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Developing a National Reference Range for Paediatric Bone Density

Doctor in Medicine

2014

Aisling Laura Dobbyn Snow

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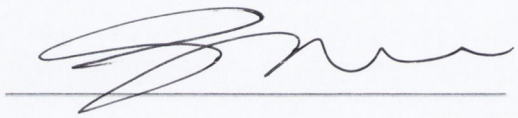

Aisling Laura Dobbyn Snow

SUMMARY

This study aimed to acquire normative dual energy x-ray absorptiometry (DXA) bone density data for Irish Caucasian children. A total of 162 healthy Irish Caucasian children (84 male, 78 female) aged between six and 16 years underwent anthropometric measurements and a single DXA examination as part of the study. 101 participants (47 male, 54 female) returned diet and lifestyle questionnaires and 48 participants (21 male, 27 female) performed self-assessment of pubertal stage. In order to take account of bone size, areal bone mineral density (aBMD) results were converted to a volumetric estimate, corrected BMD (BMDcorr) using the Kroger method of BMD_{corr} = BMC/Volume = aBMD x [4/(π x Width)]. Participants were grouped by sex and age and the Cole and Green (LMS) method was used to analyse aBMD and BMD_{corr} results by median $M(Age_i)$, coefficient of variation $S(Age_i)$ and the Box-Cox power $L(Age_i)$. Height and weight were analysed by age and compared to the Irish reference data. Body mass index (BMI) was also calculated, as weight (kg) divided by height^2 (m^2). Separate comparisons of aBMD and BMD_{corr} by Tanner stage were performed for male and female participants using a paired Student's t-test. Dietary factors were analysed by sex and duration of daily exercise was analysed by age range for males and for females and compared to aBMD and to BMD_{corr} using a paired Student's t-test.

LMS coefficients are presented in individual tables for males and females and can be used to calculate the aBMD or BMD_{corr} Z-score for an individual child using the formula $z_i = [y_i/M(Age_i)]^{L(Age_i)} - 1 / L(Age_i)S(Age_i)$. Percentile charts for aBMD are

given for males and for females. Pubertal stage, diet and lifestyle data are presented in tabulated and chart form.

As expected, aBMD, BMD_{corr} and height all increase with age in both males and females. Overall, the female participants in the study are significantly taller than the Irish reference standard; male participants also tend to be taller than the reference standard but not to a significant level. Analysis of bone density in those who exercise more than and less than one hour per day reveals a significant inverse relationship between higher levels of exercise and both aBMD and BMD_{corr} in females aged between 11 and 16 years. It is also evident that, after the age of 12 years, the female study participants became less involved in exercise whereas males continued to maintain their levels of activity.

The bone density data collected from this group of participants represents the first normative data of its kind for Irish children and it is presented in such a way as to allow clinicians and radiologists to evaluate paediatric aBMD and BMD_{corr} in context. The addition of participants in the future would both increase the accuracy and broaden the applicability of the results.

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Professor Edna Roche has generously provided me with the support and expert insight necessary to enable me to bring structure and purpose to this research. Professor Myra O'Regan has provided invaluable guidance and expertise in the formation of a solid statistical methodology for data analysis.

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Ms Inez Byrne, Ms Anne Stafford, Ms Liz Shields and Ms Darina O'Sullivan expertly performed the DXA scans for the study. Ms Bernadette Rowley provided dedicated and very much appreciated administrative support throughout. Ms Elaine O'Mullane, research nurse in the Department of Paediatrics in Trinity College Dublin, was always available to assist with practical details. Dr Alison Hurley was always ready to advise and help with practical planning. Ms Alison Clapp assisted immeasurably by lending her literature search expertise.

Sincere thanks are owed to the National Children's Hospital Foundation (NCHF) for providing the funding to allow this study to become a reality.

DEDICATION

To my parents, Joan Dobbyn and David Snow, to whom I owe endless appreciation for so many things including exceptional encouragement over my many years of study.

To my sister Fiona, who is always available with help and encouragement and inspires me with her amazing creativity, commitment to family and dedication to hard work.

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CHAPTER 1

INTRODUCTION

1.1 Bone health

Bone health is a complex concept that has many interwoven influencing factors. The strength of a bone is determined not only by the architecture of its physical structure but also by the density of the structure itself(1). In childhood the immature but growing skeleton is in a state of flux where bone shape and composition are constantly developing under a multifactorial influence(2). Many of the key determinants of bone development relate to ethnicity and other inherited variables but numerous environmental factors also play an important role. These include diet, exercise, vitamin D levels, pubertal stage, weight, body composition and presence or absence of negative impacts such as chronic ill health, bone disease, detrimental medications and hormone imbalance(3). The influence of modifiable environmental factors is of particular importance in childhood and adolescence, during which time it is possible to significantly impact bone development and, subsequently, influence bone health in adulthood(4).

The evaluation of paediatric bone health requires a detailed, rounded clinical review of all familial and environmental bone-influencing variables. This evaluation can be augmented by the objective analysis of bone by one of a number of available techniques(5). Histologic and ash analyses of bone are

accurate analytic techniques and are considered to be the gold standard in evaluation of bone structure and mass however they require, at minimum, a substantial bone biopsy and are clinically impractical, especially in the paediatric population(6, 7). The most clinically relevant options for analysis of bone density are dual energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral QCT (pQCT) and, to a lesser degree, quantitative ultrasound (QUS)(1, 5, 8).

The structure and mass of bone affect bone strength in different ways(9). QCT has been known for some time now to be a non-invasive, accurate method of assessing both the structure and density of bone(10). Until the recent advent of improved CT technology and advanced software analytic algorithms, the use of QCT had been limited due to radiation dose concerns, particularly in the paediatric population(5, 11). pQCT, where bone analysis of an anatomic periphery is the focus, initially emerged as a lower-dose CT alternative, albeit without the ability to provide spinal data or the level of detail collected by QCT(1). Both CT methods have an advantage over DXA, in that they are able to provide structural details about the bone being analysed. This can be combined with other measurements such as regional muscle strength to enable interesting methods of interpretation such as the functional approach described by Schoenau et al(12). At present however, the number of centres offering QCT imaging is limited and few paediatric reference datasets have been published. DXA, on the other hand, is widely available.

1.2 Paediatric DXA

DXA scanners are utilised by both radiologists and physicians as the most common method of measuring bone density in children. A DXA 'system' refers to the combination of a particular brand and model of scanner and the software applied to interpreting the results. DXA scans measure, amongst other parameters, bone mineral content (BMC) and bone mineral density (BMD). Results are typically presented both in 'raw' format and as a standard deviation from the mean. It is the standard deviation from the mean that provides clinicians with meaningful information about bone density in a given patient. The original normative data used to calculate standard deviations in bone densitometry were acquired from healthy adult women; this data was initially used in the interpretation of DXA results for patients of all ages and both sexes. The standard deviation from the healthy young adult female mean is referred to as a 'T-score'. It is now recognised that comparing paediatric DXA results to an adult female mean provides a misleading evaluation because bone structure and composition are different in children. Current best practice therefore dictates that DXA results for children are given both as a number and as a 'Z-score' that instead relates the result to an age- and sex-matched mean(13). Unlike a low T-score in adult women, an isolated low Z-score in children has not been shown to correlate closely to the risk of fracture. The Z-score can instead be used as part of the multi-factorial assessment of bone health, which as a whole can give the clinician a broad estimate of fracture risk. Children who are deemed to be at significantly increased fracture risk and who have low BMD are frequently treated with medications such as bisphosphonates or growth

hormone in a bid to stabilise or improve bone density. Whilst these medications can be beneficial, they are associated with clinically significant side effect profiles and it is highly desirable to limit their use to patients most likely to benefit. Misdiagnosis of low bone density on DXA can lead to the inaccurate identification of patients in need of pharmacologic intervention.

Multiple factors contribute to the misdiagnosis of low bone density in children. In one study of paediatric DXA results, up to 88% of scans had one or more interpretative errors, the most common of which was the use of a T-score rather than a Z-score (14). Other errors included technical problems with data acquisition, statistical errors in interpretation and failure to account for ethnicity, sex or bone size.

1.3 DXA and Bone Size

Correction of DXA results for bone size or height is necessary because DXA scanners obtain data by projecting an x-ray beam through the patient to a receptor on the far side. Results are based on the number of x-rays reaching the receptor; both the x-ray source and the receptor are fixed in a single plane and DXA scanners can therefore measure bone area but not bone volume. As a result, standard results are given as a two-dimensional 'areal' BMD (aBMD), expressed in g/cm^2. It has been shown that aBMD results are not accurate in children who have bones that are smaller or larger than average(15).

Many paediatric patients undergoing DXA examination have chronic conditions that place them at risk of having smaller than average bones and/or poor bone health. In order to provide accurate paediatric DXA results, it is important that imaging is performed on a well-maintained DXA scanner, that account is made of patient height and that results are put in the context of a relevant reference range. It has been suggested that deviations in body and bone size could impact aBMD to a clinically significant degree in up to 17% of children undergoing DXA imaging(16).

Volumetric estimates of aBMD results attempt to take account of variations in bone size and have been shown to carry a higher coefficient of variant to ash analysis than aBMD(7). In order to maintain an acceptable level of accuracy in the use of DXA in children it is therefore necessary to adjust areal DXA data to estimate a volumetric result. Accordingly, a guideline document published by the International Society for Clinical Densitometry (ISCD) states that pediatric aBMD results should be corrected for height(17). The ISCD guidelines, published in 2007, do not specify the method of correction that should be used. Some DXA systems provide an inbuilt option for height correction but the absence of such an option is common. DXA scans performed at the Adelaide and Meath Hospital incorporating the National Children's Hospital (AMNCH) are performed on a GE Lunar Prodigy DXA system that utilises the most recent GE paediatric analysis software. Correspondence with GE Healthcare prior to this study revealed that, whilst the machine uses advanced pediatric software, there is no available software module that will automatically adjust results for patient height. Furthermore, they do not envisage such a module becoming available.

1.3.i Correction of BMD results for Bone Size

The literature reveals two principal methods for the adjustment of aBMD results for bone size to produce a volumetric BMD (vBMD) estimate. The method described by Kroger et al, in which the vertebral body is assumed to be cylindrical in shape, provides a corrected BMD (BMD_{corr})(77):

Kroger et al: $BMD_{corr} = BMC/Volume = aBMD \times [4/(\pi \times Width)]$

The method described by Carter et al, who coined the term bone mineral apparent density (BMAD), uses bone area to estimate bone width(18, 19):

Carter et al: $BMAD = BMC/Volume = BMC/(Area)^{1.5}$

Both the Kroger and Carter methods provide densitometry results in g/cm^3 and both have been used to estimate vBMD in published studies. The Carter method initially provided an estimate of bone volume at a time before DXA scanners had the ability to automatically measure vertebral width. Vertebral width is now routinely provided as part of the result data. The Kroger method was chosen for use in this study as it requires parameters that are readily accessible in GE Lunar Prodigy reports and is used by a number of academic groups who have acquired and published normative paediatric DXA data.

In order for BMD_{corr} to be clinically useful, it needs to be reported as a Z-score in the context of a reference range that is relevant to both the patient being assessed and the DXA system performing the data acquisition and analysis.

1.4 DXA Cross Calibration

Detailed analysis of densitometry results from different DXA systems suggests that the primary cause for inter-system variation is that they employ different mathematical equations in bone detection(20). Comparison of DXA results obtained on different scanners is not straightforward and requires the calculation and implementation of complex mathematical 'cross calibration' equations(21-23). Significant differences in Z-scores have even been demonstrated among reference databases acquired on the same brand of scanner(24). In the case of some systems, for example GE DPX-L and GE Lunar Prodigy, the comparison of data is made more complicated by fundamental differences in scan acquisition technique. Older scanners, such as the DPX-L, use a 'pencil beam' technique while the Lunar Prodigy uses a more advanced 'fan beam' method of imaging. It is well documented that pencil beam and fan beam DXA systems produce differing results, which can be clinically significant(25). One study that evaluated DXA data acquired using GE DPX-L and GE Lunar Prodigy DXA systems in the same children revealed that lumbar spine BMD was 1.6% higher on the Prodigy than the DPX-L system, with $p<0.0001$(26).

