Original Article

Impact of supplementary high calcium milk with additional magnesium on parathyroid hormone and biochemical markers of bone turnover in postmenopausal women

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The aim of this study was to investigate the impact of magnesium-enriched, high-calcium milk on serum parathyroid hormone (PTH) and biochemical markers of bone turnover in postmenopausal women. We recruited 50 healthy postmenopausal women to take part in this randomised controlled study. Half of the women consumed two serves of high-calcium skim milk enriched with magnesium (milk group) and half consumed two serves apple drink per day (apple group), each for 4 weeks. The milk provided 1200 mg calcium and an additional 106 mg magnesium. We investigated the responses of serum PTH, as well as the serum and urinary calcium, magnesium and biochemical markers of bone turnover. There was no effect of time or drink on the clinical biochemistry, serum PTH or urine markers of bone resorption (free deoxypyridinoline and N-telopeptides). Serum C-telopeptides (CTX), another marker of bone resorption, did not change with time in the apple group. However, in the milk group, serum CTX decreased significantly from 0.43 ± 0.04 ng/mL to 0.32 ± 0.02 at 2 weeks ($p < 0.0001$) and 0.28 ± 0.02 at 4 weeks ($p < 0.0001$). In the milk group, urinary calcium and magnesium each increased during the night but not during the day. Overall, these data suggest that milk has an antiresorptive effect on bone, but that this is not accompanied by measurable changes in serum PTH.

Key words: apple, bone turnover, calcium, magnesium, milk, parathyroid hormone, postmenopausal women.

Introduction

It is well established that elemental calcium suppresses bone resorption.[1–9] Increased calcium intake through milk has also been shown to decrease both parathyroid hormone (PTH) and bone resorption in adult men and women,[10] although these responses were not seen in adolescent girls after 6, 12 or 18 months of milk supplementation.[11] As well as calcium, milk is a good source of magnesium and potassium. A high intake of these minerals is associated with a high bone-mineral density.[12–16]

The present study was designed to investigate the PTH response, and those of biochemical markers of bone resorption (serum C-telopeptides (CTX) and urinary free deoxypyridinoline and N-telopeptides) and bone formation (serum amino-terminal procollagen 1 extension peptides (P1NP)), to 4 weeks of high-calcium milk supplemented with magnesium, compared with an apple drink.

Materials and methods

Experimental subjects

Fifty healthy women who were at least 5 years postmenopausal were recruited. They had not taken any mineral supplements or pharmaceutical therapy known to affect bone metabolism for the 3 years before the study. The women each gave written, informed consent to take part in the study, which was approved by the Human Ethics Committee of Massey University, Palmerston North, New Zealand.

Anthropometry

Body weight was measured using a beam balance (Detecto, Cardinal Scale Manufacturing, MO, USA) to the nearest 0.2 kg and standing height was measured using a stadiometer (Institute of Fundamental Sciences, Engineering Services Workshop, Massey University) to the nearest 0.1 cm. Waist and hip circumferences were measured to the nearest 0.1 cm using a non-stretch measuring tape. Body fat (% body weight) was derived from total body bioelectrical impedance (Biodynamics Model 310, Seattle, WA, USA) after at least

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3 h without food or drink. The maximal handgrip of the non-dominant arm was measured to the nearest 0.5 kg using a conventional hydraulic handgrip dynamometer (Jamar™, Sammons Preston, Bolingbrook, IL, USA).

Food intake was assessed twice; once at the start and again in the third week of the intervention by 24 h food intake recalls. The dietary composition of macro- and micronutrient content food intakes were subsequently taken from the New Zealand Food Composition Table, which we accessed using nutrient analysis software (FOODworks v2, Xyris Software (Australia), Highgate Hill, QLD, Australia).

