

Ultrasound Bone Analysis in Children and Adolescents with Anorexia Nervosa and Related Eating Disorders

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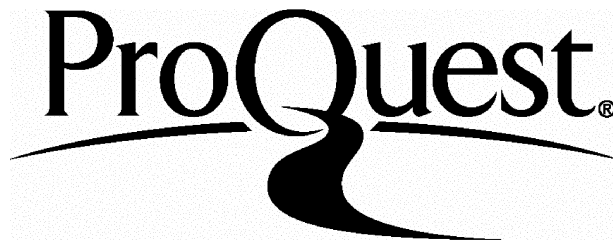
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Abstract

Since the development of the broadband ultrasound attenuation (BUA) technique [LANG1984] there has been considerable interest in its use to assess osteoporosis in adults but its application in paediatrics has been limited. The first aim of this research was to test rigorously the utility of a commercial ultrasound machine, the CUBA Clinical, for longitudinal studies in paediatrics. The second aim was to use BUA to evaluate the bone status of children with anorexia nervosa and related eating disorders (ANRED). Cross-sectional data were obtained on 52 children with ANRED and 100 healthy control children, and longitudinal data were obtained on 23 children with ANRED and 62 controls.

The precision of the CUBA Clinical, measured as the root mean square standard deviation of paired measurements on 50 children, was 6.02dB.MHz^{-1} . Using 3 multiples of the precision as the benchmark for significant clinical change in vivo, the CUBA Clinical was not precise enough to measure significant BUA changes in a group of 85 children when scanned at one-year intervals. Cross-sectional data analysis demonstrated that the 52 children with ANRED had a significantly reduced mean BUA Z score compared to age and gender matched controls (mean -0.82, 95%CI -0.52, -1.12), but when compared to weight and gender matched controls the BUA Z scores were within normal limits (mean 0.02, 95%CI 0.33, -0.29). However a sub-group of 18 children with food avoidance emotional disorder (FAED) had mean BUA Z scores both for age and gender (mean -1.43, 95%CI -0.96, -1.90) and weight and gender (mean -0.56, 95%CI, -0.03, -1.09) which were significantly reduced below zero. Almost 89% (16/18) of these FAED children were pre-pubertal which is likely to have had an impact on the low BUA Z scores seen.

In conclusion the CUBA Clinical is not precise enough to measure significant annual changes in BUA in paediatrics. Children with ANRED have significantly reduced BUA Z scores for age and gender. Pre and peri-pubertal children with FAED have both age and weight corrected BUA Z scores that are significantly reduced. They are therefore a sub-group less likely to attain an optimal peak bone mass and more likely to sustain a future osteoporotic fracture.

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List of abbreviations

Abbreviation	Term in full
AN	Anorexia nervosa
ANRED	Anorexia nervosa and related eating disorders
BMD	Bone mineral density
BMI	Body mass index
BN	Bulimia nervosa
BUA	Broadband ultrasound attenuation
CUBA	Contact ultrasound bone analyser
CV	Coefficient of variation
DXA	Dual energy X-ray absorptiometry
FAED	Food avoidance emotional disorder
QUS	Quantitative ultrasound
RMS SD	Root mean square standard deviation
SCV	Standardised coefficient of variation
SE	Selective eating
SOS	Speed of sound
UBA	Ultrasound bone analysis
VOS	Velocity of sound

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Chapter 1 Introduction and rationale for the research

Historically, scant attention has been given to the assessment of secondary osteoporosis in patients with anorexia nervosa and related eating disorders (ANRED). Furthermore no studies to date have reported the use of ultrasound to assess the risk of osteoporosis in pre-pubertal children with these eating disorders. This research project will evaluate the validity and precision of the McCue CUBA Clinical to measure broadband ultrasound attenuation through the heel during growth and development in a cohort of ANRED children and a cohort of healthy control children.

1.1 *The assessment of children and adolescents*

Children and adolescents form a heterogeneous group whose growth is evaluated in a number of ways both in health and illness. The most common measurements of growth are related to gender, age, weight, height and body mass index. These measurements can also be monitored in relation to pubertal status and ethnic background to allow for the impact of genetic differences between children along with variations in the age of onset of puberty and its progression. However, despite these many variables for the assessment of growth, the key measures used in clinical practice are age, height and weight. The varied use of these parameters for growth assessment in childhood and adolescence has a significant impact on the diagnosis and monitoring of diseases such as osteoporosis and anorexia nervosa.

1.2 *Anorexia nervosa and related eating disorders*

In 1874 Gull described a disease characterised by extreme emaciation and occurring “mostly in the female sex and between the ages of 16 and 23” [GULL1874]. He named the disease anorexia nervosa. He noted the following common features in patients with the disease: “emaciation; restless activity; amenorrhoea and a morbid mental state”. Interestingly in the same paper Gull acknowledged the incidence of the disease in males as well as females.

“We might call the state hysterical.....I prefer however the more general term ‘nervosa’ since the disease occurs in males as well as females”.

Gull recommended that the key treatment for patients with the disease was to re-feed them at regular intervals and to keep them warm.

