Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial

Joanna Cadogan, Richard Eastell, Nicola Jones, Margo E Barker

Abstract

Objectives: To investigate the effect of milk supplementation on total body bone mineral acquisition in adolescent girls.
Design: 18 month, open randomised intervention trial.
Subjects: 82 white girls aged 12.2 (SD 0.3) years, recruited from four secondary schools in Sheffield.
Intervention: 568 ml (one pint) of whole or reduced fat milk per day for 18 months.
Main outcome measures: Total body bone mineral content and bone mineral density measured by dual energy *x* ray absorptiometry. Outcome measures to evaluate mechanism included biochemical markers of bone turnover (osteocalcin, bone alkaline phosphatase, deoxypyridinoline, *N*-telopeptide of type I collagen), and hormones important to skeletal growth (parathyroid hormone, oestradiol, insulin-like growth factor I).
Results: 80 subjects completed the trial. Daily milk intake at baseline averaged 150 ml in both groups. The intervention group consumed, on average, an additional 300 ml a day throughout the trial. Compared with the control group, the intervention group had greater increases of bone mineral density (9.6% *v* 8.5 %, P = 0.017; repeated measures analysis of variance) and bone mineral content (27.0% *v* 24.1 %, P = 0.009). No significant differences in increments in height, weight, lean body mass, and fat mass were observed between the groups. Bone turnover was not affected by milk supplementation. Serum concentrations of insulin-like growth factor I increased in the milk group compared with the control group (35% *v* 25 %, P = 0.02).
Conclusion: Increased milk consumption significantly enhances bone mineral acquisition in adolescent girls and could favourably modify attainment of peak bone mass.

Introduction

Osteoporosis is a major public health problem, with over 200 000 fractures occurring annually in the United Kingdom, of which 85% occur in women.[1] It is increasingly recognised that maximising peak bone mass at skeletal maturity may provide important protection against risk of fracture in later life. By the end of the second decade, 90-95% of total body peak bone mass is attained,[2 3] with bone growth in adolescence accounting for about half of this figure.[4] Peak bone mass is determined by a combination of endogenous (genetic, hormonal) and exogenous (nutritional, physical activity) factors.[5] These exogenous factors are amenable to intervention and could thus provide a basis for public health strategies for preventing osteoporosis.

Recent studies of nutrient intake in British schoolchildren revealed that calcium intakes in teenage girls are low in comparison with recommended levels[6 7]; this age group may be consuming insufficient calcium to meet the demands of rapid skeletal growth. In addition, milk consumption per household in the United Kingdom has been declining steadily since the 1970s.[8] Government legislation introduced in the early 1970s restricted the provision of school milk,[9] which may have had a further negative impact on calcium intakes in childhood. In the last milk trial in British schoolchildren,[10] height and weight were the only outcome measures, as no reliable means of measuring bone density or bone turnover were available at that time. Our study aimed to evaluate the effect of milk supplementation on bone mineral acquisition in adolescent girls and to investigate the physiological mechanism for any effect.

Subjects and methods

Subjects

Subjects were volunteers from four local schools in the city of Sheffield. The schools were selected to give an equal representation of manual and non-manual social classes in the trial. Eighty two healthy white girls with a mean age of 12.2 (SD 0.3) years were enrolled into the study. No subjects had any history of bone disease or were taking any drugs known to influence calcium metabolism. They were all non-smokers and were not following any special dietary regimens. No subjects were taking calcium supplements.

Written informed consent was obtained from all volunteers and their parents. The study was carried out in accordance with the Declaration of Helsinki and with the approval of the ethics committee of the Northern General NHS Hospital Trust, Sheffield.

Study design

The study design was an 18 month intervention trial. The subjects were randomised by a statistician who

Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU
Joanna Cadogan, *research student*
Margo E Barker, *lecturer*
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield, Northern General Hospital
Richard Eastell, *professor*
Department of Public Health Medicine, University of Sheffield Medical School, Sheffield S10 2RX
Nicola Jones, *research officer*
Correspondence to: Dr Barker m.e.barker@sheffield.ac.uk

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took no part in the execution of the trial, using randomised permuted blocks stratified by pubertal stage, into a milk group and a control group. The intervention comprised 568 ml (one pint) of whole or reduced fat milk according to the subject's preference, which was delivered to all subjects' houses every morning. Subjects in the milk group were asked to consume as much of the pint as possible as a daily supplement to their usual food intake. The calcium contents of whole, semi-skimmed, and skimmed milks are virtually identical: 115 mg/100g, 118 mg/100g, and 120 mg/100g, respectively. Subjects in the control group were asked to continue with their habitual diet.

