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Effects of dairy food supplements on bone mineral density in teenage girls

Summary Background Bone mineral density (BMD) is largely genetically determined and this influence is most powerful in the period of rapid skeletal development in childhood and late adolescence but environmental factors such as exercise and dietary calcium intake may influence up to 20 %.

Aims of the Study The aims of the study were to examine healthy late adolescent females for the effects and benefits of a high calcium intake from dairy product foods on bone mineral density, body composition, lipids and biochemistry. The secondary aim is determine whether a

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E. Hooke Christchurch Girls High School 10 Matai Street Christchurch, New Zealand high intake of dairy product foods in the diet is acceptable for this age group long term.

Methods Ninety-one teenage girls who participated in a two-year randomised controlled study on the effect of dairy food supplementation on dietary patterns, body composition and bone density in post-pubertal teenage girls were approached one year after the cessation of the study to determine the effects of the cessation of dairy supplements on bone mineral density, dietary habits, biochemical markers, body composition and blood lipids. Bone mineral density and bone mineral content were assessed at the hip, spine and total body. Anthropometric data were collected, and exercise, Tanner, dietary assessment, preference and compliance questionnaires were administered. Lipid profiles, hydroxyproline excretion and urinary calcium and sodium excretion measurements were performed.

Results There were no significant differences between the 2 groups for height, weight, lean and fat mass.

The supplemented group had significantly higher calcium, phosphorus and protein intake during the supplementation period (p < 0.001). No differences were seen between the groups 12 months after supplementation finished.

There were no significant differences in exercise level, preference or acceptability of dairy products or in the lipids and bone markers between baseline the end of supplementation and 1 year follow-up.

There was a significant increase in trochanter (4.6%), lumbar spine (1.5%) and femoral neck (4.8%) BMD (p < 0.05) in the high calcium group at the end of supplementation. There was an increase in bone mineral content at the trochanter (p < 0.05) and lumbar spine; however the latter was not statistically significant, in the high calcium group at the end of supplementation. There was no difference in vertebral height or width at any stage of the study, indicating no influence on bone size.

Conclusions In this 3 year study (2 years of supplementation, 1 year follow-up), teenage girls, aged 15–18 years, were able to significantly increase their BMD at the trochanter, femoral neck and lumbar spine when supplemented with dairy product foods to a mean calcium intake of 1160 mg/d. There was also an effect seen on the BMC particularly at the trochanter and to a lesser extent at the lumbar spine. The dietary calcium intake achieved did not adversely affect body weight, fat and lean mass or blood lipid profiles. Twelve months after the supplementation finished the girls had returned to their baseline diet, indicating selfselection of a high dairy product diet may be hard to achieve.

Key words BMD – BMC – Adolescent – Calcium Supplementation – Female

Introduction

Osteoporosis is a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [1]. Up to 80% of bone mineral density (BMD) may be genetically determined. The greatest period of rapid skeletal development occurs in childhood and adolescence accounting for 30–40% increase in skeletal bone mass.

Environmental factors such as exercise and dietary calcium intake may influence up to 20% of BMD [2]. The peak bone mass in pre-menopausal women has been shown to be a major determinant of osteoporosis in later life [3–5].

There are two approaches to the prevention of this disease, the primary prevention strategy is increasing peak bone mass at skeletal maturity, the secondary strategy is reducing the rate of bone loss after menopause [6].

An adequate amount of dietary calcium is important for reaching skeletal maturity in adolescence. Ninety five percent of bone acquisition appears to be completed by age 18 years, and a further 0–5% by the age of 30 years [6, 7]. A meta-analysis evaluating the existing literature confirms this, showing a positive relationship between calcium intake during adolescence and bone mass in females 18–50 years of age [8].

Prospective randomised clinical trials have shown calcium supplementation can increase bone acquisition in adolescence [9–14], early adulthood and into the third decade of life [6, 7]. When calcium supplementation ceased the beneficial effect on BMD disappeared if calcium intakes were not maintained at the level achieved in the studies [15–17].

