Original Article

Postprandial metabolic responses to milk enriched with milk calcium are different from responses to milk enriched with calcium carbonate

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The purpose of this study was to assess whether there are any differences in postprandial physiological responses to skim milk powder enriched with milk calcium (SMP + milk calcium) and skim milk powder enriched with calcium carbonate (SMP + CaCO3), with each of the milks providing 1200 mg calcium. This was a randomised, controlled, crossover study involving 16 men and 29 women over 55 years of age. Measurements of calcium and bone metabolism were taken after an overnight fast before each drink, and postprandially every hour for 8 h. The impact of time and drink on the responses was analysed by repeated measures of analysis of variance. Serum calcium was significantly higher between 2 and 8 h after consumption of SMP + CaCO3 compared with SMP + milk calcium ($P < 0.0001$). Serum phosphate was significantly higher between 2 and 5 h after drinking the SMP + milk calcium compared with SMP + CaCO3 ($P < 0.0001$). The level of parathyroid hormone (PTH) was virtually unchanged after consumption of SMP + milk calcium, but decreased between 1 and 4 h after SMP + CaCO3 ($P = 0.02$). The serum C-telopeptide level, a marker of bone resorption, was significantly lower after SMP + CaCO3, compared with SMP + milk calcium, between 4 and 8 h after drinking the milk ($P < 0.05$). We conclude that serum calcium levels have a higher increase after SMP + CaCO3 consumption than after SMP + milk calcium consumption, and that this is associated with lower serum PTH concentrations and a more prolonged postprandial decrease in bone resorption.

Key Words: bone turnover, calcium, milk, parathyroid hormone, postmenopausal women, pyridinium crosslinks.

Introduction

The bone health benefit of milk is attributed, to some extent, to an inhibition of bone resorption secondary to a decrease in serum parathyroid hormone (PTH) in response to raised serum calcium.[1] A number of studies have looked at the postprandial metabolic effects of different calcium preparations. These studies have consistently found that bone resorption is rapidly suppressed by an acute oral dose of calcium.[2–7] However, there is some controversy over the postprandial bioavailability of different calcium salts. For example, Yang *et al.* reported no difference in acute metabolic responses to calcium carbonate compared with tricalcium phosphate.[6] In contrast, Reginster *et al.*, using a similar experimental protocol, reported that calcium carbonate induced larger increases in serum calcium and suppression of bone resorption than other calcium salts, including tricalcium phosphate.[5] Zikán *et al.* have shown that calcium carbonate suspended in water was more effective in reducing bone resorption than milk enriched with milk calcium.[8] The impact on bone physiology of different sources of calcium, each delivered in milk, is not known. The present study was designed to assess whether there are any differences in postprandial physiological responses to apple drink (control), skim milk enriched with milk calcium and skim milk enriched with calcium carbonate, with each of the milks providing 1200 mg calcium.

Materials and methods

The volunteers were 16 healthy men and 29 healthy women who were all over 55 years of age. The women were at least 5 years postmenopausal. None of the volunteers had taken any pharmaceutical therapy known to affect bone metabolism for the 3 years before the study. All the subjects gave written, informed consent to take part in the study, which was approved by the Manawatu-Whanganui Ethics Committee

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and the Massey University Human Ethics Committee.

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, Palmerston North) to the nearest 0.1 cm. Waist and hip circumferences were measured to the nearest 0.1 cm using a non-stretch measuring tape. Habitual physical activity was assessed using a questionnaire.[9] Although most people were retired from paid employment, we asked them to rate their work as the activity they usually did between 09:00 and 17:00 h. Calcium intake was assessed for the 24 h preceding each study (three times in all) using a short questionnaire.[10]

The volunteers attended the laboratory on three different days, each typically one week apart. They consumed 400mL of a different drink, in random order, on each day. An apple drink containing 25% fruit juice (No Frills™, Franklins, Chullora, NSW, Australia) served as the control drink. The composition of the apple drink is shown in Table 1. The test drinks each provided 1200 mg calcium and were: reconstituted skim milk powder that was enriched with milk calcium (SMP + milk calcium); and reconstituted skim milk powder that was enriched with calcium carbonate (SMP + $CaCO_3$).

The milk powders were manufactured at the New Zealand Dairy Research Institute, Palmerston North, to the specifications required for a commercially available high-calcium milk powder (Anlene™, New Zealand Milk).

The composition of the drinks was independently assessed through the New Zealand Dairy Research Institute, using conventional methods, and data are provided in Table 1. Both milks were given to the volunteers as 50 g powder, which was reconstituted with 400 mL tap water that had been filtered to remove chlorine, pesticides and insecticides. The calcium content of drinking water in Palmerston North is <36 mg/L. The volunteers fasted for at least 12 h before attending the laboratory. The experimental protocol is shown in Fig. 1. Baseline blood samples were taken with an indwelling 20GA intravenous cannula (Insyte, Becton Dickinson, Sandy, Utah, USA) at approximately 09:00 and again 30 min later. The actual time of each blood sample was recorded. The cannula was kept patent by flushing with normal saline (0.9% sodium chloride). The volunteers were then given one of the three test drinks, which they were asked to consume within 15 min They started the drinks at approximately 09: 45h; the actual time was recorded and referred to as time zero. The volunteers consumed the two milks single blind.