All measurements, other than dietary intake, were made every 6 months. The principal outcome measures were changes in bone mass and density; secondary outcome measures were anthropometric and body composition variables and biochemical indices of skeletal growth.

Dietary assessment and physical activity

Nutrient intake was assessed at baseline and at the end of the study with the 7 day weighed intake method, in which subjects were instructed to weigh (Soehnle digital scales, Murrhardt, Germany; accurate to 2 g and weighing up to 5 kg) and record all items of food and drink consumed over 7 days. On five interim occasions, subjects completed a 4 day non-weighed food diary, in which portion sizes were estimated using household measures and food models. These diaries helped to monitor compliance with the supplement and to assess longitudinal dietary intake throughout the study. Nutrient intakes were calculated from the diet records using FOODBASE dietary software (Institute of Brain Chemistry and Human Nutrition, London). Habitual levels of physical activity were measured by using a questionnaire designed for this age group.[11]

Anthropometry and pubertal staging

Height was measured to the nearest mm with a stadiometer (Holtain, Crymych, Dyfed), and weight to the nearest 100 g with a set of upright balance scales (Seca 220, Hallamshire Scales, Sheffield). All measurements were made in the morning, by the same observer at each time point. Pubertal staging was ascertained by self assessment, using line drawings and written descriptions of the five stages of puberty, according to Tanner's definitions.[12] This method has been validated in adolescents.[13]

Bone mass and body composition

Total body bone mineral content, total body bone mineral density, lean body mass, and fat body mass were measured by dual energy *x* ray absorptiometry on a Hologic QDR/1000W densitometer (Hologic, Waltham, MA, USA). This method has a precision error (coefficient of variation) of 0.9-1.0% for total body bone mineral density in children.[14] A daily quality assurance test was performed using a spine phantom supplied by the manufacturer. The reproducibility of the phantom measurement over the duration of the study was 0.4%.

Biochemistry

Samples of non-fasting morning blood and 2 hour urine were obtained at each visit. To assess bone formation, serum concentrations of osteocalcin were measured with an immunoradiometric assay (ELSA-OSTEO, Cis Bio International, Gif-sur-Yvette, France), and serum immunoreactive bone specific alkaline phosphatase was measured with an immunoradiometric assay (Tandem-R OSTASE, Hybritech Europe, Liège, Belgium). Markers of bone resorption were crosslinked *N*-telopeptides of type I collagen, measured in urine with a competitive enzyme linked immunosorbent assay (Osteomark, Ostex International, Seattle, WA, USA), and urinary immunoreactive free deoxypyridinoline crosslinks, measured by competitive enzyme linked immunosorbent assay (Pyrilinks-D, Metra Biosystems, Mountain View, CA, USA). The results were expressed as a ratio to urinary creatinine concentration. Serum parathyroid hormone was measured by an immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA, USA) and serum oestradiol by radioimmunoassay (Diagnostic Products, Los Angeles, CA, USA). Serum insulin-like growth factor I was measured by radioimmunoassay (Medgenix Diagnostics, Fleurus, Belgium) after acid-ethanol extraction, to prevent interference from the binding proteins. Urinary creatinine was measured by the Jaffé technique, using a dry slide chemistry autoanalyser (Ecktachem 950, Johnson and Johnson).

Statistical analysis

Results are reported as means (SD) unless otherwise indicated. Baseline values between the milk and control groups were compared using Student's *t* tests or χ^2 tests as appropriate. For the anthropometric measurements, *t* tests were used to compare changes from baseline between the groups. Repeated measures analysis of variance was used to compare the groups with respect to changes in bone mineral measurements, biochemical analytes, and physical activity levels, since we had measurements for all subjects at all four time points (0, 6, 12, and 18 months). An active treatment analysis was performed on the 80 subjects who completed the study. Analysis of covariance was used to test further for group differences in all biochemical analyte concentrations, for pubertal status (months after menarche) controlled for. The Wilcoxon matched pairs signed ranks test was used to analyse dietary changes over time within each group. The statistical package for the social sciences (Windows Version 6, SPSS, Chicago, IL, USA) was used for data analysis.

