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EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 1.2.5

Long term follow up of UK infants born pre term and term and randomised to formulas intended to modify the whole diet or specific nutrients with reference to bone health

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: UCLON

Final report of data from Activity 1.2.5

Background

Osteoporosis is a major cause of morbidity and mortality worldwide and expected to increase in the next generation[1]. From the public health perspective attainment of peak bone mass by early adulthood is considered to be highly important to reduce the risk of lifetime fracture and osteoporosis[2]. Nutritional factors in early life that may programme peak bone mass include the use of enriched formulas and the use of engineered lipids that mimic lipid structures found in breast milk. Enriched formulas with increased concentrations of calcium and phosphorus designed for preterm infants have been shown to improve the short term risks of metabolic bone disease of prematurity and improve bone mineralisation in mid-childhood[3] but whether the use of these formulas improve the ultimate peak bone mass by early adulthood remains untested. Similarly, synthetically engineered lipids, where the most prevalent saturated fatty acid found in formula, palmitate C16:0, is inserted in the sn-2 position of the triglyceride, as found in human breast milk, rather than the sn-1 and sn-3 position typically found in vegetable sourced fats, have also been shown to improve calcium and fat absorption in both preterm[4] and term[5] infants during infancy.

This Activity was made up of three tasks, all were follow-ups of cohorts who had previously taken part in randomised controlled trials of infant nutrition that had been designed to test experimentally diets designed to improve bone mineralisation and density during infancy, by childhood and early adulthood.

The Tasks:

Task 1:
Introduction
This was a follow-up of a cohort of young adults who were born preterm (birthweight < 1850g and gestation < 37 weeks) in the mid 1980's who took part in randomised trials of preterm infant diets[6] comparing i) enriched preterm formula, ii) standard term formula and iii) banked breast milk, in addition, mothers were encouraged to provide their own breast whenever possible. The enriched preterm formula, which was novel at the time, contained twice the level of calcium (70mg vs 35mg/100ml of formula), 18% more phosphorus (35mg vs 29mg/ 100m) and 12% more energy (80kcal vs 68 kcal /100ml) compared to standard term formula used. This cohort has been followed-up several times previously, with extensive bone measurements collected when the subjects were 9-12 years old[3]; results at this age showed that i) preterm children have lower bone mass than children bone at term but the bone mass was in proportion to their body size, ii) preterm children who showed the greatest catch-up growth in height had higher bone mass, iii) children who developed metabolic bone disease of prematurity (elevated levels of plasma alkaline phosphatase) in infancy had lower height SD at 9-12 years, and iv) higher markers of bone turnover were seen in children at 9-12 years who had the lowest nutrient intake during the neonatal period.

For this follow-up, data collected included anthropometry, weight and height measured with the subject wearing light indoor clothing. Dual energy X-ray absorptiometry (DXA) scans were performed of the whole body, lumbar spine (L2-L4) and the left hip using a Lunar Prodigy machine, data collected included bone mineral

content (BMC), bone area (BA) and bone mineral density (BMD). Both the bone and anthropometric data were converted into Z-scores as a comparison to population norms. Current calcium intake was assessed using a dedicated food frequency questionnaire and fracture history was recorded from the subject themselves. Early morning blood samples were collected to measure key markers of bone turnover including, osteocalcin and N-terminal propeptide of Type 1 collagen (P1NP).

Results from follow-up

Nine hundred and 26 infants joined the original study, of which 831 survived into childhood; these survivors were all approached by letter to take part, a total of 270 subjects initially agreed to take part however, only 202 actually took part in the measurements (24% of survivors). Most of these subjects (172) agreed to be measured in London although the areas of their births were outside London (Cambridge, Sheffield, Kings Lynn, Norwich and Ipswich), with the remaining 30 being measured in a clinic in Cambridge. Compared to the subjects not seen for follow-up, those seen were more likely to be female (57% vs 48%, $p < 0.05$) and come from a higher social class at birth (9.5% vs 5.1 %, $p < 0.05$), the proportion of infants who had a birth weight < 1250g at birth did not differ between those followed up and those not. The mean age at follow-up for these subjects was 20.25 yrs (SD 0.55), 43% were male, mean gestation was 30.9 weeks (SD 2.7) and 34% had a birth weight less than the 10% centile.