Anorexia nervosa also afflicts young children and the earliest report of a pre-pubertal child with the disease was published in *The Lancet* in the late 19th century [COLL1894]. Collins described the case of a seven and a half year old pre-menarcheal female who was “of healthy ancestry” and who “had for ten weeks persistently refused food”. He described her as “egotistical” with a “morbid vanity” and “perverted ego”. She was admitted to hospital and within nine weeks had regained sufficient weight to be discharged from clinical care. She was considered cured.

Neither report mentions precipitating factors such as major life events which could have caused otherwise healthy females to refuse food. Gull [GULL1874] does however make an interesting observation about re-feeding patients with anorexia nervosa recommending that they should be surrounded by “persons who would have moral control over them; relations and friends being generally the worst attendants”.

Nowhere in either of these early reports is there mention of an association between anorexia nervosa and bone fractures although the prevalence of hip fracture in females had been reported almost half a century earlier [COOP1842].

1.3 Osteoporosis

In the early nineteenth century when writing about the number of hip fracture patients in a London teaching hospital Cooper commented [COOP1842]:

“women are much more liable to this species of fracture than men: we rarely in our hospitals observe it in the latter but our wards are seldom without an example of it in the aged female”.

Osteoporosis has since been perceived as a disease that afflicts mainly postmenopausal and elderly women. Treatment therapies for osteoporosis have therefore concentrated on limiting bone loss in the fifth decade. However, if diagnosis of the early stages of osteoporosis could be obtained at a younger age,

before a peak bone mass has been attained, more effective intervention in the disease process may be possible. Even as early as the 19th century the attainment of a low peak bone mass caused by ANRED may have been a contributor to the incidence of osteoporotic hip fractures seen in women in the post-menopausal years.

1.4 *Assessing osteoporosis in paediatrics*

Although bone mineral density is not routinely assessed in paediatrics there is growing interest in the origins of osteoporosis and the risk factors associated with it to enable earlier intervention and treatment of the disease. This is supported by research evidence which suggests that if a genetically pre-programmed peak bone mass is not attained during adolescence there is likely to be an increased risk of osteoporosis in later life [BACH1993].

There are a number of possible reasons why the bone mineral density of children and adolescents is not routinely assessed.

- The heterogeneous nature of children makes them a difficult cohort of patients to accurately investigate and monitor because there are so many variables to consider.
- The importance of attaining a peak bone mass in late adolescence and the impact of not doing so may not be fully appreciated by all clinicians.
- Only a limited number of centres in the United Kingdom have the paediatric facilities, most particularly the paediatric software programmes, to measure bone mineral density appropriately. There is therefore a risk that centres may measure bone mineral density in clinical paediatrics without having the appropriate software to analyse it correctly and thus the information will be of limited value. The alternative being that bone mineral density is not measured at all.
- DXA scanners with their incumbent radiation burden may be the only method of assessing osteoporosis of which many clinicians are aware. The detrimental effects of ionising radiation, even at the low radiation doses seen in dual energy X-ray absorptiometry, are such that the risk from the effects of the radiation may be considered by some practitioners to outweigh the benefits of the resultant bone mineral density measurement. This risk to the child is exacerbated when a

centre does not have paediatric software, as bone mineral density results will then be misleading.

Clearly the use of ionising radiation as a method of monitoring disease in children should be avoided wherever possible. This is in keeping with the aim of achieving as low a radiation dose as is reasonably practicable as outlined in the Ionising Radiation (Protection of Persons Undergoing Medical Examinations or Treatment) Regulations 1988 [DOH1988]. Therefore if a technique of assessing osteoporosis that obviates the need for ionising radiation is available then it should be evaluated for use within paediatrics. Such a technique has been available for some years. In 1984 Langton [LANG1984] suggested that quantitative ultrasound could be a useful method of assessing osteoporosis. Since then, the suitability of quantitative ultrasound as a surrogate for the measurement of bone mineral density has been reported extensively in post-menopausal populations [HANS1996].

The limited availability of paediatric software for quantitative ultrasound may have an impact on the widespread use of the technique. Most software is designed to calculate the standard deviation comparison between a patient at risk of osteoporosis and a normal female or male adult. As a child grows older and increases in height and weight so her/his bones increase in both strength and mineral density until a peak bone mass is achieved. Only the impact of gender and age are reflected in standard normative data charts used for the assessment of osteoporosis in children. In 1995 McCue PLC was one of the first commercial ultrasound companies to publish normative data for children as well as adolescents enabling the ultrasound assessment of osteoporosis in this population.

The reported use of quantitative ultrasound in the paediatric population has been minimal although scientific interest in this population is increasing as was reflected in the popularity of the 1st international conference on children's bone health in Maastricht in May 1999.

1.5 *The McCue CUBA Clinical for paediatrics*

The CUBA Clinical by McCue was selected for this research for the following reasons.

- It is commercially available and has already been adapted for paediatric use.
- There is a paediatric normative software database on the machine.
- The relative precision error at 5% is acceptable for paediatrics.
- It uses a dry contact technique to measure the ultrasound parameters, obviating the need for multiple changes of water as in other pieces of similar equipment.
- It is relatively quick and easy to use because it is not necessary to wait for water to settle before commencing a scan.
- It is portable and can therefore easily be moved to the outpatients department of a hospital and to local schools.