Levels of dietary calcium recommended for adolescent females to reach full genetic potential in skeletal mass have been indicated to be as high as 1200–1500 mg/d [18, 19]. New Zealand has adopted the recommended dietary intake (RDI) used in Australia for this age group (15–18 years) that recommends dietary calcium intakes of 800–1000 mg/d [20]. Dietary analysis of NZ adolescent female 15–18 years has shown mean dietary intakes of 607 mg/d, which is well below the RDI [21]. Another study within a NZ adolescent female population aged 16.4 years showed 60% of this group had a mean dietary calcium intake below 800 mg/d [22].

The aims of the study were to examine healthy late adolescent females for the effects and benefits of a high calcium intake from dairy product foods on bone mineral density, body composition, lipids and biochemistry. The secondary aim is to determine whether a high intake of dairy product foods in the diet is acceptable for this age group long term.

Methods

Subjects

Two hundred and five teenage girls aged 15-16 years attending a local high school were approached and asked to complete a recruitment questionnaire. Of the 184 girls who were recruited 105 met the inclusion criteria and volunteered for the study. Exclusion criteria were thyroid disorders, renal impairment, hepatic dysfunction, pregnancy, oligomenorrhoea, amenorrhoea, current systemic illness, eating disorder, anorexia, use of glucocorticoids, anticonvulsant agents or thiazide diuretics. The girls were randomly allocated to either the control group or the supplemented group using forearm BMD at baseline for stratification. The supplemented group was supplemented with dairy food products to at least 1000 mg/d, the dairy supplements were delivered fortnightly. Dairy food products included milk, flavoured milk, dairy dessert, cheese or yoghurt; low fat options were available. The girls self-selected which products they would like. Ninety one girls completed the first two years of the study. Fourteen girls (10 in the supplemented group and 4 in the control group) failed to complete the study during the 2 year study period. In the supplemented group 6 moved out of the study area, 3 developed eczema or migraines and 1 failed to comply. In the control group 4 moved out of the study area.

The 91 teenage girls who participated in a two-year study on the effect of dairy food supplementation on dietary patterns, body composition and bone density in post-pubertal teenage girls were approached one year after the cessation of the study to determine the effects of the cessation of dairy supplements on bone mineral density, dietary habits, biochemical markers, body composition and blood lipids.

The study was approved by the Southern Regional Health Authority Ethics Committee (Canterbury), New Zealand (1993). Informed consent was obtained from the girls and their parents.

Protocol

All the girls were examined at the beginning of the study then every 6 months for the first two years; they were then followed up 12 months after the supplementation finished, as previously described [23, 24].

Dietary intake was assessed at each visit by a validated 3 day food record and a calcium food frequency questionnaire; these were filled in by the girls prior to an interview with the dietitian. The 3 day food record allowed a comparison of food groups to total energy intake in addition to nutrient intake. Bone density was measured at baseline, 12 months, 18 months, 24 months and 36 months; height and weight was also recorded at these times. Blood was drawn by venipuncture at baseline, 24 months and 36 months of the study for determination of serum lipid and calcium levels. At the same visits as for the blood sampling, over night fasting urine and 24 hour urine were collected for hydroxyproline, creatinine and urinary calcium and sodium excretion, as a measure of bone turnover. Exercise level was measured at baseline and 36 months using a New Zealandspecific questionnaire [25]. Pubertal stage was assessed by a self-administered Tanner [26] questionnaire.

Dietary compliance

The supplemented group had the dairy products delivered to them fortnightly. Each girl selected the dairy products under the guidance of the dietitian, to meet the minimal requirement of 1000 mg calcium daily. All participants were required to fill in a compliance questionnaire at 6, 12, 18 and 24 months to determine how many of the dairy products were being consumed. A preference questionnaire was completed at 36 months to compare the girls preference of dairy products before the study commenced and at the completion.