Results

Eighty of 82 girls completed the trial. One girl from the milk group was excluded after 2 months because of non-compliance with the intervention, and one girl from the control group withdrew after 10 months because of a change of school. Most (36/44) subjects in the milk group chose semi-skimmed milk for their supplement; six chose whole milk and two chose skimmed milk. Table 1 shows there were no significant differences between the milk and the control groups at baseline for any of the variables measured. The anthropometric characteristics of the cohort were within expected norms for this age group.[15]

Bone mineral acquisition throughout the 18 months was significantly greater in the group given milk supplement than in the control group (fig 1). The intervention group had greater percentage increases

Table 1 Baseline clinical characteristics of subjects. Results are means (SD) unless otherwise indicated

Characteristic	Milk group (n=44)	Control group (n=38)
Age (years)	12.2 (0.3)	12.1 (0.3)
Height (cm)	151.7 (7.8)	152.9 (6.5)
Weight (kg)	45.1 (10.1)	45.3 (9.5)
Body mass index (kg/m^2)	19.5 (3.4)	19.3 (3.5)
Body fat (kg)	11.4 (4.7)	10.9 (5.4)
Body fat (%)	24.6 (5.2)	23.3 (6.2)
Lean body mass (kg)	32.0 (5.8)	32.6 (5.2)
Total body bone mineral density (g/cm^2)	0.89 (0.07)	0.90 (0.08)
Total body bone mineral content (g)	1407 (338)	1454 (331)
No (%) at Tanner stage:		
I	7 (16)	8 (21)
II-IV	37 (84)	30 (79)
No (%) menstruating	5 (11)	7 (18)
Calcium intake (mg)	739 (218)	753 (199)
Exercise (kJ/kg/day)	40.6 (18.2)	44.4 (26.9)

of total body bone mineral density (9.6% (SD 1.9%; 95% confidence interval 9.0% to 10.2%) *v* 8.5% (2.7%; 7.6% to 9.4%); P = 0.017) and total body bone mineral content (27.0% (5.8%; 25.2% to 28.8%) *v* 24.1% (6.3%; 22.0% to 26.1%); P = 0.009). Expressed in absolute terms, the respective increases were 0.090 (0.020; 0.084 to 0.096) *v* 0.081 (0.025; 0.072 to 0.089) g/cm^2 for total body bone mineral density (P = 0.021) and 428 (88; 398 to 452) *v* 391 (107; 358 to 430) g for total body bone mineral content (P = 0.035); thus the milk group gained an extra 37 g of bone mineral during the 18 months. Table 2 shows that the milk group had significantly greater increases of pelvic and leg bone mineral density than the control group.

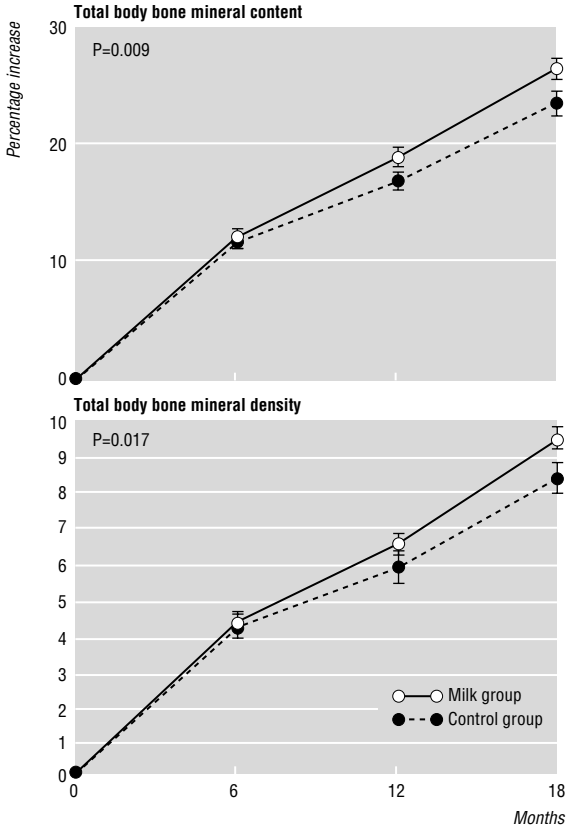

Fig 1 Mean (SE) percentage increases in total body bone mineral content and total body bone mineral density over 18 months. P values are for the differences between groups by repeated measures analysis of variance