1.6 *Osteoporosis and anorexia nervosa*

Secondary osteoporosis is a significant physical complication of anorexia nervosa which has been noted in adolescents as well as adults ([RIGO1984], [BACH1990]). However no studies have investigated the incidence of the disease in pre-pubertal children with early onset ANRED. In addition most published research on the assessment of osteoporosis in the paediatric anorexia nervosa population have reported the use of ionising radiation techniques to measure bone mineral density and assess osteoporosis. In this research, children and adolescents with ANRED will be compared to a healthy age-matched control cohort.

1.7 Hypotheses

There are two main hypotheses for this research: one technical and one clinical.

Technical hypothesis

The precision error of the broadband ultrasound attenuation technique in the McCue CUBA Clinical in children and adolescents will on average be the same as the annual increase in broadband ultrasound attenuation in children and adolescents.

The technical hypothesis tests the suitability of using the McCue CUBA Clinical to measure broadband ultrasound attenuation in longitudinal studies in children and adolescents. This involves two important research issues. The first is the precision error of the technique in children and adolescents and the second is the annual change in broadband ultrasound attenuation measurements in children and adolescents. The ratio of annual broadband ultrasound attenuation changes in a population to the precision error of the broadband ultrasound attenuation technique in the same population should be less than three to enable identification of a clinically significant change in bone status [GLUE1995]. Such longitudinal evaluation has not been performed in children using the CUBA.

Clinical hypothesis

Children and adolescents with anorexia nervosa and related eating disorders will on average have the same broadband ultrasound attenuation values through the left heel as healthy children and adolescents.

This second hypothesis is a null hypothesis assuming that there will be no difference between the two groups. However evidence in the literature confirms that adolescent and adult females with anorexia nervosa are at risk of osteoporosis [BILL1989]. This osteoporotic risk has been assessed by measuring bone mineral density or content using ionising radiation, rather than measuring broadband ultrasound attenuation. Furthermore pre-pubertal children with ANRED have not been examined. Researchers have also reported the utility of broadband ultrasound attenuation as an alternative method of assessing osteoporosis in other paediatric disease groups [MUGH1996]. The corollary being that some of these children and

adolescents with ANRED will have evidence of osteoporosis and this will be measurable by a reduction in broadband ultrasound attenuation.

Approval for this study to commence was obtained from the Local Research Ethics Committee at Great Ormond Street Hospital for Children NHS Trust in June 1996.

1.8 Summary

Anorexia nervosa is still perceived as a disease of adolescent females although it also afflicts males and young children in the pre-pubertal years. Little consideration appears to have been given to the long term implications for society of the increasing number of children who are unlikely to attain their genetically pre-determined peak bone mass as a result of ANRED.

This research has two aims. The first is to evaluate the validity and precision of the McCue CUBA Clinical as a method of measuring broadband ultrasound attenuation during growth and development in healthy children and adolescents and those with ANRED. The second is to establish whether children and adolescents with ANRED have abnormally low values of broadband ultrasound attenuation compatible with osteoporosis.

Chapter 2 Bone

This chapter is divided into three sections. This first section provides a brief overview of the composition and function of normal bone. The second section provides a critical evaluation of literature concerning the attainment of a peak bone mass and the factors contributing to it, particularly the effect of puberty, calcium intake and exercise. The third and final section is a review of the classifications used in the diagnosis of osteoporosis.

2.1 *The composition and function of bone*

A development zone (metaphysis) separates the distal portions of long bones (epiphyses) from the central tubular structure of the bone (diaphysis). In growing long bones the epiphysis and metaphysis is separated by a cartilaginous region known as the growth plate or epiphyseal cartilage. The epiphyseal cartilage is responsible for longitudinal growth and becomes entirely calcified at the end of the growth period.

There are two types of bone in the human skeleton: cortical and trabecular. Cortical bone is the outer dense layer of bone which provides a protective cover to the inner, anisotropic trabecular bone which has a predominantly metabolic role. The strength of bone is dependent on both the outer cortical shell and the inner trabecular structure. Trabecular bone is found at a number of weight-bearing skeletal sites including the vertebral bodies, the femoral neck and the calcaneum. These are the most common sites used to assess osteoporosis. Trabecular bone, although containing a smaller percentage of calcium than cortical bone, approximately 20% compared to 80% [BARO1996], is metabolically more active and will therefore demonstrate osteoporotic changes more rapidly than cortical bone. Trabecular bone is approximately 20% calcified with the inter-trabecular spaces being occupied by connective tissue, haematopoietic bone marrow and blood vessels. Typical trabecular content at various skeletal sites is shown in table 1 [EINH1996].

Table 1 Trabecular content at various skeletal sites

Region	Percentage of trabecular bone
Vertebrae	66 – 90%
Femoral neck	25 – 50%
Calcaneum	80%
Distal radius	25%
Mid radius	1%

Bone is composed of osteocytes (osteoblasts and osteoclasts) embedded in an organic matrix. The matrix, which consists of 95% type I collagen, is secreted by osteoblasts and subsequently strengthened by depositions of crystalline salts of calcium and phosphate in the form of hydroxyapatite [MARK1996]. This hydroxyapatite in the matrix confers considerable strength and rigidity to bone whilst the collagen maintains bony flexibility and elasticity. Bone is in contact with soft tissue via both its external surface (the periosteum) and its internal surface (the endosteum). These surfaces are lined with layers of osteogenic cells which are of the same structure as the cells that make up cortical and trabecular bone. Although bone matrix may be calcified it is not metabolically inert. Osteocytes are found embedded deep within the calcified matrix. These osteocytes derive from osteoblasts and have been trapped within the bone by the matrix they deposited, which later has become calcified. They are connected to each other by numerous cell micro-filaments. These filaments are organised before calcification of the matrix and they form a network of canaliculi. Osteocytes are phagocytized and digested during osteoclastic resorption.

