

Original Article

The effects of a high calcium dairy food on bone health in pre-pubertal children in New Zealand

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Childhood and adolescence is the period of most rapid skeletal growth in an individual's lifetime. A greater peak bone mass achieved in the first 2-3 decades of life, may protect against the risk of osteoporotic fracture in later life. The aim of this randomized, controlled study was to assess in pre-pubertal boys and girls (aged 8-10 years) the effect of 18 months of a calcium enriched, cocoa flavoured product on bone density, bone growth and bone size in New Zealand children. One hundred and fifty four pre-pubertal boys and girls (aged 8-10 years) were randomized to receive a high calcium dairy drink or a control drink reconstituted with water for 18 months. They were assessed at baseline and then every 6 months for the first 18 months, while they were having the supplement; they were then followed up 12 months after supplementation had finished. Bone mineral density and bone mineral content were assessed at the total body, hip and spine. Indicators of bone size (vertebral width and height) were also measured at the spine. Anthropometric data was collected, medical history questionnaires were administered (including the Tanner or pubertal stage questionnaire), dietary calcium intake was assessed with a calcium food frequency questionnaire and calcium supplement compliance was determined. There was no significant difference between the 2 groups for bone mineral density or bone mineral content at any time point. There was no difference in vertebral height or width at any stage of the study, indicating no additional influence on bone size at the lumbar vertebrae. There were no significant differences between height, weight, lean mass or fat mass at any time point. Both groups had higher habitual calcium intakes than recommended for this age group going into the study and throughout the study. In this 2½ year study (18 months supplementation, 1 year follow-up) we did not observe a difference in bone mineral density in pre-pubertal children. This was probably due to their high habitual dietary calcium intake whereby minimal addition of calcium to the diet reached the threshold level where no further benefit was seen. There were no significant differences between the two groups in body composition. Growth and the mean height and weight remained between the 50th and 75th percentile for their age. We have shown calcium supplementation in children with high habitual dietary calcium intake appears not to have additional effects on bone mass. Calcium supplementation needs to be targeted in those children with low habitual dietary calcium intake.

Key Words: BMD, BMC, pre-pubertal, calcium supplementation, bone health, New Zealand.

Introduction

The amount of calcium a child needs to support optimal growth and to maximize peak bone mass (PBM) has not been conclusively established. Childhood and adolescence is the period of most rapid skeletal growth. Increase in total body bone mass can be in the order of 7-8% per year^{1,2} with the highest bone mineral density gain in girls between the ages of 10 to 14.³ A greater PBM achieved in the first 2-3 decades of life, may protect against the risk of osteoporotic fracture in later life⁴. Accordingly, osteoporosis is sometimes termed a "paediatric disorder".

Osteoporosis is a major New Zealand (NZ) public health issue affecting approximately one in three women and one in eight men of the elderly population and costs the health care system an estimated \$200 million a year.⁵ It is therefore important that recommendations for calcium intake in children correspond with the intake required to

maximize their PBM, and therefore provide some protection against osteoporosis in later life. There are limited data on the calcium intake of children in NZ, and there have been no published surveys of nutrient intakes in this population. A 1997 National Nutrition Survey found 15-18 year old young adults have a daily average calcium intake of 870mg.⁶ Other studies have suggested calcium intake decreases from childhood to adolescence,⁷⁻⁸ so it seems probable that the intake of NZ children will be higher than this.

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Accepted 25 June 2004

Eight intervention studies⁹⁻¹⁶ in children aged 7-14 years examining the effects of calcium supplementation on bone mineral density have been published. The studies, with the exception of the study by Cadogan *et al.*, (1997)¹⁵, were all of a randomized double-blind placebo-controlled study design. This is the strongest study design to prove causality, and controlling for potentially confounding factors that are known to influence bone mass. Additional factors associated with peak bone mass are genetics, hormones, physical activity, lifestyle factors such as smoking and nutrition.¹ One of these studies has shown an increase in bone size¹¹ corresponding with an increase in bone mineral density when additional calcium is given in the form of calcium fortified foods. In addition, the studies⁹⁻¹⁵ have suggested the appendicular skeleton, particularly the regions of compact bone, appear more sensitive than the axial skeleton to the effects of calcium supplementation. The main areas of increase in bone mineralisation have been the radial and femoral diaphysis,¹¹ the midshaft radius,^{10, 12-14} total hip⁹, and leg and pelvis.¹⁵ However, some studies have also found significant differences in the spine^{10,13,16} and the total body.¹⁵⁻¹⁶