Intervention

The volunteers were allocated randomly to either a control group or a treatment group. The control group consumed an apple drink containing no more than 25% apple juice (No Frills™, Franklins, Chullora, NSW, Australia), while the intervention group consumed reconstituted, high-calcium skim milk powder (Anlene Gold, New Zealand Milk, Wellington, New Zealand) supplemented with magnesium. This was manufactured at the New Zealand Dairy Research Institute, Palmerston North, by dry blending the high calcium skim milk powder with magnesium oxide (Kirsch Pharma, Salzgitter, Germany). The final product contained 1200 mg calcium and 172 mg magnesium per 50 g milk powder (two serves). The milk was provided in single-serve sachets, with each serve providing 25 g milk powder. The volunteers were asked to reconstitute each sachet of milk powder with 200 mL tap water. The calcium content of drinking water in Palmerston North is <36 mg/L. Therefore, the tap water used to reconstitute the milk powder would have provided <7 mg calcium.

The women were asked to consume two serves/day (2 × 200 mL) of the drink that we provided (apple or milk), with one serve taken early in the evening (at approximately 18:30 h) and the second just before going to bed (at approximately 22:00 h). The volunteers were asked to keep a diary in which they recorded the actual time they consumed each drink, and any occasion when they missed a drink.

Protocol

Biochemical measurements of blood and urine were made at the start and again after 2 and 4 weeks of each 4-week period. A venous blood sample was taken at approximately 08.30 h, after fasting for at least 12 h. The actual time of blood collection was recorded. The blood was kept cold (<8°C) until it was spun at 1560 x g. for 10 min. A number of biochemical measurements were made on the same day using fresh serum, and the remaining supernatant material was removed and frozen at –70°C until it was analysed. Two timed 24 h urine collections were made on consecutive days, at the start and after 2 and 4 weeks. The samples were divided into night (time spent in bed) and daytime collection periods. The urine was kept dark and cool in insulated containers until it was taken to the laboratory and frozen until subsequent analysis.

Biochemistry

Serum calcium, magnesium, phosphorus, albumin, sodium, potassium and creatinine were measured using commercially available kits by spectrophotometry (Boehringer/Hitachi system, Roche Diagnostics, Basel, Switzerland). Serum PTH was measured by immunradiometric assay (IRMA) using the Gamma-BCT intact PTH kit (Immunodiagnostics Systems, Boldon, UK). CTX in serum were measured by electrochemiluminescence immunoassay using the Roche Elecsys 2010 system and a commercially available kit for α-crosslaps (Roche Diagnostics, Mannheim, Germany). Urine N-telopeptide cross-links of type I collagen (NTX) was measured by enzyme-linked immunosorbent assay (ELISA) using the Osteomark kit (Ostex International, Seattle, WA, USA). P1NP were measured in a random subsample of 18 women who consumed the apple drink and 14 women who consumed the milk. It was measured using a monoclonal antibody sandwich-type immunoassay by electrochemiluminescence using the Roche Elecsys 2010 system. Urinary calcium and magnesium were measured by spectrophotometry (Cobas Fara II autoanalyser, Roche Diagnostics, Switzerland) using commercially available kits. Calcium was measured using the Boehringer Mannheim 14893216 kit, and magnesium was measured using the Boehringer Mannheim 1489330 kit (Boehringer-Mannheim, Mannheim, Germany). Urinary free deoxypyridinoline (DPD) was measured by chemiluminescence with a competitive immunoassay kit using the Bayer ACS 180 (Bayer Corporation, Diagnostics Division, NY, USA). Urinary creatinine was measured by a routine automated method on the Vitros 250 (Orthoclinical Diagnostics, Rochester, NY, USA).

Statistical analyses

Comparisons between drinks and the influence of time were evaluated using a crossover repeated-measures analysis of variance (general linear models procedure) and post hoc comparisons of means were carried out using the Tukey–Kramer test. Measurements were considered to be different if $P < 0.05$. Results are expressed as mean ± SD.

