Bone

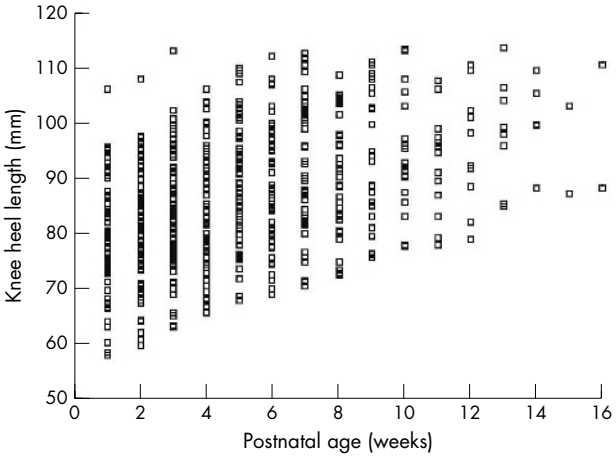
G136 A LONGITUDINAL STUDY OF LOWER LIMB LENGTH AND TIBIAL SPEED OF SOUND IN PRETERM INFANTS: RELATIONSHIP WITH BIOCHEMICAL MARKERS OF BONE TURNOVER

J. May[1], J. Dutton[2], J. Morris[3], W. D. Fraser[2], A. J. B. Emmerson[1], M. Z. Mughal[4]. [1]*Neonatal Medicine, St Mary's Hospital for Women & Children, Manchester, UK;* [2]*Clinical Biochemistry, Royal Liverpool Hospital, Liverpool, UK;* [3]*Medical Statistics, University of Manchester, Manchester, UK;* [4]*Paediatric Medicine, St Mary's Hospital for Women & Children, Manchester, UK*

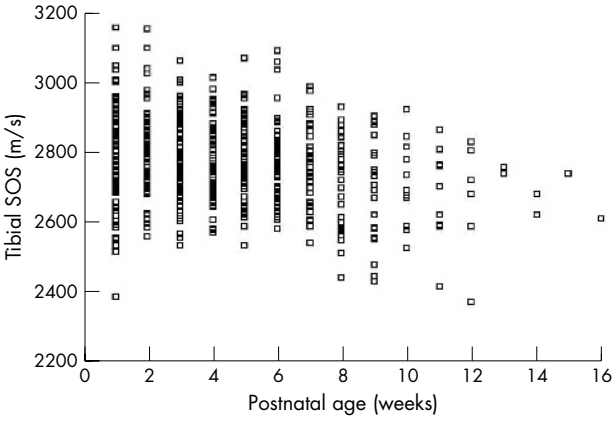
Background: In healthy singleton infants 31 to 42 weeks gestation, tibial speed of sound (m/s) measured at median age of 2.1 days after birth increased with gestational age.

Method: In this longitudinal study we measured the knee–heel limb length (mm) using an electronic neonatal knemometer (FORCE Institutes, Denmark) and tibial speed of sound using the Sunlight Omnisense quantitative ultrasound device (Sunlight Medical Ltd., Israel), in the same limb, in 82 preterm infants. The median (range) gestation and birth weight of infants was 27 weeks (23 to 37.6 weeks) and 903 grams (418 to 1495 grams), respectively. Measurements were performed weekly; median period of 4 weeks (2 to 14 weeks). There was a significant increase in lower limb length (r 0.96; $p<0.001$), (see fig 1) but a significant decrease (within subject correlation, r −0.28; $p<0.001$) in tibial speed of sound with postnatal age (fig 2).

Results: In a subgroup of 24 infants, changes in serum concentration of bone specific alkaline phosphatase (BSALP; a marker of bone formation) and urinary excretion of urinary deoxypyridinoline to creatinine ratio (DPD; a non-reducible cross link of collagen that is a marker of bone resorption) were measured on the same day and around the same time as lower limb length and tibial speed of sound measurements. The serum concentration of BSALP (r +0.44; p 0.001) and urinary DPD/creatinine (r +0.24; p 0.033) increased with postnatal age.



