


# Calcium and vitamin D fortified milk reduces bone turnover and improves bone density in postmenopausal women over 1 year

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## Abstract

**Purpose** In Malaysia, hip fracture incidence is higher in Chinese women than other ethnic groups. This study compared the effects of a high-calcium vitamin D fortified milk with added FOS-inulin versus regular milk over 1 year on aspects of bone health in Chinese postmenopausal women in Malaysia.

**Methods** One-hundred and twenty-one women (mean age 59 ( $\pm$  4) years) were randomized into two groups: control ( $n$  = 60; regular milk, 428 mg calcium per day) or intervention ( $n$  = 61; fortified milk at 1200 mg calcium, 96 mg magnesium, 2.4 mg zinc, 15  $\mu$ g vitamin D and 4 g FOS-inulin per day). At baseline, weeks 12, 24, 36 and 52, parathyroid hormone (PTH), C-Telopeptide of Type I Collagen (CTX-1), Procollagen I Intact N-Terminal propeptide (PINP) and vitamin D levels were assessed. Bone density (BMD) was measured at baseline and week 52 using a GE Lunar iDXA.

**Results** Body mass index, lumbar spine and femoral neck BMD did not differ between groups at baseline. Over 52 weeks, mean plasma 25 (OH) D<sub>3</sub> levels increased to 74.8 nmol/L (intervention group) or remained at 63.1 nmol/L (control group) ( $p$  < 0.001 between groups). PTH levels increased in the control group ( $p$  = 0.001). The intervention resulted in a significant suppression of CTx-1 and PINP at  $p$  = 0.018 and  $p$  = 0.004. Femoral neck BMD remained stable in the intervention group but decreased significantly in the controls, with a borderline treatment effect ( $p$  = 0.07). **Conclusion** Compared with regular milk, the fortified milk suppressed bone turnover markers and tended to increase femoral neck BMD.

**Keywords** Postmenopausal women · Fortified milk · Bone markers · Bone density

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## Introduction

The incidence of osteoporosis and its associated fracture risk is a worldwide concern as many countries are experiencing large-scale demographic shifts to ageing populations [1]. The burden of osteoporosis, as an age-related disease, is anticipated to disproportionately affect Asian countries due to their large population sizes, high rates of economic development and urbanisation, and projected large elderly populations. A more than twofold increase in total population and life expectancy for the elderly in the developing world is anticipated over the next 25 years, as reported in Hernlund et al. [2]. Over two decades ago [3], it was estimated that Asia would experience 50% of all hip fractures worldwide by 2050 [4, 5]. The IOF Asian audit of 2009 cites a two to threefold rise in hip fracture incidence over the past 30 years for most Asian countries

[6]. Malaysia is a multi-ethnic country and hip fracture incidence appears to be higher for Chinese women than other races [7], with Lee et al. [8] reporting an incidence of 220 hip fractures per 100,000 among Chinese females in Malaysia.

Both low calcium intakes and insufficient 25 (OH) vitamin D3 (25(OH)D3) levels have been reported as risk factors for osteoporosis in Asian women [5]. While recommendations for minimum calcium intake are similar for Asian and Caucasian women, at 1000 mg/day [9], actual intakes in Asian women are reported to fall significantly short of this minimum [7, 10–13]. As in most countries, the Malaysian Dietary Guidelines recommend an adequate intake of dairy products such as low fat milk and other foods to enable an adequate intake of calcium [14].

Most of the more recent studies in populations of Asian women investigating vitamin D status describe sub-optimal 25(OH)D3 levels, with some studies reporting negative relationships between 25(OH)D3 and PTH, and some also demonstrating further associations with bone turnover markers and bone mass [10, 12, 15–19]. The general incidence of low levels of vitamin D and low habitual dietary calcium intakes indicates that many Asian women are likely to experience high bone turnover with a concomitant increase in fracture risk. Daily consumption of milk fortified with 800 mg calcium was shown to reduce bone loss in a study of Chinese women living in Hong Kong [20, 21]. An intervention with Chinese women in Malaysia supplying a higher dose of calcium, 1200 mg, in fortified milk, significantly reduced the percentage total body, femoral neck and total hip bone loss over 24 months, compared to the control group [22]. The fortified milk also contained vitamin D 10 µg/day, which resulted in improved vitamin D status even though the average serum 25 (OH)D3 for these women was 69 nmol/L at baseline. Their status improved to 87 nmol/L over 12 months, demonstrating maintenance of a sufficient vitamin D status [23, 27].

In addition to providing additional dietary calcium and vitamin D, further micronutrients can be provided to the diet that may affect calcium absorption and bone health in Asian women. Oligofructose-enriched inulin (FOS-inulin) has been shown to increase intestinal calcium and magnesium absorption in older women [24], and reduce bone resorption [24]. Magnesium has been shown to reduce hydroxyapatite crystal size, thereby affecting bone quality [25] and increasing bone density in older women.