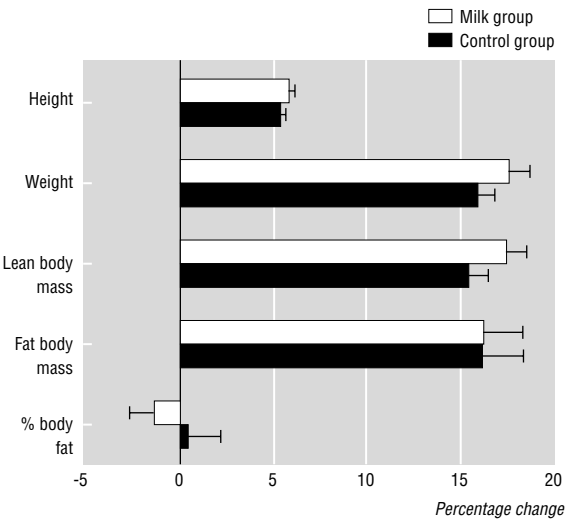

Fig 2 Mean (SE) increments in height, weight, lean body mass, fat body mass, and percentage body fat over 18 months. *t* Tests showed no significant differences between groups

The groups had similar changes over 18 months in anthropometric and body composition variables, and they did not differ significantly at the end of the study. Both groups showed similar increments in height, weight, lean body mass, and fat body mass, although the milk group showed non-significant trends towards greater gain in weight and lean body mass, and reduction in percentage body fat (fig 2). The groups made similar pubertal progression throughout the study: they did not differ significantly with respect to serum oestradiol concentrations, the number of subjects in each Tanner stage, the proportion reaching menarche, nor time (months) since menarche.

Table 3 shows that at baseline, milk intake was approximately 150 ml a day in each group. The milk group increased their mean milk intake by about half a pint a day, from 170 (SD 122) to 486 (186) ml/day. This level of compliance was corroborated by the interim food diaries completed at intervals of 3 months throughout the study. Milk consumption in the control group was unchanged. In the milk group, the milk supplement significantly increased intakes of protein, calcium, phosphorus, magnesium, and zinc (table 3) and riboflavin and thiamin (data not shown). There was a trend towards increased energy intake (P = 0.065). Nutrient intakes did not change significantly in the control group. The interim nutritional assessments from the estimated food records corroborated the

Table 2 Mean (SD) percentage increases in regional bone densities (from total body measurement)

	Milk group	Control group	P value
Head	16.1 (6.5)	14.5 (6.7)	0.39
Arms	9.9 (3.0)	9.8 (4.2)	0.54
Ribs	5.7 (2.9)	5.3 (2.7)	0.53
Thoracic spine	17.9 (5.5)	16.2 (6.0)	0.09
Lumbar spine	17.9 (6.8)	16.2 (6.7)	0.47
Trunk†	14.5 (3.7)	13.1 (4.4)	0.17
Pelvis	14.0 (5.0)	11.6 (4.3)	0.003
Legs	10.4 (3.3)	9.1 (4.0)	0.005

†Ribs, lumbar spine, and thoracic spine.

Table 3 Mean (SD) daily intake of milk, energy, and nutrients by milk and control group, assessed by 7 day weighed intake

	Milk group	Control group
Milk (g):		
Baseline	170 (122)	142 (127)
18 months	486 (186)*	160 (113)
Energy (kJ):		
Baseline	8012 (1652)	7992 (1387)
18 months	8563 (1326)	7511 (1417)
Protein (g):		
Baseline	59.1 (14.2)	55.8 (11.7)
18 months	70.7 (13.6)*	56.4 (9.9)
Fat (g):		
Baseline	78.9 (18.6)	80.9 (16.2)
18 months	81.3 (15.8)	73.6 (15.5)
Carbohydrate (g):		
Baseline	257 (57)	255 (55)
18 months	273 (44)	241 (54)
Calcium (mg):		
Baseline	739 (218)	753 (199)
18 months	1125 (294)*	703 (205)
Phosphorus (mg):		
Baseline	1020 (258)	975 (204)
18 months	1334 (274)*	967 (179)
Magnesium (mg):		
Baseline	210 (49)	208 (59)
18 months	253 (49)*	201 (37)
Zinc (mg):		
Baseline	6.6 (1.8)	6.7 (2.3)
18 months	8.2 (1.6)*	6.5 (1.2)

* Significantly different from baseline; P<0.01.

results of the final 7 day weighed intake. The two groups had similar levels of physical activity throughout the study, ascertained by questionnaire at each time point.