Abstract G136, figure 1



Abstract G136, figure 2

Conclusion: The observed increase in lower limb length during the early neonatal period is consistent with a rapid postnatal tibial growth, whereas the decrease in tibial speed of sound during this period might either be due to a lag in tibial cortical mineralisation or cortical thinning. Preliminary results also suggest that bone turnover increases progressively during the early neonatal period.

1. **Yiallourides**, *et al. Biol Neonate* 2004;**85**(4):225–8.

G137 PHALANGEAL ULTRASOUND AND FRACTURE RISK IN CHILDHOOD

K. Manias, N. J. Bishop. *University of Sheffield and Sheffield Children's Trust, Sheffield, UK*

Introduction: Quantitative ultrasound may provide information about the structure and microarchitecture of bone, and would be extremely useful in paediatric practice if proved effective in assessing bone quality and fracture risk in children. We tested the hypothesis that children who had recently sustained a fracture would have a lower amplitude dependent speed of sound (AD-SoS) and ultrasound bone profile index (UBPI) assessed by phalangeal ultrasound than fracture free controls. We also determined whether these parameters were lower in children with recurrent fractures than in those fracturing only once.

Methods: We studied 97 children aged 4–16; 31 had fractured once, 27 had sustained their second or subsequent fracture, and 39 controls. Children with metabolic bone disease were excluded. Subjects underwent anthropometry and phalangeal ultrasound (IGEA Digital Bone Measurement Sonic Bone Profiler, model BP01; software v3.0(x)) three months after fracture. Size adjusted *z* scores were created for AD-SoS derived from data from control children in order to remove size related effects. Inter group differences for the means of each variable were calculated and significance tested using 2 sample t tests and the F test of multiple means. The significance level was set at $p<0.05$.

Results: Children with one or more fractures had a significantly lower machine generated age and gender adjusted *z* scores for AD-SoS (−0.064 (SD 0.906); p 0.014), and lower mean body size adjusted *z* score for AD-SoS (p 0.0037) and UBPI (p 0.0371) than controls. There was no further significant reduction in these parameters in children with recurrent fractures compared with those who had only fractured once. There were no systematic differences between those who had fractured upper or lower limbs for either measure.

Conclusions: The phalangeal ultrasound parameters AD-SoS and UBPI are lower in apparently healthy children who have recently sustained a fracture than those remaining fracture free. This implies that having an inferior bone structure is a risk factor for fracturing in childhood. The lack of fracture site dependent variability suggests the results do not simply reflect disuse post fracture. Ultrasound values did not discriminate between children fracturing only once or recurrently. Phalangeal ultrasound may add to the assessment of bone quality and fracture risk in children.

G138 SPEED OF SOUND MEASUREMENTS BY QUANTITATIVE ULTRASOUND IN CHILDREN AND ADOLESCENTS WITH CEREBRAL PALSY: ARE THEY USEFUL?

B. Lahoti[1], A. Meyer-Heim[1], Y. Khan[1], C. Fairhurst[1], I. Kenney[3], J. E. Williams[2], K. L. Loh[2], M. S. Fewtrell[2]. [1]*Institute of Child Health, London, UK;* [2]*Chailey Heritage Clinical Service, East Sussex, UK;* [3]*RoyalAlexander Children's Hospital, Brighton, UK*

Background: Children with cerebral palsy have an increased susceptibility to fractures. Dual *x* ray absorptiometry, the most widely used technique for measuring bone density, has limitations in this population (accessibility, need for cooperation, and metal artefacts). Quantitative ultrasound is a safe, mobile technique, requires less cooperation, and provides a measure of speed of sound; measurements predict fracture risk in adults but their value in paediatric populations is unclear.

Aims: To (a) compare speed of sound measurements from children with quadriplegic cerebral palsy with those from healthy subjects; (b) identify factors that predicts speed of sound ; (c) investigate relationships between speed of sound and outcomes (fracture history and bone pain).