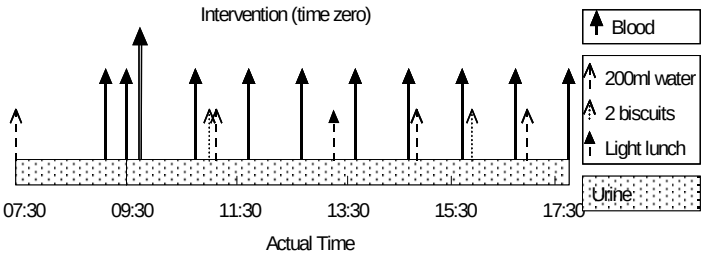

Figure 1. Experimental protocol

Table 1. Composition of the different milk drinks

Ingredient	Method of analysis	Drink consumed		
		Apple	SMP + milk calcium	SMP + $CaCO_3$
Nitrogen (% w/w)	Kjeltec	0.01	5.27	5.19
Fat (% w/w)	Roese-Gottlieb	0.06	0.44	0.46
Lactose as monohydrate (% w/w)	Autoanalyser	–	54.8	53.2
Inorganic phosphate as PO_4 (mmol/kg)	Autoanalyser	0.4	387	220
Citrate (mg/g)	Enzymatic	2.5	12.1	12.1
Calcium (mg/kg)	ICP	25	24 970	26 533
Potassium (mg/kg)	ICP	230	15 133	15 100
Magnesium (mg/kg)	ICP	19	1550	1143
Sodium (mg/kg)	ICP	30	4280	4203
Phosphorus (mg/kg)	ICP	16	16567	9670
Zinc (mg/kg)	ICP	0.1	63.7	39.4
Copper (mg/kg)	ICP	0.05	0.27	0.30
Fructose (% w/w)	GC	2.38	0	0
Galactose (% w/w)	GC	0.12	0.05	0.06
Glucose (% w/w)	GC	1.92	0.07	0.06
Sucrose (% w/w)	GC	3.85	0	0
Lactose (% w/w)	GC	0	53.4	53.3
Maltose (% w/w)	GC	0	0	0.03
Total disaccharides (% w/w)	GC	3.71	54.85	54.85
Vitamin C (mg/100 g)	HPLC	5.5	13.7	13.4
Vitamin D (µg/100 g)	HPLC	0.5	21.4	21.1
Vitamin A (µg/100 g)	HPLC	2	1000	1100

GC, gas chromatography; HPLC, high-performance liquid chromatography; ICP, induced coupled plasma technique–optical emission spectroscopy; SMP, skim milk powder.

Blood samples were taken every hour after time zero. Blood in which PTH was to be measured was kept cold (<6°C) until it was spun at 1560x g for 15 min at 4°C. The remaining blood was kept at room temperature before being spun at 15°C, again at 1560x g for 15 min. All blood samples were spun 2 h after they had been taken. The supernatant material was removed. Serum for routine clinical biochemical measurements was stored at <6°C and the remainder was frozen at –70°C.

The volunteers consumed 800 mL of filtered tap water in 200 mL aliquots at set times during the day, from the time they woke up until the final samples were collected. They were also given two plain biscuits 1.25 h and again 6.25 h after time zero. A light lunch comprising two slices of wholemeal bread, thinly spread with sunflower margarine, and 130 g canned peaches in syrup was consumed after 3.25 h. This food provided an additional 35 mg calcium, 48 mg magnesium, 156 mg phosphorus and 380 mg sodium. The energy content of the meal was 1510 kJ (68% from carbohydrate, 21% from fat and 9% from protein). These nutrient values were obtained using the New Zealand Food Composition Database, which we accessed using nutrient analysis software (FOODworks v2, Xyris Software (Australia), Highgate Hill, QLD, Australia).

Serum calcium, albumin, phosphate, cholesterol, high-density lipoprotein cholesterol and triacylglycerol were measured within 24 h in fresh serum stored at <6°C. Measurements were made in duplicate on a computer-controlled automatic analysis system (Boehringer Mannheim/ Hitachi 917 Rack, Mannheim, Germany) using commercially available kits in the Clinical Services Laboratory at Palmerston North Hospital. We corrected serum calcium for albumin.[11]

Serum PTH was measured in duplicate by immuno-radiometric assay using the Gamma-BCT intact PTH kit (Immunodiagnostics Systems, Boldon, UK), with intra- and interassay coefficients of variation (CV) of <10%. Serum C-telopeptides (CTX) were measured in duplicate by electro-chemiluminescence immunoassay using the Roche Elecsys 2010 system and a commercially available kit for α-crosslaps (Roche Diagnostics, Mannheim, Germany), with an intra-assay CV of <2% and interassay CV of <5%. Serum N-telopeptides (NTX) were measured in duplicate by competitive-inhibition enzyme-linked immunosorbent assay using a commercially available kit (Osteomark, Ostex International, Seattle, WA, USA), with an intra-assay CV of <5% and interassay CV of <7%.

Urinary calcium and creatinine were measured by spectrophotometry (Cobas Fara II autoanalyser, Roche Diagnostics, Basel, Switzerland) using commercially available kits. Urinary sodium and potassium were measured using ion selective electrodes on the Hitachi 704 Automatic analyser (Boehringer-Mannhein) with a commercially available kit.

Secondary outcome variables

In addition to the primary outcome variables that focused on bone health, we also measured a number of secondary outcome variables that focused on heart health. We measured serum lipids at baseline and again 2 and 5 h after consuming each drink.

A trained observer measured arterial blood pressure by conventional sphygmomanometry twice during the baseline period and subsequently every hour after the apple drink and after the SMP +$CaCO_3$. Blood pressure was measured in duplicate with at least 2 min between consecutive readings. Measurements were made approximately 10 min before each blood sample, with the volunteers seated.

Sample size

We assumed that a 30% difference in change in CTX level between the groups (primary outcome variable) would be biologically significant. Using data previously obtained in this laboratory, we estimated this to be 0.08 ng/mL with a standard deviation on the change being 0.11 ng/mL. To detect this difference with a power of 90% and alpha = 0.05, we required 45 subjects to consume each drink.

Statistical analyses

The integrated areas under the curve for the change from mean baseline for each serum measurement was calculated using Simpson's rule. Comparisons between the start time for each drink and comparisons between the areas under the curve were evaluated using univariate analysis of variance by general linear model (GLM), and comparisons of means were carried out using Tukey's pairwise comparisons. This method was also used to assess differences in 24 h calcium intake and changes in mineral excretion rates between studies. These statistical tests were carried out using Minitab statistical software, release 13.1 (Minitab, PA, USA). Comparisons between drinks and the influence of time were evaluated using a crossover repeated-measures analysis of variance fitted using SAS Proc Mixed (SAS Institute, Cary NC, USA), with an autoregressive structure to the measurements within each dietary intervention.