Markers of bone formation and resorption were similar in the groups throughout the trial (table 4), indicating no effect of supplementation on bone turnover. This was confirmed in analysis of covariance, adjusted for pubertal status (months after menarche). Changes in parathyroid hormone and oestradiol levels were also similar. The milk group showed a clear trend towards higher concentrations of insulin-like growth factor I over the course of the study (35% (39%) *v* 25% (43%); P = 0.080). This effect was significant (P = 0.02) after adjustment for pubertal status. Thus, throughout the trial, the milk group had consistently higher concentrations of serum insulin-like growth factor I, and this was not related to a difference in sexual maturity.

Table 4 Mean (SD) concentrations of biochemical markers of bone turnover and hormonal indices throughout the trial

		Time			
Analyte	Baseline	6 months	12 months	18 months	P value*
Bone alkaline phosphatase (μg/l):					
Milk	80.1 (19.9)	70.2 (17.8)	66.0 (20.2)	56.3 (22.4)	0.974
Control	85.9 (19.7)	72.4 (23.5)	67.7 (23.8)	56.4 (27.3)	
Osteocalcin (ng/ml):					
Milk	127 (31)	112 (28)	102 (30)	93 (31)	0.109
Control	134 (44)	117 (35)	108 (32)	94 (41)	
N-telopeptide (nmol BCE/mmol Cr):					
Milk	350 (155)	415 (186)	338 (173)	292 (163)	0.989
Control	360 (150)	492 (333)	336 (138)	322 (195)	
Deoxypyridinoline (nmol/mmol):					
Milk	17.1 (5.1)	19.3 (6.2)	17.3 (6.0)	15.8 (6.6)	0.345
Control	16.9 (5.1)	19.6 (6.0)	16.1 (4.9)	16.2 (6.7)	
Oestradiol (pmol/l):					
Milk	47.6 (39.5)	84.2 (72.6)	109.2 (114.8)	104.8 (59.5)	0.937
Control	53.2 (48.2)	76.2 (61.9)	97.6 (90.7)	125.3 (95.4)	
Insulin-like growth factor I (ng/ml):					
Milk	390 (169)	450 (179)	500 (124)	522 (104)	0.023
Control	385 (163)	408 (118)	426 (135)	448 (105)	
Parathyroid hormone (pg/ml):					
Milk	22.4 (7.6)	26.8 (13.0)	26.9 (10.4)	20.4 (7.2)	0.271
Control	24.8 (11.5)	22.6 (8.8)	26.5 (11.1)	19.4 (10.1)	

*Adjusted for pubertal status, P values are for difference between the groups, over time using analysis of covariance.

Discussion

The results of this trial indicate that increased milk consumption in adolescent girls resulted in greater total skeletal mineral acquisition over 18 months. These results are consistent with the hypothesis that milk intake has a beneficial effect on bone mass which, if sustained throughout the pubertal growth period and into adulthood, could favourably modify peak bone mass. Our findings agree with those of calcium supplementation trials in children[16-18] and adolescents[19 20] and with a dairy supplementation trial in early pubertal girls.[21]

Our results are also consistent with the evidence from retrospective studies, which have found that a high calcium intake in the form of dairy products in early life is positively associated with greater peak bone mass in adult life.[22-25] Also, supplementation with dairy products has been shown to prevent bone loss in premenopausal and postmenopausal women.[26 27]

Nutritional factors

The mean calcium intake of the group given milk supplement was 1125 mg/day. The baseline calcium intake of the study cohort, at 746 mg/day, was slightly below the United Kingdom reference nutrient intake of 800 mg/day for girls of this age group.[28] It is therefore of interest that calcium intake in excess of an amount deemed sufficient in public health policy terms (140% of the reference nutrient intake) resulted in significant gains in bone mineral. Similar findings have been reported by others.[16 19] These findings support the hypothesis that current levels of calcium intake (and therefore the current reference nutrient intake in the United Kingdom) are inadequate for maximum bone mineral accrual in rapidly growing adolescents.