Methods: Speed of sound was measured in the right tibia and radius using quantitative ultrasound (Sunlight Omnisense) in 60 children

Arch Dis Child: first published as on 21 March 2005. Downloaded from http://adc.bmj.com/ on August 19, 2022 by guest. Protected by copyright.

(34 boys) with severe quadriplegic cerebral palsy (age 4–19 years). The children were non-ambulatory. Machine reference data provided age and sex matched *z* scores. Weight and arm span were measured. Data were collected on mobility, weight bearing, feeding mode, calcium intake, medications, fracture history, and *x* ray reports.

Results: Mean speed of sound *z* scores were significantly <0 for radius (−0.97 (SD1.23)) and tibia (−0.31 (1.26)); correlation 0.35 (p 0.01), with no sex difference. *z* Score decreased with age (p<0.001 radius; p 0.07 tibia) but showed no significant association with size, activity, weight bearing, feeding mode, calcium intake, or anticonvulsant use. Fracture history and pain scores were not significantly associated with speed of sound *z* scores; there was a weak relationship between radiologically assessed osteopenia and radius *z* scores (p 0.09). Eleven children had a positive fracture history. In a multivariate analysis including speed of sound measurements, only anticonvulsant use was predictive, with fractures in 10/26 (39%) treated children *v* 1/32 (3%) untreated (p 0.001). No single drug could be implicated due to use of multiple agents in many children.

Conclusions: Children with quadriplegic cerebral palsy have low mean speed of sound *z* scores that decrease with age, but no single aetiological factor was identified. Speed of sound did not distinguish children with or without a positive fracture history. Limitations of this study include retrospective collection of activity and diet data and the relatively small number of children with fractures. Our data suggest that quantitative ultrasound may not be useful for predicting fracture risk in non ambulant children with quadriplegic cerebral palsy but a prospective study is required to confirm or refute this.

G139 DUAL *x* RAY ABSORPTIOMETRY OF THE LUMBAR SPINE IN A CLINICAL SETTING: DOES THE METHOD OF SIZE ADJUSTMENT MATTER?

M. S. Fewtrell[1], I. Gordon[2], L. Biassoni[2], T. J. Cole[3]. *[1]MRC Childhood Nutrition Research Centre, Institute of Child Health, London WCIN 1EH; [2]Department of Rodiology, Great Ormond Street Children's Hospital NHS Trust, London; [3]Department of Epidemiology and Biostatistics, Institute of Child Health, London, UK*

Dual *x* ray absorptiometry is increasingly used in a clinical setting to evaluate bone mass in children. Areal bone mineral density (aBMD) measurements are affected by body size but there is no consensus on the optimal way to deal with this for individual patients.

Aim: To compare parameters of bone mass with varying degrees of size correction and to determine the impact on the categorisation of patients as normal or abnormal.

Methods: Healthy children (n=78) and four groups of patients (n=194) underwent dual *x* ray absorptiometry scans of the lumbar spine (L2-4, GE Lunar Prodigy). Five measures of bone mass were derived, all adjusted for age and sex: aBMD, BMAD (BMC/BA $^{1.5}$), BMCh (BMC/height^3), BMCa (BMC adjusted for BA), and BMCt (BMC adjusted for BA and height). Standard deviation (SD) scores were calculated for each parameter for patients using data from healthy controls.

Results: Compared with healthy children, all patient groups had significantly reduced BMD SD scores (p<0.001). Mean BMAD, BMCa, and BMCt SD scores were significantly lower in only 2/4 patient groups, while BMCh SD scores were low only in one group. BMCt showed no advantage over BMCa. The proportion of patients with SD scores ⩽2 was 27% for aBMD but between 10–13% for BMAD, BMCh, and BMCa.