Osteoblasts

Osteoblasts originate from mesenchymal stem cells and undergo proliferation and differentiation into preosteoblasts and then mature osteoblasts. These cells never appear or function individually, but are always found in clusters of cuboidal cells along the bone surface (100-400 per activation site).

The plasma membrane of osteoblasts is rich in alkaline phosphatase, which is often used as a marker of bone formation and is related to the maturity of the matrix. Osteoblasts also secrete osteocalcin, a non-collagenous protein - another marker of

osteoblastic activity and bone formation. In addition osteoblasts have receptors for oestrogen, parathyroid hormone and vitamin D3 in their nuclei. These cells produce osteoid tissue which following a ten day lag period (osteoid maturation period) is calcified into the recognisable bone matrix.

Osteoclasts

Osteoclasts are large multinucleated cells (containing between 4 and 20 nuclei) which derive from cells of the mononuclear/phagocytic lineage [BARO1996]. They are found in contact with calcified bone surfaces in resorptive sites and are generally seen alone or in pairs, very occasionally there may be up to five at any one site. Most often they are seen at endosteal bone surfaces. One of the distinctive features of the osteoclast is the ruffled border which attaches the cell to the surface of the bone and seals off the resorptive site. This ruffled border attaches to the surface of bone and forms an extracellular resorption area. A low pH in the extracellular area dissolves the hydroxyapatite crystals and mobilises calcium from the bone.

Skeletal modelling/remodelling

Throughout life, bone is continuously formed and resorbed in what is described as the modelling/remodelling cycle. Once peak bone mass is attained approximately 3% of cortical bone and 25% of trabecular bone is renewed annually. Both trabecular and cortical bone rely on a well developed vascular supply to maintain this activity.

Osteoclasts within the interstitial spaces aggregate onto the surfaces of trabeculae and resorb bone, a process which takes between two and four weeks. Osteoblastic bone formation is however, a longer process, generally lasting approximately three to four months.

There are three distinct phases of skeletal modelling/remodelling. The first occurs during growth and reflects the increase in osteoblastic activity compared with osteoclastic activity where bone is initially formed and then resorbed. This is sometimes referred to as bone modelling. Subsequent to the attainment of peak bone mass the function of osteoblasts and osteoclasts reaches a steady state of equilibrium and the remodelling cycle commences where bone is first resorbed and then formed.

In postmenopausal women, after approximately fifty years of age, the balance swings in the opposite direction to that seen during growth and the bone remodelling cycle causes a gradual reduction in bone mineral density. At this stage the activity of osteoclasts surpasses that of osteoblasts. Osteoporosis is caused by an imbalance in bone remodelling. As trabecular bone is metabolically more active than cortical bone the rate of remodelling of trabecular bone is higher than that of cortical bone. Early osteoporotic changes will therefore be apparent within trabecular bone prior to any change in cortical bone.

2.2 Genetically pre-determined peak bone mass

Factors that influence bone accretion during growth, and therefore the consequent attainment of a peak bone mass, include age, body mass, ethnicity, gender, genetics, nutrition, physical activity and pubertal stage. Bachrach [BACH1993] explains the importance of attaining a genetically pre-determined peak bone mass during adolescence as analogous to savings in a bank. She explains that the bone mineral deposited during adolescence may be used as a reserve in later life when the mass of bone begins to reduce as a result of primary osteoporosis (menopause-type I osteoporosis and/or age-type II osteoporosis). Bachrach further argues that the potential to reach peak bone mass is genetically pre-determined and it will only be attained if all those factors that contribute to achieve it are optimised. It is thought that a genetically determined peak bone mass is achieved by the end of the second decade, although the completion of bone accretion varies within and between the sexes and the final age of attainment of peak bone mass is variable [BACH1990]. Females tend to attain a peak bone mass slightly earlier than boys, at the age of approximately 14-15 or two years post menarche [BONJ1991]. Though boys attain their peak bone mass slightly later than girls they reach a higher final peak bone mass [IBID]. The bone mineral density of children is however difficult to evaluate because their body is in a perpetual state of change as they increase in age, height and weight as they progress through puberty.

There are some factors related to bone health over which a child has no control, such as her/his genes, ethnicity and gender. There are however two key factors over which they do have some control: nutrition and exercise. Nutrition is specifically relevant if food is plentiful and a child chooses not to eat, as is the case in early onset

ANRED. Exercise outside school hours is often an optional activity for children. Some choose to be more physically active than others who may prefer to play video games and/or watch television. The effect of weight as a result of nutrition and exercise also has an impact on the onset of puberty.

Children affected by a disease state, (psychiatric and/or physical in origin) which influences their height, weight or pubertal status are at risk of not attaining their genetically pre-determined peak bone mass and therefore developing secondary osteoporosis.

