *et al.* 2000). Several studies have shown positive effects of additional Ca and/or vitamin D on BMD of the lumbar spine and hip. These effects are associated with decreased fracture risk (Prince *et al.* 1995; Dawson-Hughes *et al.* 1997; Riggs *et al.* 1998; Kanis, 1999). The amounts of Ca and vitamin D necessary for maximum effect are debated (McKane *et al.* 1996; Dawson-Hughes, 1998; Vieth 1999). The Ca source used may influence the final effect on BMD.

Chicken eggshell powder is a rather unknown Ca source. It contains about 39% (w/w) elemental Ca with a bioavailability as high as from CaCO₃ (Schaafsma & Beelen, 1999). Since it is widely available, it may be a cheap and effective solution for many Ca-deficient populations. It is of interest to know that chicken eggshell powder also contains Sr (on average 380 µg/g). This micronutrient may have an anabolic effect on bone (Reginster *et al.* 1999). In small studies, eggshell powder showed positive effects on BMD of the lumbar spine and hip in post-menopausal women with osteoporosis (Makai & Chudacek, 1991; Schaafsma & Pagan, 1999).

Suitable parameters to measure effects of nutritional intervention on bone in a healthy population are BMD for bone strength (Kanis, 1999), deoxypyridinoline (D-Pyr) for bone resorption (Delmas, 1991), and bone-specific alkaline phosphatase and the amino-terminal propeptide extension of type I collagen (P1NP; Cabrera *et al.* 1998) for bone formation. Carboxylated osteocalcin, expressed as a percentage from total osteocalcin, is a marker of bone mineralisation and vitamin K status (Boskey *et al.* 1998). Parathyroid hormone (PTH) and calcitonin are regulators of Ca metabolism and provide information about bone remodelling (Aurbach, 1988). Vitamin D status is reflected by serum levels of calcidiol (25(OH)D) which is the precursor of calcitriol (McKenna & Freaney, 1998).

The present paper describes a double-blind, placebo-controlled, randomised study with healthy Caucasian post-menopausal women. The aim of the study was to investigate the effects of two different Ca sources, chicken eggshell powder and purified CaCO₃, on BMD of the lumbar spine and hip. To support the BMD measurements, changes in biochemical markers of bone formation (P1NP, bone-specific alkaline phosphatase, osteocalcin), bone resorption (D-Pyr) and vitamin K status in bone (under-carboxylated osteocalcin) were measured. In addition markers of vitamin D status (25(OH)D) and Ca metabolism

(PTH and calcitonin) were also measured. Both Ca sources were given in combination with other minerals and vitamins, including Mg and the vitamins cholecalciferol and phylloquinone. Comparisons were made between the two Ca sources, with baseline, and with placebo.

Methods

Subjects

Apparently healthy Caucasian post-menopausal women (*n* 85) were selected from databases of three general practitioners in the village of Eelde in The Netherlands. Selection criteria used were age (≥ 50 and < 70 years), absence of acute and chronic illnesses, and no use of medicines known to interfere with bone metabolism. The general practitioner assistants asked selected women, by telephone, if they were interested to participate in the study. The research assistant visited interested women for the final inclusion criteria. Women included were at least 5 years post-menopausal, did not use vitamin and/or mineral preparations known to interfere with bone metabolism, and they were asked to abstain from artificial sunbathing during the study period. Finally, following the first dual-energy X-ray absorptiometry, women with a lumbar spine T-score < -2.5 or evident arthrosis of the lumbar spine or hip, confirmed by X-ray photography, were excluded. Post-menopausal age was determined by recall. Participants were included after informed consent was obtained. Baseline characteristics are given in Table 1. During the study twelve women dropped out because of the need for medical treatments not related to the intervention. The Medical Ethical Committee of the University Hospital of Groningen approved the study.