The need for cross calibration can be determined by scanning phantoms that are

relevant to the body region in question. This allows an estimation of the over- or under-estimation of DXA parameters measured on one system but analysed using a reference range acquired on another(27). Cross calibration equations therefore differ depending on the specific DXA scanners concerned. The implementation of cross calibration equations reduces, but does not eliminate, the variation in these results(28).

1.5 Influences of Bone structure and Health

1.5.i Ethnicity

Ethnic differences in BMC and aBMD were confirmed in the Bone Mineral Density in Childhood Study (BMDCS)(29). Bone size has been shown to be the primary determinant of differences in BMC between ethnicities; a secondary effect is mediated by extrinsic factors such as diet and exercise(30).

1.5.ii Nutrition

The 2003 World Health Organisation (WHO) report on diet, nutrition and prevention of chronic diseases addresses the prevention of osteoporosis, making recommendations about the consumption of calcium, vitamin D, sodium, fruit and vegetables and about body weight(13, 31). Calcium supplementation provides a modest improvement in BMD in adolescent females(32). Low serum calcium has been shown to be present in a significant number of girls thought to

be otherwise healthy.

Vitamin D3 (cholecalciferol) is generated in the skin when it is exposed to ultraviolet light. Vitamin D2 (ergosterol), on the other hand, is ingested from dietary sources. Both cholecalciferol and ergosterol undergo initial hydroxylation in the liver, resulting in metabolically active 25-dihydroxycholecalciferol (25(OH)D) compounds. Some 25(OH)D is converted in the kidneys to calcitriol, which has a regulatory effect on the level of calcium in the blood and has a positive impact on bone growth and remodeling. It is 25(OH)D that is used as a measure of systemic Vitamin D; plasma 25(OH)D less than 50nmol/l is considered low in Ireland.

Low vitamin D levels, especially common in winter and spring in Europe, may exacerbate the detrimental effect of hypocalcaemia on bone mineralisation(33). Vitamin D deficiency has been shown to correlate with increased body fat and with reduced height, but does not appear to be directly linked to lower peak bone mass(34). There are conflicting reports of the indirect impact of low circulating plasma 25(OH)D on size-adjusted BMC but meeting the criteria for normal vitamin D status in childhood has been shown by some to positively affect bone mass(35). Despite a previously held belief that low maternal vitamin D levels may adversely impact infant BMC, recent studies have failed to support this theory, with no demonstrable association detected(36-39).

The effect of fruit and vegetable consumption on BMC and BMD has been widely studied. Overall, the evidence suggests a positive association between consumption of fruit and, to a lesser degree, vegetables, and bone mineral values as assessed by DXA(40). Childhood dairy consumption positively impacts bone

health independent of gender, exercise, height, weight, BMI and body fat; milk protein consumption in childhood appears to confer benefit to the bone mineralisation process (41-43). Studies have failed to show a direct positive effect of breastfeeding over and above formula feeding on bone density later in childhood(44).

1.5.iii Body Composition, Birth Weight

Body composition broadly refers to an individual's relative amounts of fat and lean tissue mass; it provides more detailed information than body mass index, which does not differentiate between fat and lean mass. As well as measuring bone density parameters, DXA scanners can provide body composition data. It is important to take into account both lean body mass (which may have a positive association with BMD) and fat body mass (which may have a negative association with BMD) when evaluating bone health in children(45). Whole body DXA is one method of estimating lean and fat mass. Although adolescents who have higher body fat also tend to have higher bone mass, the association appears to be mediated by their concomitantly higher lean body mass; it has been shown that fat mass alone does not improve BMD in overweight adolescent boys(46, 47). In girls who are overweight or obese, BMC and BMD increase in proportion to the increased lean component of their body mass rather than to the fat component(48). In fact, although increased weight is, overall, beneficial to bone health, fat mass (as opposed to lean mass) may actually have a negative effect; in two individuals of the same weight and sex BMC has been found to be lower in the subject with the higher percentage fat(49, 50). Increased total body fat mass

in girls has a negative association with vBMD in the femur and tibia(51). It has also been shown that raised BMI in childhood can lead to early puberty, thus negatively affecting the achievable peak bone mass(52).

Bone is composed of a dense outer rim of cortical bone and a less dense inner core that is referred to as 'cancellous' or 'trabecular' bone. Trabecular bone has a latticework structure, with a fine solid matrix that is surrounded by bone marrow. Bone marrow varies in its composition; in childhood, haematopoietic marrow predominates but, as skeletal development proceeds, this is gradually converted to more fatty marrow. In addition to total body fat having an impact on bone density, the amount of fat within the bone marrow also appears to have an effect. The specific impact of marrow fat on bone density has been studied with QCT analysis of marrow composition. Whereas DXA cannot differentiate the relative percentages of trabecular and cortical bone, QCT easily distinguishes these components(53). One study using QCT demonstrated that bone strength was increased and marrow fat was decreased in female athletes, with the authors suggesting that increased osteoblast activity, triggered by reduced marrow fat levels, may play a key role in the enhanced bone strength conferred by exercise(54).

Birth weight has been shown to influence bone parameters in later childhood and adulthood however this influence does not persist after correction for body size(55-57). It has been postulated that body measurements at birth may influence the likelihood of subsequent involvement in weight bearing sports, thus positively influencing bone health(58). Similar to isolated low birth weight, prematurity alone does not appear to be a risk factor for low bone density in adulthood(59). However, in comparison to the apparent lack of correlation

between uncomplicated prematurity and low bone mass in adulthood, prematurity associated with very low birth weight (VLBW) (birth weight less than 1,500g) or other complications has been shown to be associated with impaired BMD at the point of peak bone mass in early adulthood(60). In addition to the findings regarding birth weight and later bone mass, low BMD in childhood tends to persist over medium-term follow up(61). It has also been shown that, without bone-modifying interventions, bone mass tracks along percentile curves in adolescence(62, 63).

1.5.iv Exercise

Given the malleability of the developing skeleton and the tendency of BMD to follow percentile curves over time, exercise during the period of childhood bone growth is of particular benefit in maximizing bone strength and minimizing fracture risk in adulthood(64). Some increase in BMC and BMD continues even beyond the age of puberty; it has been shown that exercise in young men can also increase both BMC and bone volume(65). However, the time of peak effect of exercise on bone structure and mineralisation is in childhood and adolescence(66). The period of maximal benefit of exercise on bone health in girls has been suggested to be Tanner stage I, before the period of accelerated skeletal growth associated with puberty(67). Early moderately vigorous physical activity has been shown in other studies to have a long-term beneficial effect on BMC in boys(68). In fact, the benefit of weight bearing exercise in childhood can be seen well into adulthood, in the form of measured structural and mineral parameters(69, 70). In males, participation in sports during childhood confers a

benefit in terms of bone health parameters into adulthood even if participation in physical activity ceases(71). Similarly, continuation of childhood exercise participation from adolescence into early adulthood assists in maintaining peak bone mass in females(72).

In addition to the timing of exercise in childhood, the type, intensity and daily duration of physical activity are important determinants of bone health. The type of exercise chosen determines not only whether there will be a benefit to bone health but also which bones, if any, will be affected. Aerobic but non-weight bearing sports reduce BMI but do not tend to confer an increase in bone mass(73, 74). Population-based exercise programmes for school aged children have been shown to have a beneficial effect on bone mass and bone size(75). In a Swedish study, school aged boys partaking in the recommended 60 minutes of school exercise per week were compared with boys partaking in 40 minutes of school exercise per day; those with the higher exercise participation had increased lumbar BMC after two years(76, 77). One study determined that a positive effect on bone mass was seen with just 28 minutes per day of vigorous activity or with 78 minutes per day of moderate-to-vigorous activity(78). Another study of a school-based physical activity programme that involved just 10 minutes of vigorous exercise per day showed a small benefit to both BMC and BMD(79). The absence of significant benefit on bone structure or mineral content from light or moderate daily physical activity has been confirmed by a number of studies(80). Although these levels of activity may be beneficial to health and wellbeing for other reasons, more vigorous weight-bearing activity is required before a beneficial effect on bone health is seen. A systematic review of articles addressing the effect of physical activity in school aged children and adolescents concluded that 60 minutes or more or moderate to vigorous physical activity

should be performed every day(81).

1.5.v Smoking

In addition to the more established non-hereditary influences on bone health, there is limited evidence to support an association between passive exposure to household cigarette smoke in early adulthood and reduced premenopausal bone mass in adult women(82). There is little in the literature that addresses the impact of passive cigarette smoke on bone health in children.

1.6 Reference Ranges

In adult women, significant differences between BMD z-scores calculated from US and UK reference data suggest that the populations cannot be used interchangeably for the purpose of calculating Z-scores(83). This may also apply to paediatric DXA scanning. Analysis of Irish and UK height and weight reference data by Hoey also revealed significant differences in the trajectory of the percentile curves between the Irish and UK populations(84).

In the lead up to this research an initial audit was undertaken to determine whether it would be worthwhile developing an Irish reference range for pediatric aBMD and BMD_{corr}(85). The aBMD results of 66 children who were scanned over a two-year period were reviewed retrospectively. Two groups

of patients were included; those born small for gestational age and had failed to demonstrate catch-up growth (n=19), and a group of patients with cystic fibrosis (CF) (n=47). BMD_{corr} was calculated for each patient using the Kroger method and subsequently compared to age- and sex-matched reference data from the Netherlands(86). The Dutch study was chosen because its cohort closely resembled the prospective Irish cohort of healthy Caucasian children. In addition, the study published the standard deviation required to allow calculation of a Z-score. The patients' original Z-score, calculated from aBMD, and their height-adjusted Z-score, calculated from BMD_{corr}, were compared by applying Student's t-test. We found that the Z-scores for BMD_{corr} differed significantly from Z-scores for aBMD in both groups studied. In the SGA group, the mean aBMD Z-score was -1.1 and the mean BMD_{corr} Z-score was 0.1 (p=0.000). In the CF group, the mean aBMD Z-score was -1.3 and the mean BMD_{corr} Z-score was -0.4 (p=0.002). Overall, the mean aBMD Z-score was -1.1 and the mean BMD_{corr} score was -0.2 (p=0.000). With conversion of aBMD to BMD_{corr}, three patients who initially had Z-scores that were low enough for them to be considered candidates for bisphosphonate or growth hormone therapy were found to have BMD_{corr} Z-scores above the threshold for treatment. This audit was limited both by the absence of an Irish reference range against which to compare the results and by the fact that the Dutch dataset was acquired on a different DXA system (a GE scanner but of the DPXL rather than Lunar Prodigy subtype). Despite its limitations, the audit provided some support for the international best practice position that pediatric aBMD results should be corrected for height. In the case of the patients studied, correction of aBMD using the Kroger method significantly altered their results when the non-

Irish, non-Lunar Prodigy reference dataset was used to evaluate standard deviations from the mean.

1.7 Research question

The aim of this study is to acquire normative DXA data for Caucasian Irish children scanned on a GE Lunar Prodigy DXA system. This is with a view to providing a relevant local reference range that facilitates contextualisation of paediatric DXA results, allows adjustment for bone size and minimises the risk that paediatric DXA results will erroneously lead to pharmacologic intervention for low bone mineral density.

CHAPTER 2

METHODOLOGY

2.1 Participants

Participants were recruited from the Dublin area through the National Children's Hospital (NCH) in Tallaght. The NCH is a 65-bed paediatric teaching hospital associated with Trinity College Dublin and has Accident and Emergency, inpatient, operative and outpatient services; approximately 65,000 children attend the hospital every year. Advertisements were placed in the waiting areas of the paediatric outpatient clinic, operating room and radiology department in An electronic advertisement visible to all staff members at AMNCH was also placed on the hospital intranet noticeboard; this was renewed every 6 months for the first 18 months of the study. The majority of respondents contacted the administrative staff of the paediatric department of the NCH in person or by telephone; the remainder contacted the principle investigator by email. At least three attempts were made by the principal investigator to contact each respondent by telephone.