The aim of this randomized, controlled study is to assess in pre-pubertal boys and girls (aged 8-10 years) the effect of 18 months of a calcium enriched, cocoa flavoured product (New Zealand Milk Limited, Wellington, New Zealand) on bone mineral density, bone growth and bone size at the vertebrae in NZ children. This age group was chosen because previous studies have shown this is a period of most rapid development.² Supplementation with both milk calcium and calcium salts have been shown to increase bone mineral density in this age group.⁹⁻¹⁶ There are limited data on supplementation with calcium on bone development in male pre-pubertal children. With the increase in osteoporosis in both males and females, it is also important to consider the effect nutrition may play on their bone development.

Subjects and Methods

Three hundred and ninety children aged 8-10 years were approached at three local primary schools and asked to attend an information evening with their parents. Of the 211 who attended, 159 satisfied the inclusion criteria and agreed to participate. Exclusion criteria included allergy to dairy products, any major disease states including significant psychological problems. If the child was on any medication that influenced bone growth or metabolism [e.g steroids (inhaled or oral), anti-convulsants, thiazide diuretics or vitamin D] they were excluded. Of the 159 randomised children, 5 (2 controls and 3 treatment) did not complete the baseline questionnaires after randomisation and took no further part in the study.

The children at each school were randomly allocated to either the treatment group or the control group using heel ultrasound values at baseline for stratification. The study was approved by the Southern Regional Health Authority Ethics Committee, (Canterbury) New Zealand in 1997. Informed consent was obtained from the children and their parents.

Protocol

All the children visited the research centre and met with

the research nurses, at baseline and then every six months for the first 18 months, while they were having the supplement; they were then followed up 12 months after the supplementation finished. Bone mineral density, bone mineral content and bone size were measured at baseline, 6 months, 12 months, 18 months and 30 months; height and weight were also recorded at these times. In addition, the children completed a calcium food frequency questionnaire²² to determine dietary calcium intake at each visit. A medical questionnaire was completed at baseline and at 30 months to check medication use, medical history, previous fractures, family history and caffeine intake; for the females there were also questions asked about menarche history. Pubertal stage (breast and genital development) was assessed by a self-administered Tanner questionnaire¹⁷ at baseline, 18 and 30 months.

Dietary supplement and compliance

The children had sachets of the product (Table 1) delivered to them either fortnightly at school or monthly at home over the 18 months of supplementation. Each child was required to have one sachet of the product in the morning and afternoon mixed with hot or cold water as a drink. The children were blinded to the study product as both looked and tasted the same. The high calcium milk provided 600mg of calcium per 40g serve or 1200mg per day. The control milk provided 200mg of calcium per 40g serve or 400mg per day - this is equivalent to two glasses of milk. The children completed a tick sheet after they had consumed the drink, and returned it to the study coordinator each month as a measure of compliance. Compliance was assessed as the number of drinks actually consumed expressed as a percentage of those that should have been taken. The study product was initially chocolate flavoured, however other flavours were introduced to maintain compliance.

Table 1. Nutritional composition of 2 sachets (80g) of high calcium supplement compared to 2 sachets (80g) of the placebo supplement

	Treatment	Placebo
Energy	1529 kJ 364 kcal	1650 kJ 393 kcal
Protein	10.2 g	10.6 g
Carbohydrate	34.7 g	34.7 g
Fat	21.4 g	24.5 g
Vitamin A	656 µg*	656 µg*
Vitamin D	336 IU	320 IU
Vitamin C	52 mg	58 mg
Calcium	1200 mg	400 mg
Phosphorus	776 mg	320 mg

*= µg retinol equivalents

Procedures

Bone mineral density, bone mineral content and body composition were determined by dual-energy x-ray absorptiometry (DPX-IQ; Lunar Radiation Corp., Madison, Wisconsin). The total body, lumbar spine and total hip were measured for bone mineral density and bone mineral content; lean muscle mass and fat mass were also measured from the total body scan. The scans were analysed using the Lunar paediatric database (version 4.79).