Supplements and diet

Women were randomly allocated to one of three intervention groups. The three supplements used (Table 2) were chicken eggshell powder (Biomim a.s., Cifer, Slovak Republic) combined with Mg, cholecalciferol, phylloquinone, and a standard mixture of minerals and vitamins (supplement A), or purified CaCO₃ (Calcipural GP; Scora S.A., Caffiers, France) combined with the same amounts of minerals and vitamins as supplement A (supplement B), or a placebo product containing 2.5 g skimmed milk powder (supplement C). The subjects were instructed to take the supplements twice daily, suspended in water, yoghurt or milk. Compliance, expressed as the percentage of sachets taken, was assessed on the basis of emptied sachet counts and written checklists. A minimum compliance of 75% was necessary for evaluation of results. The intake of Ca was recorded at three occasions during the study period by means of a food frequency questionnaire (National Osteoporosis Foundation, 1999). The aim was to classify Ca intake as low (< 800 mg/d), median (800–1000 mg/d) or high (> 1000 mg/d).

Materials and methods

Evening weight and height were measured by standardised

Table 1. Baseline characteristics of the study subjects
(Mean values with their standard deviations)

Group*...	A		B		C	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	60.5†	3.6	59.5††	3.8	63.5	4.0
Post-menopausal age (years)	9.9††	4.0	12.1	6.4	14.3	4.6
Weight (kg)	71.4	9.0	75.5	10.4	76.9	11.9
Height (cm)	164.2	5.4	164.5	5.7	163.9	6.1
BMI (kg/m ²)	26.5	3.2	28.1	4.8	28.7	4.4
Lumbar spine (AP)						
T-score	-0.97	0.96	-0.73	0.89	-0.72	1.17
Z-score	0.51	1.00	0.68	0.87	0.98	1.16
Femoral neck						
T-score	-1.54	0.95	-1.53	0.95	-1.06	1.12
Z-score	0.26	1.04	0.05	0.78	0.86	1.07
Total proximal femur						
T-score	-0.92	0.83	-0.77	1.01	-0.49	1.00
Z-score	0.20	0.86	0.29	1.03	0.81	0.92

AP, anteroposterior position.

*Group A (n 24), eggshell powder-enriched supplement in combination with minerals and vitamins; Group B (n 22), purified calcium carbonate-enriched supplement in combination with minerals and vitamins; Group C (n 27), placebo containing 2.5 g skimmed milk powder.

Mean values were significantly different from those of group C: †P<0.05, ††P<0.01.

methods, and BMI was calculated (kg/m²). BMD of the lumbar spine (L1–L4) in anteroposterior position and of the hip (total proximal femur, femoral neck, trochanter, intertrochanter, and Ward's triangle) was assessed by dual-energy X-ray absorptiometry on a Hologic QDR 1000 (Hologic Inc., Waltman, MA, USA). The CV was 1% for the spine and 1.6% for the hip. Those measurements were done at the start and after 6 and 12 months of intervention. Blood samples were obtained between 19.00 and 21.00 hours at baseline (September–December) and after 6 (March–June) and 12 months (September–December). Serum was stored at -20°C within 1 h. Second morning urine samples were collected before

food intake and stored in a freezer until analysis. For standard monitoring, serum albumin, total Ca, phosphate, creatinine, γ glutamyl transferase, alanine amino transferase and aspartate amino transferase were measured (Mega automated analyser; Merck, Darmstadt, Germany or Vitros; Johnson & Johnson Diagnostic, Rochester, NY, USA). Bone formation was studied by assessment of P1NP (RIA; Orion Diagnostica, Espoo, Finland; intra- and inter-assay CV were 8.4% and 9.0% respectively), bone-specific alkaline phosphatase (Immuno assay; Metra Biosystems, Mountain View, CA, USA; intra- and inter-assay CV were 4.9% and 5.5% respectively), and total osteocalcin and undercarboxylated osteocalcin before and