Most of the studies investigating nutrition and bone mass have focused exclusively on calcium, whereas we evaluated the efficacy of a milk supplement on bone acquisition. Since milk contains other nutrients essential for bone growth, our results may be due, at least in part, to nutrients other than calcium. Milk supplementation resulted in significantly higher intakes of protein, calcium, phosphorus, magnesium, zinc, and a range of other micronutrients. In public health terms, such a "multi-nutrient" approach may not be without merit. Studies examining dietary patterns associated with low calcium intakes have shown that diets deficient in calcium are also low in a range of other nutrients, after energy intake is controlled for.[29]

Growth factors

The increased protein intake from the milk may partly explain our findings. Serum insulin-like growth factor I

increased over the 18 months in both groups as expected but showed a greater increment in the milk group. Serum concentrations of insulin-like growth factor I are influenced by nutritional status and are particularly responsive to changes in protein intake and, to a lesser degree, energy intake.[30]

Insulin-like growth factor I has potent anabolic effects on growing skeletal tissue. At birth, concentrations of insulin-like growth factor I are about half adult levels; they increase gradually during childhood, reaching a peak at pubertal stages 3 and 4.[31] The insulin-like growth factor I enhances chondrocyte proliferation in the growth plate; in bone tissue it stimulates osteoblast proliferation and differentiation and matrix formation, including the synthesis of type I collagen and other protein components.[32] However, whether changes in circulating concentrations of the factor reflect local (bone tissue) concentrations is uncertain, and the amount in bone tissue may be more important for bone metabolism.[33]

The milk supplement may have predominantly enhanced acquisition of bone in the legs and pelvis; growth in the lower body segment seems to depend more on growth hormone than does growth in the upper segment (more dependent on sex hormones).[34] Insulin-like growth factor I may also have had a role in the greater (although non-significant) increase in lean body mass observed in the milk group. In human muscle tissue, insulin-like growth factor I stimulates all anabolic processes.[35]

Bone gain

Milk had no detectable effect on concentrations of biochemical markers of bone formation and resorption. Supplemental calcium given alone to postmenopausal women suppresses bone turnover (via reduced parathyroid hormone secretion), resulting in a contraction of the remodelling space[36] and a reduced rate of bone loss.[37] A similar mechanism may underlie enhanced bone gain in children given calcium supplements.[16] However, with milk, a fall in parathyroid hormone concentrations mediated by serum calcium enrichment could be offset to some extent by the increased phosphate intake, resulting in no net effect on either parathyroid hormone concentrations or bone remodelling.

Alternatively, insulin-like growth factor I may have stimulated periosteal bone apposition, resulting in a slightly larger skeletal envelope in the milk group. The 2% difference in the gain in total body bone area (17.6% in the milk group *v* 15.7% in the control group) is consistent with this effect. Against this hypothesis is the fact that the bone formation markers were not raised in the milk group, although this assumes that these biochemical measures are sensitive enough to detect subtle changes in envelope size.

Conclusion

This study has shown that a modest increase in milk consumption augments bone mineral acquisition in adolescent girls. The small difference in bone mass observed in this trial and others (1-3% per year), if maintained, could have a substantial impact on future incidence of fractures.[38] However, short term increases in calcium or dairy food intake in children or adolescents may not be sufficient to sustain an increase in bone mass over several decades. Preliminary evidence from calcium supplementation trials indicates that the benefits may be lost once supplements are stopped,[39 40] although other trials have shown that the effect persists one year after stopping supplementation with a milk calcium extract.[18]

Short term intervention trials may indicate causality, but their findings must be interpreted together with the evidence from retrospective studies, which have shown that high calcium intakes throughout all of early life are associated with higher peak bone mass.[22-25] One study has shown a (non-significant) trend towards higher bone mass in young adults who had received milk in an intervention trial as very young children.[41] Finally, although the importance of calcium nutrition to bone density has been established in children and adolescents, our results additionally suggest that protein may have mediated some of the skeletal anabolic effects of the milk. Within the context of a public health strategy designed to reduce osteoporotic fracture rates, an increase in milk consumption could represent an important contribution.