Table 2. Baseline characteristics of both groups, mean values (\pm SEM)

	Treatment (N = 74)	Control (N = 80)	P value
Age (years)	9.4 (0.1)	9.4 (0.1)	0.952
Ratio males to females	36:38	39:41	
Height (cm)	135.1 (0.8)	135.4 (0.8)	0.767
Weight (kg)	31.5 (0.7)	32.4 (0.9)	0.399
Dietary calcium intake (mg)	934 (44)	985 (53)	0.461
Total Body BMD (g/cm ²)	0.881 (0.006)	0.881 (0.007)	0.951
Total Body BMC (g)	1152 (23)	1172 (29)	0.603
L1-L4 Spine BMD (g/cm ²)	0.742 (0.009)	0.730 (0.009)	0.329
L1-L4 Spine BMC (g)	23 (1)	23 (1)	0.800
Total Hip BMD (g/cm ²)	0.813 (0.014)	0.792 (0.015)	0.305
Total Hip BMC (g)	18 (0.4)	17 (0.5)	0.727
Total Fat (kg)	5.8 (0.4)	6.3 (0.5)	0.428
Total Lean (kg)	23.8 (0.3)	24.3 (0.4)	0.357
Tanner 1 (breast/genital)#	1 (1-2)	1 (1-2)	
Tanner 2 (pubic hair)#	1 (1-2)	1 (1-2)	

The region of interest was adjusted to match previous scans and anatomical changes due to growth. Quality assurance was carried out three times per week during the study. The coefficient of variation for repeated scans of the lumbar spine was 1%, the femoral neck 2.5% and 0.5% for the total body. The coefficient of variation for repeated scans of lean muscle mass was 1.1% and 1.9% for total fat mass. The hip bone areas were not analysed because of growth, positioning and coefficient of variance factors.

Height was measured using a wall mounted stadiometer (Harpentin); children were measured three times to within 2mm and the average obtained. Weight was measured on electronic calibrated scales (Salter). All measurements were undertaken by assessors blinded to the treatment received by each child. Dietary calcium intake was calculated from a calcium food frequency²² questionnaire using data from the New Zealand food composition tables.

Table 3. Height, Weight, Total Body Lean Mass and Fat Mass for the Treatment and Control group at baseline, 6, 12, 18 and 30 months (\pm SEM)

	Baseline (N _{txi} =74, N _{con} =80)	6 Months (N _{txi} =45, N _{con} =58)	12 Months (N _{txi} =56, N _{con} =60)	18 Months (N _{txi} =60, N _{con} =65)	30 Months (N _{txi} =58, N _{con} =65)	P value (0-30)
<i>Height (cm)</i>						
Treatment	135.1 (0.8)	138.4(1.2)	141.6 (1.0)	144.3 (1.0)	151.0 (1.0)	0.607
Control	135.4 (0.8)	138.9 (1.0)	142.8 (1.0)	145.3 (1.0)	151.8 (1.1)	
<i>Weight (kg)</i>						
Treatment	31.5 (0.7)	33.5 (1.1)	36.3 (1.0)	38.4 (1.0)	42.9 (1.2)	0.548
Control	32.4 (0.9)	33.8 (1.0)	37.4 (1.2)	39.0 (1.2)	44.0 (1.4)	
<i>Lean Mass (g)</i>						
Treatment	23806 (346)	24995 (548)	26458 (468)	27783 (488)	30978 (577)	0.823
Control	24318 (421)	25337 (501)	26874 (545)	28114 (580)	31178 (672)	
<i>Fat Mass (g)</i>						
Treatment	5775 (411)	6794 (643)	7932 (658)	8391 (650)	9532 (740)	0.531
Control	6317 (534)	6751 (608)	8340 (825)	8628 (790)	10260 (874)	

Statistical analysis

Preliminary power calculations indicate that 75 subjects in each group will provide sufficient power (80%) to detect a differential effect of approximately 4% ($\alpha = 0.05$). Comparisons between the two groups at baseline were made using independent *t*-tests; ANOVA for repeated measures was used to compare the changes over time between the two groups. When ANOVA indicated a significant interaction between time and treatment group, comparisons between groups at individual times were made using Fisher's Least Significant Difference test. All analyses were performed on an intention to treat basis. The statistical package used was SPSS (Statistical Package for Social Sciences) 10.0.1 for Windows (1999).