Results

Anthropometry and diet

The physical characteristics of the women are shown in Table 1. Except for height, there were no differences in

Table 1. Physical characteristics of the postmenopausal women

Characteristic	Control group	Milk group
Age (years)	68.2 ± 6.9	67.0 ± 6.6
Weight (kg)	69.8 ± 12.3	72.2 ± 12.7
BMI (w/h^2)	27.7 ± 4.6	27.3 ± 4.5
Waist : hip ratio	0.83 ± 0.04	0.83 ± 0.06
Handgrip (kg)	21.1 ± 4.9	23.1 ± 4.5
Body fat (% body weight)	31.6 ± 6.6	30.7 ± 5.6

BMI, body mass index; w/h^2, weight (kg)/height (m^2). Values are mean ± SD, $n = 25$ in each group. There was no statistical difference between the two groups in any of these variables.

any of the anthropometric variables that we measured between the two groups. The women who consumed milk were, on average, 4.1 cm taller than the women who consumed the apple drink, at 162.7 ± 4.8 cm compared with 158.6 ± 5.7 cm ($P < 0.01$).

Table 2 shows the 24 h food intakes of both groups. The milk group consumed significantly more energy, protein, calcium, magnesium, phosphorus, sodium, potassium and zinc than the control group. The baseline calcium intakes were 0.90 ± 0.09 and 0.85 ± 0.09 g/day (±SD) for the apple and milk groups, respectively (not significant).

Time of consuming the drinks

There was no difference between groups in the actual times at which the drinks were consumed. The control group consumed the apple drink at –15 ± 30 min relative to 18:30 h and 6 ± 11 min relative to 22:00 h. The intervention group consumed the milk at –6 ± 26 min relative to 18:30 h and –13 ± 9.7 min relative to 22:00 h.

Compliance

Compliance was virtually 100%. The number of missed serves was 0.9 ± 0.4 (range 0–9 serves) and for the milk it was 0.4 ± 0.2 (range 0–3 serves). There was no difference in compliance between the two groups.

Time of blood samples

There was no difference between groups in the actual times at which the blood samples were taken. The control group blood samples were taken at –3 ± 9 min relative to 08:30 h and the intervention group blood samples were taken at 5 ± 10 min relative to 08:30 h.

Clinical biochemistry

There was no effect of time or treatment on any of the routine clinical biochemical measurements that we made, and all were within the normal reference range (Table 3).

PTH and biochemical markers of bone turnover

There was no significant effect of time or drink on serum PTH, although it tended to be lower after 2 weeks in the milk group (Fig. 1). However, there was a significant effect of time and drink on serum CTX ($P < 0.0001$). There was no difference in baseline serum CTX between the two groups (Fig. 2), but serum CTX decreased significantly after the milk, from 0.43 ± 0.03 at baseline to 0.28 ± 0.02 ng/mL ($P < 0.001$) at 4 weeks. There was no effect of time on serum CTX in the apple group. There was no significant effect of time and drink in either daytime or night time measurements of urinary free DPD or urinary NTX (Table 4). There was no difference in serum P1NP either with time or between

Table 2. Nutrient intakes of the postmenopausal women over 24 h

Nutrient	Apple group		Milk group		*P*-value
	Baseline	3 weeks	Baseline	3 weeks	
Energy (mJ)	6.16 ± 0.30	6.67 ± 0.45	6.65 ± 0.34	7.90 ± 0.44	<0.05
Carbohydrate (g)	184 ± 10	199 ± 17	190 ± 12	214 ± 12	NS
Fat (g)	50 ± 4	55 ± 4	59 ± 4	61 ± 6	NS
Protein (g)	70 ± 5	76 ± 6	72 ± 4	118 ± 5	<0.001
Calcium (g)	0.90 ± 0.09	0.81 ± 0.09	0.85 ± 0.09	2.02 ± 0.08	<0.001
Magnesium (mg)	278 ± 12	268 ± 14	283 ± 17	394 ± 16	<0.001
Phosphorus (g)	1.31 ± 0.89	1.31 ± 0.80	1.35 ± 0.80	2.53 ± 0.85	<0.001
Sodium (g)	1.75 ± 0.11	2.06 ± 0.21	2.09 ± 0.15	2.58 ± 0.20	0.01
Potassium (g)	3.37 ± 0.20	3.09 ± 0.20	3.39 ± 0.20	5.16 ± 0.15	<0.001
Zinc (mg)	9.07 ± 0.64	8.46 ± 0.69	9.57 ± 0.61	14.87 ± 0.83	<0.001