Table 2. Characteristics of subjects involved in this study

	N	Age (years)	Weight (kg)	Body mass index (weight/height^2)	Waist: hip ratio
Women	29	68 ± 1	69.9 ± 2.0	26.8 ± 0.8	0.83 ± 0.01
Men	16	69 ± 2	75.0 ± 1.5	25.2 ± 0.7	0.92 ± 0.01

Table 3. Serum calcium and phosphate values measured after drinking different milk drinks

Time after drink (h)	Drink consumed		
	Apple	SMP + milk calcium	SMP + $CaCO_3$
Total serum calcium (mmol/L)*			
Baseline 1	2.32 ± 0.01	2.30 ± 0.01	2.32 ± 0.01
Baseline 2	2.32 ± 0.01	2.31 ± 0.01	2.32 ± 0.01
1	2.30 ± 0.01	2.31 ± 0.01	2.32 ± 0.01
2	2.31 ± 0.01	2.33 ± 0.01	2.36 ± 0.01
3	2.31 ± 0.01	2.33 ± 0.01	2.38 ± 0.02
4	2.29 ± 0.01	2.33 ± 0.02	2.37 ± 0.02
5	2.28 ± 0.01	2.31 ± 0.02	2.36 ± 0.02
6	2.29 ± 0.01	2.33 ± 0.01	2.37 ± 0.02
7	2.27 ± 0.02	2.30 ± 0.01	2.34 ± 0.02
8	2.29 ± 0.01	2.32 ± 0.01	2.37 ± 0.02
Serum calcium adjusted for albumin (mmol/L)*			
Baseline 1	2.32 ± 0.01	2.30 ± 0.01	2.31 ± 0.01
Baseline 2	2.32 ± 0.01	2.31 ± 0.01	2.32 ± 0.01
1	2.30 ± 0.01	2.32 ± 0.01	2.32 ± 0.01
2	2.31 ± 0.01	2.34 ± 0.01	2.36 ± 0.01
3	2.30 ± 0.01	2.34 ± 0.01	2.38 ± 0.01
4	2.29 ± 0.01	2.34 ± 0.01	2.38 ± 0.01
5	2.28 ± 0.01	2.33 ± 0.01	2.37 ± 0.01
6	2.28 ± 0.01	2.33 ± 0.01	2.37 ± 0.01
7	2.27 ± 0.01	2.31 ± 0.01	2.34 ± 0.01
8	2.28 ± 0.01	2.31 ± 0.01	2.35 ± 0.01
Serum phosphate (mmol/L)*			
Baseline 1	1.11 ± 0.02	1.10 ± 0.02	1.10 ± 0.02
Baseline 2	1.11 ± 0.02	1.10 ± 0.02	1.10 ± 0.02
1	1.04 ± 0.02	1.08 ± 0.02	1.10 ± 0.02
2	1.06 ± 0.02	1.14 ± 0.02	1.10 ± 0.02
3	1.08 ± 0.02	1.24 ± 0.02	1.16 ± 0.02
4	1.09 ± 0.02	1.23 ± 0.02	1.16 ± 0.02
5	1.11 ± 0.02	1.25 ± 0.02	1.20 ± 0.02
6	1.16 ± 0.02	1.26 ± 0.02	1.24 ± 0.02
7	1.14 ± 0.02	1.23 ± 0.02	1.20 ± 0.02
8	1.18 ± 0.02	1.23 ± 0.02	1.22 ± 0.02

Values are mean ± SEM. *Statistical significance between drink and time was $P < 0.0001$. SMP, skim milk powder.

Post-hoc comparisons were carried out using least significant differences, which were calculated by the program. These statistical tests were performed using the SAS system for Windows, release 8.01 (SAS Institute). Measurements were considered to be significantly different if $P < 0.05$. Results are expressed as mean ± and standard errors.

Results

Physical characteristics of the volunteers

The physical characteristics of the volunteers are shown in Table 2. The mean calcium intake was 731 ± 37 mg/day, with reported intakes of 681 ± 43 mg in the 24 h preceding the apple drink study, 718 ± 43 mg in the 24 h preceding the milk calcium study and 795 ± 48 mg in the 24 h preceding the calcium carbonate study. The 24 h calcium intake preceding the calcium carbonate study was significantly higher than for the apple drink ($P<0.05$), but there were no other differences.

The volunteers were generally active, with 22 of the 45 people participating in at least one sporting activity per week. The sporting activities were distributed fairly evenly between swimming, table tennis, walking, bowls, golf and dancing. Over half of the group reported that they never, seldom or sometimes watched television ($n = 29$), whereas only 16 people reported that they watched television often or very often. The Baecke habitual physical activity indices were as follows: work, 2.71 ± 0.05; sport, 2.51 ± 0.11; and leisure, 3.04 ± 0.11.

Baseline times

The actual times when the first blood samples were taken were: apple, 08:58 ± 2 min; SMP + milk calcium, 08:58 ± 2 min; and SMP + $CaCO_3$, 9:01 ± 2 min. There were no differences in the time of the first blood sample between interventions. The actual times when the second blood samples were taken were: apple, 09:38 ± 2 min; SMP + milk calcium, 09:37 ± 2 min; and SMP + $CaCO_3$, 09:38 ± 2 min. There were no differences in the time of the second blood

sample between interventions. The actual times when the drinks were started (time zero) were: apple, 09:47 ± 2 min; SMP + milk calcium, 09:46 ± 2; and SMP + $CaCO_3$, 09:44 ± 2 min. There were no differences in starting times between interventions.

Serum calcium

The type of drink combined with the influence of time (drink*time) had a significant effect on total calcium ($P < 0.0001$), albumin ($P < 0.01$), and serum calcium adjusted for albumin (Ca_{adj}; $P < 0.0001$) (Table 3). Serum Ca_{adj} decreased after the apple drink but increased after each of the milk drinks. Serum Ca_{adj} was significantly higher after consumption of SMP + $CaCO_3$ compared with SMP + milk calcium, between 2 and 8 h after drinking the milk ($P < 0.0001$). Figure 2 shows the changes in serum Ca_{adj}. The data shown were calculated as the difference between the value at each time and the mean of the two baseline measurements. There was a significant effect of drink and time on the change in serum Ca_{adj} ($P < 0.0001$ for drink*time). The increase in serum Ca_{adj} was significantly greater after each of the milk drinks than after the apple drink at each time point ($P = 0.01$ at 1 h and $P < 0.0001$ at each subsequent time for SMP + $CaCO_3$ compared with apple and and for SMP + milk calcium compared with apple). The increase in Ca_{adj} was significantly greater after SMP + $CaCO_3$ compared with SMP + milk calcium between 3 and 8 h after drinking the milk ($P = 0.01$ at each of these times). There was a significant difference in the integrated responses (area under curve) for Ca_{adj} between apple and SMP + milk calcium ($P < 0.0001$), apple and SMP + $CaCO_3$ ($P < 0.0001$) and between SMP + milk calcium and SMP + $CaCO_3$ ($P = 0.002$) (Table 4).