The aims of the current study were to compare the effects of a high-calcium vitamin D fortified milk with added FOS-inulin and magnesium versus regular milk on vitamin D status, bone biomarkers and serum parathyroid hormone (PTH) as well as bone density in Chinese postmenopausal (PM) women living in Malaysia, over 52 weeks.

## Methods

The study was approved by the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia.

### Study population, inclusion and exclusion criteria

Women over 55 years of age, at least 5 years post-menopause, and with a body mass index (BMI) between 17 and 32 kg/m<sup>2</sup> were recruited. Exclusion criteria included a history of metabolic bone disease; abnormal liver or kidney function tests; diagnosed diabetes mellitus or insulin resistance; lactose intolerance/milk allergy; regular use of calcium and/or vitamin D supplements; regular use of anti-acids containing calcium; more than two units of alcohol per day; smoking; regular use of medication that may influence bone mass; rheumatoid arthritis/autoimmune disease and fractures in the last 6 months.

### Randomisation criteria, procedures and intervention

#### Recruitment

A total of 975 postmenopausal community dwelling women were initially screened for eligibility using a structured questionnaire, and finger-prick cholesterol and glucose tests. From these, 416 women passed the initial screening exercise and were invited to a second stage screening (Fig. 1).

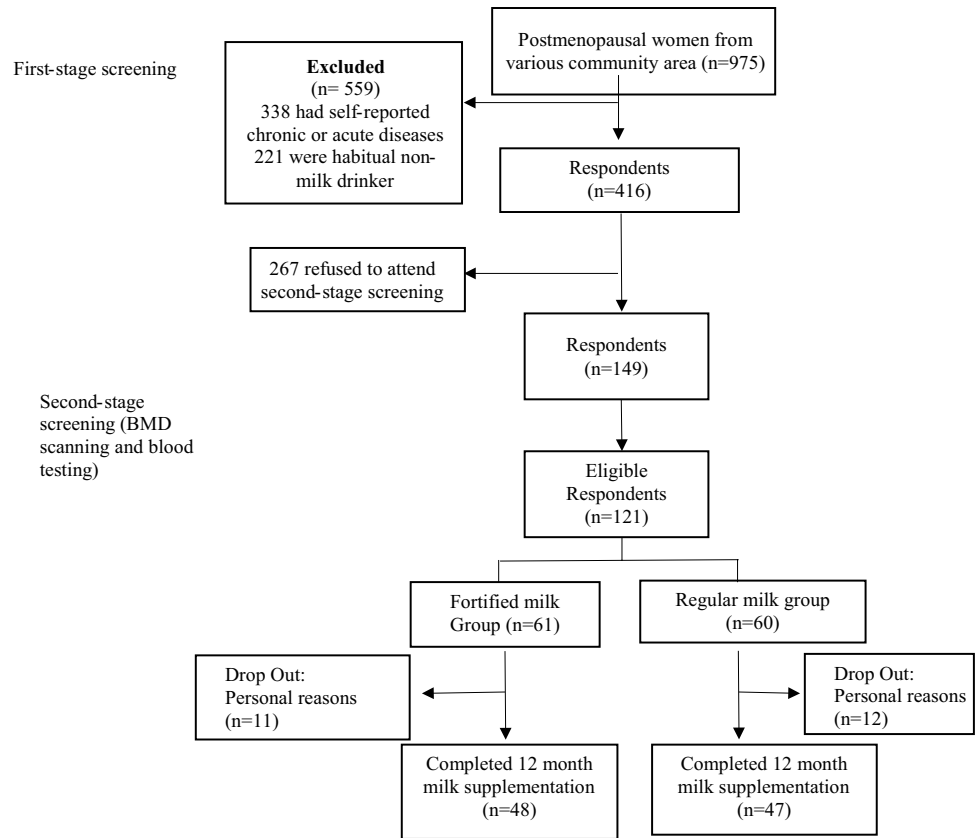
#### Screening visit

A screening questionnaire on general health was administered at an initial visit. A blood sample was taken between 8 and 10 a.m., after an overnight fast, and haematology, blood minerals and metabolic markers were assessed. If the blood test results were normal, study subjects were asked to have a bone mineral density (BMD) measurement of whole body, femoral neck and spine (L1–L4) (using a GE Lunar iDXA, GE Healthcare, Madison, WI, USA). Body weight was measured to the nearest 0.1 kg using SECA scales and standing height was measured to the nearest 0.1 cm using a stadiometer. The medical history of each subject was recorded at this visit.