The study rationale, structure, logistics, risks and benefits were discussed by telephone with each contactable respondent. A comprehensive information leaflet (Appendix 1) was offered to each respondent and was either posted or emailed to those that wished to receive it. The demographic details and relevant medical history of each child being volunteered for participation were then recorded. These details included name, gender, ethnicity, date of birth,

gestational age at birth, birth weight, medical conditions, medications, allergies and fracture history. Prospective participants were excluded if they were not Caucasian, had a diagnosed chronic medical condition (including bone disease, cystic fibrosis, inflammatory bowel disease and severe asthma), used medications that impact bone health (for example oral steroids), had a significant family history of primary osteoporosis or had a history of fracture(s) associated with no or minimal trauma (for example following a fall from standing height). Female participants who had begun menstruation were scheduled for DXA imaging between day one and day ten of their menstrual cycle. This was in compliance with the local 'Ten-Day Rule' regulation governing the use of ionising radiation in females of childbearing potential.

2.2 Consent, Auxiology

Ethical approval for the study was granted by the joint AMNCH – St James's Hospital (SJH) Research Ethics Committee. At the time of their child or children's scan each parent or guardian signed a form consenting to their participation. The study radiographers, who are trained in the correct procedure, performed Auxiological assessment. Height was measured with a 'Harpenden' stadiometer using a standardized technique, with the head in the Frankfurt plane. Weight was assessed using a self-zeroing Seca (Hamburg, Germany) electronic scales, with the participants wearing light indoor clothing and no shoes. Height and weight were analysed by age and compared to the Irish reference data. Body mass index (BMI) was also calculated as weight (kg) divided by height^2 (m^2).

2.3 Pubertal Assessment

Formal assessment of pubertal stage was offered to male participants nine years and older and to female participants eight years and older who had not yet had a menstrual period. Due to very low rates of acceptance to undergo formal clinical assessment, optional self-assessment of pubertal stage was offered as an alternative; pubertal self-assessment forms were given to the parent or guardian of relevant participants for completion at home. The standardised male and female forms asked participants or their parent or guardian to assess which of a series of standardised photographs corresponding to Tanner stages I to V most closely resembled their pubertal stage (Appendix 2)(87). The relationship of Tanner stage to aBMD and BMD_{corr} was evaluated.

2.4 Diet and Lifestyle Questionnaire

Diet and lifestyle were assessed by postal questionnaire. Participants were asked to detail their dietary intake (dairy products, vegan or vegetarian diet and dietary supplements), physical activity levels, sedentary habits, use of portable digital devices and exposure to environmental smoke over the period leading up to their DXA assessment. Completed forms were returned to the principal investigator in the NCH radiology department. Dietary factors were analysed by sex.

2.5 Bone Mineral Density Assessment

Bone densitometry was assessed using DXA imaging. DXA scanning was performed by one of four paediatric DXA radiographers, all of whom perform paediatric and adult DXA scans on a weekly basis in AMNCH and are trained in the use of the hardware and software. All scans were acquired using a GE Lunar Prodigy DXA scanner (GE Healthcare, Chalfont St Giles, United Kingdom). The scanner undergoes daily quality assurance (QA) using a standard GE phantom (a phantom is a precisely standardised model that can be used to test the accuracy of a piece of imaging equipment). Weekly QA using the specific lumbar spine phantom (Lunar 18562) is also performed and the scanner is serviced regularly according to the GE service schedule. A single anteroposterior (AP) DXA scan of the lumbar spine was performed on each participant, with each DXA scan limited to the AP projection of the lumbar spine in order to minimise the dose of radiation imparted to the participants while still providing the data required to complete the study.

The standard DXA bone data was acquired for each participant, including BMC, BMD and vertebral width for each vertebral level from L1 to L4. The DXA scanner software automatically presents the data relative to the pre-programmed normal range, providing a Z-score based on the patient's age and sex. The data were transferred automatically to the picture archiving and communication system (PACS) of the hospital radiology department and stored as a permanent part of the participant's medical record. Scans were reported using the standard reporting system in the AMNCH radiology department. Patients who were found

to have low aBMD (a Z-score of ≤1 SD compared to the age and sex matched mean) were referred for clinical paediatric review in the NCH.

2.6 BMD Correction for Bone Size

BMD results were adjusted for bone size producing corrected BMD (BMD_{corr}) by using the method published by Kroger et al(18):

$$BMD_{corr} = BMC/Volume = aBMD \times [4/(\pi \times Width)]$$

2.7 Statistical Analysis of Bone Density

Participants were grouped by sex and age (in single years). The LMS (Cole and Green) method was used to determine the percentile curves for aBMD and for BMD_{corr} for each gender using the Generalised Additive Models for Location, Scale and Shape (GAMLSS) statistical modelling package in the R software language(88-90). The distribution of aBMD and BMD_{corr} at each group was summarised by the median $M(Age_i)$, coefficient of variation $S(Age_i)$ and the Box-Cox power $L(Age_i)$. The Box-Cox power was used to transform the data to make them normally distributed within a particular age group. A simpler model using only means and variances was also built, in which only the M and S components of the LMS model were used. Worm plots were used to check the model. Bootstrapping the model was attempted in order to measure the uncertainty in

the results but due to small sample sizes, bootstrapping of the LMS model was not possible. Bootstrapping of the simpler MS model was found to be possible. The relationship of both aBMD and BMD_{corr} to birth weight was analysed and depicted using scatter plots. Separate analysis of the relationship of bone density to breastfeeding was performed. Duration of daily exercise was analysed by age range for males and for females and compared to aBMD and BMD_{corr} using a paired Student's t-test.

In order to calculate the standard deviation for aBMD or BMD_{corr} in an individual child the relevant LMS values for their age and gender are used. The following formula calculates the Z-score for a particular y_i (in this case aBMD score) at age Age_i:

$$z_i = [y_i/M(Age_i)]^{L(Age_i)} -1 \; / \; L(Age_i)S(Age_i).$$

CHAPTER 3

RESULTS

3.1 Study population

Over the period of the study (January 2010 to June 2012), a request for further information about the study was received from approximately 180 parents or guardians, many of whom had more than one child within the age range covered by the study. In total, the parents or guardians of approximately 260 children between the ages of 6 and 16 years responded to either the printed or electronic advertisement. The study could not be discussed with the parents or guardians of approximately 40 children due to incorrect contact details being provided, the respondent no longer having interest in the study or the respondent being unable to discuss the study at the time of contact.

The total study population consisted of 162 healthy Irish Caucasian children (84 male, 78 female) aged between six and 16 years (Table 1). All performed scans were technically adequate, without significant artifact or other limitation.

Age (years)	Gender		Total
	Female (n)	Male (n)	
6-6.9	7	8	**15**
7-7.9	8	6	**14**
8-8.9	9	4	**13**
9-9.9	8	11	**19**
10-10.9	6	9	**15**
11-11.9	10	7	**17**
12-12.9	10	9	**19**
13-13.9	9	11	**20**
14-14.9	6	9	**15**
15-15.9	1	7	**8**
16-16.9	4	3	**7**
Total	**78**	**84**	**162**

Table 1. Study participants by age and sex.

3.2 Bone densitometry

The aBMD of all female participants was found to lie within two standard deviations of the mean for age when evaluated using the inbuilt DXA software; all female participants were therefore included in the statistical analysis. Two male participants (one aged six and one aged 15 years) were found to have an aBMD less than or equal to two standard deviations below the automatically-calculated mean for age; they were ultimately included in the reference data as both had a BMD_{corr} with the normal range. Due to the very small number of male participants aged 16, these participants were omitted in the analysis using the Cole and Green method. Three further points were also omitted from the male LMS analysis; one low aBMD result in a 14 year old and two low aBMD results in 15 year olds.

Both aBMD and BMD_{corr} increased with age in males and in females; the results are presented as scatter plots by age and sex (Figure 1, 2). The LMS coefficients are presented in individual tables for males (Table 2, 3) and females (Table 4, 5) and can be utilised in conjunction with the formula $z_i = [y_i/M(Age_i)]^{L(Age_i)} - 1 / L(Age_i)S(Age_i)$ to calculate the aBMD or BMD_{corr} Z-score for an individual child. The results of the simpler MS model using only means and variances are presented as percentile curves for males (Figure 3) and females (Figure 4); these demonstrate greater variation for females than for males.

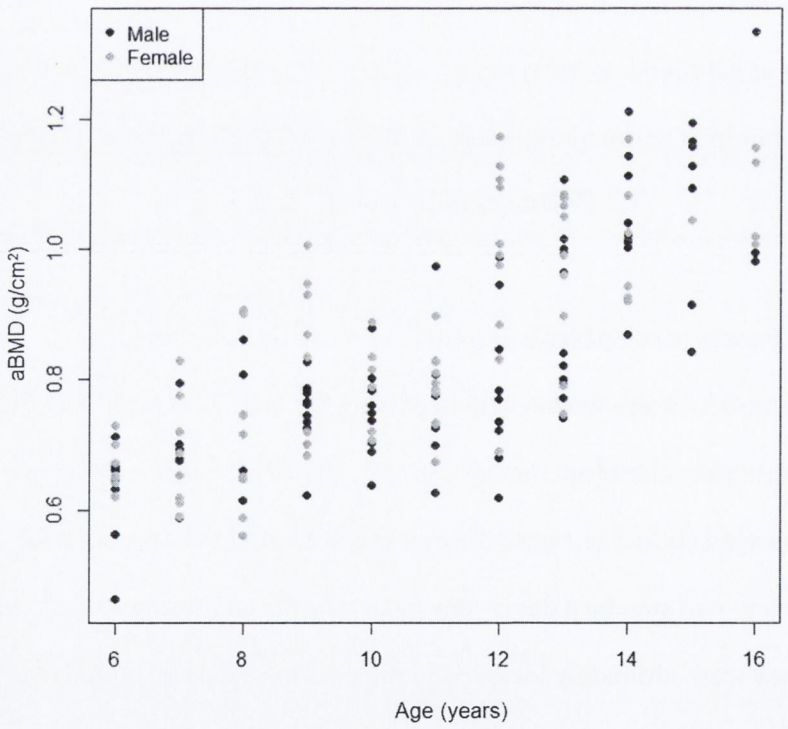


Figure 1 . Age by aBMD by gender.

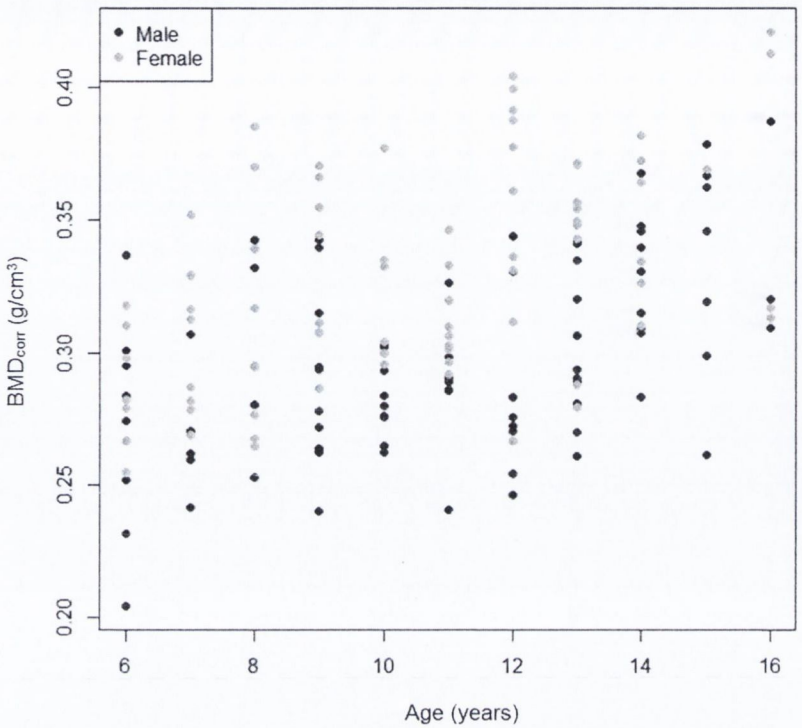


Figure 2. Age by BMD_{corr} by gender.

aBMD for Males			
Age (years)	L	M	S
6-6.9	4.4744	0.6412	0.1451
7-7.9	3.2363	0.6949	0.1405
8-8.9	2.1516	0.7333	0.1284
9-9.9	1.2635	0.7477	0.1143
10-10.9	0.5507	0.7501	0.1236
11-11.9	0.0394	0.7636	0.1567
12-12.9	-0.2644	0.8096	0.1765
13-13.9	-0.4308	0.8996	0.1439
14-14.9	-0.5284	1.0209	0.0806
15-15.9	-0.6007	1.1497	0.0806

Table 2. LMS data for aBMD in males.