Bone development and structure in children

In children, little is known about the changing structure of trabecular and cortical bone during growth. Most literature relates to adult females and Type I osteoporosis which occurs after the menopause. This is because the changing structure of trabecular bone is normally established by examining samples of bone which have been surgically removed from the iliac crest of patients and because of the invasive nature of the technique such biopsies in children are rare [JONE1999]. In post-menopausal women the predominant change is of the trabecular struts becoming thinner and in severe cases losing their connections to other trabeculae [LIND1995].

There is evidence to demonstrate the positive effect of oestrogen on bone mineral density during growth (see the section entitled the effect of oestrogen) but little to inform us about what happens physiologically at the structural level. Atkinson [ATKI1967] examined the architecture of trabeculae in post-mortem samples of vertebral bodies from 68 subjects aged from 5 to 90 years. He found changes in vertebral trabecular bone associated with increasing age and noted a loss of horizontal trabeculae throughout life. He argued that this might be because horizontal trabeculae are not as important as vertical trabeculae for weight-bearing activities and are therefore more expendable. Jones et al [JONE1999] examined the structure of bone in 58 normal children using backscattered electron imaging of iliac crest biopsies and noted a gradual increase in cortical thickness and width of the trabecular region in the second decade of life. A more recent publication of normative paediatric reference data using bone histomorphometry [GLOR2000] demonstrated that trabecular struts became significantly thicker during growth with

no significant change in the distance between the struts from 1.5 years of age to 22.9 years. The authors noted that gender differences were only seen in those over 14 years of age and postulated that they resulted from differences in the timing of puberty.

The effect of puberty

It is well reported that the period of maximum increase in bone accretion occurs during, and is thought to be as a result of, the growth spurt of puberty with its associated rise in oestrogen, testosterone and Growth Hormone ([GILS1988], [GLAS1990], [BONJ1991], [DESC1991], [KATZ1991], [LLOY1992], [SEEM1992], [THEI1992], [BACH1993], [HERG1995]). There is debate however about whether this increase in bone mineral density is real or apparent. That is, as is argued by Katzman et al [KATZ1991], Schönau et al [SCHO1993a] and Cochat et al [COCH1996], the increase in bone mineral density seen during childhood may actually only be an apparent increase. It is seen as a result of the positive relationship between increasing bone mineral and increasing bone volume during childhood growth. Seeman [SEEM1997] goes further and describes as “erroneous” the view that bone density increases with age. He argues that this apparent correlation with age is caused by the failure of densitometric methods to account for variations in bone size caused by the measurement of an areal rather than a true bone mineral density. It is logical to assume that as a bone increases in volume during growth, in normal circumstances there should be a concurrent increase in the mineral that constitutes that growing bone. The corollary being that the larger the bone under examination the greater will be its apparent mineral density. This would explain the differences that are seen as a result of ethnicity, whereby those from Afro-Caribbean races are reported to have a greater bone mineral density than Caucasians, who in turn have a greater bone mineral density than those from Asian races [SEEM1997]. Despite this debate about whether the increase in bone mineral is real or apparent there is a significant amount of literature to support the notion that a genetically pre-determined peak bone mass is attained during late adolescence. This does however depend upon the maintenance of a healthy lifestyle and of major concern is the potential impact in adult life of not attaining a genetically pre-determined peak bone mass in adolescence.

The effect of oestrogen

There is evidence to demonstrate the negative impact on bone mineral density of the absence of oestrogen in women with amenorrhoea [DAVI1990]. The precise mechanism for the activity of oestrogen in relation to bone tissue is however unclear. Oestrogen is thought to have a rate-limiting effect on the formation and activity of osteoclasts, hence the loss of bone density seen at the menopause [SCHI1998]. Oestrogen also stimulates the secretion of insulin-like growth factor-1, which plays a vital role in augmenting linear growth of bone as outlined in the section on Growth Hormone. In addition androgen and oestrogen receptors have been identified on osteoblasts and insufficient androgen levels have been seen in men with osteoporosis [ANDE1998]. Oestrogen is also thought to have a significant role to play in the maintenance of bone health in males since oestradiol is detectable in healthy men at levels comparable to those seen in post-menopausal women [IBID], whereas considerably lower levels of oestradiol are seen in men with hypogonadism induced osteoporosis.

The effect of Growth Hormone

Growth Hormone (GH) is secreted by the anterior lobe of the pituitary gland in response to a number of physiological stimuli including deep sleep, physical exercise, stress and food [BROO1982]. Growth Hormone together with its major mediator, insulin-like growth factor-1 (IGF-1), augments linear growth, which occurs at the epiphyseal plates of bones [INZU1996]. GH has been shown to have a greater effect on long bones such as the femur than short bones such as the vertebrae [SEIN1999]. GH stimulates the production of IGF-1, which is produced both in growing bone and in the liver. The production of IGF-1 is also affected by nutrition being reduced in cases of malnutrition.

In addition to the increase in linear growth, GH also has a significant impact on improving bone mineral density by both enhancing osteoblastic function and by increasing renal 1 α -hydroxylase [PFEI1997]. These two main pathways for the action of GH on bone are outlined below.