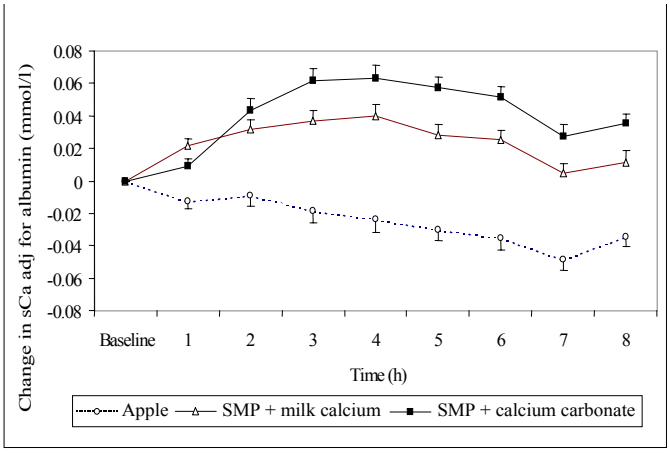

Figure 2. Change in serum calcium adjusted for albumin (values are mean mean ± SEM). Effect of drink by time, P < 0.0001.

Serum phosphate

There was a significant drink*time effect on serum phosphate ($P < 0.0001$) (Table 3). Serum phosphate was significantly higher between 2 and 5 h after the SMP + milk calcium compared with SMP + $CaCO_3$ ($P < 0.0001$ for 2–4 h and $P < 0.01$ at 5 h). There was also a significant drink*time effect on the change in serum phosphate ($P < 0.0001$). The increase in serum phosphate was significantly greater after each of the milk drinks than after the apple drink at each time point, except at 2 h for SMP + $CaCO_3$ compared with apple. The increase in serum phosphate was significantly greater after the SMP + milk calcium compared with SMP + $CaCO_3$ between 2 and 5 h after drinking the milk ($P = 0.001$ at each of these times) and again after 7 h ($P < 0.05$). There was a significant difference in the integrated responses for serum phosphate between apple and SMP + milk calcium ($P < 0.0001$), apple and SMP + $CaCO_3$ ($P < 0.0001$) and between SMP + milk calcium and SMP + $CaCO_3$ ($P = 0.0006$) (Table 4).

Parathyroid hormone

There was a significant drink*time effect on PTH levels ($P < 0.0001$), with PTH increasing after the apple drink ($P = 0.02$ from 1 to 8 h), being virtually unchanged after the SMP + milk calcium, and decreasing between 1 and 4 h after SMP + $CaCO_3$ ($P = 0.02$) (Table 5). Figure 3 shows the changes in serum PTH. There was a significant drink*time effect observed for the change in serum PTH ($P = 0.02$ for drink*time), with serum PTH being significantly lower after SMP + $CaCO_3$ consumption compared with SMP + milk calcium between 3 and 6 h after drinking the milk ($P = 0.05$ at each of these times). There was a significant difference in the integrated responses between apple and SMP + milk calcium ($P < 0.0001$), and between apple and SMP + $CaCO_3$ ($P < 0.0001$). There was no difference in the integrated PTH response between SMP + milk calcium and SMP + $CaCO_3$ ($P = 0.06$), although the difference in PTH between the two milks almost reached a significant level (Table 4).

Table 4. Area under curves

Volunteers	Drink consumed		
	Apple	SMP + milk calcium	SMP + $CaCO_3$
Δ Serum calcium†	–0.20 ± 0.03[a]	0.19 ± 0.04[b]	0.32 ± 0.04[c]
Δ Serum phosphate	–0.05 ± 0.07[a]	0.83 ± 0.10[b]	0.47 ± 0.08[c]
Δ Serum PTH	5.22 ± 0.69[a]	–0.06 ± 0.77[b]	–2.66 ± 0.74[b]
Δ Serum CTX	–0.61 ± 0.09[a]	–1.08 ± 0.09[b]	–1.24 ± 0.10[b]
Δ Serum NTX	–3.88 ± 1.13[a]	–7.17 ± 1.53[a]	–7.53 ± 1.52[a]

†Adjusted for albumin. Different superscript letters ([a–c]) indicate significant differences between measurements ($P < 0.05$). CTX, serum C-telopeptide; NTX, serum N-telopeptide; PTH, parathyroid hormone; SMP, skim milk powder.

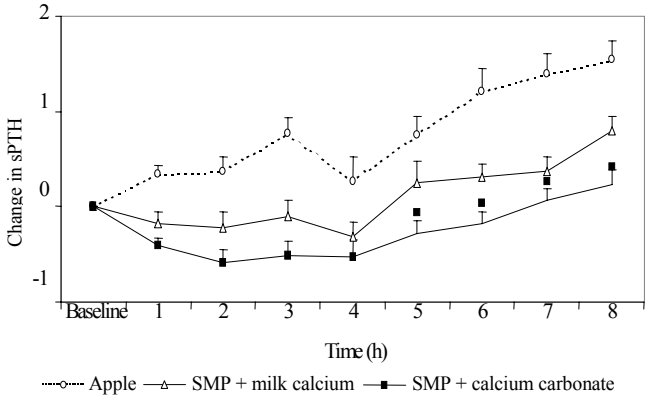

Figure 3. Change in serum parathyroid hormone (PTH) (values are mean ± SEM). Effect of drink by time, $P = 0.02$

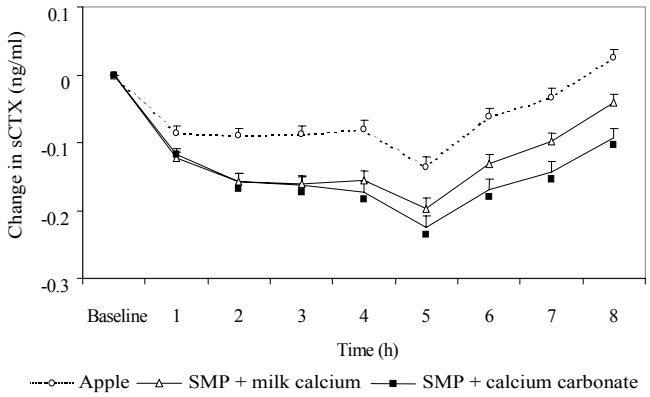

Figure 4. Change in serum C-telopepdtides (CTX) (values are mean ± SEM). Effect of drink by time, $P < 0.0001$.