BMD_{corr} for Males			
Age (years)	**L**	**M**	**S**
6-6.9	1.0701	0.2739	0.4607
7-7.9	-0.0069	0.2772	0.4283
8-8.9	-0.7272	0.2799	0.3991
9-9.9	-0.9303	0.2813	0.3737
10-10.9	-0.6933	0.2824	0.3502
11-11.9	-0.2341	0.2851	0.3264
12-12.9	0.3035	0.2923	0.2996
13-13.9	0.9528	0.3061	0.2692
14-14.9	1.7363	0.326	0.2378
15-15.9	2.606	0.349	0.209

Table 3. LMS data for BMD_{corr} in males.

aBMD for Females			
Age (years)	**L**	**M**	**S**
6-6.9	-0.9908	0.656	0.088
7-7.9	-0.7609	0.698	0.1591
8-8.9	-0.531	0.74	0.2072
9-9.9	-0.3012	0.7821	0.184
10-10.9	-0.0713	0.8242	0.1502
11-11.9	0.1586	0.8664	0.1527
12-12.9	0.3885	0.9087	0.1654
13-13.9	0.6184	0.951	0.1383
14-14.9	0.8483	0.9934	0.0962
15-15.9	1.0782	1.0357	0.068
16-16.9	1.308	1.0781	0.0533

Table 4. LMS data for aBMD in females.

BMD_{corr} for Females			
Age (years)	**L**	**M**	**S**
6-6.9	-3.6708	0.2887	0.3157
7-7.9	-2.9743	0.297	0.3125
8-8.9	-2.2779	0.3052	0.3097
9-9.9	-1.5815	0.3134	0.3072
10-10.9	-0.8851	0.3217	0.3047
11-11.9	-0.1887	0.3299	0.3026
12-12.9	0.5077	0.3381	0.3007
13-13.9	1.2041	0.3463	0.299
14-14.9	1.9005	0.3546	0.2974
15-15.9	2.5969	0.3628	0.296
16-16.9	3.2933	0.371	0.2947

Table 5. LMS data for BMD_{corr} in females.

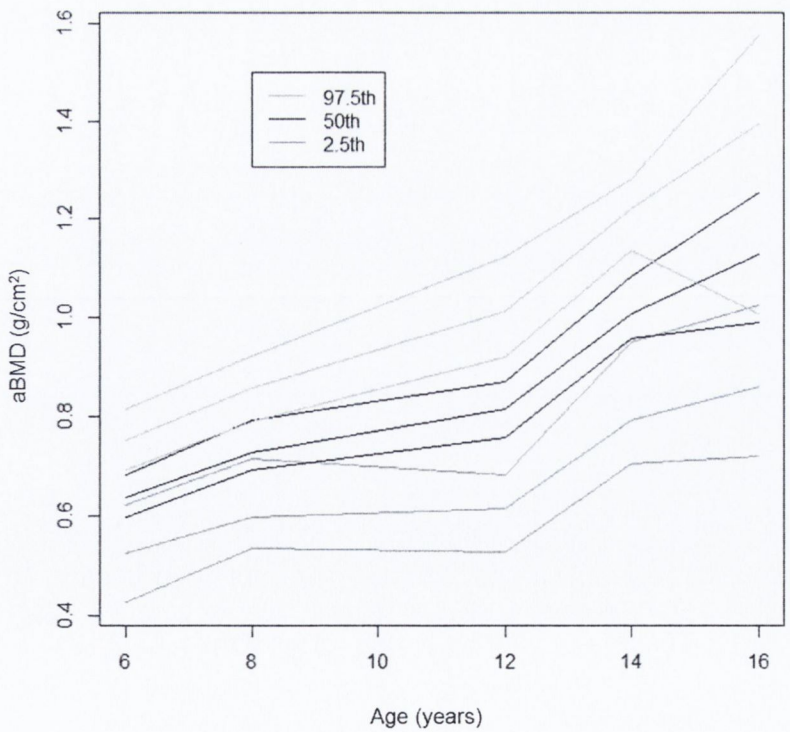


Figure 3. aBMD percentiles for males.

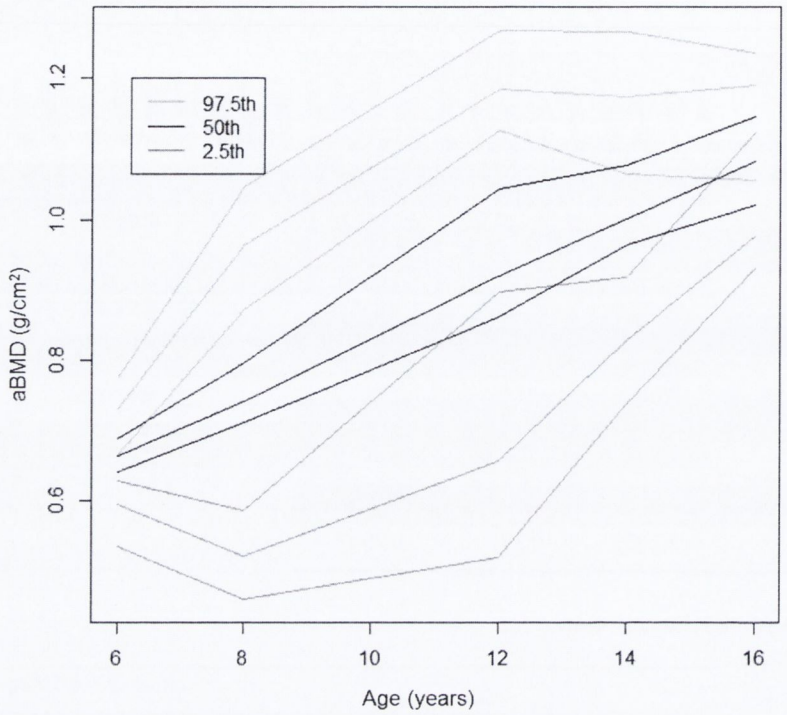


Figure 4. aBMD percentiles for females.

3.3 Birth Weight, Height, Weight, BMI

Birth weight did not correlate with aBMD or BMD_{corr} in later childhood (Figure 5, 6). As expected, height increased with age for both male and female participants (Figure 7, 8). Overall the female participants in the study cohort were significantly taller than the Irish reference standard (p=0.00035); the male participants were also taller but this was not significant (p=1.75). Height, weight and BMI data are given in Appendix 5.

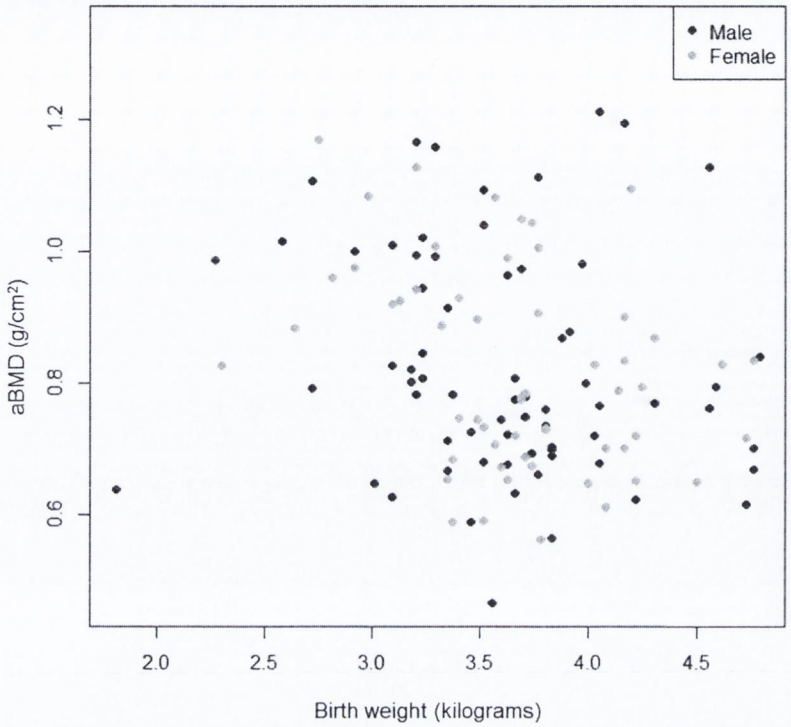


Figure 5. aBMD by birth weight by gender.

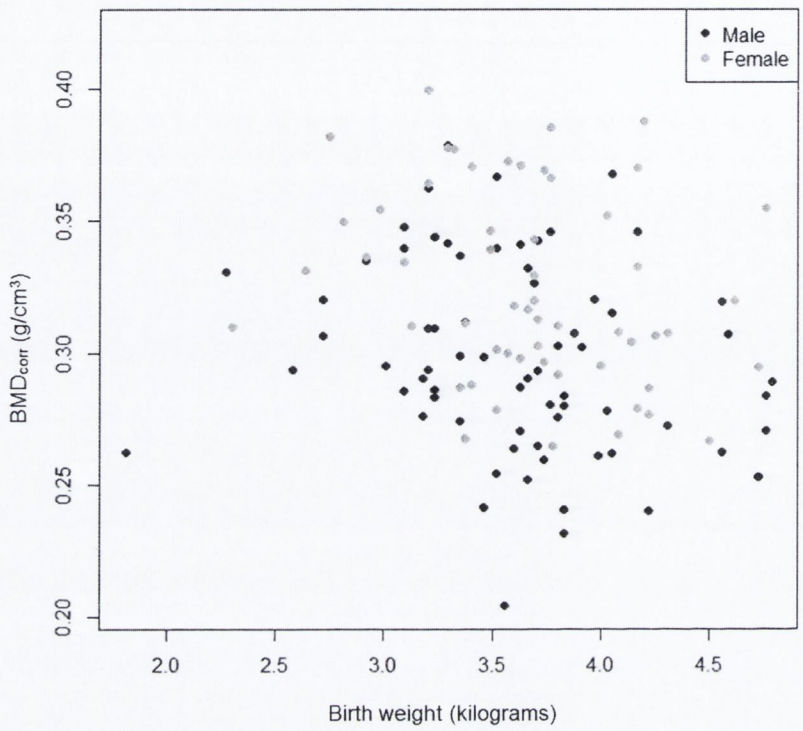


Figure 6. BMD_{corr} by birth weight by gender.

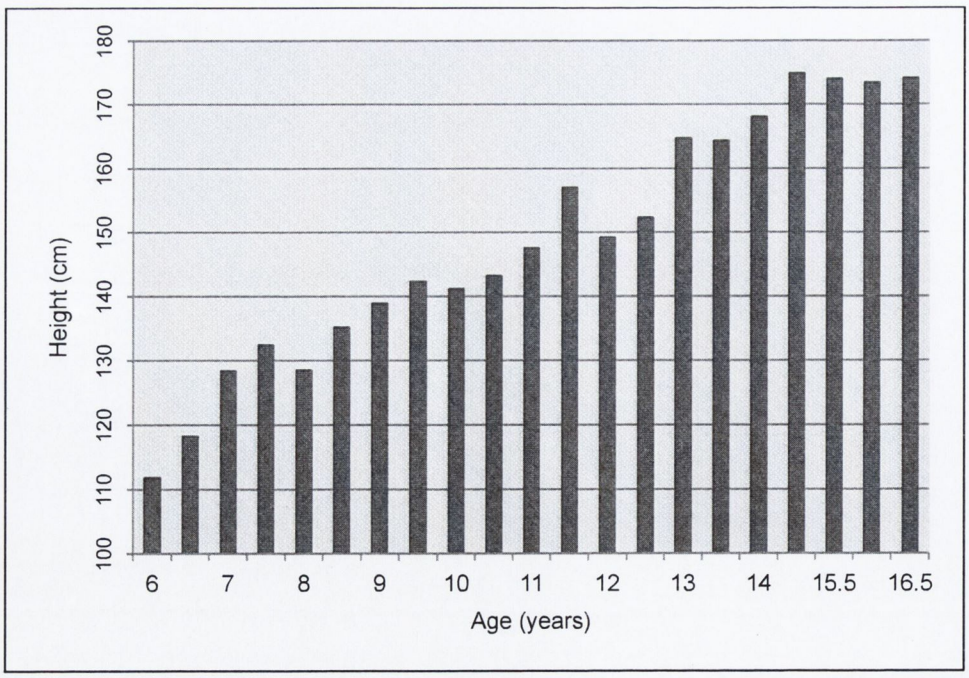


Figure 7. Height by age in males.