Procedures

Dietary records were analysed for total energy, calcium, protein, fat, vitamin D, phosphorus and magnesium by a computer software dietary package (DIET 1) [27], based on the New Zealand food composition tables. Bone mineral density, BMC and body composition was determined by dual-energy x-ray absorptiometry (DPX-L; Lunar Radiation Corp., Madison, Wisconsin). The total body, lumbar spine and 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 coefficient of variation for repeated scans of the lumbar spine is 1%, the femoral neck 2.5% and 0.5% for the total body. The coefficient of variation for repeated scans for lean muscle mass is 1.1 %, and 1.9 % for total fat mass [28]. The biochemical markers where analysed using an enzymatic method with a coefficient of variance of 3 % [29].

Statistical analysis

Repeated measures of analysis of variance were used to compare the changes in bone mineral density, bone mineral content, bone size and body composition between the two treatment groups. All p values were two tailed when checking for significance. The statistical package used was SYSTAT [30].

Results

From the 91 girls who completed the supplementation study 73 girls were followed up. The reasons for withdrawal included moved out of the area (n=9), unable to be contacted (n=7) and did not wish to participate (n=2). There were no differences between the two groups in terms of this attrition.

No differences were seen in the changes between the two groups from baseline, the end of supplementation or 1 year follow-up for height, weight, body fat and lean muscle (Table 1). Both groups were matched for Tanner stage at the beginning of the study.

The supplemented group achieved a higher calcium intake (p < 0.001) than the control group at all times during the supplementation period (Table 2). Protein and phosphorus were also statistically significant at the end of supplementation. No difference was seen in the daily mean intake of energy, fat, vitamin D and magnesium. There were no significant differences between the groups in any nutrient 12 months after the supplementation finished. During the supplementation part of the study the girls appeared to reduce the amount of baked products in their diet to enable them to consume the higher volume of dairy products, this finding was reversed twelve months after the supplementation finished.

No significant differences were seen in exercise level (17.4±1.6 vs 20.1±2.3 hours at baseline and 22.9±1.9 vs

Table 1 Weight, height, body fat and lean muscle mass for the control and supplemented group at 0, 2 years and follow-up (SEM)

	Baseline	2 years	Follow-up
	(n=105)	(n=91)	(n=73)
Weight (kg)			
Control	58.7(1.1)	62.7(1.0)	62.4(0.2)
Supplemented	55.8(0.9)	60.4(1.1)	61.8(2.8)
Height (cm)			
Control	165.0(0.8)	166.6(0.8)	166.2(0.9)
Supplemented	164.4(0.9)	165.7(0.9)	165.2(0.9)
Body Fat (g)			
Control	16764(814)	19320(722)	19987(824)
Supplemented	14748(656)	17364(706)	18066(668)
Lean Muscle (g)			
Control	38533(559)	38654(503)	38689(573)
Supplemented	37688(622)	38408(714)	38443(733)
Tanner 1			
Control	4.03(0.09)		
Supplemented	4.17(0.08)		
Tanner 2			
Control	4.33(0.09)		
Supplemented	4.34(0.08)		

No significant differences seen in the anthropometric data between either group. Tanner 1 = breast development, tanner 2 = pubic hair development.

Table 2 Dietary analysis of mean calcium, protein, vitamin D, phosphorous and magnesium from the 3 day food record for the control group and the supplemented group at 0,2 years and follow-up (SEM)