Conclusions: All size corrected parameters of bone mass performed similarly and classified significantly fewer patients as abnormal than did aBMD. The use of one of these parameters should reduce the number of patients diagnosed inappropriately with low bone mass. However, without validation against an outcome measure such as fracture or gold standard of bone density or structure, it is not possible to determine which parameter is most correct.

G140 PRELIMINARY OBSERVATIONS FROM TIME LAPSE VIDEOMICROSCOPY OF CELL FUSION IN RAW264.7 CELLS STIMULATED WITH RANK LIGAND

P. S. Grabowski, S. Smith, N. Jenkins, H. Moore, N. J. Bishop. *University of Sheffield, Sheffield, UK*

Aim: To develop a method to characterise cell fusion events leading to osteoclast formation.

Introduction: Osteoclast polykaryons develop from monocytic precursors. While current antiresorptive agents, for example bisphosphonates, target osteoclast activity, inhibition or enhancement of osteoclast formation may provide alternative strategies to treating diseases of high or low bone turnover, respectively. Considerable progress has been made in determining molecular mediators driving osteoclastogenesis and regulating osteoclast function, but little is known about cell membrane fusion leading to polykaryon formation. Here we have used time lapse video microscopy to characterise cell fusion during polykaryon formation in RAW264.7 murine monocytic cells stimulated with RANK ligand.

Methods: RAW 264.7 cells were cultured in DMEM containing 10%(v/v) fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and recombinant murine RANK ligand (30 μg/ml; Peprotech). Cells were cultured for 6 days, refreshing the medium on days 3 and 6. Cells were transferred to an Olympus IX70 inverted microscope, equipped with motorised stage and focus control encased in a perspex chamber at 37˚C and 5% CO_2 in air (v/v). Images were captured every 10 minute from randomly selected fields over 40 hours using a CCD camera controlled by SimplePCI software. Image stacks were analysed for cell activity and fusion.

Results: The majority of cells (ca. 80–90%) were uniformly round and exhibited little motility. Most of the remaining cells were motile, extending leading and trailing cytoplasmic processes as they moved around. Fusion events occurred almost exclusively between motile cells. Not all motile cells underwent fusion and not all cell–cell contacts resulted in fusion events. Prior to fusion, cell edges remained in contact for from 10 to >100 minutes. The fusion process itself was very rapid (generally <10 min). Upon formation polykaryons slowed down, took up a more defined leading edge with distinct lamellopodia, while many developed well defined intracellular vacuoles.

Conclusions: Cell fusion in RANKL stimulated RAW264.7 cells is a rapid process that occurs between a subset of motile cells. Time lapse videomicroscopy can provide substantial information on cell fusion events leading to polykaryon formation. Studies are ongoing to further characterise cell fusion in these cells.

G141 SKELETAL HEALTH AND BODY COMPOSITION IN CHILDREN WITH CHRONIC KIDNEY DISEASE

R. Rashid, E. Neill, H. Maxwell, S. F. Ahmed. *Royal Hospital for Sick Children, Glasgow, UK*

Aim: To examine bone mineral content and body composition in children with chronic kidney disease.

Methods: In a LREC approved observational study, dual energy *x* ray absorptiometry was used to examine the relationship between bone mineral content, lean mass, and fat mass in 60 children with chronic kidney disease; 30 children (22M, 8F) with chronic renal insufficiency (median age: 11.6 years, 10th, 90th centiles: 6.7, 15.8) and 30 children (20M, 10F) post-transplantation (Tx) (median age 13.4 years (7.8,17.5)). Median duration post-Tx was 3.4 years (1.8,5.4). LM and FM were adjusted for height and expressed as LM HtSDS and FM HTSDS. Lean mass and FM were also corrected for height by dividing by height squared and expressed as Lean Mass Index (LMI) and Fat Mass Index (FMI). Bone mineral content was measured for total body (TB) and lumbar spine (L1-L4) (LS). Bone mineral content was adjusted for height age and expressed as percentage predicted height bone mineral content (ppHtBMC). Data were also analysed for differences between sex and pubertal status.