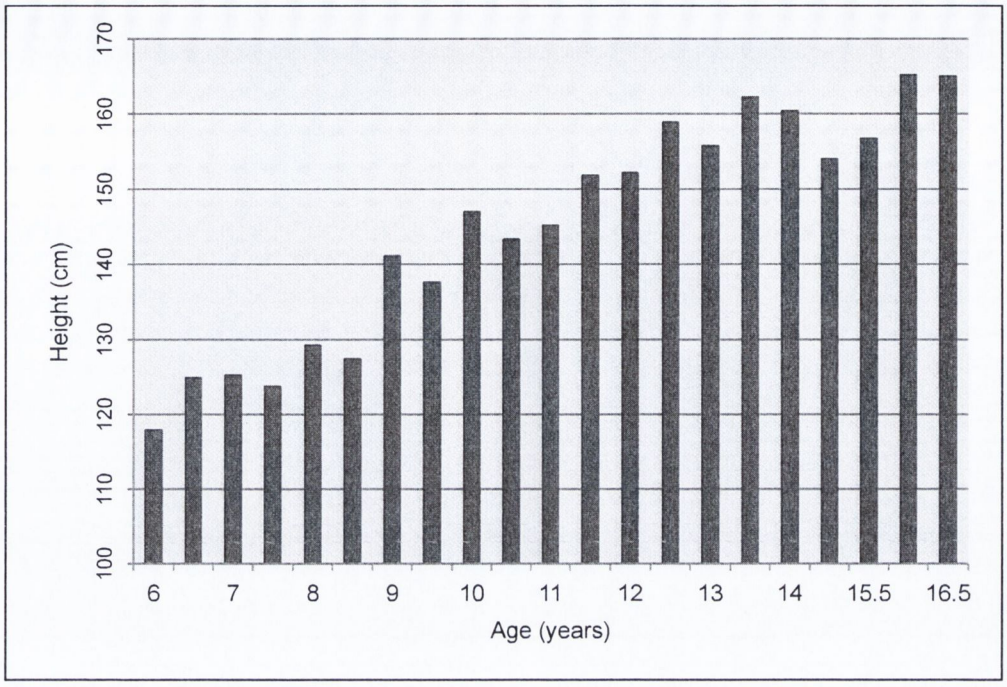


Figure 8. Height by age in females.

3.4 Pubertal Stage

Pubertal self-assessment was completed by 27 females who were 8 years or older and by 27 males who were 9 years or older at the time of their DXA examination. Pubertal stage data is summarised in Appendix 6. Male aBMD and BMD_{corr} results for Tanner stages 0-I and II+ are shown below (Table 6, 7). All females who completed pubertal self-assessment were Tanner stage II or above.

	Tanner Stage	N=	Mean aBMD (g/cm^2)	CI for Mean	P=
Males >9 years	0-I	11	0.776	0.6836-0.8677	
	II+	16	0.853	0.7768-0.9295	0.19

Table 6. aBMD by Tanner stage in males. CI = confidence interval.

	Tanner Stage	N=	Mean BMD_{corr} (g/cm^2)	CI for Mean	P=
Males >9 years	0-I	11	0.281	0.2605-0.3011	
	II+	16	0.296	0.2790-0.3127	0.25

Table 7. BMD_{corr} by Tanner stage in males. CI = confidence interval.

3.5 Diet

There was no significant association between breastfeeding and bone density in males or females (Table 8, 9). Dietary intake results are summarised in Figure 9 and in the data provided in Appendix 7.

		N	aBMD	95% CI	P
Females	Breastfed	29	0.829	0.7673-0.8913	
	Not breastfed	25	0.809	0.7451-0.8737	0.66
Males	Breastfed	33	0.287	0.2738-0.2992	
	Not breastfed	14	0.288	0.2686-0.3076	0.89

Table 8. Mean aBMD in breastfed and non-breastfed males and females. CI = confidence interval.

		N	BMD_{corr}	95% CI	P
Females	Breastfed	29	0.327	0.312-0.3413	
	Not breastfed	25	0.321	0.305-0.3365	0.59
Males	Breastfed	33	0.287	0.2738-0.2992	
	Not breastfed	14	0.288	0.2686-0.3076	0.89

Table 9. Mean BMD_{corr} in breastfed and non-breastfed males and females. CI = confidence interval.

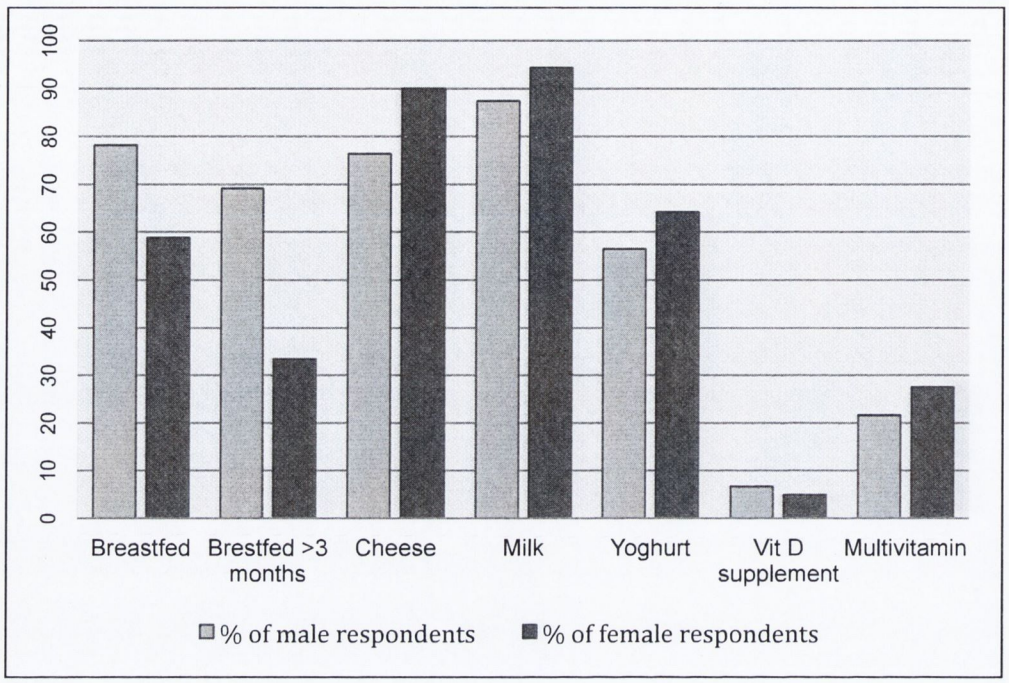


Figure 9. Summary of dietary intake results in male and female respondents.

3.6 Exercise

A total of 54 females and 47 males returned exercise data. For males of all ages and females in the 6-10 year age range there was no significant difference in aBMD or BMDcorr between those who exercised less than one hour per day and those who exercised more than one hour per day (Table 10, 11). For females aged 11-16 years there was a significant inverse relationship between both aBMD and BMD_{corr} and exercise of one hour or more per day ($p=0.0009$ and $p=0.001$))(Table 12,13).

	Exercise	N=	Mean aBMD (g/cm^2)	CI for Mean	P=
All males	<1 hour/day	20	0.786	0.7181-0.8542	
	>1 hour/day	27	0.783	0.7244-0.8415	0.94

Table 10. aBMD and exercise in males. CI = confidence interval.

	Exercise	N=	Mean BMD_{corr} (g/cm^2)	CI for Mean	P=
All Males	<1 hour/day	20	0.285	0.2685-0.3011	
	>1 hour/day	27	0.289	0.2746-0.3027	0.72

Table 11. BMD_{corr} and exercise in males. CI = confidence interval.

	Exercise	N=	Mean aBMD (g/cm^2)	CI for Mean	P=
All females	<1 hour/day	23	0.909	0.8492-0.9694	
	>1 hour/day	31	0.753	0.7015-0.8051	0.66
6-10.9 years	<1 hour/day	9	0.768	0.6854-0.8500	
	>1 hour/day	21	0.729	0.6750-0.7828	0.43
11-16.9 years	<1 hour/day	14	1	0.9323-1.068	
	>1 hour/day	10	0.804	0.7240-0.8850	0.0009

Table 12. aBMD and exercise in females. CI = confidence interval.

	Exercise	N=	Mean BMD_{corr} (g/cm^2)	CI for Mean	P=
All females	<1 hour/day	23	0.342	0.3270-0.3572	
	>1 hour/day	31	0.31	0.2974-0.3234	0.0024
6-10.9 years	<1 hour/day	9	0.315	0.2904-0.3387	
	>1 hour/day	21	0.31	0.2942-0.3259	0.75
11-16.9 years	<1 hour/day	14	0.36	0.3426-0.3771	
	>1 hour/day	10	0.311	0.2908-0.3316	0.001

Table 13. BMD_{corr} and exercise in females. CI = confidence interval.

Males continued to exercise in both the home and school environments throughout the age range studied (Figure 10). Females, on the other hand, tended to reduce their daily exercise (both at home and at school) after the age of 12 years (Figure 11).

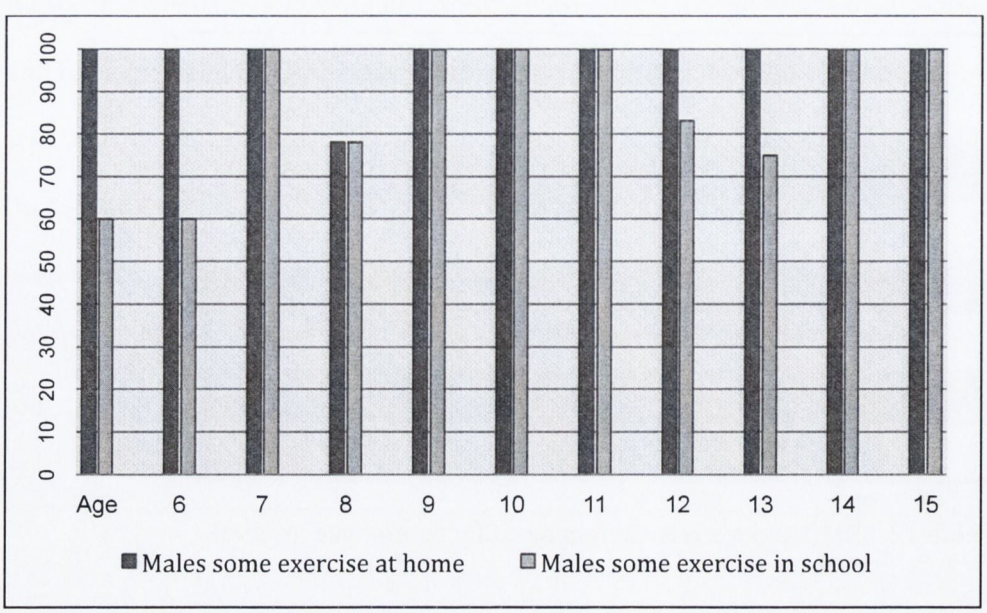


Figure 10. Exercise at home and in school by age in males.