0 years 2 years Follow-up (n=105)(n=91)(n=73)Calcium (mg/d) Control 765.3(54.5) 683.9(47.1) 651.6(35.0) Supplement 744.1(54.1) 1155.1(52.3)** 695.0(59.0) Energy (kJ/d) Control 7591.4(375.7) 7376.3(459.9) 7364.5(300.9) Supplement 8080.9(372.6) 8634.4(409.0) 7524.7(375.5) Protein (g/d) Control 66.2(3.5) 62.4(3.5) 64.4(2.6) 62.5(3.5) 81.2(4.1)** Supplement 64.7(3.3) Fat(g/d)Control 72.3(4.9) 62.7(4.8) 67.7(3.4) Supplement 78.6(4.6) 78.9(5.0) 69.7(4.3) Vitamin D (µg/d) Control 1.77(0.21)1.09(0.13)1.09(0.14)Supplement 1.91(0.23) 1.46(0.20)1.11(0.15) Phosphorus (mg/d) Control 1122.3(65.2) 1078.5(64.2) 1047.6(42.2) Supplement 1127.4(67.2) 1436.1(64.4)** 1084.1(58.1) Magnesium (mg/d) Control 222.3(12.0) 243.7(17.7) 218.8(10.5) Supplement 245.4(13.6) 267.6(13.3) 226.2(12.6)

A significant difference was seen in the dietary calcium, protein and phosphorus intake in the supplemented group at the end of the supplementation period.

26.6±2.5 hours at follow-up), preference or acceptability of dairy products between the two groups 12 months after the supplementation finished.

There were no differences in blood lipid results or changes in bone markers (Table 3). Creatinine levels were significantly different at the end of supplementation between the two groups (p < 0.05).

There were significant increases seen in the bone density at the trochanter, lumbar spine and femoral neck (p < 0.05) at the end of supplementation; no effect was seen 12 months after supplementation finished (Fig 1).

A significant increase in bone mineral content (p < 0.05) was seen at the trochanter after the 2 years of supplementation (Table 4). There was also an increase in the lumbar spine that did not achieve statistical significance (p=0.054). At 1 year follow-up there was no change in all BMCs. There was no difference between the groups for changes in bone size, this was measured by comparing vertebral width and height.

Table 3 Serum lipids and biochemical markers of bone turnover at 0, 2 years and follow-up in the control group and supplemented group (SEM)

	0 years (n=105)	2 years (n=91)	Follow-up (n=73)
Total abalasta:-1			(/
Total cholesterol			
(mmol/L)	4.500(0.12)	4.570(0.12)	4.006(0.70)
Control	4.590(0.12)	4.570(0.12)	4.906(0.79)
Supplemented	4.566(0.21)	4.721(0.22)	5.006(0.25)
Triglycerides			
(mmol/L)	0.010(0.05)	1 211(0 10)	1.062(0.07)
Control	0.812(0.05)	1.311(0.10)	1.062(0.07)
Supplemented	0.912(0.07)	1.234(0.12)	0.956(0.08)
HDL (mmol/L)		4.447.000	4 700/000
Control	1.346(0.05)	1.267(0.04)	1.589(0.06)
Supplemented	1.320(0.06)	1.269(0.06)	1.644(0.08)
LDL (mmol/L)			
Control	2.873(0.11)	2.713(0.11)	2.840(0.14)
Supplemented	2.846(0.17)	2.903(0.17)	2.932(0.20)
Chol/HDL ratio			
Control	3.530(0.12)	3.705(0.13)	3.203(0.14)
Supplemented	3.551(0.20)	3.856(0.22)	3.212(0.21)
Calcium (mmol/L)			
Control	1.893(0.23)	2.306(0.30)	1.888(0.24)
Supplemented	1.754(0.23)	2.329(0.30)	2.120(0.24)
Hydroxyproline			
$(\mu mol/L)$			
Control	723.57(69.3)	395.16(39.0)	309.63(25.4)
Supplemented	611.77(62.5)	482.10(46.9)	343.43(34.0)
Calcium			
creatinine ratio			
Control	0.134(0.01)	0.203(0.02)	0.151(0.02)
Supplemented	0.134(0.01)	0.161(0.02)	0.163(0.02)
Hydroxyproline			
Creatinine Ratio			
Control	0.047(0.002)	0.048(0.010)	0.023(0.001)
Supplemented	0.047(0.002)	0.032(0.002)	0.025(0.001)
Creatinine	(()	()
(mmol/L)			
Control	16.128(1.45)	12.843(1.27)*	13.940(1.12)
Supplemented	13.869(1.54)	15.253(1.43)	14.036(1.29)