NS, not significant. Values are mean ± SD, $n = 25$ in each group. Where $P = 0.05$, only the 3 weeks value for the milk group is different from the other three measurements.

Table 3. Biochemical analysis of nutrients in the serum of postmenopausal women

Nutrient	Apple group			Milk group		
	Baseline	2 weeks	4 weeks	Baseline	2 weeks	4 weeks
Magnesium (mmol/L)	0.83 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.83 ± 0.01
Calcium (mmol/L)	2.38 ± 0.02	2.35 ± 0.02	2.35 ± 0.02	2.37 ± 0.02	2.36 ± 0.01	2.34 ± 0.02
Albumin (g/L)	41.20 ± 0.50	40.30 ± 0.40	40.00 ± 0.30	40.60 ± 0.40	40.50 ± 0.40	40.30 ± 0.40
Calcium adjusted for albumin (mmol/L)	2.35 ± 0.02	2.34 ± 0.02	2.36 ± 0.02	2.36 ± 0.02	2.35 ± 0.01	2.31 ± 0.03
Phosphorus (mmol/L)	1.13 ± 0.02	1.14 ± 0.02	1.14 ± 0.02	1.20 ± 0.02	1.23 ± 0.02	1.20 ± 0.02
Sodium (mmol/L)	137.90 ± 0.40	138.60 ± 0.30	138.80 ± 0.50	138.40 ± 0.30	138.00 ± 0.30	138.70 ± 0.30
Potassium (mmol/L)	4.46 ± 0.11	4.64 ± 0.09	4.56 ± 0.06	4.36 ± 0.06	4.50 ± 0.05	4.47 ± 0.08
Creatinine (mmol/L)	0.076 ± 0.003	0.073 ± 0.003	0.074 ± 0.003	0.076 ± 0.002	0.073 ± 0.002	0.073 ± 0.003
Total protein (g/L)	71.50 ± 0.80	70.50 ± 0.80	70.60 ± 0.70	71.60 ± 0.80	71.90 ± 0.80	71.50 ± 1.00

Values are mean ± SD, $n = 25$ in each group. There was no statistical difference between the two groups in any of these variables.

Table 4. Biochemical analysis of urinary markers of bone resorption, calcium and magnesium in postmenopausal women

Sample (mmol/L)	Apple group			Milk group		
	Baseline	2 weeks	4 weeks	Baseline	2 weeks	4 weeks
Night free DPD/cr	4.44 ± 0.51[a]	3.61 ± 0.60[a]	4.98 ± 0.41[a]	3.75 ± 0.38[a]	3.31 ± 0.38[a]	4.37 ± 0.45[a]
Day free DPD/cr	3.34 ± 0.39[a]	4.58 ± 0.43[a]	3.95 ± 0.39[a]	4.48 ± 0.47[a]	4.60 ± 0.34[a]	4.06 ± 0.45[a]
Night uNTX/cr	50.67 ± 15.39[a]	53.36 ± 20.32[a]	51.39 ± 17.04[a]	53.24 ± 19.95[a]	50.41 ± 22.45[a]	47.44 ± 23.91[a]
Day uNTX/cr	43.17 ± 10.43[a]	44.09 ± 15.83[a]	41.54 ± 13.31[a]	44.42 ± 14.90[a]	42.39 ± 26.54[a]	42.51 ± 30.05[a]
Night Ca/cr	0.47 ± 0.18[a]	0.36 ± 0.18[b]	0.40 ± 0.17[a]	0.43 ± 0.20[a]	0.52 ± 0.24[c]	0.53 ± 0.20[c]
Day Ca/cr	0.44 ± 0.21[a]	0.37 ± 0.17[b]	0.42 ± 0.20[a]	0.36 ± 0.15[a,b]	0.36 ± 0.16[a,b]	0.36 ± 0.18[a,b]
Night Mg/cr	0.42 ± 0.13[a]	0.42 ± 0.13[a]	0.39 ± 0.13[a]	0.43 ± 0.12[a]	0.56 ± 0.19[b]	0.54 ± 0.18[b]
Day Mg/cr	0.38 ± 0.12[a]	0.36 ± 0.09[a]	0.36 ± 0.12[a]	0.36 ± 0.12[a]	0.37 ± 0.09[a]	0.36 ± 0.14[a]