#### Intervention

One-hundred and twenty-one postmenopausal Malaysian Chinese women were successfully screened and provided written informed consent for the trial. The women were randomized into two groups with 60 in the control group and 61 in the intervention (Int) group. The control group was assigned to receive two servings per day of regular milk

**Fig. 1** Flow diagram of the recruitment process



**Table 1** Composition of the high-calcium, vitamin D, FOS-inulin fortified milk powder and the control milk powder, given as dose per day (from two serves)

Content	Control	Intervention*
Fat (g)	13	1.6
Calcium (mg)	428	1200
Vitamin D (µg)	0	15
Zinc (mg)	0	2.4
Magnesium (mg)	0	96
FOS-inulin (g)	0	4

\*Anlene™, Fonterra Brands (Singapore) Pve Ltd

powder (~ 428 mg calcium) and the Int group was assigned to receive 2 servings per day of fortified milk powder (1200 mg calcium, plus 96 mg magnesium, 2.4 mg zinc, 15 µg vitamin D and 4 g FOS-inulin) (Anlene™, Fonterra Brands (Singapore Pte Ltd.)) for 52 weeks (Table 1).

**Baseline**

*Blood sampling*

Blood samples were taken between 8 and 10 a.m. (after an overnight fast) for the baseline measurements. Samples

were taken to measure plasma markers of bone metabolism [C-Telopeptide of Type I Collagen (CTX-1) and Procollagen I Intact N-Terminal propeptide (PINP)] as well as serum calcium, magnesium, phosphorus, lipid profile, glucose, insulin, parathyroid hormone (PTH) and 25 (OH) vitamin D3. The samples for measuring minerals and metabolic health were collected, processed and analysed immediately by local diagnostic laboratories. Plasma samples for CTx-1, PINP, PTH and 25 (OH) vitamin D3 were snap frozen and stored at -80°C before being analysed by Canterbury Health Laboratories, Christchurch, New Zealand.

**Anthropometry**

Methodology as per screening visit.

**Questionnaires completed at baseline**

A 3-day diet record, food frequency questionnaire, demographics, physical activity level, sun exposure and a list of all medication taken in the last 6 months were recorded at baseline.

## Follow-up measurements

At weeks 12, 24, 36 and 52, blood samples were taken between 8 and 10 a.m. (after an overnight fast) for 25 (OH) vitamin D<sub>3</sub> and bone marker measurements. In addition, blood samples at weeks 12, 24 and 52 blood samples were also analysed for blood minerals, lipid profile and glucose. Collection and analysis of the samples were conducted as per the baseline visit. At week 52, anthropometry, DXA, and questionnaires were completed as at baseline. Retrospectively, a number of DXA scans were of insufficient quality/consistency to be analysed resulting in low final numbers for DXA data.

### Blood and bone marker analyses

PTH, CTx-1 and total PINP were analysed by electrochemiluminescence immunoassay using the Roche COBAS® e411 system (Roche Diagnostics, Indianapolis, IN, USA). 25 (OH)D<sub>3</sub> was analysed using isotope-dilution liquid chromatography–tandem mass spectrometry (ID-LC–MS–MS) [26].

### Compliance

Milk powder was dispensed to each subject at baseline and on a monthly basis thereafter. In addition, phone calls were made to monitor milk consumption of the subjects. Each subject was provided with a monthly diary and asked to record their milk powder intake each day.

### Statistical analyses

Four subjects (one from the Int and three from the control group) were retrospectively found to meet exclusion criteria and were excluded from the analyses [regular use of vitamin D supplements ( $n = 1$ ) or signs of metabolic bone disease ( $n = 3$ )]. Hence, a total of one-hundred and seventeen subjects ( $n = 60$  for Int and  $n = 57$  for control) were included in the analyses.

Based on published data, the within-subject standard deviation for one of the primary outcome variables, CTx-1 was estimated to be 0.045 ng/mL and a representative mean CTx-1 value was taken to be 0.23 ng/mL. The corresponding standard deviation of the treatment difference was calculated as 0.064 ng/mL. To detect a difference of 20% (i.e. 0.046 ng/mL) with a power of 90% and an alpha of 5%, we required 42 subjects per group. To allow for dropouts, and/or potentially a lower difference (16.5%), the number of volunteers required was increased to 60 per group.

SAS (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Outcome measures: the primary outcome variables were difference from baseline for Vitamin D and CTx-1,

secondary outcomes were difference from baseline for PINP and PTH, vitamin D status, as well as absolute values of vitamin D, CTx-1, PINP, PTH, BMD and *T* scores, and serum calcium, magnesium, phosphorous, glucose, insulin, and total cholesterol. Mixed models approach or repeated measures analysis of variance was used and the reported *p* values for the effects of treatment group, time, and their interaction were based on a compound symmetry covariance pattern model. Data were analysed as (a) raw data, (b) difference from baseline, and (c) percentage change from baseline. For (b) and (c), the baseline results (week 0) were included in the model as a covariate and the repeated measures analysis was based on the results from during the intervention (i.e. weeks 12–52). ANOVA was followed by post hoc comparisons of treatment means using the Tukey–Kramer test. Data were log<sub>10</sub> transformed if required to achieve homogeneity of variance but all results are shown in the original units. Measurements were considered to be significantly different if  $p < 0.05$ .