^{*}p < 0.05

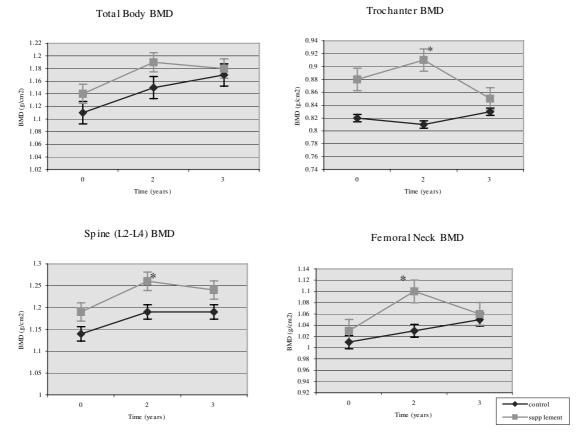
A significant difference was seen in the creatinine levels at the end of supplementation.

Discussion

Our study demonstrates the beneficial effect of dairy food calcium supplementation on bone mineral density in teenage girls. The effect is most noticeable at the sites of trabecular bone, namely the femoral neck (4.8%), trochanter (4.6%) and the lumbar spine (1.5%) (p < 0.05).

^{**} p < 0.001

Fig. 1 The mean (SEM) trochanter, lumbar spine, femoral neck, and total body bone mineral density in teenage girls with or without dairy supplementation.



* p < 0.05The p value indicates significant differences in the change from baseline between the two groups after supplementation.

It is known that skeletal mass doubles through childhood, puberty and adolescence, with increases up to 7–8% per year [31]. This gain appears to be highest between 11 and 14 years of age in girls [32]. The age of peak bone density is controversial but some authors have suggested it may be as late as 23 years [33] or even later at 29 years [7]. In this study it appears peak bone mass occurred at the femur and trochanter by age 17 years of age, while the bone density at the spine was still increasing at 18 years of age.

Bone mineral content may be a better indicator of accretion in bone mineralisation than bone density. We found a significant increase in the bone mineral content at the trochanter after 2 years in the supplemented group. There was also an increase in the lumbar spine; however this was not significant. There were no differences in vertebral height or width indicating calcium supplementation at this age appears not to affect bone size.

There is an indication that hip fracture rates may be higher in women who have had lower dietary calcium intakes [1]. Previous studies have used dietary calcium to demonstrate increases in bone density. In a study using 9 to

13 year old girls Chan et al. demonstrated, after a 12 month period of time, increases in bone density at the spine and total body in 48 subjects [10]. Cadogan et al. demonstrated in 82 pubertal girls aged 11 to 12 years, using a milk supplement, similar significant increases in bone density at the hip and spine [12].

Throughout the supplementation period of the study, calcium intake in the supplemented group differed significantly from the control group (1155 mg/day vs 684 mg/day). This returned to similar levels 12 months after supplementation finished (695 mg/day vs 652 mg/day). It was also evident the girls in the supplemented group had returned to a diet similar to their baseline diet; hence we were unable to positively influence their dairy product intake long term. Other nutrients (protein and phosphorus) were also significantly higher during supplementation. In previous studies where dairy products were used to supplement there was an increase in vitamin D that paralleled the increase in calcium, phosphorus, protein and energy; however in New Zealand dairy products are not fortified with vitamin D and we did not expect to see an increase. It

Table 4 Change in bone mineral content at the total body, lumbar spine, femoral neck and trochanter, and change in vertebral height and width from 0–2 years and 2–3 years (SEM)