The first effect is a direct action of GH and IGF-1 on the activity of bone remodelling units. Osteoblasts and bone marrow cells contain GH and IGF-1

receptor sites. Thus GH acts directly on these receptor sites to increase the activity of bone remodelling units which causes a net increase rather than decrease in bone mineral density and biomechanical strength [PFEI1997].

The second effect is an indirect action of GH on bone caused by the enhancement of those other factors that contribute to bone mineral density. GH/IGF-1 increase the production of 1 α -hydroxylase which is the key enzyme in the vitamin D pathway and is produced in the kidney. Vitamin D is essential for the resorption of calcium from the digestive system.

The effect of ingested calcium

The mineral content of bone and therefore its strength is related to the amount of calcium absorbed [LLOY1996]. In a young adult's diet comprising 1000 milligrammes of calcium daily approximately 20% is absorbed by the intestine, whereas in a child approximately 30% is absorbed to allow for the higher rate of bone formation [PEAC1998].

In 1998 the Department of Health published a report on calcium and vitamin D levels and their impact on bone health [DOH1998]. This report was prepared by a sub-group of the Committee on Medical Aspects of Food and Nutrition Policy (COMA). The sub-group published two levels of nutrient intake for calcium: the reference nutrient intake and the lower reference nutrient intake.

Reference nutrient intake (RNI)

This is the amount of calcium that is enough for almost every individual, and those consuming the RNI are most unlikely to be deficient. The RNI is set at two standard deviations above the estimated average dietary requirement.

Lower reference nutrient intake (LRNI)

This is the amount of calcium that is enough for only the small number of people who have the lowest needs, and those consistently consuming less than the LRNI will almost certainly be deficient. The LRNI is set at two standard deviations below the estimated average dietary requirement.

Table 2 RNI and LRNI for calcium

	RNI (milligrammes.day ⁻¹)	LRNI (milligrammes.day ⁻¹)
1-3 years	350	200
4-6 years	450	275
7-10 years	550	325
11-18 years (male)	1000	480
11-18 years (female)	800	450

A number of studies have been conducted on children and adolescents to evaluate the impact of calcium intake on bone mineral density. Lloyd et al [LLOY1996] reported increases of between 12 and 24% in bone mineral density in 12 year old children who received calcium supplements of 500 milligrammes daily for two years when compared to matched controls. In the USA Chan [CHAN1991] also demonstrated this positive relationship in a cohort of 164 healthy white children aged 2 – 16 years. He reported that the bone mineral content of those consuming at least 1000 milligrammes of calcium daily was significantly higher than the bone mineral content of those who consumed less. A study which provoked much media coverage was one by Cadogan et al [CADO1997] published in the British Medical Journal. They demonstrated that 44 female 12 year olds who had a diet supplemented with one pint of milk daily for 18 months had higher bone mineral density than a matched group of 38 female 12 years olds who did not receive this supplement. Interestingly they also noted that the milk supplemented group had significantly higher levels of IGF-1 over the course of the study, which raises the possibility that this may have had an impact on the improved bone mineral density in this group. At the end of the study the mean calcium intake of the milk group was 1125 milligrammes.day⁻¹ compared to 703 milligrammes.day⁻¹ in the control group. These data are somewhat at odds with the recommended RNI and LRNI levels of calcium for females aged between 11 and 18 as outlined in table 2. These RNI levels are set at two standard deviations above normal dietary requirements. The mean calcium intake of the control group in Cadogan's study [IBID] was much higher than the LRNI level of calcium for age and close to the RNI level of calcium for age. In comparison the mean calcium intake of the milk group in the same study exceeded both the LRNI and the RNI of calcium for age. Therefore, assuming calcium absorption was similar for the two groups, one would have expected there to be a minimal difference between them in terms of their measured bone mineral density, as both mean values

were either above or close to the RNI for age. However, because the milk group, at 1125 milligrammes.day⁻¹, had a higher bone mineral density than the control group (700 milligrammes.day⁻¹) this provides some evidence that the RNI value for 11-18 year old females at 800 milligrammes.day⁻¹ may be too low.

In a more recent study by Wang et al [WANG1999] the broadband ultrasound attenuation measurements of 18-19 year old women were correlated to their dietary data at age 9-11 years. Wang et al demonstrated a positive correlation between these two parameters and concluded that pre-adolescent diet therefore had a positive impact on quantitative ultrasound measurements post-adolescence. Despite Wang's findings it is evident that although calcium supplementation in childhood can increase bone mineral density, there are no data currently available to support the hypothesis that the initial increase in bone mineral density will be maintained to increase final peak bone mass. A long-term longitudinal study is needed to evaluate this more fully.

One of the lifestyle measures to promote bone health, recommended by nutritionists and endorsed by the Department of Health, is an adequate intake of dietary calcium, particularly in adolescence. The daily allowance of calcium recommended by the National Osteoporosis Society is 1,000 milligrammes of calcium [NOS1998], which is approximately the amount of calcium found in a pint and a third of milk. In the USA the recommended daily allowance of calcium for adolescents is 50% higher at 1,500 milligrammes [MATK1999]. In comparison, the Royal College of Physicians and the Department of Health in the UK recently recommended a daily dose of calcium for females of only 800 milligrammes [RCP1999] in adolescence. This range of recommended daily allowance of calcium in paediatrics demonstrates the need for further research into this subject so that consensus may be reached.