Ca/cr, calcium (mmol)/creatine (mmol); DPD/cr, deoxypyridinoline/creatinine; Mg/cr, magnesium/creatine; uNTX/cr, urinary N-telopeptides/creatinine. Values are mean ± SD, $n = 25$ in each group. [a–c]Within each row, different superscript letters indicate significant differences ($P < 0.05$) between measurements.

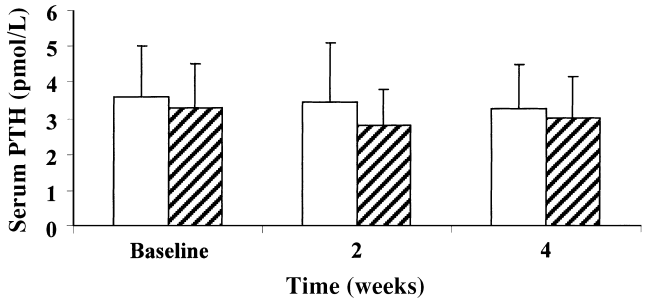

Figure 1. Influence of an apple drink or high-calcium skim milk on serum parathyroid hormone (PTH). $n = 25$ in each group, values are mean ± SD. There was no significant time by treatment effect.

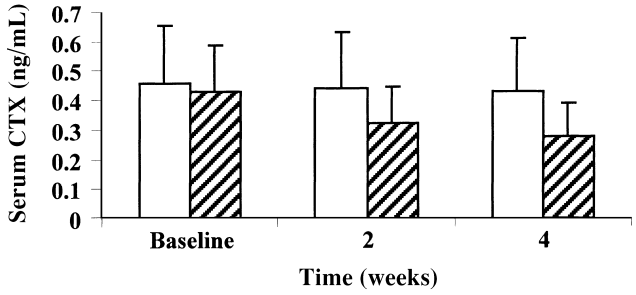

Figure 2. Influence of an apple drink or high calcium skim milk on serum C-telopeptides (CTX). $n = 25$ in each group, values are mean ± SD. There was a significant time by treatment effect ($P < 0.0001$).

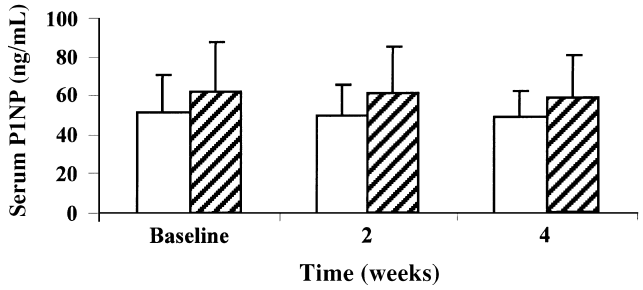

Figure 3. Influence of an apple drink or high calcium skim milk on serum procollagen 1 extension peptide (P1NP). $n = 18$ in the apple group and $n = 14$ in the milk group, values are mean ± SD. There was no significant time by treatment effect ($P = 0.8664$).