Table 5. Levels of serum parathyroid hormone (pmol/L) measured after drinking different milk drinks*

Time after drink (h)	Drink consumed		
	Apple	SMP + milk calcium	SMP + $CaCO_3$
Baseline 1	3.18 ± 0.25	3.41 ± 0.27	3.22 ± 0.25
Baseline 2	3.25 ± 0.26	3.57 ± 0.28	3.32 ± 0.25
1	3.55 ± 0.31	3.32 ± 0.30	2.88 ± 0.26
2	3.58 ± 0.31	3.26 ± 0.31	2.74 ± 0.29
3	4.01 ± 0.36	3.39 ± 0.30	2.76 ± 0.28
4	3.61 ± 0.30	3.27 ± 0.31	2.74 ± 0.30
5	4.00 ± 0.36	3.80 ± 0.39	2.98 ± 0.28
6	4.43 ± 0.40	3.78 ± 0.31	3.08 ± 0.27
7	4.61 ± 0.36	3.85 ± 0.29	3.33 ± 0.25
8	4.78 ± 0.37	4.34 ± 0.33	3.52 ± 0.30

Values are mean ± SEM. *Statistical significance between drink by time was $P < 0.0001$. SMP, skim milk powder.

Table 6. Levels of serum C-telopeptides and N-telopeptides measured after drinking different milk drinks

Time after drink (h)	Drink consumed		
	Apple	SMP + milk calcium	SMP + $CaCO_3$
Serum CTX (ng/mL)*			
Baseline 1	0.44 ± 0.03	0.44 ± 0.03	0.42 ± 0.03
Baseline 2	0.43 ± 0.03	0.43 ± 0.03	0.42 ± 0.03
1	0.35 ± 0.03	0.31 ± 0.02	0.30 ± 0.02
2	0.34 ± 0.03	0.28 ± 0.02	0.27 ± 0.02
3	0.34 ± 0.03	0.27 ± 0.02	0.26 ± 0.03
4	0.35 ± 0.03	0.28 ± 0.03	0.25 ± 0.02
5	0.29 ± 0.02	0.23 ± 0.02	0.20 ± 0.02
6	0.37 ± 0.03	0.31 ± 0.03	0.25 ± 0.03
7	0.40 ± 0.03	0.33 ± 0.03	0.28 ± 0.03
8	0.46 ± 0.03	0.39 ± 0.03	0.33 ± 0.03
Serum NTX (nmol/L)**			
Baseline	15.22 ± 1.69	15.61 ± 1.86	14.91 ± 1.55
2	14.52 ± 1.73	13.48 ± 1.66	11.75 ± 1.08
4	13.52 ± 1.66	13.48 ± 1.67	12.53 ± 1.48
6	13.99 ± 1.79	13.57 ± 1.79	12.95 ± 1.50
8	14.88 ± 1.76	13.74 ± 1.17	13.90 ± 1.62

Values are mean ± SEM. Statistical significance between drink by time was *P<0.0001 and **not significant. CTX, serum C-telopeptide; NTX, serum N-telopeptides; SMP, skim milk powder.

Serum CTX and NTX

There was a significant effect of drink*time on serum CTX ($P < 0.0001$) (Table 6). Serum CTX decreased after each drink, but this decrease was significantly greater each time for each of the milks compared with apple ($P = 0.01$). Serum CTX was significantly lower after SMP + $CaCO_3$ compared with SMP + milk calcium between 4 and 8 h after drinking the milk ($P < 0.05$ at 4 h, $P < 0.01$ at 5 h and $P < 0.0001$ at 6–8 h).

Figure 4 shows the changes in serum CTX. There was a significant drink*time effect for changes in serum CTX ($P < 0.0001$). The decrease in serum CTX was significantly greater after each of the milk drinks than after the apple drink at each time point ($P = 0.001$ at 1 h and $P < 0.0001$ at each subsequent time for each of the milk *vs* apple drinks). The decrease in serum CTX was significantly greater after SMP + $CaCO_3$ compared with SMP + milk calcium between 5 and 8h after drinking the milk (P=0.01 at 5h, and P<0.0001 at 6-8 h).

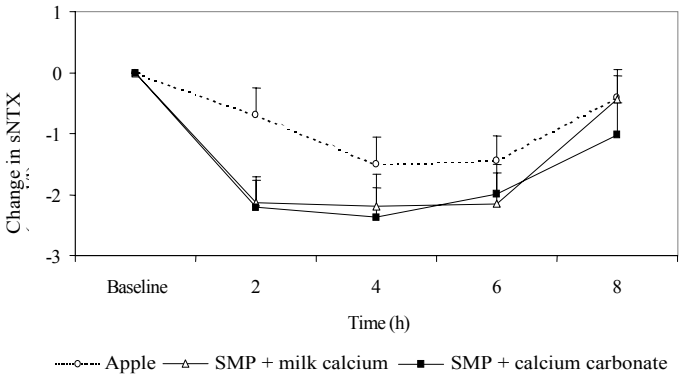

Figure 5. Change in serum N-telopeptides (NTX) (values are mean ± SEM). Effect of drink by time, not significant.

There was a significant difference in the integrated responses between apple and SMP + milk calcium (P<0.0001), and between apple and SMP + $CaCO_3$ (P<0.0001). There was no difference in the integrated CTX response between SMP + milk calcium and SMP + $CaCO_3$ ($P = 0.076$). The latter *P*-value suggests that the integrated response of CTX to SMP + $CaCO_3$ tended to be greater than the response to SMP + milk calcium (Table 4).

There was no significant drink*time effect on serum NTX. The changes in serum NTX are shown in Fig. 5, and the areas under the curve for each drink are show in Table 4. Although there was no significant effect of drink*time for changes in serum NTX, and no significant difference in areas under the curve for each drink, there was a trend for serum NTX to have a greater decrease after each of the milk drinks than after the apple drink.

Urinary calcium, sodium and potassium

The urinary calcium, sodium and potassium responses were not different between the milk drinks, but the responses to each of the milks were different from the responses to the apple drink. Urinary calcium increased by 0.14 ± 0.03 mmol/mmol Cr after SMP + milk calcium and by 0.17 ± 0.03 mmol/mmol Cr after SMP + $CaCO_3$, but decreased by 0.06 ± 0.04 mmol/mmol Cr after the apple drink ($P < 0.0001$). Urinary sodium decreased by 0.70 ± 0.90 mmol/mmol Cr after SMP + milk calcium and by 0.89 ± 0.93 mmol/mmol Cr after SMP + $CaCO_3$, but decreased by 4.35 ± 1.38 mmol/mmol Cr after the apple drink ($P < 0.01$). Urinary potassium decreased by 0.70 ± 0.51 mmol/mmol Cr after SMP + milk calcium and by 0.72 ± 0.49 mmol/mmol Cr after SMP + aCO_3, but decreased by 1.47 ± 0.68 mmol/mmol Cr after the apple drink ($P < 0.05$).