Results: In the chronic renal insufficiency group, median Ht SDS was −1.6 SDS (−3.1 to 0.3). Median %calorie and %protein intake were 95% (84 to 119) and 100% (93 to 120). LMHtSDS and %FMHtSDS were −1.0 SDS (-1.8 to 0.3) and 1.1 SDS (0.1 to 2.4), respectively. LMI was 13.8 (11.8 to 17.6), FMI was 3.6 (1.6 to 8.2), and ppHtBMC was 104% (96 to 114). There was a significant correlation between ppHtBMC (TB & LS) and LMI (r 0.5, p<0.05) but not with FMI. This correlation was strongest in boys. Both pre- and post-pubertal children showed an association between ppHtBMC (TB) and LMI (r, 0.5, p<0.05). In the Tx group, median Ht SDS was −1.7 SDS (−3.3 to 0.1). Median percentage calorie and percentage protein intake were 102% (88 to 118) and 124% (98 to 140). LMHtSDS and %FMHtSDS were −0.82 SDS (−1.8 to 0.3) and 1.3 SDS (0.3 to 2.5), respectively. LMI was 14.3 (12.4 to 16.8), FMI was 4.3 (2.1 to 10.4), and ppHtBMC was 92% (71 to 108). In boys, there was an association between ppHtBMC (TB) and LMI (r, 0.6, p<0.05) and FMI (0.6, p<0.05). This was not observed in girls. The correlation between ppHtBMC (TB) and LMI was stronger in the pubertal children than the pre-pubertal group. There was no association between bone mineral content and nutritional intake in either group.

Conclusion: Adjustment for height is crucial when studying bone and body composition in children with chronic disease. When adjusted for height, bone mineral content is lower in the Tx group than in the chronic renal insufficiency group and there is a strong association between bone mineral content and LM in children with chronic kidney disease. Interventions to improve bone mineral content or LM may prove beneficial for both measures.

Arch Dis Child: first published as on 21 March 2005. Downloaded from http://adc.bmj.com/ on August 19, 2022 by guest. Protected by copyright.

Abstract G142

		6 or 8 mg/kg/yr		12 mg/kg/yr	
		Mean	SD	Mean	SD
OI type					
Age at entry (yrs)		0.51	0.33	0.32	0.36
Initial:-	LS BMC (g)	1.05	0.50	1.1	0.96
	LS Area (cm^2)	5.56	0.99	3.15	2.05
	LS Ht (cm)	3.23	0.31	2.82	0.35
	LS BMD (g/cm^2)	0.149	0.035	0.170	0.075
Percentage change	LS BMC (g)	170	97	350	192
after 12 months	LS Area (cm^2)	62	28	69	40
	LS Ht (cm)	28	13	42	7
	LSBMD (g/cm^2)	108	60	153	59

G142 PAMIDRONATE FOR INFANTS WITH OSTEOGENESIS IMPERFECTA: COMPARISON OF DIFFERENT DOSES

S. Senthilnathan, E. Walker, R. Cousins, B. Silverwood, N. J. Bishop. *Academic Unit of Child Health and Sheffield Children's Hospital, Sheffield, UK*

Background and Aims: Current regimens of intravenous pamidronate, given in cycles at intervals for children and infants with osteogenesis deliver 9 to 12 mg/kg/year of drug. We wished to ascertain the effect on bone mass of administering lower doses of pamidronate when therapy was started in the first year of life.

Methods: We aimed to recruit 22 infants over a two year period aiming to show a one standard deviation score difference in bone mass after one year's therapy. However, we recruited only 11 infants in three years; recruitment was then halted. Infants were randomised to receive either 6 or 12 mg/kg/year in total of pamidronate, at intervals of 6, 6, 8, 10, 10, and 12 weeks. Measurements of lumbar spine bone size and mass (DXA Lunar Prodigy, software v2.1) and biochemical measures of formation (bone specific alkaline phosphatase) and resorption (urine NTx, Osteometer) were carried out at each infusion. Skeletal surveys were undertaken at baseline and 12 months.