Comparison with population data.
Anthropometric and bone data were compared to population reference data. Subjects born both small for gestational age (SGA) and with a birthweight <1250g were significantly shorter and lower lumbar spine BMD scores; those born appropriate for gestational age (AGA) but with a birthweight < 1250g were shorter and those born SGA with birthweight > 1250g were shorter with higher BMI, those who were born AGA and >1250 had higher weight and BMI z-scores. The markers of bone turnover showed a negative correlation with whole body BMD z-scores and BMC/lean tissue ratio.

Analysis by randomised groups:
The bone data was analysed by feeding group (term formula, preterm formula and banked breast milk), No differences between feeding groups were seen in any bone parameters; this was also the case for the bone turnover markers.

Non-randomised analyses
The effect of receiving ‘any human milk’, as BBM or mother’s breast milk or the two together, was examined. Subjects who received more than 90% human milk had significantly higher whole body BA (3.5%, $p = 0.01$) and BMC (4.8%, $p = 0.01$) than those who received <10%, although this effect was not seen in the lumbar spine or BMC data. There was a ‘dose-response’ relationship between the proportion of human milk in the neonatal diet and later whole body bone mass. The neonatal intakes of calcium and phosphorus were calculated and compared to bone and bone turnover outcomes but there was no correlation seen between early intake and later bone mass or turnover.

Conclusion:
Increasing dietary nutrient calcium and phosphorus intake for preterm infants whilst on the neonatal unit had no influence on the bone health and markers of turnover by early adulthood. However, the intake of human milk (either as mother's breast milk or banked breast milk) appears to provide beneficial effects by increasing the WBBA and BMC despite the lower intake of early calcium and phosphorus associated with the provision of unsupplemented breast milk in this study , suggesting that there maybe non-nutritive factors such as growth factors found in breast milk that improve bone health.

Task 2:
Introduction
This was a follow-up of a cohort of formula fed term SGA children (birthweight < 20th centile in this study), born 1999-2002 who took part in a randomised controlled trial of a control vs nutrient enriched formula (40% more protein and 12% more energy and a higher proportion of palmitic acid in the sn-2 position) for the first six months of life. Children born SGA are at risk of having reduced adult height when compared to children who are born appropriate for gestational age[7]. At the end of original intervention period, infants randomised to the enriched formula showed faster growth compared to infants on control formula, although this did not reach significance.

For this follow-up, subjects were approached by letter at least twice and invited to attend a bone clinic at Yorkhill Sick Children's Hospital in Glasgow from 2007 to 2009. Primary outcomes collected were bone size and bone geometry data, measured by dual X-ray absorptiometry (DXA), peripheral quantitative tomography, (pQCT) and quantitative ultrasound (QUS). DXA data collected included lumbar, whole body and hip bone area and bone mineral content in contrast the QUS measures as speed of sound (SOS) from the radius the data is associated with fracture risk, cortical thickness, density, microstructure and elasticity[8] independent of BMD reported from the DXA. The pQCT provided data on the structural measurements of tibia (cortical and trabecular tissue) and parameters of strength as the strength strain index[9]. In addition data on exercise, calcium intake and anthropometry were collected.

Results
Forty-five of the original cohorts of 246 subjects took part (18%). Twenty four of the participants had been randomised to the enriched formula and 21 to the control group in the original trial. Subjects who were followed-up were not different to those who were not followed-up with respect to birthweight or gestational age. The percentage of boys was also similar between groups, and the level of education of the mothers was the same for each group, however mothers of the children who were followed up were significantly older (30.5 (6.05) yrs vs 26.6 (6.4) yrs, $p < 0.001$). The mean age of the subjects at follow-up was 6.7 years, which was the same for both randomised groups.