<u>-</u>			
	0–2 years	2–3 years	
Total Body (g)			
Control	167.4(16.2)	9.3(10.7)	
Supplemented	168.9(24.7)	13.6(11.7)	
Lumbar Spine (g)			
Control	2.58(0.36)	0.05(0.53)	
Supplemented	3.83(0.53)	0.44(0.21)	
Femoral Neck (g)			
Control	0.06(0.05)	0.04(0.02)	
Supplemented	0.12(0.06)	0.04(0.02)	
Trochanter (g)			
Control	0.24(0.13)	-0.12(0.10)	
Supplemented	0.75(0.16)*	-0.05(0.11)	
Vertebral Height (cm)			
Control	0.14(0.04)	0.08(0.02)	
Supplemented	0.11(0.03)	0.10(0.02)	
Vertebral Width (cm)			
Control	0.08(0.02)	0.03(0.01)	
Supplemented	0.07(0.01)	0.03(0.01)	

^{*}p < 0.05

There is a significant difference in the change in BMC from 0–2 years between the control and supplemented group. No significant differences were seen in any other measurement.

is uncertain which nutrient or combination of nutrients is responsible for changes in BMD when dairy products are used as the supplement, as energy, protein, calcium, phosphorus and vitamin D are known to be associated with bone health [2]. High protein intake has produced negative calcium balance from increased urinary calcium excretion if phosphorus intake is kept low, but if the phosphorus intake increases with the protein intake, the effect of a high protein intake on calcium metabolism is minimised [10].

Most studies on diet and health are based on the assumption that dietary assessments are able to obtain valid measures of long-term average dietary intake. Many studies have compared one dietary assessment method with another, but with the absence of external validation it is not possible to conclude which method measures the true or valid intake [34–36]. The magnitude and direction of the errors or bias remains largely unexplored. For these reasons we used a variety of dietary intake measures, with the hope of reducing some of the error.

There is concern dairy products may lead to a higher dietary fat intake. In this study fat intake was similar in both groups, throughout supplementation and 1 year after sup-

plementation had finished. There were no differences in lean and fat mass, or weight. This is consistent with findings by Chan et al. [10]. Supplementation did not adversely affect blood lipids with no differences seen between the groups at any stage. There was a significant difference in creatinine levels at the end of supplement between the groups. It is unclear why this occurred as there were no changes in weight or lean muscle mass, and these are the most common causes of altered creatinine levels.

Height increased in both groups at the same rate. The girls were matched for pubertal stage at baseline, they were still growing and growth parameters remained similar throughout the study. There was no difference between either group in their exercise level at baseline and follow-up.

A number of longitudinal randomised studies have supported increasing dietary calcium through calcium tablets or dairy product foods benefits BMD in young adolescents [6, 9–14]. The majority of these studies have been done in girls younger than the participants in our study. In the younger girls BMD acquisition is occurring at a much higher rate than in the older adolescents used in our study. When dairy product foods were used as a supplement, BMD changes were greater than when calcium was used as the only supplement [10]. One study found no significant difference in BMD changes in girls who were post-pubertal or went through puberty during the study using calcium tablets [9]. However in another study of 20 pubertal girls, positive effects on BMD were found with calcium intakes of 1640 mg/d in the supplemented group versus 750 mg/d in the control group when both calcium tablets and milk were used as the supplement [6]. Similar results were found in a two year calcium supplementation study using calcium citrate malate to increase calcium intakes to 1300 mg/d in Caucasian girls [13].

Follow-up studies after the cessation of calcium supplementation have found the beneficial effect of calcium supplementation on BMD disappears when calcium levels are not maintained at the higher levels as in the original study [15, 16].

In this 3 year study (2 years of supplementation, 1 year follow-up), teenage girls, aged 15–18 years, were able to significantly increase their BMD at the trochanter, femoral neck and lumbar spine when supplemented with dairy product foods to a mean calcium intake of 1160 mg/d. There was also an effect seen on the BMC at the trochanter and the lumbar spine, although the latter was not significant. The dietary calcium intake achieved did not adversely affect body weight, fat and lean mass or blood lipid profiles. Twelve months after the supplementation finished the girls had returned to their baseline diet, indicating self-selection of a high dairy product diet may be hard to achieve.

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