The effect of vitamin D

The major source of Vitamin D in humans arises from the conversion of 7-dehydrocholesterol in the skin to cholecalciferol (Vitamin D₃). This conversion is initiated by the action of light of wavelengths 290 – 310 nanometres on the skin. Cholecalciferol however has no useful effect until it is converted into the active metabolite 1,25-dihydroxyvitamin D₃ in the kidney. This active metabolite controls

plasma calcium concentrations by modulating calcium absorption in the small intestine, phosphate retention in the kidney and calcium release from bone. Deficiencies of 1,25 dihydroxyvitamin D₃ will lead to reductions in serum calcium subsequently leading to secondary osteoporosis. Cholecalciferol is converted into 25 hydroxyvitamin D₃ in the liver and this is then converted to the active metabolite 1,25 dihydroxyvitamin D₃ by the action of 1 α -hydroxylase in the kidney.

Interestingly, for those aged between 4 and 64 years the sub group of COMA do not recommend a reference nutrient intake for vitamin D [DOH1998]. This, they argue, is because healthy individuals in this age range will synthesise adequate vitamin D by exposure of the skin of the face and arms to as little as 30 minutes of sunlight each day between April and October. They do however recommend that particular attention be given to those citizens who may be deficient by virtue of their culture or ethnicity.

The effect of exercise

Observational studies have been used to provide evidence that the bone density of subjects who actively exercise is higher than the bone density of those who are sedentary ([BASS1998], [KHAN1998], [UUSI1998], [HOSH1996], [KARL1996], [SALA1996]). Furthermore the exercise groups in such observational studies can be divided into those, who by the nature of their activity have a large muscular stature, for example gymnasts and those who have a less muscular stature, such as long-distance runners ([TAAF1997], [ETHE1996]). The gymnasts in these studies have an increased bone mineral density compared to both other athletes and controls. This difference is emphasised when the bone mineral density is normalised for height and weight [DYSO1997]. This fits well with Frost's model of the mechanostat theory [FROS1996] where he emphasises the importance of this dominating muscular mechanical effect on bone mineral density. Frost proffered a hypothesis that the degree of bone mineral density is dependent not only on the type of exercise undertaken but also on the quantity of muscle placing a strain on the bone. This explains why weight-lifters and gymnasts have a higher bone mineral density than long distance runners; the size of the muscle needed to motivate the bones for weight-lifting or gymnastics is significantly greater than that needed for running. Hergenroeder [HERG1995] endorses this explaining that the magnitude of strain

across a bone is more important than the number of repetitions of a particular exercise/movement. When bone reaches a threshold level called the “minimum effective strain for modelling” mechanically controlled bone modelling switches on [FROS1997].

There are also exercise studies demonstrating that not all exercise is beneficial to bone health. For example athletic individuals who have less muscle bulk and a smaller physical stature and undertake activities such as ballet-dancing ([KHAN1998], [PEAR1996]) or long-distance running [TAAF1997] are reported to have a lower bone mineral density than healthy matched controls.

The bone mineral density of pre-pubertal children who undertake weight bearing exercise (gymnastics) has been compared to that of children who undertake non-weight bearing exercise (swimming) and the positive effects of the former in terms of increased bone mineral density have been demonstrated [COUR1998].

Surprisingly paediatric exercise intervention studies have demonstrated that children who follow a weight-bearing exercise regime for as short a period of time as ten months [BRAD1998] have a higher bone mineral density than that of healthy matched controls. This is particularly marked if the weight-bearing exercise (whether as a planned intervention study or undertaken of the child’s own volition) is in the pre-pubertal years ([BAIL1999], [BASS1998], [BRAD1998], [COUR1998], [MORR1997], [SLEM1994]). Exercise intervention programmes normally include a mixture of weight-bearing activities such as aerobics, football and weight-training ([MORR1997], [BRAD1998]).

The velocity of sound and broadband ultrasound attenuation have also been used to evaluate the bone status of pre-pubertal children, but with conflicting results. In Daly’s study [DALY1997] the velocity of sound through the calcaneum, radius and phalanges of pre-pubertal gymnasts was predictably higher than that obtained on matched controls. In contrast there was no significant difference in broadband ultrasound attenuation through the calcaneum in the two groups. In an observational study by Lappe et al [LAPP1998] on normo-active pre-pubertal children the velocity of sound through the patella was, surprisingly, reduced rather than raised in those

undertaking the most exercise. VOS is related to the elasticity of bone whereas BUA provides additional information since it is related to both structure of bone and density (see section 4.3). One could therefore postulate that had BUA been measured instead of VOS a different result may have been observed, however evidence from Daly's study [DALY1997] suggests that this would not be the case. It is possible however that the results are misleading because the patella, which was measured in Lappe's study, is not a weight-bearing bone and if VOS through the calcaneum had been measured then an increase rather than decrease may have been noted. Another potential confounder in Lappe's study is the measurement of the exercise patterns which may not be representative of the population as a whole since they were undertaken on one day during winter.