Table 2. Composition of the supplements (per sachet) for groups A, B and C*

Nutrient	Group A	Group B	Group C	US RDA
Energy (kJ)	3.4	3.4	34	7980
Protein (g)	0.2	0.2	0.7	50
Ca				1200
From eggshell (mg)	500			
purified CaCO ₃ (mg)		500		
from milk (mg)			25	
Mg (mg)	175	175	2.8	320
Zn (mg)	5	5	0.08	12
Si (μ g)	1000	1000	about 70	†
Vitamin A (μ g RE)	160	160	3	800
Cholecalciferol (μ g)	5	5	0.005	10
Vitamin E (mg α -TE)	5	5	0.007	8
Phylloquinone (μ g)	40	40	0.23	65
Vitamin C (mg)	30	30	0.12	60
Thiamine (μ g)	700	700	4.6	1000
Riboflavin (μ g)	800	800	35	1200
Vitamin B ₆ (μ g)	1000	1000	9.2	1600
Vitamin B ₁₂ (μ g)	0.5	0.5	0.09	2.0

RDA, recommended dietary allowances for women aged 51–70 years (National Research Council, 1989; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997); RE, retinol equivalents; α -TE, α -tocopherol equivalents.

*For details of subjects and procedures, see Table 1 and pp. 268–269.

†No RDA has been given. Si levels in cows' milk were found to be about 400 μ g/l (Anderson, 1992).

after extraction with hydroxyapatite, respectively (IRMA; Medgenix-H-Ost, Fleurus, Belgium; intra- and inter-assay CV were 3.1% and 6.4%, respectively). Carboxylated osteocalcin is calculated as the difference between under-carboxylated osteocalcin and total osteocalcin. Calcitonin (RIA; Nichols Institute, San Juan Capistrano, CA, USA; intra- and inter-assay CV were 3.5% and 6.3% respectively), intact PTH (IRMA; Nichols Institute; intra- and inter-assay CV were 3.5% and 6.0%, respectively) and 25(OH)D (competitive protein binding assay (Chen *et al.* 1990); intra- and inter-assay CV were 10.0% and 16.0%, respectively) were determined because they are important (pre-) hormones in bone metabolism. Urine was analysed for Ca, phosphate, creatinine (Mega, Merck or Vitros; Johnson & Johnson) and for D-Pyr (HPLC (Beardsworth *et al.* 1990); intra- and inter-assay CV was 6.0%, the CV within and between 3-week analyses were <12% and <15%, respectively). The urinary excretion of D-Pyr and Ca was corrected for urinary creatinine. Premenopausal D-Pyr reference value (University Hospital Groningen) was 11.7 $\mu\text{mol/mol}$ creatinine (95% CI 6.8, 23.1).

Statistics

Sample size per group was calculated based upon a small pilot study (Schaafsma & Pakan, 1999). For this study, we calculated that at a 95% level of significance and 90% power, twenty-one women per group were needed to detect a difference of 1.4% in BMD of the femoral neck between intervention and placebo group. We aimed to include twenty-five women per group.

Allocation to the different intervention groups was based on computer-generated random numbers. Data were analysed using SPSS 7.0 (SPSS Inc., Chicago, USA).

Before the main analysis, P1NP, body weight and post-menopausal years at baseline were identified as potential confounders by forward multiple regression. However, the variables appeared not to be of significant importance in the univariate general linear model, using the change

in femoral neck BMD as dependent variable. Therefore, these confounders were not taken into consideration during the main analysis, in order to keep the model as simple as possible.

Some of the parameters (post-menopausal age, weight, BMI, P1NP, calcitonin, PTH, and BMD lumbar spine) were found to have non-parametric distributions according to the Kolmogorov–Smirnov normality test. For that reason, all parameters were evaluated with non-parametric, two-tailed statistical tests. Differences between the groups were studied with the Kruskal–Wallis test combined with the Mann–Whitney U-test. Differences between repeated measurements within the groups were identified with the Wilcoxon signed-rank test. Associations between baseline values of parameters and their changes after 12 months were studied with the Spearman rank test. Differences were considered significant when $P \leq 0.05$. Changes in parameters were calculated as percentage change from baseline.