Anthropometric outcomes were compared by randomised groups. There were no differences between groups with respect to weight, height, BMI, head circumference or the corresponding z-scores, although on all these parameters the enriched group tended to have greater values. DXA results showed no differences between groups

but the speed of sound suggested that the SOS data for the distal radius from the control groups were higher than the enriched group, (control 3631m/s (116) vs enriched 3553m/s (108)m, p = 0.09). Body composition data, measured as fat and fat free mass from the DXA scans were compared; as with anthropometric data the groups showed no differences, however the same tendency remained for the enriched group to have greater values, most markedly in the fat variables. For example for % fat mass, groups means were 24.0% (10.3) compared to 19.3% (8.7), p = 0.12 and fat mass index (fat mass/height^2) were 4.17 (2.62) m^2 vs 3.14 (2.02) m^2 , p = 0.16 whereas for fat free mass the differences the values for the two groups were very similar (17.17kg (2.61) vs 17.28kg (2.28), p = 0.9). However, when the analysis was adjusted for factors also known to effect fat mass such as age and gender these tendencies for greater fat mass in the enriched group diminished.

Conclusion:
The low follow-up rate for this study was a limiting factor for the interpretation of the results; the small groups provided insufficient power to say with confidence that any differences were significant. Other studies have found that enriching the diet for SGA infants to improve early catch-up growth have been shown to increase the fat mass and % fat seen in mid-childhood, however, as most enriched formula diets will have increased levels of calcium and phosphorus further follow-ups are needed to established whether the increased levels of micro-nutrients provide benefits not associated with increased levels of energy and protein.

Task 3:
Introduction
This was a follow-up of a term cohort of 203 formula fed infants who had been randomised at birth to receive either a control formula or a control formula with a significant proportion of the palmitate in the sn-2 position, (known as 'Betapol'), for the first 12 weeks of life. There was also a control group of 120 breast fed (breast-fed for 12 weeks) infants[5]. At the end of the intervention, the infants were measured using DXA and single photon absorptiometry (SPA) and stool characteristics were assessed both biochemically and by parent kept diaries. The results showed that the infants who received the Betapol formula had increased whole body bone mineral content (when adjusted for body size) by 5.8% (p = 0.05), softer stools with fewer calcium soaps compared to the infants on control formula. Whilst the presence of Betapol reduced stool harness in the formula fed infants, these softer stools were still significantly harder than those seen in the breast fed infants.

Follow-up of Task 3:

This Task was to follow-up as many of this cohort as possible when around 10 years old, and collect data on i) bone parameters (BMC, BA and BMD at the lumbar spine, hips and whole body, as raw data and SD scores), ii) anthropometry, iii) calcium intake and iv) habitual activity levels. Parents were contacted by letter and invited to take part in the follow-up as one clinic visit; the visits took place either in a clinic in Cambridge or at Great Ormond St Hospital, London.

Results
Fifty seven formula fed children (27 had been randomised to control formula + 30 randomised to Sn-2 formula as infants) and 34 breast fed infants took part in the follow-up. There were no differences in baseline characteristics between the children who took part in the follow-up and those who did not. Children randomised to Sn-2 formula who were followed up had lower birthweight SDs compared to the control group, (-0.10 (0.80) vs 0.36 (0.84), p = 0.04) but no other differences between the groups were significant.

Comparison of outcome measures by randomised groups showed that there were no differences with respect to BMC, BMD, BA and z-scores for both the lumbar spine (L2-L4) and whole body measurements. In non-randomised analysis, breast fed subjects were compared to formula fed subjects; SD scores needed to be applied to bone and anthropometrc outcomes as the formula fed children were significantly younger than the breast fed children (10.67 (0.59) yrs vs 11.22 (0.36), p < 0.001). Comparison of the groups showed again there were no apparent differences in the size-adjusted bone outcomes between the groups, although the formula-fed children had higher weight SD scores (0.75 (1.13) vs 0.22 (1.08), p = 0.03); no differences in height were seen.