Results

Of the children randomized and starting intervention ($N=154$) 51% were female and 49% were male. There was a mixture of ethnic identities in the study population, however the majority of participants identified themselves as NZ European or Pakeha (81%). Other nationalities were Australian, Dutch, English, NZ Maori, Scottish and Other.

The groups were very well matched at baseline on all variables (Table 2). The withdrawal rate was high in this study, 53.9% withdrew at some point in the 18 months from taking the supplement. Of these children 66.3% continued to be monitored at each data collection point. The main reason for the children withdrawing was a dislike of the study products, both treatment and control, and an inability to have 2 cups a day. We have included the data points for the children who did not finish the supplement in the analysis. Both groups were evenly matched in terms of withdrawals from the study ($N_{\text{control}} = 39$, $N_{\text{treatment}} = 44$). Most withdrawals occurred in the first six months of starting the intervention. The mean compliance for those who finished treatment ($N=71$) was 80.6%.

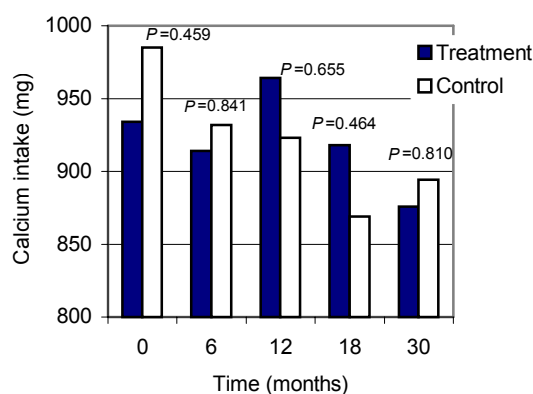
No significant differences were seen in the changes in the anthropometric values between the two groups and the minimum *P* value (0-30) from repeated measures of ANOVA = 0.531. Throughout the study the mean value for both the males and females in both groups remained between the 50th and 75th percentile for both height and weight [National Child Health Survey (NCHS) growth curves for children].

Table 4. Percentage change from baseline for the Total Body, Lumbar Spine, Total Hip, Trochanter and Femoral neck bone mineral density for the Treatment and Control groups at, 6, 12, 18 and 30 months (\pm SEM)

	6 Months ($N_{\text{txt}}=45, N_{\text{con}}=58$)	12 Months ($N_{\text{txt}}=56, N_{\text{con}}=60$)	18 Months ($N_{\text{txt}}=60, N_{\text{con}}=65$)	30 Months ($N_{\text{txt}}=58, N_{\text{con}}=65$)	<i>P</i> value (0-30)
<i>Total Body (%)</i>					
Treatment	1.6(0.9)	4.4(0.9)	5.1(0.9)	9.4(1.0)	0.737
Control	1.6(0.9)	3.3(1.0)	4.3(1.0)	8.9(1.1)	
<i>L1-L4 Spine (%)</i>					
Treatment	2.3(1.6)	5.9(1.5)	8.4(1.5)	16.3(1.9)	0.616
Control	4.1(1.5)	5.8(1.5)	8.6(1.5)	16.8(2.1)	
<i>Total Hip (%)</i>					
Treatment	1.0(2.1)	4.8(1.7)	6.8(1.6)	14.0(1.9)	0.081
Control	2.4(2.0)	3.9(1.8)	5.4(1.6)	12.4(2.0)	
<i>Trochanter (%)</i>					
Treatment	0.9(2.1)	6.2(1.9)	8.6(1.9)	15.8(2.2)	0.088
Control	2.8(2.2)	5.1(2.0)	7.5(2.0)	14.9(2.2)	
<i>Femoral Neck (%)</i>					
Treatment	0.7(2.1)	6.7(1.7)	8.9(1.9)	15.4(1.9)	0.447
Control	3.4(1.9)	7.0(1.8)	8.2(1.7)	15.3(1.7)	

Table 5. Percentage change from baseline in parameters of bone size; L1-L4 lumbar spine width, area, height and volumetric density for 6, 12, 18 and 30 months (\pm SEM)