Table 7. Levels of serum lipids measured after drinking different milk drinks

Time after drink (h)	Drink consumed		
	Apple	SMP + milk calcium	SMP + $CaCO_3$
Serum cholesterol (mmol/L)*			
Baseline	5.43 ± 0.12	5.49 ± 0.12	5.43 ± 0.13
2	5.47 ± 0.12	5.37 ± 0.12	5.35 ± 0.12
5	5.37 ± 0.12	5.27 ± 0.12	5.29 ± 0.12
Serum HDL-C (mmol/L)**			
Baseline	1.48 ± 0.05	1.46 ± 0.05	1.51 ± 0.05
2	1.50 ± 0.05	1.44 ± 0.05	1.48 ± 0.05
5	1.45 ± 0.05	1.39 ± 0.05	1.44 ± 0.05
Serum triacylglycerol (mmol/L)*			
Baseline	1.23 ± 0.10	1.25 ± 0.10	1.24 ± 0.10
2	1.18 ± 0.09	1.21 ± 0.10	1.20 ± 0.11
5	1.57 ± 0.11	1.66 ± 0.13	1.64 ± 0.13
Serum LDL-C (mmol/L)			
Baseline	4.66 ± 0.11	4.67 ± 0.12	4.64 ± 0.12
Serum cholesterol : HDL ratio			
Baseline	3.87 ± 0.16	3.95 ± 0.17	3.78 ± 0.16

There was no difference in the baseline values for serum LDL-C or cholesterol : HDL ratio between interventions (univariate ANOVA by GLM). Statistical significance between drink and time was *not significant and **$P < 0.01$. GLM, general linear model; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; SMP, skim milk powder.

Subgroup analyses

Serum calcium adjusted for albumin, PTH and serum CTX were reanalysed by repeated measures analysis of variance, with the program modified to assess whether there were any gender differences. The drink*time effect was not significantly different between men and women for these data.

Secondary outcome variables

Blood lipid data are shown in Table 7. Total serum cholesterol tended to decrease after each of the milk drinks, but overall there was no significant drink*time effect. However, serum high-density lipoprotein cholesterol (HDL-C) increased significantly 2 h after the apple drink ($P < 0.05$), but was significantly lower than baseline after 5 h ($P < 0.001$). In contrast, HDL-C decreased at both 2 h ($P < 0.05$) and 5 h ($P < 0.0001$) after each of the milk drinks. Serum HDL-C was significantly higher at each time point in the SMP + $CaCO_3$ study than in the SMP + milk calcium ($P < 0.01$ at each time point). Serum triacylglycerol increased at 5 h after all three drinks, but there was no drink*time effect. Blood pressure decreased significantly during the study ($P < 0.0001$), but there was no significant drink*time effect (Table 8).

Table 8. Arterial blood pressure measured after drinking different milk drinks

Time after drink (h)	Drink consumed	
	Apple	SMP + $CaCO_3$
Systolic blood pressure (mm/Hg)*		
Baseline 1	142 ± 3	138 ± 3
Baseline 2	136 ± 3	136 ± 3
1	131 ± 3	134 ± 3
2	124 ± 3	126 ± 3
3	127 ± 3	127 ± 3
4	124 ± 3	124 ± 3
5	123 ± 3	124 ± 3
6	124 ± 3	127 ± 3
7	131 ± 3	130 ± 3
8	136 ± 3	138 ± 3
Diastolic blood pressure (mmHg)*		
Baseline 1	83 ± 1	83 ± 2
Baseline 2	80 ± 2	82 ± 2
1	76 ± 2	77 ± 2
2	72 ± 1	73 ± 2
3	74 ± 2	76 ± 2
4	71 ± 2	72 ± 2
5	72 ± 2	73 ± 2
6	74 ± 2	75 ± 2
7	77 ± 2	78 ± 2
8	80 ± 2	81 ± 2

*Statistical difference between drink and time was not significant.
SMP, skim milk powder.

Discussion

We have shown, for the first time, that the physiological responses to two serves of SMP + milk calcium and two serves of SMP + $CaCO_3$ are not equivalent. However, the differences in metabolic responses to the two milk formulations were, biologically, small. The changes in serum calcium suggest that the calcium in SMP + $CaCO_3$ is more rapidly bioavailable than the calcium in SMP + milk calcium. However, this is not to infer differences in fractional absorption between the two drinks, which this study was not designed to test. The more rapid increase in serum calcium may be associated with solubilisation of the different calcium salts in the gastrointestinal tract. Milk calcium tends to be larger and more particulate than $CaCO_3$, which might lead to a slower release of bioavailable calcium for the body, as well as a concomitant lag in calcium uptake and hence a slower response of serum calcium to the beverage. The differences in serum calcium between drinks were associated with expected differences in serum PTH, with lower concentrations seen after SMP + $CaCO_3$ consumption. Our data suggest that SMP + $CaCO_3$ induces a more prolonged postprandial decrease in bone resorption than SMP + milk calcium. We compared the responses to each of the milks against the responses to an apple drink. The small amount of energy from the apple drink may have impacted on bone resorption, and especially on serum CTX because it is strongly influenced by fasting and feeding.[12] This food effect probably explains why serum CTX decreased abruptly but transiently, approximately 1 h after the light lunch on all three laboratory visits.