## Results

### Baseline characteristics

Table 2 summarises the characteristics of the women. The mean ages for the control and the Int group were 59 and 60 years, respectively, and the mean BMI for the control group was 24.5 versus 23.4 kg/m<sup>2</sup> for the Int group ( $p = 0.363$ ). Lumbar spine BMDs were slightly lower in the Int group with *T* scores of  $-0.28$  for the control and  $-0.75$  for the Int groups ( $p = 0.070$ ). For the femoral neck, BMDs were normal with *T* scores at  $-0.48$  for the control and  $-0.92$  for the Int group. Mean calcium intake from the food frequency questionnaire for these groups was 547 mg

**Table 2** Baseline characteristics of the women

	Control ( $n = 57$ )	Int ( $n = 60$ )	<i>p</i> value
Age	60 (4.3)	59 (3.9)	0.793
BMI	24.5 (2.94)	23.4 (2.94)	0.363
Lumbar spine BMD (g/cm <sup>2</sup> )	1.08 (0.113)	1.02 (0.113)	0.065
Lumbar spine <i>T</i> score	$-0.28$ (1.023)	$-0.75$ (1.023)	0.070
Femoral neck BMD (g/cm <sup>2</sup> )	0.86 (0.108)	0.80 (0.108)	0.162
Femoral neck <i>T</i> score	$-0.48$ (0.894)	$-0.92$ (0.894)	0.177
Calcium intake (mg)	547 (246.0)	527 (246.0)	> 0.999
25 (OH) vitamin D <sub>3</sub> (nmol/L)	64.8 (18.89)	62.3 (1.89)	> 0.999

Values are given as means (SD)

SD standard deviation, BMI body mass index, BMD bone mineral density, Int intervention

(control) and 527 mg/day (Int). Baseline 25 (OH)D3 was 64.8 nmol/L for the control group versus 62.3 nmol/L for the Int group. No significant differences in age, BMI, femoral neck BMD, calcium intake or 25 (OH)D3 levels were detected.

## Impact of intervention

### Compliance, general health and blood minerals

The women's compliance with taking the products varied between 86 and 90% over the 52 weeks. General measures of health including fasting blood glucose and fasting lipid profiles were within normal ranges at weeks 0 and 52 (Table 3) and all blood minerals including calcium, magnesium, and phosphorus were within normal ranges. No significant changes were observed for the blood minerals over time. There was an overall treatment effect ( $p = 0.012$ ) for the change in total cholesterol, with a reduction in total cholesterol in the Int group and no change in the control

group. There was a treatment by time effect on the change in LDL levels ( $p = 0.033$ ) where levels reduced in the Int group compared to baseline but remained stable in the control group (Table 3).

### Vitamin D status

For assessment of the vitamin D status of the women, the Institute of Medicine Guidelines (IOM) were used where a level of  $\geq 50$  nmol/L is considered adequate, and  $< 50$  nmol/L is inadequate [27]. For the control group, 28% had inadequate levels at baseline and over the 52 weeks of supplementation the percentage remained at 21%. In the Int group, 32% had inadequate 25 (OH)D3 levels at baseline and after 52 weeks of supplementation all the women in the Int group were sufficient.

Mean serum 25 (OH) vitamin D3 levels at baseline were sufficient (equal to or  $> 50$  nmol/L) [27] in the control as well as the Int groups (Table 4). Over the 52 weeks, serum 25 (OH)D3 levels remained constant in the control group

**Table 3** General health measures and blood minerals over the 52 weeks of intervention

	Control			Int		
	Week 0	Week 24	Week 52	Week 0	Week 24	Week 52
Calcium-Corr (mmol/L)	2.39 (0.009)	2.40 (0.009)	2.38 (0.010)	2.34 (0.009)	2.35 (0.009)	2.34 (0.009)
Magnesium (mmol/L)	0.98 (0.012)	0.93 (0.013)	0.91 (0.013)	0.97 (0.011)	0.97 (0.012)	0.96 (0.013)
Phosphorus (mmol/L)	1.13 (0.020)	1.11 (0.020)	1.15 (0.021)	1.10 (0.020)	1.09 (0.019)	1.10 (0.020)
Total cholesterol (mmol/L)	5.57 (0.107)	5.51 (0.108)	6.00 (0.113)	5.67 (0.102)	5.55 (0.105)	5.81 (0.110)
LDL (mmol/L)	3.63 (0.654)	3.72 (0.787)	3.61 (0.736)	3.26 (0.705)	3.20 (0.562)	3.13 (0.652)
Fasting blood glucose (mmol/L)	5.12 (0.068)	5.13 (0.069)	5.09 (0.072)	4.97 (0.065)	5.09 (0.067)	5.04 (0.070)

Values are given as least squares means (SEM).  $n = 42$ –60 per group

Calcium-Corr calcium corrected for albumin, Int intervention

**Table 4** Response (change from baseline) of PINP over 52 weeks to intervention with either regular milk powder (Control,  $n = 60$ ) or fortified milk powder (Int,  $n = 55$  to 57)