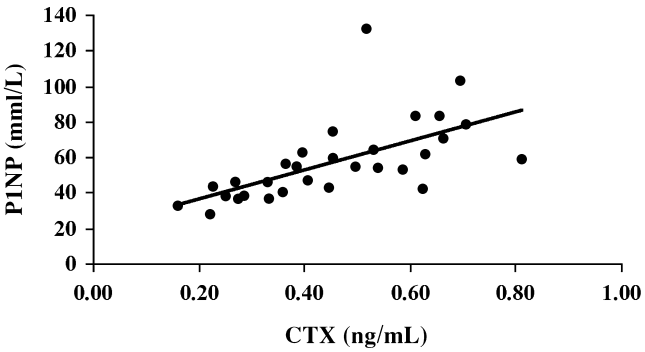

Figure 4. Correlation between baseline C-telopeptides (CTX) and procollagen 1 extension peptide (P1NP). $n = 32$; $r = 0.62$; $P < 0.001$.

treatments ($P = 0.8664$; Fig. 3). Serum P1NP was positively correlated with serum CTX. At baseline, the correlation between serum P1NP and serum CTX for both groups combined ($n = 32$) was 0.62 ($P < 0.001$; Fig. 4).

Urinary calcium and magnesium

The levels of urinary calcium and magnesium are shown in Table 4. There was a significant interaction between time and drink for urinary calcium expressed in relation to creatinine excretion ($P < 0.0001$). There was a significant decrease in calcium excretion, both in the daytime and overnight, after 2 weeks of apple-drink consumption. After milk consumption, urinary calcium excretion increased at night ($P < 0.05$ at 4 weeks) but there was no significant change in daytime urinary calcium excretion. There was no difference in night time excretion of calcium between drinks, either at baseline or at 2 weeks, but at 4 weeks the calcium excretion was significantly higher in the milk group, compared with the apple group ($P < 0.01$). There were no differences in daytime calcium excretion between groups.

There was a significant interaction between time and drink for urinary magnesium expressed in relation to creatinine excretion ($P < 0.0001$). There was no significant difference in

magnesium excretion, either in the daytime or overnight, with time after apple-drink consumption. Urinary magnesium excretion increased at night in the milk group ($P < 0.001$ at 2 weeks and $P = 0.01$ at 4 weeks). There was no difference in baseline night excretion of magnesium between drinks, but at 2 and 4 weeks the magnesium excretion was significantly higher in the milk group when compared with the apple group ($P < 0.0001$ for each). There were no differences in daytime magnesium excretion levels between the groups.

Discussion

The serum CTX data from this study suggest that consuming two serves of high-calcium milk enriched with magnesium decreases bone resorption in postmenpausal women with calcium intakes in the order of 900 mg. This was associated with an increased nocturnal urinary excretion of calcium and magnesium. Daytime excretion of these minerals was not different between the two groups. This suggests that the influence of milk on bone resorption may be limited to the postprandial period and may not persist all day. The fact that we did not also see a decrease in urinary free DPD after milk is consistent with previous findings that free DPD is not sensitive to calcium-mediated inhibition of osteoclast activity.[17] The NTX results suggested that night time measurements tended to be higher than daytime measurements, but this did not reach statistical significance. There was also a tendency for overnight urine NTX to decrease after the consumption of milk, but not after apple drink. Again, this did not reach statistical significance. The discrepancies between the serum and urine markers of bone resorption suggest that, under the circumstances of this study, serum CTX may be a more sensitive marker for bone resorption than either urine free DPD or urine NTX.