Results: One infant died three weeks after the first infusion. Of the remaining 10, an administrative error (child changed name completely) resulted in a child randomised to 12 mg/kg/year receiving 8 mg/kg/year in total. There were no statistically significant differences between the groups. An independent effect of dose on LS BMD after 12 months therapy, after adjusting for age at study entry, initial LS BMD, and serum alkaline phosphatase activity (p 0.04). Serum alkaline phosphatase fell progressively from 239 (SD 49) IU/l at baseline to 171 (43) IU/l at 12 months.

Conclusions: We failed to show a clear effect of pamidronate dose on spine bone mass acquisition in infants with osteogenesis imperfecta, although there were positive results after adjusting for potential confounders. Pamidronate did not result in over suppression of bone turnover.

G143 A RANDOMISED CONTROLLED TRIAL OF CALCIUM SUPPLEMENTATION IN PRE-PUBERTAL GYMNASTS *V* CONTROLS

K. A. Ward[1], S. A. Roberts[1], S. New[2], J. E. Adams[1], M. Z. Mughal[3]. *[1]University of Manchester, Manchester, UK; [2]University of Surrey, Surrey, UK; [3]St Mary's Hospital, Manchester, UK*

Background and Aim: During childhood, the positive effects of physical activity and calcium upon the skeleton are well documented. However, studies of the interaction between calcium and physical activity in a group of regular exercisers have not previously been reported. Therefore, the aim of this study was to investigate the effects of 12 months supplementation of 500 g calcium carbonate (Calcichew™, Shire Pharmaceuticals) or placebo, upon the peripheral and axial skeleton of elite, pre-pubertal gymnasts (14 hours of weight bearing activity per week) and controls (6 hours of weight bearing activity per week). We hypothesised that there would be an interaction between exercise and calcium supplementation in the gymnasts leading to greater increases in a) distal bone area and trabecular and total volumetric bone mineral density (TrBMD, TotBMD) and b) mid-diaphyseal bone cross sectional area, bone mineral content (BMC) and cortical vBMD (CtBMD) in both the radius and tibia. Secondary outcomes of cortical area, periosteal and endosteal circumferences, stress strain index, and muscle area were also measured.

Methods: Eighty six subjects (49 females) participated in the double blind randomised controlled trial (44 gymnasts: 42 controls) and 75 subjects completed the trial (45 females; 39 gymnasts, 36 controls). All participants had peripheral quantitative computed tomography (Stratec XCT-2000) measurements of their radius and tibia before and after the intervention period. In the intention to treat cohort analysis of covariance was used to test differences in response to treatment between calcium supplementation and placebo groups in gymnasts *v* controls. All bone outcomes were log transformed and adjusted for covariates of gender, pubertal status at end of trial, baseline height, baseline measurement, and delay between baseline measurement and start of intervention. Baseline median calcium intakes in the groups were 775 (controls – placebo), 904 (controls – calcium carbonate), 746 (gymnasts – placebo), and 888 (gymnasts – calcium carbonate) mg/day.

Results: At the tibia, significant interactions were found for TrBMD (calcium carbonate + controls 5.5%, gymnasts + controls −1.5%; p 0.02) and CtBMD (calcium carbonate + controls 0.61%, gymnasts + controls −0.63%; p 0.01). At the radius no significant interactions were found, likewise for all secondary outcome measures.

Conclusion: We conclude that in gymnasts consuming the RNI of calcium for their age (800 mg/day), extra calcium supplements have no beneficial effect upon their skeletal health. Calcium supplements have a positive effect on the volumetric density of the peripheral skeleton of the control group. A synergistic action of calcium and exercise upon the bone may only occur in children who do not participate in high intensity, weight bearing exercise prior to calcium intervention.

Arch Dis Child: first published as on 21 March 2005. Downloaded from http://adc.bmj.com/ on August 19, 2022 by guest. Protected by copyright.