Group	25 (OH) Vit D <sub>3</sub> (nmol/L)		PTH (pmol/L)		CTx-1 (μg/L)		PINP (μg/L)	
	Control	Int	Control	Int	Control	Int	Control	Int
Week 0	64.8 (2.52)	62.3 (2.45)	3.6 (0.14)	3.7 (0.14)	0.44 (0.02)	0.45 (0.02)	47.9 (1.85)	47.9 (1.79)
Week 12	52.9 (2.70)	62.7 (2.55)	3.8 (0.152)	3.61 (0.146)	0.41 (0.018)	0.35 (0.018)*	42.6 (1.91)	40.9 (1.82)
Week 24	58.3 (2.84)	67.0 (2.67)	3.8 (0.15)	3.70 (0.149)	0.38 (0.019)	0.35 (0.018)	43.4 (1.95)	39.3 (1.5)
Week 36	66.2 (2.87)	78.7 (2.74)*	3.7 (0.15)	3.63 (0.15)	0.38 (0.18)	0.35 (0.02)	41.5 (1.95)	39.5 (1.87)
Week 52	63.1 (2.87)	74.8 (2.74)*	4.1 (0.15)	3.98 (0.15)	0.39 (0.02)	0.37 (0.02)	43.8 (1.95)	41.9 (1.87)
$p$ value week 0 vs 52	$> 0.999$	$< 0.002$	$< 0.001$	0.925	$< 0.006$	$< 0.001$	0.347	$< 0.001$
Postmenopausal reference values								
			PTH (pmol/L)		CTx-1 (μg/L)		PINP (μg/L)	
			3.7 (1.5)		0.45 (0.10)		60.9 (3.66)	

PTH parathyroid hormone, CTx-1 C-Telopeptide of Type I Collagen, PINP Procollagen I Intact N-Terminal propeptide, Int intervention

\* $p < 0.05$  between groups at the same time point

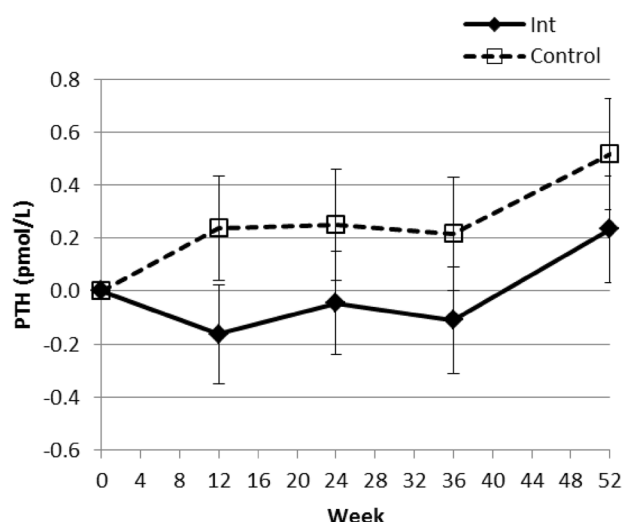
(64.8–63.1 nmol/L) but increased significantly in the Int group (62.3–74.8 nmol/L,  $p = 0.002$ ) (Fig. 2). The change over time was significantly different between the groups ( $p < 0.001$ ).

*Changes in PTH*

Figure 3 shows the changes from baseline in PTH levels in the two groups of women over time, while Table 4 shows the actual PTH levels for the different time points. PTH levels in the control group increased from 3.56 to 4.10 pmol/L over the 52 weeks ( $p < 0.001$ ). In the Int group, however, PTH decreased from 3.76 to 3.61 pmol/L but then increased again to 3.98 ( $p > 0.05$ ) at week 52. The change from baseline in PTH in the Int group was significantly different from the change from baseline in the control group ( $p = 0.001$  between groups, Fig. 3)

*Change in CTx-1 and PINP*

Figures 4 and 5 show the changes from baseline in CTx-1 and PINP over the 52 weeks of supplementation and Table 4 shows CTx-1 and PINP levels for the different time points. Both the control as well as the Int group of women exhibited bone turnover within the typical range for postmenopausal women with mean plasma CTx-1 values at 0.44–0.45  $\mu\text{g/L}$  at baseline. In the control group, plasma CTx-1 reduced significantly between baseline and week 52 (0.44–0.39  $\mu\text{g/L}$ ,  $p = 0.006$ ) and in the Int group, CTx-1 reduced from 0.45 to 0.35  $\mu\text{g/L}$  ( $p < 0.001$ ) over the first 12 weeks and remained at this level till week 52 (0.37  $\mu\text{g/L}$ ). The changes from baseline in CTx-1 for the Int group were significantly different