	6 Months ($N_{\text{txt}}=45, N_{\text{con}}=58$)	12 Months ($N_{\text{txt}}=56, N_{\text{con}}=60$)	18 Months ($N_{\text{txt}}=60, N_{\text{con}}=65$)	30 Months ($N_{\text{txt}}=58, N_{\text{con}}=65$)	<i>P</i> value (0-30)
<i>L1-L4 Width (%)</i>					
Treatment	2.3 (1.4)	4.9 (1.3)	7.2 (1.2)	12.1 (1.2)	0.676
Control	2.6 (1.1)	5.5 (1.2)	7.2 (1.1)	12.1 (1.3)	
<i>L1-L4 Area (%)</i>					
Treatment	2.9 (2.4)	8.1 (1.7)	13.7 (1.8)	25.0 (2.0)	0.511
Control	4.9 (2.0)	10.4 (1.9)	14.3 (2.0)	25.8 (2.4)	
<i>L1-L4 Height (%)</i>					
Treatment	1.9 (0.8)	3.8 (0.7)	6.2 (0.7)	10.1 (1.6)	0.293
Control	2.7 (0.7)	5.0 (0.8)	7.1 (0.8)	12.1 (1.1)	
<i>L1-L4 Volumetric Density (%)</i>					
Treatment	6.5 (5.6)	19.6 (4.9)	28.3 (5.0)	54.3 (6.5)	0.603
Control	14.0 (5.3)	20.9 (5.2)	32.6 (5.6)	60.5 (7.3)	

**Figure 1.** Comparison between the habitual calcium intake in the treatment and control groups throughout the study.

There were no significant differences in the bone mineral density changes between the groups, although some trends were evident at the total hip and trochanter. The changes 0-18 months also showed no significant advantage to either treatment (minimum *P* value =0.567). There were no significant differences in the indicators of bone size between the groups. The changes 0-18 months

also showed no significant advantage to either treatment (minimum *P* value =0.675)

Throughout the study habitual calcium intake did not change, indicating that the supplement did not influence food choice e.g substituting calcium rich foods for other foods (mean_{treatment} 30 months = 876, mean_{control} 30 months = 894). There were no significant differences in the percentage change in bone mineral content between the groups (minimum *P* value =0.268). There was no difference in the change in pubertal stage for the children between groups (Tanner stage 1 at baseline, Tanner stage 2 at 30 months). Fifteen girls started menstruating during the study duration, seven in the treatment group and eight in the control group.

Discussion

Our study shows there is no beneficial effect of taking a high calcium dairy supplement when the habitual dietary calcium intake is already high. There was no difference between the groups, although trends in the total hip and trochanter were evident. There may be a limit at which calcium supplementation has a positive effect on bone mineralisation. In the control group the mean intake during supplementation was 1395mg per day, and the