Results

Age, compliance, calcium intake and routine parameters

Although the women were recruited from the same population and randomly allocated to one of the three groups, women in groups A and B were significantly younger than those in group C. Furthermore, group A had a significantly lower post-menopausal age compared with group C (Table 1). The number of women with a post-menopausal age less than 10 years in groups A, B and C accounted for 58, 45 and 15%, respectively. These differences in age and post-menopausal age, however, were not associated (Spearman rank test) with changes in BMD at any site. Mean compliance with intervention in groups A, B and C was 97 (range 90.8–100)%, 98.4 (93.4–100)% and 97 (82.4–100)%, respectively. The calculated Ca intake of the participants in the present study was unexpectedly high (1294 (SD 421) mg/d) when compared with the mean Dutch (Dutch National Food Consumption

Table 3. Bone mineral densities (g/cm^2) at baseline and their percentage changes after 12 months of intervention*

(Mean values with their standard deviations)

Group...	A		B		C	
	Mean	SD	Mean	SD	Mean	SD
Site of measurement						
Lumbar spine: Baseline	0.936	0.105	0.966	0.099	0.968	0.129
Change (%)	0.10	2.14	0.52	2.07	0.13	2.31
Femoral neck: Baseline	0.741	0.095	0.741	0.095	0.792	0.114
Change (%)	1.75††	3.91	1.16	5.09	-0.60	3.50
Total proximal femur: Baseline	0.864	0.099	0.883	0.121	0.917	0.120
Change (%)	0.23	3.59	0.23	3.57	-0.14	4.34
Trochanter: Baseline	0.649	0.073	0.671	0.092	0.696	0.091
Change (%)	-0.31	3.00	-0.46	5.24	0.57	6.47
Intertrochanter: Baseline	1.016	0.126	1.038	0.156	1.073	0.159
Change (%)	0.20	3.94	0.29	4.53	-0.28	4.85
Ward's triangle: Baseline	0.592	0.138	0.539	0.125	0.609	0.129
Change (%)	3.38††	5.07	4.08	10.76	-0.50	6.24

* For details of subjects and procedure, see Tables 1 and 2 and pp. 268–269.

Mean values at 12 months were significantly different from those at baseline: † $P=0.013$, †† $P=0.006$.

Mean change at 12 months was significantly different from that of group C: ‡ $P=0.014$.

Survey, 1998) daily Ca intake by 50–65-year-old women (995 mg). Habitual Ca intake did not differ between the groups at baseline and did not change during the study. No significant differences were found in body weight, height, and BMI between the groups at baseline (Table 1) nor were changes observed during the study. Serum concentrations of Ca, phosphate, creatinine, albumin and the enzymes γ glutamyl transferase, alanine amino transferase and aspartate amino transferase also did not change during the study (result not shown).

Bone mineral density

Despite the age differences between the groups, BMD baseline values were not significantly different (Table 3). After 12 months of intervention, BMD of femoral neck and of Ward's triangle had increased significantly in group A compared with baseline. No significant changes were seen in BMD at other sites nor in groups B and C. No differences were found between groups A and B. The increase in BMD of the femoral neck in group A was significantly different from the decrease in group C, which is in accordance with the calculated statistical power of the study.

Effects of baseline BMD on BMD changes after 12 months were studied for the combined groups A and B, since these groups only differed in Ca source. Low baseline BMD in groups A and B were associated ($P < 0.01$) with larger increases or smaller losses after 12 months in case of the intertrochanter ($r -0.45$), total proximal femur ($r -0.39$), and the Ward's triangle ($r -0.41$). In contrast, lumbar spine BMD increased more or decreased less when baseline BMD was higher ($r 0.39$, $P < 0.01$). No

significant correlations were found for the femoral neck and trochanter.