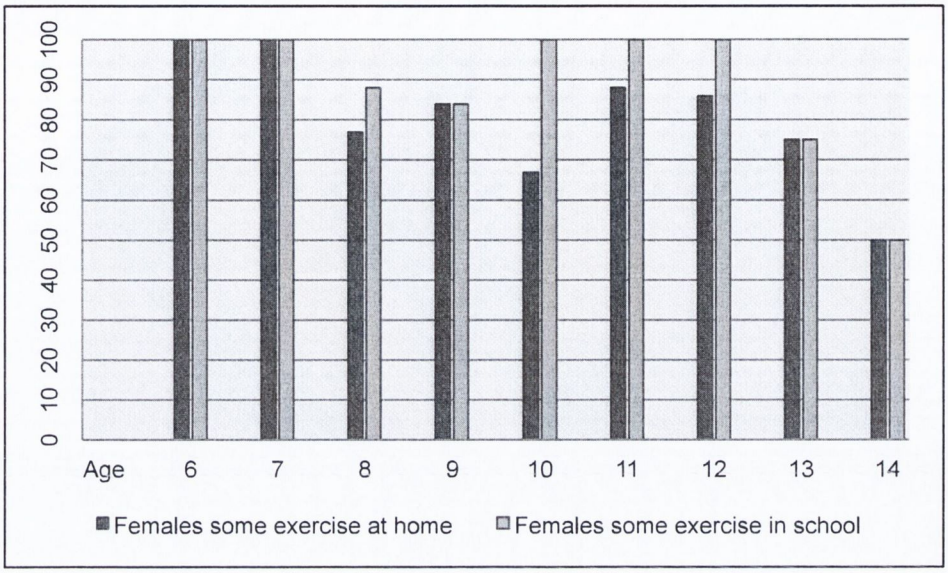


Figure 11. Exercise at home and in school by age in females.

3.7 Sedentary pastimes

Analysis of time spent in sedentary pastimes revealed that the majority of both males and females watched between one and three hours of television per day (Figure 12). Approximately half of participants of both sexes also played up to an hour of computer games per day (Figure 13).

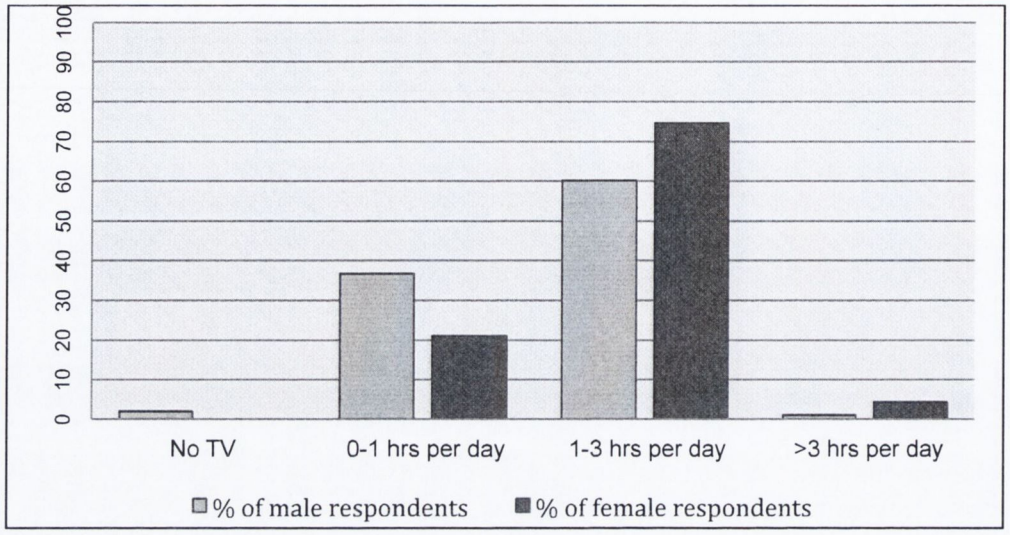


Figure 12. Daily television watching in male and female respondents.

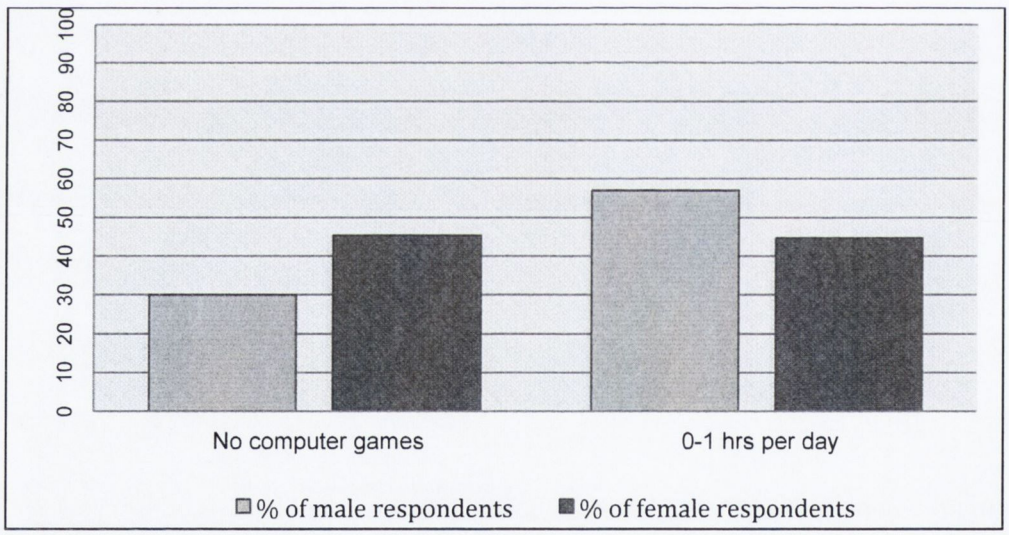


Figure 13. Daily computer games in male and female respondents.

3.8 Cigarette Smoke Exposure

Very few respondents reported being exposed to smoke in the home and none reported exposure to smoke in the car (Figure 14).

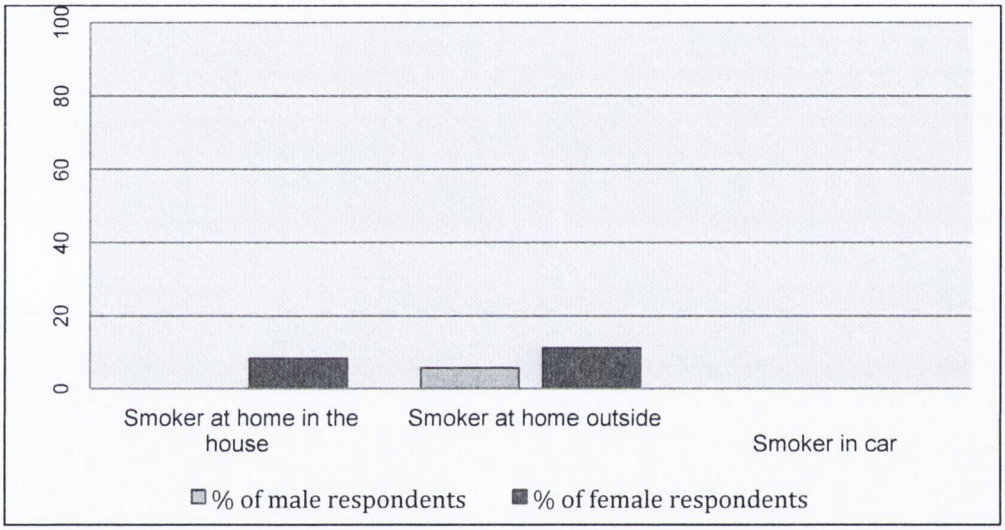


Figure 14. Passive cigarette smoke exposure in male and female respondents.

CHAPTER 4

DISCUSSION

Despite advances in QCT technology, DXA remains a very important tool in the evaluation of children at risk of low bone density, not least because of its ubiquity. Radiologists and clinicians who oversee paediatric DXA scanning have a responsibility to optimise patient care by making the results they generate as accurate and as relevant as possible. The use of DXA in the paediatric setting requires a thorough knowledge of the complexity of bone development and bone health. Attention to detail is required in the acquisition and interpretation of DXA data. Paediatric DXA recommendations clearly address a number of key areas, including the use of Z-scores rather than T-scores in children, the importance of correction of results for bone size and the need to compare results to a local reference range. Many DXA practitioners understand these recommendations but the fact that DXA imaging is available in so many settings and is subject to relatively few regulations means that paediatric DXA interpretation is still not universally adequate. The results of this study provide the first opportunity for Irish physicians to adjust paediatric DXA results for bone size and to relate results to relevant, local normative data. It is hoped that this data can assist in enhancing the accuracy of DXA analysis of bone density in Irish children and therefore minimise unnecessary bone modulating pharmacotherapy.

4.1 Findings in our Study Cohort

4.1.i Nutrition

The majority of participants throughout all age ranges in this study consume dairy products on a regular basis, suggesting that milk protein/calcium consumption should be sufficient in the group as a whole. Our knowledge of the group's dietary and sunlight-mediated exposure to Vitamin D is limited. Very few participants regularly take a multivitamin or Vitamin D supplement. There is limited knowledge of the effect of regular multivitamin consumption or Vitamin D supplementation in childhood on bone development. Based on the current literature there seems to be insufficient evidence to suggest that children who are eating a normal diet require routine vitamin supplementation for bone health. The recent publication of results that indicate that, despite previously seemingly convincing evidence to the contrary, maternal Vitamin D deficiency is not a precursor of low infant BMC serves as a reminder of the complexity of this topic. Our knowledge of the myriad dietary and physiologic determinants of paediatric bone health remains far from complete. If possible, measurement of serum Vitamin D would be beneficial to future bone density studies.

4.1.ii Body Composition

Interestingly, a comparison of our study cohort to the Irish reference ranges for height and weight shows the participants to be taller and heavier than their

peers of three decades ago (the normative data were collected in the 1980s); this was a significant finding for height in the female subset of patients. In their analysis of the UK reference data for the Hologic QDR Discovery DXA scanner, Ward et al(88) also found that their study population was taller and heavier than the standard UK reference data(89, 91). It is difficult to know whether children and adolescents are now taller and heavier in general or whether it is our cohort in particular that demonstrates this trend.

4.1.iii Pubertal Stage

The Irish weight and height reference data published in 1987(84) showed a significantly later pubertal growth spurt than in the United Kingdom or United States but no significant difference in the final adult height or weight. Although we found our cohort to be taller and heavier than the Irish reference range, determination of pubertal stage was very limited by the refusal of formal assessments, the known inaccuracies of self-assessment and the relatively low numbers of self-assessments returned. Further evaluation of bone density in the setting of formal pubertal stage assessment would bring an added benefit to potential future extensions to this study. However, the non-invasive nature of DXA imaging is in contrast to the more personal nature of pubertal stage assessment and, as we found in this study, those willing to undergo the former are commonly unwilling to subject themselves to the latter.

4.1.iv Exercise

The literature makes it clear that it is not only the duration but also the intensity of exercise undertaken that is important to bone health. Many of the published studies that aim to determine the amount of exercise required to make a positive impact on bone density seem to suggest that the current Irish recommendation of 60 minutes of physical activity per day should be adequate to provide a benefit to bone health, provided it is sufficiently vigorous. A number of DXA-based studies evaluating the impact of childhood exercise on bone density fail to estimate vBMD or to utilise analytic tools other than DXA to determine bone structure or strength(92). Further studies, perhaps with both DXA and QCT, may be beneficial to assist in determining the levels of childhood exercise that are required to deliver optimal benefit to developing bones. The inverse relationship between bone density and the amount of exercise performed by adolescent females in this study may have a number of causes. Of note, the mean BMI of females in the same age range also increased; it is possible that although the beneficial effects from exercise are reduced, there is a counter-effect of increased weight. Investigation of body composition in this age group may help to elaborate. Increasing the numbers of scans performed in this particular age range would be beneficial in confirming the finding.

4.1.v Cigarette Smoke Exposure

Interestingly, it has been demonstrated that the negative effects of first-hand cigarette smoke inhalation may reduce with age(93). If this is the case it would seem logical that the deleterious effect of exposure to cigarette smoke on bone health may be maximal in childhood and adolescence however this remains to be studied. The population in our cohort reported low rates of exposure to environmental smoke. The Office of Tobacco Control reported that among adults between the ages of 25 and 54 years there was a cigarette smoking prevalence of 22.1-30.1% in the 12 months to June 2012; this prevalence is considerably higher than is implied for the household members of participants in our study group(94). There are many potential reasons for this, including discrepancy in social class between the two groups (we did not record social class) and the fact that the parents or guardians who were interested in participating in the study were probably more likely to be motivated and to have an interest in their own and their families' health.