The most likely explanation for the mechanism by which milk reduces bone resorption is through increasing serum calcium. An increase in serum calcium would be expected to decrease circulating PTH. However, we did not detect any statistical difference in serum calcium or PTH either between groups or with time in either group in this study. We have previously reported that high-calcium milk, with and without added magnesium, induces a significant postprandial increase in serum calcium and decrease in serum PTH.[18] The results of the present study suggest that these postprandial effects are no longer evident 12 h after consuming the milk. Therefore, in order to assess the physiological impact of milk on serum calcium and PTH it is probably necessary to take samples within the first few hours of consuming it. The fact that PTH was not decreased, despite reductions in serum CTX could indicate that high calcium milk may reduce bone resorption via mechanisms that are not dependent on PTH.

It is unlikely that the additional magnesium in the product that we tested had any impact on bone resorption. Magnesium supplementation transiently decreases both serum PTH and biochemical markers of bone turnover in young people during the first 5–10 days of 30 days of magnesium supplementation, but is no longer evident after 2 weeks.[19–21] We have recently reported no difference in the postprandial PTH and bone resorption responses to either high calcium skim milk or high calcium skim milk enriched with magnesium.[18]

The significant correlation between serum P1NP and serum CTX at baseline is consistent with the coupling of bone formation and bone loss. We did not detect any changes in serum P1NP after either drink. This may be because the duration of our intervention was too short. It is well known that markers of bone formation respond much later than markers of bone resorption.[22] It is also possible that we did not have sufficient statistical power to detect changes in P1NP after supplementary milk consumption, given the limited number of women in whom we made these measurements.

Increasing the dietary intake of milk and other dairy foods is a simple way of improving the diet for bone-health benefits. Cross-sectional studies suggest that frequent milk consumption in adolescence and young adulthood is associated with a higher bone-mineral density at various sites.[23,24] Intervention studies provide experimental evidence that milk or dairy products enhance bone-mineral density in adolescents[26,26] and reduce bone loss in pre- and postmenopausal women.[27,28] There is some evidence to suggest that magnesium supplementation increases bone-mineral density[30,30] and relieves back pain and movement restrictions in osteoporotic patients.[31]

An adequate intake of calcium and magnesium is associated with good bone health, and therefore milk that is fortified with these minerals provides a strategy for improving bone health in the community. This is especially the case for people with habitually low calcium intakes.

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References

1. Goddard M, Young G, Marcus R. Short-term effects of calcium carbonate, lactate, and gluconate on the calcium-parathyroid axis in normal elderly men and women. Am J Clin Nutr 1986; 44: 653–658.
2. Reid IR, Hannan SF, Schooler BA, Ibbertson HK. The acute biochemical effects of four proprietary calcium preparations. Aust NZ J Med 1986; 16: 193–197.
3. Woo J, Swanminathan R, Lau E, MacDonald D, Pang CP, Nordin BEC. Biochemical effects of a single oral dose of calcium on bone metabolism in elderly Chinese women. Calcif Tissue Int 1991; 48: 157–160.
4. Reginster JY, Denis D, Bartsch V, Deroisy R, Zegels B, Franchimont P. Acute biochemical variations induced by four different calcium salts in healthy male volunteers. Osteoporosis Int 1993; 3: 271–275.