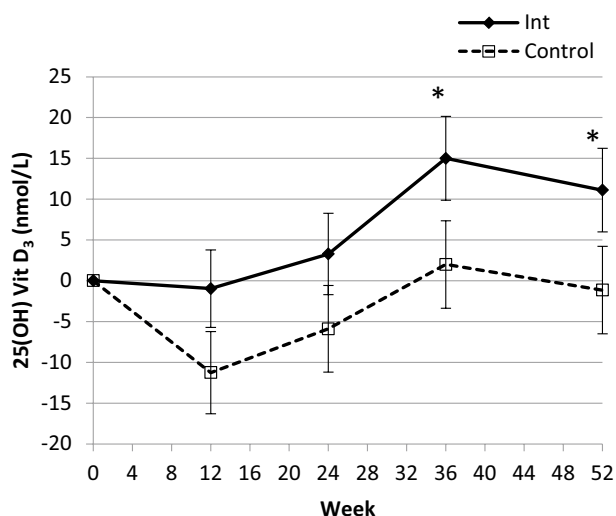


**Fig. 3** Change from baseline for PTH over 52 weeks of intervention with either regular milk powder (Control,  $n = 60$ ) or fortified milk powder (Int,  $n = 55$  to 57)

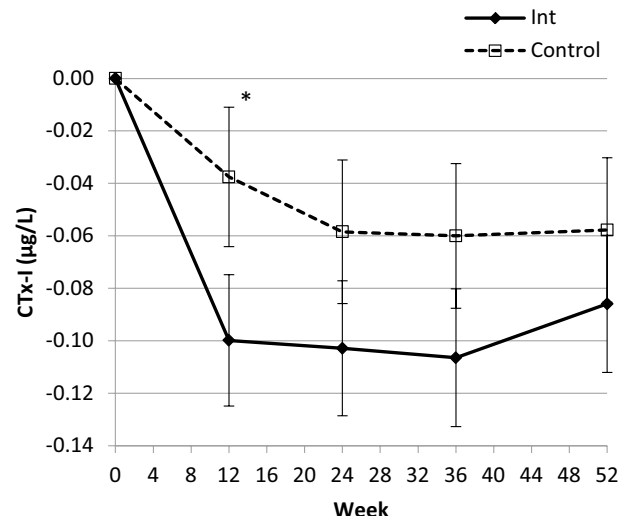
from the control group ( $p = 0.018$ ). PINP decreased in both groups, but the decrease was greater in the fortified than the regular milk group ( $p = 0.004$  for fortified vs regular). PINP was significantly reduced in the Int group from 48 to 42  $\mu\text{g/L}$  ( $p = 0.001$ ) over the 52 weeks.

*Change in bone density*

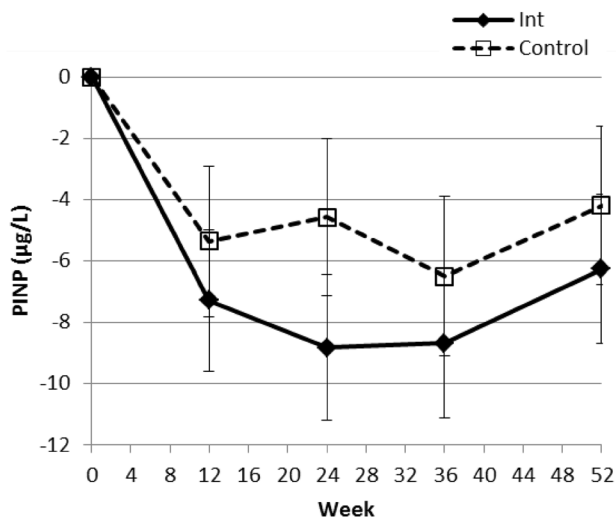
Lumbar spine bone density (BMD) and the  $T$  scores remained constant, with no significant changes in both



**Fig. 2** Change from baseline for 25 (OH) vitamin D3 over 52 weeks intervention with either regular milk powder (Control,  $n = 60$ ) or fortified milk powder (Int,  $n = 55$ –57). \* $p \leq 0.05$  for difference between control and Int



**Fig. 4** Response (change from baseline) of CTx-1 over 52 weeks to intervention with either regular milk powder (Control,  $n = 60$ ) or fortified milk powder (Int,  $n = 55$ –57). \* $p \leq 0.05$  for difference between control and Int



**Fig. 5** Response (change from baseline) of P1NP over 52 weeks to intervention with either regular milk powder (Control,  $n = 60$ ) or fortified milk powder (Int,  $n = 55$ – $57$ )

groups of women over 52 weeks. Femoral neck BMD decreased in the control group over the 52 weeks and was significantly different from baseline ( $p = 0.009$ ). The femoral neck BMD remained stable in the Int group with a borderline treatment effect ( $p = 0.07$ ). The  $T$  scores remained stable in the Int group but reduced significantly in the control group ( $p = 0.610$  for Int and  $p = 0.009$  for control for difference from baseline) and  $0.084$  for Int versus control. The  $Z$  score for the control group decreased significantly over the 52 weeks ( $p = 0.015$ ), and the difference from baseline was significantly different between the two groups at  $p = 0.046$ . The  $Z$  scores for whole body and lumbar spine increased throughout the study period, when compared to baseline in both groups indicating a beneficial effect of milk on overall bone density (Table 5).