Although this study was completed over the time period when smoking in cars with children became illegal in Ireland, the numbers of participants reporting exposure to second hand cigarette smoke is too low to draw any conclusion about the efficacy of the ban.

4.2 DXA Analysis

The LMS method of calculation of standard deviation allows data to be smoothed by accounting for 'skewness' or variation in results(90). It also produces results in a manner that allows clinicians to easily calculate a standard deviation for an individual patient. Statistical analysis of the study data using the LMS method has produced useable tabulated results but the need to omit some of the male data underlines the problems associated with the small sample size. Increasing the number of children scanned would strengthen the male and female LMS data, along with the conclusions that can be drawn from their use.

The optimal method of estimation of vBMD using DXA is difficult to determine. The Kroger method provides a reasonable and well-founded method of correction but it should be remembered that it remains an estimate rather than a direct measurement of vBMD. In an attempt to increase the accuracy of DXA evaluation of bone density, Molgaard et al proposed a three-step method of bone analysis that aims to assist in interpretation of results by accounting for bone shape as well as patient height(15). Implementation of an approach such as that of Molgaard et al seems reasonable as it provides the clinician with a framework for the evaluation of patients' bones that goes beyond a single measurement.

Despite lower rates of correlation between DXA and ash analysis than between QCT and ash analysis, DXA can be a clinically useful diagnostic tool in the evaluation of bone density and body composition in children if used carefully. However, it is becoming increasingly evident that no single measure of bone parameters can provide a rounded evaluation of bone health. Wells et al have

proposed a multifaceted approach to analysis of body composition, emphasising the move toward the use of DXA and other technologies in conjunction with other measurements such as bioelectric impedance (a measurement of the impedance to electrical flow in the body and thus an estimate of relative body water and fat content), rather than in a stand-alone fashion(95). Although DXA measurements of bone density can be manipulated in an attempt to account for confounding factors such as bone size, DXA scanners cannot provide detail of bone structure. On the other hand, DXA currently provides a realistic, low radiation dose, option for evaluation if whole-body composition. Most modern DXA systems have the ability to analyse regional and whole body composition, determining relative bone, fat and lean mass. Whilst QCT easily determines bone density, shape and structure using very low dose focused CT examinations, more extensive whole-body imaging for body composition is prohibited by dose considerations.

As QCT becomes more prevalent, new strategies for analysing bone health will be required. In centres where there is access to multiple options for bone assessment, a full standard assessment of bone health could now reasonably consist of lumbar QCT for bone structure and density, a whole-body DXA for body composition, serum analysis of Vitamin D, clinical evaluation of pubertal stage, a review of diet and exercise and, in patients with relevant conditions, regional muscle strength measurements. Until low dose QCT is commonly available, DXA evaluation of BMC and BMD will continue to hold a position of high importance in the overall paediatric bone health assessment.

4.3 DXA Bone Density Reference Ranges

To be most accurate, paediatric DXA results need to be compared to a relevant reference range. Although large collections of normative paediatric DXA data exist, the published data sets are all non-Irish and most were acquired on DXA systems that have been shown to produce different results to the GE Lunar Prodigy. Although cross calibration equations can go some way to allowing such data to be more relevant to the Irish paediatric population, the accuracy of DXA cross calibration in children has not been well studied. The majority of published DXA cross calibration studies are based on adult data and although some inferences can be drawn from these studies, adult cross calibration does not require consideration of bone growth or other paediatric-specific factors such as pubertal stage or exercise. When the added complexity of the paediatric setting is considered, it is clear that dedicated, local, system-specific normative data, which avoid the need to use cross calibration, represent a more accurate approach to densitometry interpretation. We have collected data that provide the means to present Irish paediatric DXA results in a local context. With an increasingly diverse ethnic population in Ireland, there will also be an increasing need for normative paediatric DXA data that is relevant to other subsets of the population.

4.4 Conclusion

The normative data collected during our study represents the first paediatric DXA reference range in Ireland and provides an important framework for the contextualisation of Irish paediatric DXA results. Although the results need to be

viewed in the context of a relatively small sample size, these new Irish data nonetheless have the ability to add value to the evaluation of bone health in Caucasian Irish children scanned on GE Lunar Prodigy DXA systems, particularly at the NCH. It is hoped that the reference range can be expanded in due course to both improve its accuracy and broaden its applicability.

APPENDICES

Appendix 1

Parent/Guardian Information Sheet

SJH / AMNCH RESEARCH ETHICS COMMITTEE

Parent/Guardian Information Sheet

1. **Title of study:**

   Developing a national reference range for paediatric bone density in Ireland

2. **Introduction:**

   Children with certain chronic illnesses are at risk of breaking bones due to a condition called osteoporosis. Paediatric specialists use a scan called a DXA (pronounced 'dexa') scan to diagnose this condition in their patients. At the moment, making this diagnosis in Ireland isn't as accurate as it could be because we don't know what the normal values are for Irish children. This project aims to find out what those normal values are and provide doctors with what is called a 'reference range'.

   To come up with our reference range we need to perform DXA scans on about 250 healthy boys and girls between the ages of 6 and 16. Each child will have one scan in the Radiology department in the National Children's Hospital during a 30-minute appointment.

3. **Procedures:**

   Children who can participate in this study need to be Irish, white and generally healthy. The reason for restricting who can participate in this way is that ethnicity is known to affect bone density readings.

   If you are happy to allow your child to take part in the study you will need to bring them to our x-ray department once, for a 40 minute appointment. You will be met by one of the study's doctors who will run through a short, confidential questionnaire with you in order to make sure that your child can be included in the study.

   You will then discuss the risks and benefits of participation with the doctor and, if you are happy to do so, sign a consent form to allow the scan to go ahead. Your child will have height and weight measurements recorded, and then have their DXA scan done.

   In order to make our results as accurate as possible, it would be ideal to know whether your child is going through puberty and, if so, what stage of puberty they are at. This applies to girls from about 8 years of age until they get their first period. This also applies to boys between 9 years of age and 16 years of age or older. In order to check this, we are offering a 'pubertal assessment' to children in the relevant age range. This involves a 5-minute examination performed by either a consultant paediatrician, a senior registrar or a nurse in paediatric endocrinology with special training in the area. The examination involves checking for hair in the armpits and in the pubic area. For girls the examination also involves checking for breast growth. For boys, growth of the testicles is checked. This part of the scan procedure is strictly voluntary and both you and your child must be in agreement that you would like to opt into it. You can still be part of the bone density study without undergoing this examination.

If you, or your child, choose not to undergo pubertal assessment, there is a questionnaire that you could fill out instead which gives us more limited information. The questionnaire asks questions about growth of hair in the armpits and in the pubic region, and about breast development. This questionnaire is also completely voluntary and won't be presented to you if you decide you would rather not complete it.

4. Benefits:

The biggest benefit of your child's participation in this study is to our society as a whole. Once completed, the study will provide doctors with a valuable set of data that will allow them to improve the care they can offer to children with a range of serious conditions.

Your child's scan results will be sent to your GP. The vast majority of all scans performed will be normal. In the unlikely event that your child's results are abnormal, your GP will consider the correct action to take. They may recommend further tests or a visit to a specialist.

5. Risks:

DXA scans involve the use of a very small amount of radiation. Radiation can harm human cells. One useful way of measuring radiation is to calculate the 'effective dose' measured in Sieverts (Sv). The effective dose represents the amount of radiation affecting the human body.

We all encounter radiation all of the time without being aware of it. This is because radiation is created naturally and released into the environment. This is called background radiation. The amount of background radiation we're each exposed to in Ireland is approximately 3000 microSv (μSv) per year (about 8μSv per day). Taking a return flight from Ireland to America exposes us to between 40 and 60μSv.

A DXA scan of the sort needed for this study involves a radiation dose of approximately 2μSv. This equals about 6 hours worth of background radiation.

So what risk does 6 hours of background radiation or 1 DXA scan carry? Risk from radiation is sometimes described as the risk of developing cancer because of the radiation. The risk of developing a fatal cancer from a DXA scan is between 1 in 2,000,000 and 1 in 20,000,000. This is considered by most to be insignificant, especially when put in the context of our everyday background radiation dose.

The risk to an unborn child from radiation is significantly higher than the risk to older children. As such it is essential that we don't scan any female that could be pregnant. This is why we follow the 10-day rule and only scan females who get periods when they are between day 1 and day 10 of their cycle, counting the first day of a period as day 1. **If you have any concern that your daughter could be pregnant you must contact the study doctor immediately.**

6. Exclusion from participation:

An important group of children in our study are girls who have started to have periods. To complete our reference range we need to include them in our study. As mentioned above, to include these girls safely and to comply with the rules that govern the use of x-rays, we need to make sure that

their scans are done between day 1 and day 10 of a period. **Any girl who is or could be pregnant cannot be scanned because the scan could pose a risk to the unborn child. Any girls taking forms of contraception that prevent them from having regular periods will also be excluded from the study.**

Certain other children will not be able to participate as their results could artificially alter the results of the study:

- **Children who are not Irish and white** (Ethnicity makes a difference to bone density. If possible, we would eventually like to make reference ranges for Irish children of other ethnicities too)
- **Children with serious chronic illnesses** (They are more likely to have low bone density)
- **Children taking steroid medications** (These can reduce bone density)
- **Children with known bone problems** (They are more likely to have abnormal bone density)
- **Children who have had broken bones after minimal injury** (They are more likely to have low bone density)

7. **Alternative treatment:**

Your child does not have to be a part of this study in order to have a DXA scan performed. If you feel your child needs to have a DXA scan but you do not want them to participate in this study, speak to your GP, who can discuss the issues with you. DXA scans for children in Ireland are currently measured against results from other countries. Following this project we hope to make results more accurate.

8. **Confidentiality:**

Your child's identity will remain confidential. Your child's name will not be published and will not be disclosed to anyone outside the hospital.

9. **Compensation:**

Participation in this study is covered by an approved policy of insurance in the name of the Adelaide & Meath Hospital incorporating the National Children's Hospital (AMNCH). In addition the medical practitioners involved in this study have current medical malpractice insurance cover. AMNCH will comply with the ABPI guidelines and Irish Law (statutory and otherwise) in the unlikely event of your becoming ill or injured as a result of participation in this clinical study. Nothing in this document restricts or curtails your rights.

10. **Voluntary Participation:**

You have volunteered your child to participate in this study. You may withdraw your child's participation at any time.

11. Stopping the study:

The Project doctors may stop your child's participation in this study at any time without your consent.

12. Permission:

This study has the approval of the joint St James's Hospital/AMNCH Research Ethics Committee

13. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Dr Aisling Snow who can be telephoned at 01-4143762 or emailed at paediatricdxa@gmail.com. If your doctor learns of important new information that might affect your desire to remain in the study, she will tell you.

Developing a paediatric reference range for bone density in Ireland
Parent/guardian information leaflet
June 2011

Appendix 2

Pubertal Self-Assessment Forms

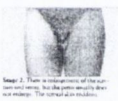
PUBERTY ASSESSMENT FORM

GENITALIA –

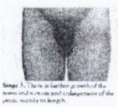
PLEASE CIRCLE WHICH PICTURE (1-5) MATCHES YOU BEST:

1.

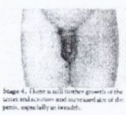
2.



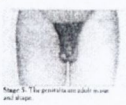
3.



4.



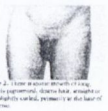
5.



PUBIC HAIR –

PLEASE CIRCLE WHICH PICTURE (1-4) MATCHES YOU BEST:

1.



2.

3.

4.

Male Pubertal Self-Assessment Form

PUBERTY ASSESSMENT FORM

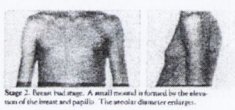
BREAST SIZE -

PLEASE CIRCLE WHICH PICTURE (1-5) MATCHES YOU BEST:

1.