Biochemical markers

Baseline values of biochemical markers and changes (%) after 12 months are reported in Table 4. Only one woman in group C had a marginal (25–40 nmol/l (Vieth, 1999)) 25(OH)D level at baseline. All three groups had increased their serum 25(OH)D levels significantly at the end of the study. For group C, the increase is probably due to the summer, since this group was not supplemented with vitamin D. During the winter months, the mean 25(OH)D level in group C decreased by 26% to 67 nmol/l. Both intervention groups did not show a winter dip of 25(OH)D. In both supplemented groups, serum levels of P1NP and PTH decreased significantly compared with baseline. The percentage changes in P1NP and PTH were also different from the percentage changes in group C. No differences were found in levels of total osteocalcin. Carboxylated osteocalcin increased during the study in group B. A significant decrease in D-Pyr excretion was found in group A. This group also had the highest baseline excretion of D-Pyr. Group C showed a small but significant increase in bone-specific alkaline phosphatase compared with baseline and compared with groups A and B. No differences were found between groups A and B in changes and absolute levels of biochemical parameters at 12 months. Only at 6 months was the decrease in D-Pyr significantly ($P = 0.04$) greater in group A compared with group B (result not shown).

When groups A and B were combined, all baseline

Table 4. Biochemical parameters at baseline and percentage changes from baseline after 12 months of intervention* (Mean values with their standard deviations)

Group...	A		B		C		P value†	
	Mean	SD	Mean	SD	Mean	SD	A v. C	B v. C
b-ALP (U/l) Baseline	16.2	4.5	17.4	6.4	14.8	3.8		
Change (%)	2.2	12.5	-2.8	21.6	9.9###	13.3	0.015	0.012
P1NP (μ g/l) Baseline	45.9	14.3	47.2	20.5	37.0	13.3		
Change (%)	-17.6##	23.2	-18.2###	19.5	-7.0	21.0	0.029	0.015
Calcitonin (ng/l) Baseline	8.1	3.5	8.0	2.6	6.4	2.5		
Change (%)	8.7	37.4	-4.0	17.7	8.4	25.5		
Intact-PTH (pmol/l) Baseline	5.5	1.3	5.8	1.9	5.4	1.7		
Change (%)	-15.4‡	26.6	-19.5‡	42.6	6.5	35.3	0.019	0.010
Calcidiol (nmol/l) Baseline	97.1	24.1	83.1	22.4	91.0	36.5		
Change (%)	25.1###	29.8	43.8###	27.3	11.1‡	22.7		0.000
D-Pyr (μ mol/mol Creatinine) Baseline	15.0	4.3	12.6	3.2	12.2	3.8	0.023	
Change (%)	-14.9##	25.1	-2.4	26.9	1.6	23.6	0.011	
%carbOC (%) Baseline	67.3	9.0	67.1	7.4	66.4	10.2		
Change (%)	5.0	12.3	6.9‡	14.3	-1.8	15.8		0.014
Total osteocalcin (μ g/l) Baseline	13.9	5.2	13.9	4.1	12.4	4.4		
Change (%)	-1.7	26.7	-2.4	24.8	0.4	17.7		
Urinary Ca:creatinine (mol/mol) Baseline	0.44	0.31	0.42	0.18	0.37	0.23		
Change (%)	10.9	70.9	23.4	74.5	-0.2	56.7		

b-ALP, bone-specific alkaline phosphatase; P1NP, amino-terminal propeptide extension of type I collagen; PTH, parathyroid hormone; D-Pyr, deoxypridinoline; %carbOC, carboxylated osteocalcin.

* For details of subjects and procedures, see Tables 1 and 2 and pp. 268–269.

† Mann-Whitney U test between the groups.

Baseline and 12 month levels of parameters were significantly different within the group † $P \leq 0.05$, ## $P \leq 0.01$, ### $P \leq 0.001$ (Wilcoxon signed rank test).

values of biochemical markers were significantly and negatively correlated ($r -0.33$ to -0.80) with the changes after 6 (except for calcitonin) and 12 months of supplementation. For the parameters P1NP, D-Pyr and PTH this means that decreases were stronger where baseline values were higher (Fig. 1). No correlation was found between baseline PTH and baseline 25(OH)D, nor between their changes after 12 months of study. However, in group B alone, larger increases in 25(OH)D during the study were associated with larger decreases in PTH ($r -0.42$, $P \leq 0.05$).