Conclusion
The use of modified fat blend with sn-2 palmitate had no effect on bone parameters during mid-childhood, suggesting that the beneficial effects of this intervention may be confined to the cohort term improvements in stool characteristics. In addition, this study showed no differences in bone mass between breast-fed and formula fed term infants at age 10 years.

Overall conclusions for Activity 1.2.5
The three tasks in this activity suggest that the benefits seen early in life associated with the use of infant formulas designed to improve bone outcomes do not appear to extend into mid-childhood and beyond. The longer follow-up of the preterm cohort, did however, suggest that breast milk, given either as mother's milk or banked breast milk was associated with improved bone outcomes by early adulthood; this is an interesting observation given the fact that human milk in this study was fed without mineral supplements or fortification, and suggests that this effect may result from non-nutrient components of human milk, such as one of the many growth factors or hormones. Interestingly, we did not detect any beneficial effect of breastfeeding on childhood bone mass in out term cohort. One previous observational study in term subjects[10] reported that those breast-fed for 3 months or more had higher bone mass at age 8 years than formula-fed subjects. It is not clear why our results should differ. One possibility is that it reflects our relatively small sample size. Another is that there is a dose-response relationship between human milk intake and later bone health (as shown in our preterm study and suggested by the Jones study); subjects in our term study were not breast-fed exclusively for long periods.

Publications generated from this Activity:

Task 1: Fewtrell MS, Williams JE, Singhal A, Murgatroyd PR, Fuller N, Lucas A. Early diet and peak bone mass: 20 year follow-up of a randomised trial of early diet in infants born preterm. Bone 45; 2009: 142-149.

Task 3: Submitted for publication: Fewtrell MS, Kennedy K, Murgatroyd PR, Williams JE, Chomtho S, Lucas A. Calcium absorption during infancy and later bone health: 10-year follow-up of a randomised trial of a term infant formula containing synthetic triacylglycerol (Betapol)' by was submitted to Archives of Diseases in Childhood on 6th September 2010 ref: ADC/2010/201608

1. Torgerson D et al. The effective management of osteoporosis. In: Barlow DH, Francis RM, Miles A. The economics of fracture prevention; 2001. p 111-21.

2. Fewtrell MS, 2006 Osteoporosis; is primary prevention possible? In: Primary prevention by nutrition intervention in infancy and childhood. Eds LucasA, Sampson HA. Nestle Nutrition Workshop Series Pediatrc Program 57. Karger 135-152.

3. Fewtrell MS et al. Bone mineralisation and turnover in preterm infants at 8-12 years of age: the effects of early diet. J Bone Miner Res 1999; 14: 810-20.

4. Lucas A et al. Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. Arch Dis Child Fet Neonatal Ed 1997; 77: F178-84.

5. Kennedy K et al. Double-blind randomised trial of a synthetic triglyceride (Betapol) in formula fed term infants: effects on stool biochemistry, stool characteristics and bone mineralisation. AJCN 1999; 70: 920-927.

6. Lucas A et al. Multicentre trial on feeding low birth weight infants: effects of diet on early growth. Arch Dis Child 1984; 59: 722-30.

7. Karlberg J et al. Growth in full term small-for-gestational-age infants: from birth to final height. Pediatr Res 1995; 38: 733-9.

8. Nguyen TV et al. Bone mineral density independent association of quantitative ultrasound measurements and fracture risk in women. Osteoporosis Int. 2004; 15: 942-7.

9. MacDonald H et al. Bone strength and its determinants on pre- and early pubertal boys and girls. Bone 2006; 39: 598-608.

10. Jones G et al. Breastfeeding in early life and bone mass in prepubertal children: a longitudinal study. Osteoporosis 2000; 11: 146-52