*Linear regression analyses*

Using linear regression analysis, we found that the greater the decrease in CTx-1 between baseline and week 36, the

lower the loss of BMD in the femoral neck and the lumbar spine over the intervention period ( $p = 0.010$  and  $p < 0.001$ , respectively). Changes in Vitamin D status also showed some relationship to femoral neck BMD but it was not significant. Regression analyses using baseline calcium intake and the outcome measures did not indicate any significant relationships.

**Discussion**

Supplementation of healthy postmenopausal Chinese women with calcium, vitamin D and FOS-inulin fortified milk for 52 weeks significantly reduced bone resorption and improved vitamin D status.

The habitual mean calcium intake for the cohorts of women in the present study was just over 500 mg/day. In a short communication, Haines et al. [28] reported calcium intake of 390 mg/day in older women attending a clinic in Hong Kong which was below the recommended intake for women in Asia. The current recommended intake for Chinese women in Hong Kong is 900 mg [29] and according to Heaney [9] should be closer to 1000 mg for Asian women in general. Chee et al. [7] recorded a calcium intake of 470 mg/day in their cohort of Chinese women in Malaysia and Kruger et al. [30, 31] reported that postmenopausal women living in Beijing had a calcium intake of less than 500 mg/day. Calcium intakes in several Asian countries are, therefore, significantly lower overall than the daily requirement for Asian women and the current recommended nutrient reference value of 800 mg/day for Malaysia (Table 2).

The women who participated in the study were mostly vitamin D sufficient. This is in contrast to a study which reported increased incidence of vitamin D inadequacy in Eastern Asia [33]. The minimum level of serum 25 (OH) D3 estimated by some researchers to be optimal for fracture prevention varies between 50 and 80 nmol/L [32, 34], while the IOM concluded that 40 nmol/L seem to be a threshold for optimal bone health [27]. Twenty-eight percent of the control group and 32% of the Int group had inadequate levels at baseline (based on sufficiency = 50 nmol/L [27]).

**Table 5** Lumbar spine and femoral neck bone mineral density at baseline and at week 52

Measurement	LS BMD (g/cm <sup>2</sup> )	LS $T$ score	FN BMD (g/cm <sup>2</sup> )	FN $T$ score
Control week 0	1.078 (0.0165)	- 0.28 (0.137)	0.857 (0.0198) <sup>a</sup>	- 0.48 (0.164) <sup>a</sup>
Control week 52	1.080 (0.0167)	- 0.27 (0.140)	0.838 (0.0197) <sup>b</sup>	- 0.63 (0.163) <sup>b</sup>
Int week 0	1.021 (0.0161)	- 0.75 (0.134)	0.803 (0.0163) <sup>ab</sup>	- 0.92 (0.134) <sup>ab</sup>
Int week 52	1.015 (0.0162)	- 0.780 (0.136)	0.802 (0.0161) <sup>ab</sup>	- 0.92 (0.132) <sup>ab</sup>

Values are given as least square means (SEM).  $n = 18$ – $59$  per group. Superscripts refer to differences between all 4 means; means with different superscripts in the same column are significantly different at  $p < 0.05$

LS lumbar spine, FN femoral neck

The percentage of women with inadequate levels of 25 (OH) D3 remained at 21% over the 52 weeks of supplementation in the control group, but at week 52 all women in the Int group were sufficient. The improvement in vitamin D status in the Int group could be an indication of study adherence and results and further relationships should be interpreted with caution. The levels we measured in the women compare well with those reported for Chinese women living in Malaysia [7, 22], where both studies reported a mean of 68.8 nmol/L and as well as a significant inverse relationship between vitamin D status and PTH levels. In our study, we did not observe a significant inverse relationship between 25(OH)D3 levels and PTH. Shibli-Rahhal and Paturi [35] assessed variations in PTH levels in patients with low vitamin D status. They concluded that less than half of patients with low vitamin D status will have an elevated PTH and improving vitamin D status does not immediately result in changes in PTH. In their study, only 30% of the patients with 25 (OH)D3 levels below 75 nmol/L had an elevated PTH. The association between vitamin D status and PTH levels is influenced by dietary calcium intake and in some of our participant's intake may have been sufficient to perturb the association between 25(OH)D3 levels and PTH [35, 36].

Huang et al. [37] reported a reduction in PTH levels, bone resorption and bone loss in postmenopausal Chinese women in response to 1000 mg calcium and 10 µg of vitamin D for 12 months. Chee et al. [22] reported significantly less bone loss at the femoral neck as well as total hip over 2 years of supplementation of postmenopausal Chinese women with fortified milk. Femoral neck BMD indeed increased in the intervention group at months 18 and 24 of supplementation. Similarly, Lau et al. [20, 21] reported increases in femoral neck BMD at months 18 and 24 of supplementation with fortified milk. In our Int group, bone density and the *T* score of the femoral neck remained stable with no significant changes over 52 weeks, while BMD as well as the *T* score reduced significantly in the control group.