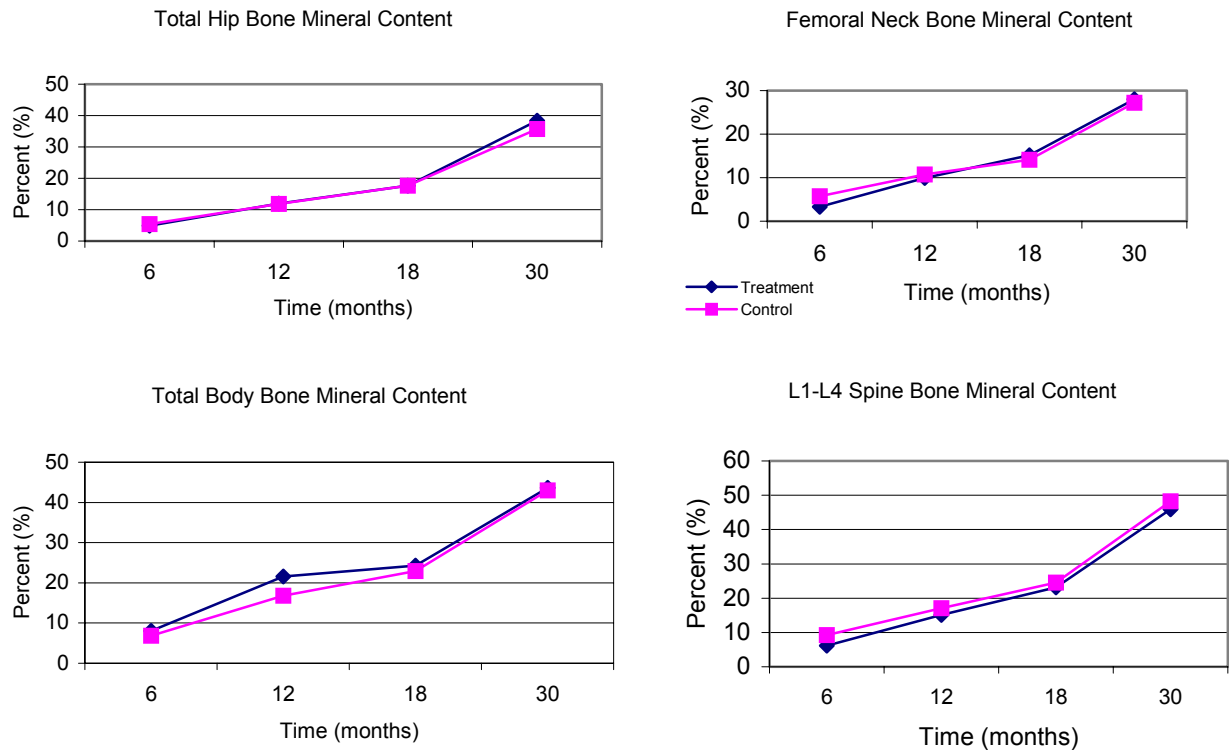


Figure 2. Percentage change from baseline for total body, L1-L4 lumbar spine, total hip and femoral neck bone mineral content at 6, 12, 18 and 30 months.

treatment group was even higher at 2106 mg per day. Weaver *et al.*,¹⁸ and Wastney *et al.*,¹⁹ have shown through calcium balance studies that maximum retention of calcium occurred at a level of 1300mg per day, which has since become the recommended intake in the USA for this age group. This group has estimated that an increase in calcium intake from 918mg to 1300mg per day could increase skeletal mass by 4% per year in pre-pubertal children. This is consistent with the increase in bone mineral density we saw in both the treatment and control groups. The previous studies that have looked at the effect of calcium supplementation on bone health and have reported varying levels of habitual calcium intake

(Fig. 3). Three studies had comparable baseline calcium intakes to this study,^{10-11,16} in that they were approximately 900mg/d; three studies^{9, 14-15} had intakes between 600mg/d and 750mg/d and the other two studies had low intakes of around 300mg/d.¹²⁻¹³ In contrast to the studies with the high intakes of calcium, our study used a placebo product containing an additional 400mg/d of calcium - this lifted the control group to around 1300mg. This was the only study to lift both the control group and the supplemented group over the “threshold” level for dietary calcium. This is an obvious flaw in the study design and would need to be addressed if this study was repeated. The main reason for the control group receiving calcium

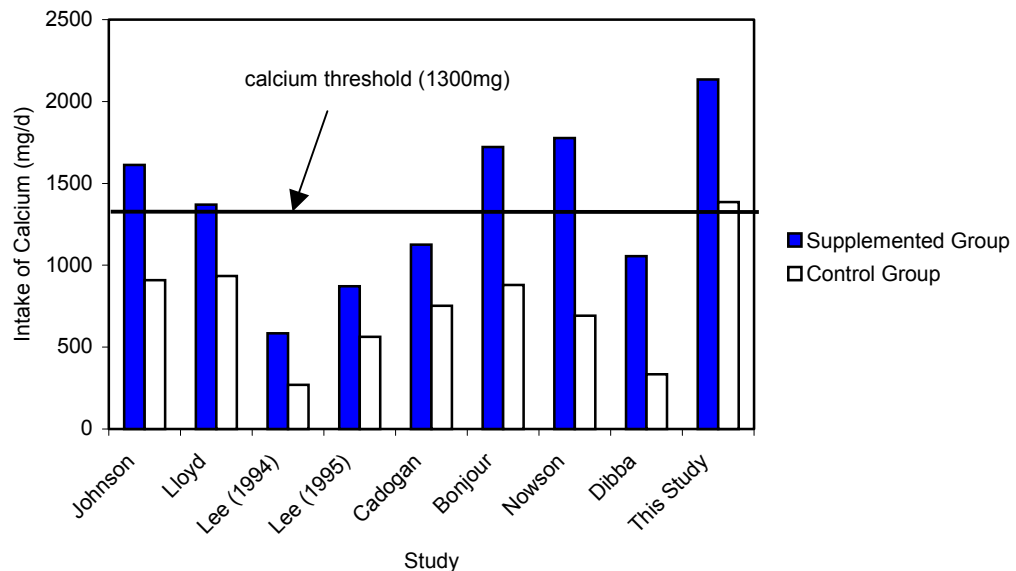


Figure 3. Differences in the calcium intakes in the intervention trials that have been carried out in pre-pubertal children