Only two significant correlations were found between BMD and biochemical parameters in the combined groups A and B. Firstly, the increase in femoral neck BMD was associated with a decrease in D-Pyr excretion

($r -0.32$, $P < 0.05$). Secondly, BMD of the trochanter showed a higher increase or a smaller decrease when serum PTH decreased more ($r -0.34$, $P < 0.05$). These correlations were especially an effect of group B.

Discussion

We investigated the effects of daily supplements with different Ca sources (chicken eggshell powder: group A, $n 24$; purified CaCO_3 : group B, $n 22$) but equal amounts of cholecalciferol, phylloquinone, Mg and other vitamins and minerals (Table 2), in healthy post-menopausal women aged 50–70 years (Table 1) during 1 year. Untreated post-menopausal women (group C, $n 27$) served as controls. The major finding at the study end

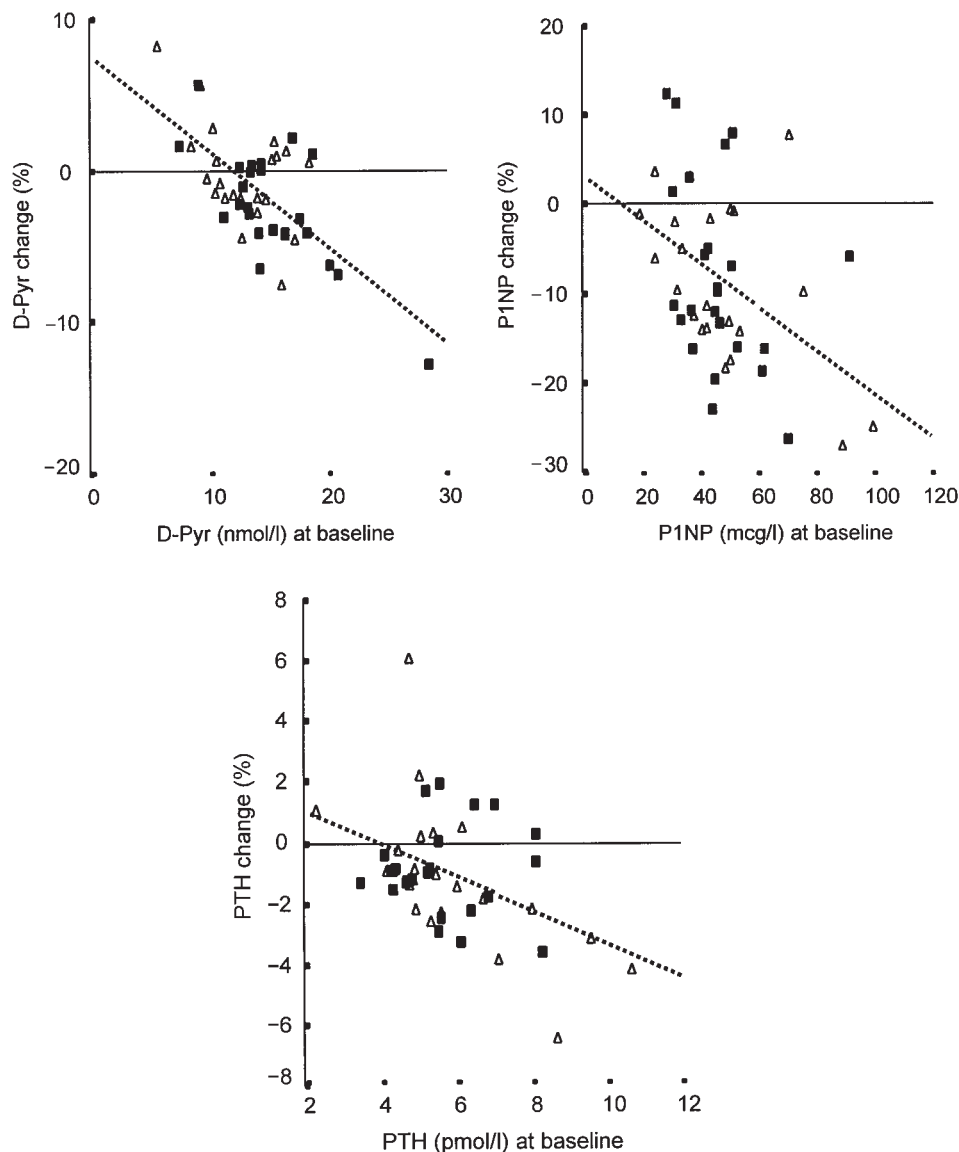


Fig. 1. Correlations between baseline levels of deoxypyrimidoline (D-Pyr; $R^2 0.46$, $r -0.46$, $P < 0.01$), amino-terminal propeptide extension of type I collagen (P1NP; $R^2 0.18$, $r -0.42$, $P < 0.01$) and parathyroid hormone (PTH; $R^2 0.19$, $r -0.36$, $P < 0.05$) and their percent changes after 12 months of intervention. Changes in parameters were obtained by subtracting baseline from 12 month values. The calculated difference was expressed as a percentage from baseline. For a description of the groups A and B, see Table 1; for the supplements, see Table 2. The regression line (---) accounts for the combined groups A and B. (■) Group A; (△) Group B.

was an increased BMD of the femoral neck in group A compared with group C. Compared with baseline, group A exhibited an increase of BMD in the Ward's triangle and femoral neck (Table 3). Changes in BMD were not different between groups A and B. Both groups exhibited changes in serum markers of bone resorption (D-Pyr), bone formation (PINP) and the 25(OH)D-PTH axis (Table 4), all together suggesting a reduction of bone