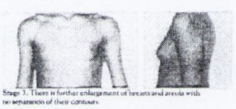
Stage 1. The breasts are prepubescent. There is elevation of the papilla only.

2.



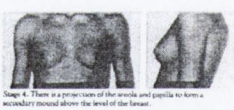
Stage 2. Breast bud stage. A small mound is formed by the elevation of the breast and papilla. The areolar diameter enlarges.

3.



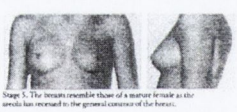
Stage 3. There is further enlargement of breasts and areola with no separation of their contours.

4.



Stage 4. There is a projection of the areola and papilla to form a secondary mound above the level of the breast.

5.



Stage 5. The breasts resemble those of a mature female as the areola has receded to the general contour of the breast.

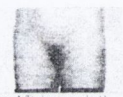
PUBIC HAIR –

PLEASE CIRCLE WHICH PICTURE (1-4) MATCHES YOU BEST:

1.

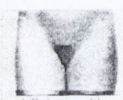
Stage 2. There is sparse growth of long, slightly pigmented, downy hair, straight or only slightly curled, primarily along the labia.

2.



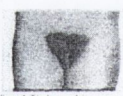
Stage 3. The hair is considerably darker, coarse, and more curled. The hair spreads sparsely over the junction of the pubes.

3.



Stage 4. The hair, now adult in type, covers a smaller area than in the adult and does not extend onto the thighs.

4.



Stage 5. The hair is adult in quantity and type, with extension onto the thighs

Female Pubertal Self-Assessment Form

Appendix 3

Diet and Lifestyle Questionnaire

NATIONAL CHILDREN'S BONE DENSITY STUDY

Breastfeeding	Not breasfed	0-1 month	1-3 months	3-6 months	>6 months
Duration of breasfeeding					
Diet & Supplements	**Yes**	**No**			
Are you vegetarian?					
Are you vegan?					
Do you eat cheese?					
Do you take vitamin D?					
Do you take any other vitamin supplements?			Which one? _____.		
Do you take a fish oil supplement?					
	None	**1-2 pints**	**2-3 pints**	**3-4 pints**	**>4 pints**
How many pints of milk do you drink per week?					
How many yoghurts do you eat per week?					
Exercise	**None**	**0-1 hour**	**1-2 hours**	**2-3 hours**	**>3 hours**
Hours of swimming per day on average					
Hours of other exercise per day on average					
Hours of exercise not arranged by school per day					
Other Activities	**None**	**0-1 hour**	**1-2 hours**	**2-3 hours**	**>3 hours**
Hours of TV watched per day on average					
Hours of computer games per day on average					
Hours of other computer use per day on average					
	Laptop	**Smart Phone**	**Tablet Device**	**Desktop**	**School Computer**
Which of the following do you use regularly					
How many hours per day on each (indicate number)					
Smoking	**In the house**	**Outside the house**	**In the Car**		
Does anyone in the house smoke…					

Additional Comments:

Appendix 4

Bone Density Results

	Age	aBMD L1-L4	Width L1-L	BMD_{corr} L1-L4
Males	**(years)**	**Mean (g/cm^2)**	**Mean (cm)**	**Mean (g/cm^3)**
	6-6.9	0.628	3.0	0.272
	7-7.9	0.689	3.2	0.271
	8-8.9	0.737	3.1	0.302
	9-9.9	0.751	3.4	0.286
	10-10.9	0.749	3.4	0.282
	11-11.9	0.773	3.4	0.288
	12-12.9	0.788	3.5	0.288
	13-13.9	0.895	3.8	0.303
	14-14.9	1.048	4.1	0.327
	15-15.9	1.072	4.1	0.333
	16-16.9	1.105	4.1	0.339

Table 14. Bone density results by age in males.

	Age	aBMD L1-L4	Width L1-L4	BMD_{corr} L1-L4
Females	**(years)**	**Mean (g/cm^2)**	**Mean (cm)**	**Mean (g/cm^3)**
	6-6.9	0.668	3.0	0.287
	7-7.9	0.687	2.9	0.303
	8-8.9	0.719	2.9	0.312
	9-9.9	0.837	3.2	0.331
	10-10.9	0.793	3.1	0.324
	11-11.9	0.787	3.2	0.313
	12-12.9	0.989	3.5	0.357
	13-13.9	0.963	3.6	0.340
	14-14.9	1.011	3.7	0.348
	15-15.9	1.044	3.6	0.369
	16-16.9	1.080	3.8	0.366

Table 15. Bone density results by age in females.

Appendix 5

Height, Weight and Body Mass Index Results

	Age	Study Cohort Height	Hoey Reference Height	Study Cohort Weight	Hoey Reference Weight
	(years)	**Mean (cm)**	**Mean (cm)**	**Mean (kg)**	**Mean (kg)**
Male	6	111.8	114.2	21.7	20.6
	6.5	118.3	116.8		
	7	128.44	120.2	29.5	22.5
	7.5	132.5	122.2		
	8	128.5	125.9	31.8	24.8
	8.5	135.2	127.7		
	9	138.9	132.1	36.6	27.8
	9.5	142.3	134.3		
	10	141.2	136	37.4	29.8
	10.5	143.2	137.9		
	11	147.5	141.2	47.7	33.9
	11.5	157	144.3		
	12	149.2	146.4	41.8	36.7
	12.5	152.3	149.9		
	13	164.8	151.9	61.5	40.0
	13.5	164.4	154.3		
	14	168.1	158.8	63.2	45.9
	14.5	174.9	162.8		
	15.5	174	168.5	71.1	53.2
	16	173.5	171.4		
	16.5	174.1	171.4	60.0	60.2

Table 16. Height and weight by age in males – study cohort and Irish reference data.

	Age	Study Cohort Height	Hoey Reference Height	Study Cohort Weight	Hoey Reference Weight
	(years)	Mean (cm)	Mean (cm)	Mean (kg)	Mean (kg)
Female	6	117.9	113.1	23.1	20.3
	6.5	124.9	116.2		
	7	125.2	117.9	26.4	21.7
	7.5	123.7	120.6		
	8	129.24	124	28.6	24.4
	8.5	127.4	127.4		
	9	141.1	129.7	37.4	27.0
	9.5	137.6	133.6		
	10	147	135.3	36.5	29.9
	10.5	143.4	138.7		
	11	145.2	140.9	41.6	33.0
	11.5	151.9	144.7		
	12	152.2	147	48.7	37.2
	12.5	158.9	151		
	13	155.8	153.4	50.4	42.3
	13.5	162.3	157.1		
	14	160.4	158.3	56.8	48.5
	14.5	154	159.9		
	15		161.5	57.2	52.2
	15.5	156.8	162.7		
	16	165.2	161.5	58.4	53.2
	16.5	165	163.6		

Table 17. Height and weight by age in females – study cohort and Irish reference data.

	Age	Study Cohort BMI	Hoey Reference BMI
	(years)	Mean (kg/m²)	Mean (kg/m²)
Male	6	15.9	15.2
	7	17.6	14.8
	8	17.8	15.2
	9	18.6	15.2
	10	18.4	15.9
	11	21.5	16.2
	12	18.2	16.9
	13	22.6	18.0
	14	21.3	18.8
	15	23.4	18.4
	16	19.8	18.1

Table 18. Mean body mass index (BMI) of the study cohort and of the Hoey reference data – males.

	Age	Study Cohort BMI	Hoey Reference BMI
	(years)	Mean (kg/m²)	Mean (kg/m²)
Female	6	15.2	15.4
	7	16.9	15.2
	8	17.2	15.4
	9	19.2	15.6
	10	17.5	15.9
	11	19.3	16.2
	12	20.4	16.8
	13	19.7	17.5
	14	23.0	19.1
	15	23.3	19.8
	16	21.3	20.1

Table 19. Mean body mass index (BMI) of the study cohort and of the Hoey reference data – females.

Appendix 6

Pubertal Stage Results

Males	Age (years)	Height (cm)	aBMD L1-L4 (g/cm^2)	BMD_{corr} L1-L4 (g/cm^3)	Tanner Stage
	9	137.5	0.746	0.264	II
	9	135.6	0.721	0.278	I
	9.1	137.6	0.623	0.240	I
	9.4	145.2	0.763	0.263	II
	9.8	127.3	0.78	0.342	I
	9.9	154	0.736	0.240	I
	9.9	149.9	0.784	0.294	0
	10.4	147	0.878	0.302	I
	10.7	143.2	0.749	0.265	I
	10.8	136.6	0.639	0.262	I
	11.1	140	0.628	0.326	II
	11.1	154.2	0.809	0.286	II
	11.1	149	0.7	0.291	II
	11.9	157	0.776	0.286	III
	12.1	151.2	0.722	0.270	III
	12.2	142.1	0.68	0.255	I
	12.5	148.7	0.784	0.312	II
	13	164.2	0.773	0.281	V
	13.2	168.1	0.84	0.289	IV
	13.3	173	0.993	0.342	IV
	13.7	164.6	0.821	0.290	IV
	14.2	157.3	0.87	0.308	IV
	14.4	173.1	1.002	0.283	IV
	14.8	179.4	1.021	0.310	III
	15.8	173.7	1.196	0.346	I
	15.8	168.3	1.167	0.362	IV
	16	173.5	0.981	0.320	III

Table 20. Self-assessed pubertal stage in individual male respondents.

Females	Age (years)	Height (cm)	aBMD L1-L4 (g/cm^2)	BMD_{corr} L1-L4 (g/cm^3)	Tanner Stage
	8.0	128.3	0.747	0.317	I
	8.1	114.6	0.589	0.268	I
	8.3	137.1	0.652	0.277	0
	8.4	131	0.907	0.385	II
	8.4	135.2	0.901	0.370	I
	8.5	121.5	0.562	0.265	I
	8.6	126	0.746	0.339	II
	8.6	129	0.717	0.294	II
	9.0	138.8	0.931	0.370	I
	9.2	136.3	0.931	0.370	0
	9.5	136.3	0.721	0.287	II
	9.5	142	0.869	0.307	III
	9.8	145.5	1.007	0.366	II
	9.9	132.4	0.702	0.308	III
	10.6	141.5	0.836	0.333	II
	10.7	148	0.816	0.335	III
	11.0	152.1	0.733	0.292	II
	11.1	133.4	0.779	0.320	II
	11.1	137	0.734	0.301	III
	11.1	150.5	0.795	0.307	III
	11.3	145	0.898	0.346	III
	11.4	147.7	0.829	0.320	II
	11.9	148.4	0.676	0.297	II
	12.0	150	1.008	0.377	III
	12.0	145.1	0.884	0.331	III
	13.1	148.3	0.747	0.288	II
	14.0	152.1	0.944	0.364	IV

Table 21. Self-assessed pubertal stage in individual female respondents.

Appendix 7

Dietary factors

	Age (years)	N=	Breastfed	Breastfed >3 months	Vegetarian	Eat Cheese	Drink Milk	Eat Yoghurt	Vitamin D	Multivitamin
			%	%	%	%	%	%	%	%
Female	6	5	80	60	0	100	100	80	20	20
	7	7	43	43	14	100	100	71	0	0
	8	9	56	33	0	100	100	67	0	22
	9	6	50	0	0	100	83	67	0	33
	10	3	67	67	0	67	100	100	0	33
	11	8	50	13	0	63	75	75	0	13
	12	7	43	43	0	71	86	57	29	29
	13	4	75	50	0	100	100	50	0	0
	14	4	25	25	0	100	100	75	0	25
	16	1	100	0	0	100	100	0	0	100
Male	6	5	100	60	0	80	80	40	0	20
	7	5	60	60	0	80	80	80	20	40
	8	1	100	100	0	100	100	100	0	0
	9	9	33	11	0	67	78	67	0	0
	10	6	100	88	0	50	100	67	0	33
	11	5	100	100	0	80	100	60	20	20
	12	3	67	67	0	33	67	33	0	0
	13	6	50	50	0	100	83	50	33	50
	14	4	50	25	0	50	75	25	0	25
	15	2	100	100	0	100	100	100	0	50
	16	1	100	100	0	100	100	0	0	0

Table 22. Breastfeeding and dietary habit results in males and females by percentage of respondents.

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