It would be interesting to know if the short-term metabolic differences in responses to the two products are still evident when the responses are integrated over a full 24 h, or if the differences are to some extent a function of the time of day that the products are consumed. However, to ensure a 24 h reduction in bone resorption, a calcium supplement should be given in a divided dose, with most of it being consumed at night.[13]

The sample size for the present study was planned to give 90% power. This assumed a maximum difference (seen after 5 h) in serum CTX of 0.08 ng/mL between the milk drinks. However, we actually observed a maximum difference of only 0.06 ng/mL, which gives only 73% power. The greatest differences in serum CTX between the milk drinks were between 6 and 8 h. Using data at 8 h, we estimate the power to be 87%, and therefore close to the planned level of 90%. Bone markers are useful for monitoring the effectiveness of antiresorptive therapies for osteoporosis, and early changes in biochemical markers for bone resorption may predict subsequent changes in bone mineral density (BMD) in response to antiresorptive therapy.[14–17] Milk and calcium supplementation are each much more weakly antiresorptive than any of the pharmaceutical therapies and, despite improvements in BMD, may not be associated with changes in bone turnover.[18,19] In the present study, the decrease in serum NTX after each milk drink was not significantly different from the decrease after the apple drink. In contrast, serum CTX did decrease more after each milk drink than

after the apple drink. These results suggest that serum CTX is a more sensitive marker for bone resorption than serum NTX.

Lau *et al.* have recently reported bone mineral density responses to one of the milk powders that we have used in the present study, but bone marker data were not included.[20] They showed that after 2 years of consuming two serves/day of SMP + milk calcium, postmenopausal women displayed a reduced rate of bone loss compared with a matched control group. Because our data demonstrate an antiresorptive effect for both skim milk powder preparations, it is likely that the long-term consumption of either of these products would impact favourably on BMD.

Milk calcium is prepared from whey protein and contains minerals other than just calcium; magnesium and phosphorus in particular. Most nutritional studies have focused on the role of calcium in bone health. However, there is increasing evidence that other micronutrients play a role. The additional magnesium and phosphorus in milk calcium may have their own effects on bone metabolism.

Additional magnesium may have a beneficial effect on bone, although research on the impact of magnesium on biochemical markers of bone turnover is limited. Dimai *et al.* reported that both serum PTH and bone markers were transiently reduced during the first 5–10 days of magnesium supplementation in young men.[21] However, there was no correlation between bone turnover and serum PTH, suggesting that it was unlikely that the decrease in bone resorption was secondary to a decrease in serum PTH. Doyle *et al.* found no significant change in any parameter of bone and calcium metabolism in young women given magnesium supplements for 4 weeks.[22] We have recently found no differences in the postprandial physiological responses (serum calcium, PTH and markers of bone resorption) to high-calcium skim milk with or without additional magnesium in postmenopausal women.[23]

Long-term intervention studies have suggested that supplementary magnesium may increase bone mineral density.[24,25] Also, there are now a number of studies that suggest dietary intake of magnesium is correlated with BMD.[26–30] Tranquilli *et al.* studied 194 women who were all 5–7 years postmenopausal. Seventy of these women were osteopenic (BMD less than 2 SD of mean corrected for sex, age and age at menopause) and these women had significantly lower dietary intakes of magnesium, phosphorus and calcium than the women with normal BMD.[26] Magnesium intakes greater than the recommended amounts were associated with higher bone mineral content. The authors suggested that this was consistent with the involvement of magnesium, both in bone crystal and organic matrix formation.

In addition to having a higher magnesium content, the SMP + milk calcium had a higher phosphorus content than the SMP + $CaCO_3$. Intuitively, one might expect the higher concentration of phosphorus in SMP + milk calcium to be beneficial for bone as phosphate is a major bone constituent. Indeed, supplementation with 1.2 g/day tricalcium phosphate and vitamin D_3 reduced the risk of fracture in elderly women.[31] However, a high phosphorus intake combined with a low calcium intake leads to a persistent increase in serum PTH,[32] which would be expected to be detrimental to bone health. Indeed, almost 25 years ago, Bell *et al.* reported that adults consuming 0.7g calcium/day who switched to a high-phosphate diet (2.1g/day) displayed decreases in serum and urinary calcium, as well as an increase in urinary hydroxyproline (a non-specific marker of bone resorption).[33]

Silverberg *et al.* subsequently showed that oral phosphate (2 g daily for 5 days) led to a decrease in total serum calcium and a 50% increase in serum phosphate.[34] Two more recent studies have reported no change in bone related hormones or bone resorption in response to a high-phosphorus diet.[35,36] However, the participants in both of these studies had adequate or high calcium intakes. Earlier work in sheep has suggested that high-phosphorus diets are unlikely to have an adverse effect on the skeleton if calcium intake is adequate.[37]

These experimental studies suggest the importance of having an adequate calcium intake when phosphate intake is high. Similar conclusions come from population-based cross-sectional studies.[38,39] Brot *et al.* have reported that both calcium and phosphorus intakes are independently correlated with bone mineral density in perimenopausal women. This is perhaps not surprising because they also report a correlation between calcium and phosphorus intake. However, they also showed that the calcium : phosphorus ratio was an independent predictor both of bone mineral content (BMC) and BMD. The relationship between dietary calcium, phosphorus and the calcium: phosphorus ratio is complex. Teegarden *et al.* have provided regression equations for estimating BMC or BMD for given calcium, phosphorus and calcium: phosphorus ratios in young women.[38] Using these equations it can be predicted that for a given calcium intake, increasing the phosphorus intake will lead to a reduction in BMD or BMC. If these equations are applied to the milk formulas that we have studied, then with the same background diet, we can predict that the milk enriched with calcium carbonate should be associated with a higher BMC and BMD.

Heart health indices were of tangential interest in this study. There is some evidence that calcium may act on lipid metabolism[40–43] and on a variety of blood pressure-regulating mechanisms.[44] We observed a small but significant decrease in HDL-C after each of the milk drinks compared with the apple drink, and a trend for cholesterol also to decrease after milk consumption. This may be due to the formation of insoluble calcium soaps in the gut, thereby reducing fat absorption. Any dietary fat would have come from the food that we gave our volunteers and not from the drinks (which were essentially fat free). It would be interesting to determine whether milk has a more marked effect on blood lipids following meals with higher fat contents. Evidence for a role of calcium in the control of blood pressure comes from both epidemiological and intervention studies, but to our knowledge there is no published study of the acute effect of a high calcium load on blood pressure. Despite differences in calcium, sodium and potassium excretion, we did not see any

differences in blood pressure between the apple and milk drinks.

In conclusion, while both milks modified indices of calcium metabolism in ways that would be expected to be beneficial for bone health, our data suggest that the acute physiological responses to SMP + milk calcium are different from the responses to SMP + $CaCO_3$. Our data show that serum calcium is increased to a greater extent after SMP + $CaCO_3$ than it is after SMP + milk calcium, and this is associated with lower serum PTH concentrations and a more prolonged postprandial decrease in bone resorption. Long-term intervention studies are needed to evaluate whether the acute differences in calcium and bone metabolism that we describe result in important differences in BMC and BMD.