5. Yang R-S, Liu T-K, Tsai K-S. The acute metabolic effects of oral tricalcium phosphate and calcium carbonate. Calcif Tissue Int 1994; 55: 335–341.
6. Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomized controlled trial. Am J Med 1995; 98: 331–335.
7. Rubinacci A, Divieti P, Polo RM, Zampino M, Resmini G, Tenni R. Effects of an oral calcium load on urinary markers of collagen breakdown. J Endocrinol Invest 1996; 19: 719–726.
8. Fardellone P, Brazier M, Kamel S, Guéris J, Graulet A-M, Liénard J, Sebert J-L. Biochemical effects of calcium supplementation in postmenopausal women: influence of dietary calcium intake. Am J Clin Nutr, 1998; 67: 1273–1278.
9. Ginty F, Flynn A, Cashman KD. The effect of short-term calcium supplementation on biochemical markers of bone metabolism in healthy young adults. Br J Nutr 1998; 80: 437–443.
10. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI, Rosen CJ. Dietary changes favourably affect bone remodelling in older adults. J Am Diet Assoc 1999; 99: 1228–1233.
11. Cadogen J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ 1997; 315: 1255–1260.
12. Freudenheim JL, Johnson NE, Smith EL. Relationships between usual nutrient intake and bone-mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. Am J Clin Nutr 1986; 44: 863–876.
13. Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. Bone Mineral 1988; 4: 265–277.
14. Tranquilli AL, Lucino E, Garzetti GG, Romanini C. Calcium, phosphorus and magnesium intakes correlate with bone mineral content in postmenopausal women. Gynecol Endocrinol 1994; 8: 55–58.
15. New SA, Boton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross sectional study in premenopausal women. Am J Clin Nutr 1997; 65: 1831–1839.
16. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PFW, Kiel DP. Potassium, magnesium and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 1999; 69: 727–736.
17. Rubinacci A, Melzi R, Zampino M, Soldarini A, Villa I. Total and free deoxypyridinoline after acute osteoclast activity inhibition. Clin Chem 1999; 45: 1510–1516.
18 Green JH, Booth C, Bunning R. Acute effects of high calcium milk with or without additional magnesium, or calcium phosphate on parathyroid hormone and biochemical markers of bone resorption. Eur J Clin Nutr in press.
19. Dimai H-P, Porta S, Wirnsbirger G, Lindschinger M, Pamperl I, Dobnig H, Wilders-Truschnig M, Lau K-HW. Daily oral magnesium supplementation suppresses bone turnover in young adult males. J Endocrinol Metab 1998, 2001; 83: 2742–2748.
20. Doyle L, Flynn A, Cashman K. The effect of magnesium supplementation on biochemical markers of bone metabolism or blood pressure in healthy young adult females. Eur J Clin Nutr 1999; 53: 255–261.
21. Basso LE, Ubbink JB, Delport R, Spies J, Hayward Vermaak WJ. Effect of magnesium supplementation on the fractional intestinal absorption of 45 $CaCl_2$ in women with a low erythrocyte magnesium concentration. Metabolism 2000; 49: 1092–1096.
22. Christenson RH. Biochemical markers of bone metabolism: An Overview. Clin Biochem 1997; 30: 573–593.
23. Murphy S, Khaw K-T, May H, Compston JE. Milk consumption and bone mineral density in middle aged and elderly women. BMJ 1994; 308: 939–941.
24. Soroko S, Holbrook TL, Edelstein S, Barrett-Connor E. Lifetime milk consumption and bone mineral density in older women. Am J Public Health 1994; 84: 1319–1322.
25. Bonjour J-P, Carrie A-L, Ferrari S, Clavien H, Slosman D, Theinz G, Rizzoli R. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest 1997; 99: 1287–1294.
26. Renner E, Hermes M, Stracke H. Bone mineral density of adolescents as affected by calcium intake through milk and milk products. Int Dairy J 1998; 8: 759–764.
27. Baran D, Sorensen A, Grimes J, Lew R, Karallas A, Johnson B, Roche J. Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: a three-year prospective study. J Clin Endocrinol Metab 1990; 70: 264–270.
28. Lau EMC, Woo J, Lam V, Hong A. Milk supplementation of the diet of postmenopausal Chinese women on a low calcium intake retards bone loss. J Bone Min Res 2001; 16: 1704–1709.
29. Abraham GE, Grewal HA. Total dietary program emphasizing magnesium instead of calcium. Effect on the mineral density of calcaneous bone in postmenopausal women on hormonal therapy. J Reprod Med 1990; 35: 503–507.
30. Stendig-Lindberg G, Tepper R, Leichter I. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. Magnesium Res 1993; 6: 155–163.
31. Driessens FCM, Steidl L, Ditmar R. Therapeutic effect of magnesium lactate supplementation on different forms of osteoporosis. Magnesium Bull 1990; 12: 155–157.