turnover. Since both an increase of BMD and a reduction of bone turnover (Lauritzen, 1996; Kanis, 1999) are associated with increased bone strength and reduced fracture risk (Compston, 1995; Dawson-Hughes, 1998), we conclude that the supplements used in groups A and B are likely to be beneficial for bone health. It must be pointed out that BMD changes did not reach significance in group B, which might be due to somewhat smaller subject numbers.

Negative correlations between baseline BMD of the hip and changes after 12 months indicate a higher responsiveness of women with low baseline BMD to additional minerals and vitamins. Biochemical parameters of bone turnover also showed such negative correlations between baseline and changes after 12 months (Fig. 1). These associations suggest that especially women with high bone turnover and/or lower baseline BMD of the hip benefited from the supplementation. The encountered positive correlation between baseline BMD of the lumbar spine and change after 12 months can possibly be explained by a higher transient remodelling effect (Heaney, 1996). It is possible that a higher BMD accounts for a larger surface area of trabecular bone and consequently more resorption cavities to fill.

Although increases in BMD of the femoral neck and Ward's triangle in group A suggest an increased femoral strength we did not observe effects on BMD of the trochanter. This might be of importance, since the strength of the femoral neck seems rather predicted by an increase in BMD of the trochanter than by that of the femoral neck itself (Cheng *et al.* 1997). Our results are in line with those of Reid *et al.* (1995), who also found no changes in trochanter BMD, following a 1 year supplementation of women 10 years past menopause with 1000 mg Ca/d. A slight increase from baseline was however noticed after 4 years of supplementation. It is therefore possible that prolonged use of the present supplements may also have favourable effects on trochanter BMD.