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References

1. Heaney RP. Calcium, dairy products and osteoporosis. J Am Coll Nutr 2000; 19: 83S–99S.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
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. Zikán V, Roubal P, Haas T, Štepán JJ. Acute effects of calcium carbonate and milk on the calcium-parathyroid axis and bone resorption in healthy women. In: Burckhardt P, Dawson-Hughes B, Heaney RP, eds. Nutritional Aspects of Osteoporosis: Proceedings of the 4th International Symposium, Lausanne, May 2000. London: Academic Press, 2001; 131–140.
9. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982; 36: 936–942.
10. Green JH, Booth CL, Bunning RLW. Assessment of a rapid method for assessing adequacy of calcium intake. Asia Pacific J Clin Nutr; 2002; 11: 147-150.
11. Phillips P, Pain R. Correcting the calcium [editorial]. BMJ 1977; 1: 598.
12. Christgau S, Bitsch-Jensen O, Hanover Bjarnason N, Gamwell Henriksen E, Qvist P, Alexandersen P, Bang Henriksen D. Serum crosslaps for monitoring the response in individuals undergoing antiresorptive therapy. Bone 2000; 26: 505–511.
13. Scopacasa F, Need AG, Horowitz M, Wishart JM, Morris HA, Nordin BEC. Effects of dose and timing of calcium supplementation on bone resorption in early menopausal women. Horm Metab Res 2002; 34: 44–47.
14. Herd RJ, Balena R, Blake GM, Ryan PJ, Fogelman I. The prevention of early postmenopausal bone loss by cyclical etidronate therapy: a 2-year double-blind, placebo-controlled study. Am J Med 1997; 103: 92–99.
15. Ravn P, Hosking D, Thomson D, Cizza G, Wasnich RD, McClung M, Yates AJ, Bjarnason NH, Christianson C. Monitoring of alendronate treatment and prediction of effect on bone mass by biochemical markers in the early postmenopausal intervention cohort study. J Endocrinol Metab 1999; 84: 2363–2368.
16. Fink E, Cormier C, Steinmetz P, Kindermans C, Le Bouc Y, Souberbie JC. Differences in the capacity of several biochemical bone markers to assess high bone turnover in early menopause and response to alendronate therapy. Osteoporosis Int 2000; 11: 295–303.
17. Greenspan SL, Rosen HN, Parker RA. Early changes in serum N-telopeptide and C-telopeptide cross-linked collagen type I predict long-term response to alendronate therapy in elderly women. J Clin Endocrinol Metab 2000; 85: 3537–3540.
18. Cadogan 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.
19. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. NEJM 1997; 337: 670–676.
20. Lau EM, 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 Miner Res 2001; 16: 1704–1709.
21. 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; 83: 2742–2748.
22. 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.
23. Green JH, Booth C, Bunning R. Impact of supplementary high calcium milk with additional magnesium on parathyroid hormone and biochemical markers of bone turnover in post-menopausal women. Asia Pacific J Clin Nutr 2002; 11: 268-73.
24. Abraham GE, Grewal H. A total dietary program emphasizing magnesium instead of calcium: Effect on the bone mineral density of calcaneus bone in postmenopausal women on hormonal therapy. J Reprod Med 1990; 35: 503–507.
25. Stendig-Lindberg G, Tepper R, Leichter I. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. Mag Res 1993; 6: 155–163.

26. 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.
27. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, 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.
28. New SA, Bolton-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.
29. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health. Am J Clin Nutr 2000; 71: 142–151.
30. Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. Bone Mineral 1988; 4: 265–277.
31. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ. Vitamin D3 and calcium to prevent hip fractures in elderly women. NEJM 1992; 327: 1637–1642.
32. Calvo MS, Kumar R, Heath H. Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. J Clin Endocrinol Metab 1990; 70: 1334–1340.
33. Bell RR, Draper HH, Tzeng DY, Shin HK, Schmidt GR. Physiological responses of human adults to foods containing phosphate additives. J Nutr 1977; 107: 42–50.
34. Silverberg SJ, Shane E, Clemens TL, Dempster DW, Segre GV, Lindsay R, Bilezikian JP. The effect of oral phosphate administration on major indices of skeletal metabolism in normal subjects. J Bone Min Res 1986; 1: 383–388.
35. Whybro A, Jagger H, Barker M, Eastell R. Phosphate supplementation in young men: lack of effect on calcium homeostasis and bone turnover. Eur J Clin Nutr 1998; 52: 29–33.
36. Grimm M, Muller A, Hein G, Funfstuck R, Jahreis G. High phosphorus intake only slightly affects serum minerals, urinary pyridinium crosslinks and renal function in young women. Eur J Clin Nutr 2001; 55: 153–161.
37. Wan Zahari M, Scott D, Loveridge N, Buchan W, Milne J. The effect of high phosphorus intake on calcium and phosphorus retention and bone turnover in growing lambs. Exp Physiol 1994; 79: 175–181.
38. Brot C, Jørgensen N, Madsen OR, Jensen LB, Sørensen OH. Relationships between bone mineral density, serum vitamin D metabolites and calcium: phosphorus intake in healthy perimenopausal women. J Intern Med 1999; 245: 509–516.
39. Teegarden D, Lyle RM, McCabe GP, McCabe LD, Proulx WR, Michon K, Knight AP, Johnston CC, Weaver CM. Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. Am J Clin Nutr 1998; 68: 749–754.
40. Yacowitz H, Fleischman AI, Bierenbaum ML. Effects of oral calcium upon serum lipids in man. BMJ 1965; 1: 1352–1354.
41. Ritz E, Heuck C-C, Boland R. Phosphate, calcium and lipid metabolism. Adv Exp Med Biol 1980; 128: 197–208.
42. Buonapane GJ, Kilara Am Smith JS, McCarthy RD. Effect of skim milk supplementation on blood cholesterol concentration, blood pressure, and triglycerides in a free-living human population. J Am Coll Nutr 1992; 11: 56–67.
43. Eichhozer M, Stähelin H. Is there a hypocholesterolaemic factor in milk and milk products? Int J Vit Nutr Res 1993; 63:159–167.
44. Hatton DC, McCarron DA. Dietary calcium and blood pressure in experimental models of hypertension. Ann Rev Hypertension 1994; 23: 513–530.