Key messages

- Osteoporosis is a major public health problem; 40% of women will sustain an osteoporotic fracture
- Maximising peak bone mass at skeletal maturity may be one of the most important protective measures against fracture in later life
- Adolescence is a critical time for bone mineral acquisition
- An increase in milk consumption among adolescent girls resulted in significant gains in bone mineral over an 18 month period
- This simple intervention indicates that increased milk consumption may be associated with higher peak bone mass

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1 Kanis JA, Pitt FA. Epidemiology of osteoporosis. *Bone* 1992;13:S7-15.
2 Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, et al. Timing of peak bone mass in caucasian females and its implication for the prevention of osteoporosis. *J Clin Invest* 1994;93:799-808.
3 Teegarden D, Proulx WR, Martin BR, Zhao J, McCabe GP, Lyle RM, et al. Peak bone mass in young women. *J Bone Miner Res* 1995;10:711-5.
4 Bonjour J-P, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. *J Clin Endocrinol Metab* 1991;73:555-63.
5 Ott SM. Bone density in adolescents. *N Engl J Med* 1991;325:1646-7.
6 Adamson A, Rugg-Gunn A, Butler T, Appleton D, Hackett A. Nutritional intake, height and weight of 11-12 year-old Northumbrian children in 1990 compared with information obtained in 1980. *Br J Nutr* 1992;68:543-63.
7 Department of Health. *The diets of British schoolchildren.* London: HMSO, 1989. (Report on health and social subjects No 36.)
8 Ministry of Agriculture, Fisheries and Food. *Fifty years of the national food survey.* London: HMSO, 1991. (J M Slater, ed.)
9 Parliament: school milk [editorial]. *Lancet* 1971;i:1359.
10 Baker IA, Elwood PC, Hughes J, Jones M, Moore F, Sweetnam PM. A randomised controlled trial of the effect of the provision of free school milk on the growth of children. *J Epidemiol Community Health* 1980;34:31-4.
11 Riddoch C, Savage JM, Murphy N, Cran GW, Boreham C. Long term health implications of fitness and physical activity patterns. *Arch Dis Child* 1991;66:1426-33.

12 Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291-303.
13 Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980;66:918-20.
14 Katzman DK, Bachrach LK, Carter DR, Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *J Clin Endocrinol Metab* 1991;73:1332-9.
15 Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child* 1995;73:17-24.
16 Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, et al. Calcium supplementation and increases in bone mineral density in children. *N Engl J Med* 1992;327:82-7.
17 Lee WTK, Leung SS, Leung DMY, Tsang HSY, Lau J, Cheng JC. A randomized double-blind controlled calcium supplementation trial, and bone and height acquisition in children. *Br J Nutr* 1995;74:125-39.
18 Bonjour J-P, Carrie A-L, Ferrari S, Clavien H, Slosman D, Theintz G, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *J Clin Invest* 1997;99:1287-94.
19 Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, et al. Calcium supplementation and bone mineral density in adolescent girls. *JAMA* 1993;270:841-4.
20 Matkovic V, Fontana D, Tominac C, Goel P, Chestnut CH III. Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *Am J Clin Nutr* 1990;52:878-88.
21 Chan GM, Hoffman K, McMurray M. Effects of dairy products on bone and body composition in pubertal girls. *J Pediatrics* 1995;126:551-6.
22 Bauer DC, Browner WS, Cauley JA, Orwoll ES, Scott JC, Black DM, et al. Factors associated with appendicular bone mass in older women. *Ann Intern Med* 1993;118:657-65.
23 Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BEC. Bone status and fracture rates in two regions of Yugoslavia. *Am J Clin Nutr* 1979;32:540-9.
24 Murphy S, Khaw K, May H, Compston JE. Milk consumption and bone mineral density in middle aged and elderly women. *BMJ* 1994;308:939-41.
25 Sandler RB, Slemenda CW, LaPorte RE, Cauley JA, Schramn MM, Barresi ML, et al. Postmenopausal bone density and milk consumption in childhood and adolescence. *Am J Clin Nutr* 1985;42:270-4.
26 Baran D, Sorensen A, Grimes J, Lew R, Karellas A, Johnson B, et al. Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: a three-year prospective study. *J Clin Endocrinol Metab* 1989;70:264-70.
27 Prince R, Devine A, Dick I, Criddle A, Kerr D, Kent N, et al. The effects of calcium supplementation (milk powder or tablets) and exercise on bone density in postmenopausal women. *J Bone Miner Res* 1995;10:1068-75.
28 Department of Health. *Dietary reference values for food energy and nutrients for the united kingdom*. London: HMSO, 1991. (Report on health and social subjects No 41.)
29 Barger-Lux MJ, Heaney RP, Packard PT, Lappe JM, Recker RR. Nutritional correlates of low calcium intake. *Clin Appl Nutr* 1992;2:39-44.
30 Ross RJM, Buchanan CR. Growth hormone secretion: its regulation and the influence of nutritional factors. *Nutr Res Rev* 1990;3:143-62.
31 Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, et al. Serum insulin-like growth factor I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size and body mass index. *J Clin Endocrinol Metab* 1994;78:744-52.
32 Price JS, Oyajobi BO, Russell RGG. The cell biology of bone growth. *Eur J Clin Nutr* 1994;48:S131-49.
33 Rodan GA, Rodan SB. The cells of bone. In: Riggs BL, Melton LJ III, eds. *Osteoporosis: etiology, diagnosis, and management*. 2nd ed. Philadelphia: Lippincott-Raven, 1995.
34 Tanner JM, Whitehouse RH, Hughes PCR, Carter BS. Relative importance of growth hormone and sex steroids for the growth at puberty of trunk length, limb length, and muscle width in growth hormone-deficient children. *J Pediatr* 1976;89:1000-8.
35 Langford KS, Miell JP. The insulin-like growth factor-I/binding protein axis: physiology, pathophysiology and therapeutic manipulation. *Eur J Clin Invest* 1993;23:503-16.
36 Heaney RP. The bone remodeling transient: implications for the interpretation of clinical studies of bone mass change. *J Bone Miner Res* 1994;9:1515-23.
37 Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomised controlled trial. *Am J Med* 1995;98:331-5.
38 Matkovic V, Illich JZ, Skugor M, Saracoglu M. Primary prevention of osteoporosis. *Phys Med Rehab Clin N Am* 1995;6:595-627.
39 Slemenda CW, Reister TK, Peacock M, Johnston Jr, CC. Bone growth in children following the cessation of calcium supplementation [abstract]. *J Bone Miner Res* 1993;8(suppl 1):S154.
40 Lloyd T, Rollings N, Andon MB, Eggli DF, Mauger E, Chinchilli V. Enhanced bone gain in early adolescence due to calcium supplementation does not persist in late adolescence [abstract]. *J Bone Miner Res* 1996;11(suppl 1):S154.
41 Fehily AM, Coles RJ, Evans WD, Elwood PC. Factors affecting bone density in young adults. *Am J Clin Nutr* 1992;56:579-86.