Both the Ward's triangle and lumbar spine are mostly composed of trabecular bone, whereas the femoral neck contains more cortical bone (Wood & Fleet, 1998). Trabecular bone often responds better to Ca supplementation than cortical bone (Kanis, 1999), and it is therefore not clear why we merely observed increases in BMD of the Ward's triangle and femoral neck and not of the lumbar spine. The relatively low post-menopausal age of the present study population might be a factor in this respect. It is known that oestrogen deficiency during the first 5–10 years past menopause results primarily in a loss of trabecular bone and this loss is poorly responsive to nutritional intervention (Compston, 1995; Ohta, *et al.* 1996). Studies (Dawson-Hughes *et al.* 1990; Nelson *et al.* 1991) with 3–13 years post-menopausal women support this statement

but also show that the effect of oestrogen deficiency on trabecular bone might even persist longer than 10 years past menopause. Fifty-eight percent of group A and 45 % of group B had post-menopausal ages below 10 years. This interpretation does not, however, provide us with an explanation for the observed increase of Ward's triangle BMD. It is also possible that the increase of femoral neck BMD, but not the lumbar spine, is caused by an increased intake of Mg and/or a decreased serum level of PTH. Mg supplementation of post-menopausal women with 500 mg Mg/d for 2 years gave rise to an increase of especially the BMD of the femoral neck (Rude & Olerich, 1996). A high PTH level in post-menopausal women is caused by oestrogen deficiency, low vitamin D status and/or low Ca intake and is regarded to decrease especially BMD of cortical bone (Compston, 1995; McKenna & Freaney, 1998). In this respect it is important to note that serum 25(OH)D levels in the supplemented groups reached values above 100 nmol/l, which did not decline during the winter months. These levels are regarded to cause the lowest achievable PTH levels at a given Ca intake (McKenna & Freaney, 1998). Furthermore, the decrease of PTH may partially be due to the increased Ca intake (up to 2300 mg/d), since a daily intake of over 2000 mg Ca by elderly women was found to reduce PTH to premenopausal values (McKane *et al.* 1996).

Directly after menopause, bone turnover increases with an excess of bone resorption (Allolio, 1999) reflected in a higher urinary D-Pyr excretion. The older women in the placebo group therefore probably lost less bone than was to be expected in the younger women in group B and group A in particular. In agreement with their lower post-menopausal age, higher D-Pyr levels in group A at baseline, compared with group C (Table 4), probably indicate a higher bone turnover. At the same time, the lower levels of biochemical markers in group C may be explained by a gradual decrease of oestrogen deficiency-related bone turnover with advancing post-menopausal age (Ohta *et al.* 1996). The urinary D-Pyr excretion in group A decreased within 6 months to levels found in groups B and C, which were close to premenopausal reference values as used by the University Hospital of Groningen. Both groups A and B showed a decrease of PINP levels, which is a marker of bone formation and therefore of bone turnover. Taken together these data suggest that the supplements taken by groups A and B, reduce bone turnover. Recently it has been reported that short-term changes in biochemical markers may predict long-term changes in BMD (Ravn *et al.* 1999). The supplements are therefore likely to change the balance of bone resorption and bone formation towards the latter especially when baseline turnover is high. This effect is shown particularly in group A with increases in BMD at certain sites.

Although the focus of this discussion is on Ca, Mg, cholecalciferol and phyloquinone, other vitamins and minerals were present, and we cannot exclude that they did not influence the final results in some way. However, effects can be explained for the major part by the four key ingredients (Schaafsma *et al.* 2001). A second point of discussion is the compositional difference between chicken eggshell powder and purified CaCO₃. A beneficial

characteristic of chicken eggshell powder may be the relatively high levels of Sr; normal daily Sr intake increases 15–40%/g eggshell powder consumed. Although this chronic loading with Sr may be stimulatory for bone formation in the long-term, the intakes are 400 to 800 times lower than the amounts used in therapeutic studies (Schaafsma *et al.* 2001).

We conclude that in healthy women, close to 10 years past menopause, supplementation with minerals and vitamins, and Ca, Mg, vitamin D and vitamin K in particular, increases BMD of the femoral neck and Ward's triangle. Favourable effects on BMD are supported by decreases in parameters of bone turnover. The present study also suggests, based on changes from baseline and compared with placebo, that eggshell powder may be preferred over purified CaCO₃ as a source of Ca. This suggestion, however, warrants further research with statistical power based on the outcome of the present study.

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