The main reason for the control group receiving calcium in the supplement was that the ethics committee at the time felt there was sufficient scientific evidence that calcium has an effect on bone health and that it is unethical to not provide some calcium to the growing skeleton. In addition, the researchers did not know how high baseline calcium intake was. In contrast, other studies²² into calcium intake in NZ children and adolescents have reported much lower levels than this study. This may be one of the main reasons no differences were seen in indicators of bone health.

We used a dairy calcium food product to supplement our subjects. Two previous studies^{11,15} looking at this age group have also used a calcium-containing food product to supplement their experimental group. Fluid milk provides an ideal source of calcium because it is inexpensive; the lactose in milk aids in calcium absorption, and it provides other nutrients that support growth and development in children.

Our baseline calcium intake was in line with the recommended intake of 800-900mg per day. However, the method used to determine habitual intake may not have been sufficient as we only used a single food frequency questionnaire. The most accurate method of dietary assessment is a weighed food collection and then assays to determine calcium mineral content. Studies have shown that even if food record diaries are collected for 9 days for girls and 18 days for boys the "true" calcium intake may be inaccurate.^{20,21} Thus, the values we obtained for habitual calcium intake may potentially be slightly over or under estimated.

Bone mineral content may be a better indicator of accretion in bone mineralisation than bone density, especially in this age group where the bones are still growing. We found no difference in bone mineral content between the two groups. Differences between the two groups were not observed in bone size during the supplementation period or during follow-up. This is different to results found by Bonjour *et al.*,¹¹ where they found an increase in bone size between the supplemented and control groups. In their study the results of the children in the lowest quartile for baseline dietary calcium intake were compared to those in the highest quartile. It was only in the children with a low dietary calcium intake that an effect on bone size and height was evident.

There is a common perception that dairy products are high in fat and consequently should be limited in the diet, or excluded. They are often thought to be the major cause of weight gain and undesirable changes in anthropometric measures. We found no adverse changes in the anthropometric measures, lean mass, fat mass, height and weight between the groups. This is consistent with previous findings in teenage girls.²²

Pubertal stage is a known determinant of bone mass. Thus using subject groups closely matched in this respect reduced the likelihood that differences in pubertal progression would obscure interpretation of bone measurements. The two groups in our study were matched at baseline and remained matched. There were a large number of the children who stopped taking the supplement during the supplementation period due to a dislike of the product. Product acceptability is important

in an intervention study and a prolonged compliance run-in period in this study may have reduced the high drop-out.

Compliance in the children that liked the supplement was high. This may be due to supervision from either the parent or the teacher in the classroom. A variety in the flavours was important for maintaining compliance and reducing the rate of withdrawals. Results from previous studies^{7,8} have suggested there is a decrease in dietary calcium intake from childhood to adolescence. By developing dietary habits to include the frequent intake of milk during childhood and adolescence it is likely that calcium intakes will be higher in later years. Studies that look at compliance are important to determine whether children can achieve the recommended daily intake from diet.

In this 2½ year study (18 months supplementation, 1 year follow-up) we did not observe a difference in bone mineral density in pre-pubertal children. We feel this is due to a high habitual dietary calcium intake, that even with minimal addition of calcium to the diet a threshold level was reached where no further benefit was seen. There were no adverse effects on body composition from the children taking the high calcium dairy product as throughout the study the children grew normally, and the mean height and weight remained between the 50th and 75th percentile for their age. Calcium supplementation in children needs to be targeted in those children with low habitual dietary calcium intake.

Acknowledgement

We would like to thank the children and their parents who participated in the study. The three schools and their staff that participated in the study: Redcliffs Primary School, St Martins School and Cashmere Primary School, and New Zealand Milk Limited, Wellington, New Zealand for their assistance with funding.

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