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Birth defects in infants conceived by intracytoplasmic sperm injection: an alternative interpretation

Jennifer J Kurinczuk, Carol Bower

See editorial by Mitchell

TVW Telethon Institute for Child Health Research, PO Box 855, West Perth, WA 6872, Australia
Jennifer J Kurinczuk, *epidemiologist*

Western Australian Birth Defects Registry, King Edward Memorial Hospital, Bagot Road, Subiaco, WA 6008, Australia
Carol Bower, *clinical associate professor*

Correspondence to: Dr Kurinczuk

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Abstract

Objective: To test the hypothesis that liveborn infants conceived by intracytoplasmic sperm injection are at an increased risk of having a major birth defect.
Design: Reclassification of the birth defects reported in infants born after intracytoplasmic sperm injection in Belgium and comparison with prevalence estimated in Western Australian population by means of same classification system.
Setting and subjects: 420 liveborn infants who were conceived after intracytoplasmic sperm injection in Belgium and 100 454 liveborn infants in Western Australia delivered during the same period.
Main outcome measures: Estimates of birth prevalence of birth defects and comparisons of odds ratios between cohort conceived after intracytoplasmic sperm injection and Western Australian infants.
Results: Infants born after intracytoplasmic sperm injection were twice as likely as Western Australian infants to have a major birth defect (odds ratio 2.03 (95% confidence interval 1.40 to 2.93); P = 0.0002) and nearly 50% more likely to have a minor defect (1.49 (0.48 to 4.66); P = 0.49). Secondary data-led analyses, to be interpreted with caution, found an excess of major cardiovascular defects (odds ratio 3.99), genitourinary defects (1.33), and gastrointestinal defects (1.84), in particular cleft palate (5.11) and diaphragmatic hernia (7.73).
Conclusions: These results do not confirm the apparently reassuring results published by the Belgian researchers of intracytoplasmic sperm injection. Further research is clearly required. Meanwhile, doctors practising intracytoplasmic sperm injection should bear this alternative interpretation in mind when they counsel couples and obtain informed consent for the procedure.

Introduction

Intracytoplasmic sperm injection, the selection and injection of a single spermatozoon into an oocyte, is probably the most important development in assisted reproduction since the birth of the first "test tube baby" in 1978. This procedure offers, for the first time, real