The effects of the fortified milk on BMD are supported by the significant reduction in bone resorption represented by the changes in CTx-1 over time. CTx-1 levels reduced in both groups of women, demonstrating the beneficial effects of milk on bone health, but the change induced by the fortified milk was more pronounced and was significantly different from controls at week 12 ( $p < 0.05$ ) while the change in CTx-1 in the control group was significant at week 52 ( $p < 0.006$ ). Increasing calcium intake has significant effects on CTx-1 levels as has been shown by several researchers over time. [20, 21, 29–32]. High-calcium milk supplementation reduced CTX-1 levels more than 30% over 4 weeks in South East Asian women [38], and by 25% in Chinese women living in Beijing, China [30]. Aloia et al. illustrated in 2010 that supplementation with up to 100 µg vitamin D per day had no effect on bone turnover or PTH levels [39].

In a more recent study, this group ran an intervention study in 159 postmenopausal women for 28 weeks. The women were supplemented with 100 µg vitamin D, with or without 1200 mg calcium, calcium alone or with a placebo. CTx-1 was significantly reduced by the calcium alone or in combination with vitamin D while vitamin D alone had no effect [39].

The significant decrease in CTx-1 between baseline and week 52 was also associated with less bone loss at the lumbar spine ( $p < 0.001$ ). Four prospective studies have shown that increased levels of bone resorption markers are associated with an increased risk of fracture [40]. CTx-1 can be predictive of future bone loss [41, 42] and future risk of fracture [43, 44] as well as predict long-term changes in BMD [43]. Tamaki et al. [45] demonstrated that increased levels of bone resorption markers in women more than 5 years past menopause were associated with increased risk of vertebral fractures independent of BMD. While lumbar spine BMD did not change significantly in both control and Int groups, femoral neck BMD remained stable in the Int group but was reduced in the control group, with significant changes in the *T* scores as well. Femoral neck bone density is a better predictor for hip fracture than any other site in the skeleton; thus, the maintenance of femoral neck BMD in the Int group should indicate some protection from future fractures [44–46].

Our findings are in agreement with the study reported by Chee et al. [22] where milk supplementation of older Chinese women in Malaysia resulted in a reduction of bone loss at several sites; a follow-up of the same women 18 months later indicated that in the group that had previously consumed fortified milk, the beneficial effects were maintained while bone loss was increased in the control group [47]. In a meta-analysis on the effects of milk intake on bone biomarkers as well as bone density, milk supplementation reduced bone resorption significantly even in interventions less than 6 months in duration [48]. In addition, the analyses indicated that total body bone mineral content increased significantly in the intervention groups compared to controls while total body BMD increased by 0.01 g/cm<sup>2</sup> with borderline significance. Whether these relatively small changes could result in a reduction of fracture risk is not known, and an earlier meta-analysis reported no reduction in risk for hip fracture due to milk intake [49].

Our study had some limitations: Firstly, the fortified milk delivered 4 g of FOS-inulin, 96 mg magnesium and 2.4 mg zinc per day. FOS-inulin has been shown to increase calcium and magnesium absorption but with no significant effects on bone markers [24 and the FOS-inulin could have contributed to the positive effects observed in the Int group by supporting calcium bioavailability and bone mineralisation [50]. Some epidemiological studies indicate that magnesium intake is associated with increased bone density in



older women, and limited supplementation studies in adults indicate a correlation between magnesium intake and bone density [51]. Zinc supplementation trials reported increased bone density after up to 18 months of supplementation [52, 53] but at much higher levels of 24–40 mg/day. It is not possible to ascertain which of the additional ingredients in the fortified milk contributed to the effect on markers and bone density, thus the effects shown relate to the complete formulation rather than to specific ingredients. Secondly, up to 90% of Chinese adults are lactose intolerant [54]. As intolerance was one of our exclusion criteria, our population and findings may not be representative of Chinese postmenopausal women.

In summary, in our study, both regular and fortified milk reduced bone turnover but the effect of the fortified milk was greater. No significant effects were observed for PTH levels of both groups. While BMD did not increase in any of the groups, the fortified milk maintained BMD of the femoral neck with a borderline treatment effect. Osteoporosis is a significant health problem resulting in loss of independence due to fractures resulting in morbidities. The incidence of osteoporosis is increasing yearly and has a large impact on individuals, society and escalating healthcare costs. Consumption of milks fortified with additional calcium and vitamin D provides a means to reduce poor bone health and the subsequent risk of fractures.

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#### Compliance with ethical standards

**Conflict of interest** L.M Schollum and JM Todd are employees of Fonterra Cooperative Ltd. B Kuhn-Sherlock is a consultant statistician engaged by Fonterra Cooperative Group Ltd, New Zealand. All other authors do not have a conflict to declare.

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