The type and extent of physical activities undertaken in childhood and adolescence can be used to predict the long-term benefits of exercise. Retired ballet dancers have the same bone mineral density as controls [KHAN1998]; retired short distance runners have an increased bone mineral density compared to retired swimmers [ETHE1996] and retired gymnasts have a significantly higher bone mineral density than age-matched controls [BASS1998]. Therefore exercise undertaken in childhood, particularly in the pre-pubertal years may reduce the risk of osteoporotic fracture in later life.

2.3 Osteoporosis

Osteoporosis is a significant public health risk and in the United Kingdom alone it costs the National Health Service approximately £940 million annually [RCP1999]. These costs are predominantly indirect costs arising from the hospitalisation and treatment of patients following osteoporotic fracture. There are also significant personal costs to the patient her/himself in terms of a reduction in quality of life because of the disease. The problem is likely to exacerbate as it is predicted that the yearly incidence of hip fracture in the European Community will more than double over the next 50 years from 414,000 to 972,000 [EURO1998]. This figure is based on the future rise in size of the ageing population leading to a coincident increase in the number of people at risk of hip fracture. Approximately 90% of hip fractures occur in people aged 50 years or over and 80% occur in women [DOH1994]. In

comparison the most common fractures in young people are of the long bones [IBID].

Table 3 outlines the classification of primary and secondary osteoporosis. Other than the idiopathic juvenile form, osteoporosis generally affects children as the secondary state via other disease processes.

Table 3 Classification of osteoporosis

Primary

Idiopathic juvenile osteoporosis

Idiopathic osteoporosis in adults

Involutional osteoporosis

Type I (“postmenopausal” osteoporosis)

Type II (“senile” osteoporosis)

Secondary

Anorexia nervosa

Chronic renal failure

Hypocortisolism

Hypogonadism

Hyperthyroidism

Hyperparathyroidism

Definitions of osteoporosis

There are two interlinked radiographic definitions of osteoporosis on which the provision of osteoporosis diagnostic services are based. These criteria were developed to identify those members of the population at significant risk of osteoporotic fracture. The first and most relevant to this thesis concisely describes osteoporosis in terms of both structure as well as bone mineral density. This definition is the one advocated by practitioners who use ultrasound to assess osteoporosis and was published by the Consensus Development Conference in 1993 [CHRI1993].

Osteoporosis is a disease characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture.

The second radiographic definition of osteoporosis bases the classification on bone mineral density alone and tends to be cited by practitioners who use ionising radiation techniques such as dual energy X-ray absorptiometry (DXA) to assess osteoporosis. This definition of osteoporosis was developed by a study group of the World Health Organisation [WHO1994].

Osteoporosis is described as a bone mineral density at least 2.5 standard deviations below the peak bone mass in normal young women. **Osteopenia** is described as a bone mineral density between 1 and 2.5 standard deviations below the peak bone mass in normal young women.

Those patients with a bone mineral density within 1 standard deviation of normal young women are deemed to have normal bone mineral density. The standard deviation when compared to peak bone mass in normal healthy young Caucasian women is known as the t score, whereas when it is compared to the bone mass of an age matched population it is referred to as a Z score. The terms mass and density are not strictly interchangeable because mass is a measure of how much matter a body contains, whilst density is a measure of mass per unit volume. However peak bone mass is the term used in osteoporosis scientific literature to describe the target level of mineral in healthy bone and bone mineral density is the most frequently used measure of this. Therefore these terms have been used in this thesis.

Although the WHO definition is the most widely accepted internationally, it is misleading to those with responsibility for the purchase of diagnostic radiographic equipment, as there is no acknowledgement within the statement of those methods of assessing osteopenia and osteoporosis that do not measure bone mineral density. The effect to the patient of the wide acceptance of the WHO definition was exemplified at an international bone ultrasonometry symposium in 1997. At the symposium, speakers from Korea [HAN1997], Germany [WUST1997], Japan [YAMA1997], Canada [TENE1997] and Australia [FORM1997] described the use of ultrasound in their respective countries as a research tool or for use in private practice only. The rationale for such use is based on government policy not to reimburse the cost of ultrasound bone assessment examinations, as the technique

does not measure bone density directly. Rather, it has been argued that ultrasound measures the strength and structure of bone as well as the density ([GLUE1992], [LANG1994]), which ironically can be argued to be a more valid predictor of fracture risk than bone density alone. This adherence to the WHO criteria may therefore limit innovation in health technology design. However such governmental policies are likely to change as a result of recent acceptance of some quantitative ultrasound machines by the Food and Drugs Administration in the United States of America.

2.4 Summary

Bones continuously grow throughout childhood and become structurally moulded according to their weight bearing or non-weight bearing functions. Trabecular bone has a predominantly metabolic function contributing to calcium and phosphate homeostasis in the body. Cortical bone has a limited metabolic function but is predominantly responsible for the strength of bones. The two types of bone are found throughout the skeleton in different proportions. Diagnostic instruments have been developed to measure the amount of calcium in bone to provide an indication of osteoporotic fracture risk. The bones most commonly measured are those with the highest trabecular content and/or the highest fracture risk.

There is minimal data on the changing structure of trabecular and cortical bone during growth however it appears that trabecular struts become thicker during childhood and adolescence with no significant change in the distance between them [GLOR2000].

The maintenance of healthy bone is dependent upon a number of inter-related hormones including Growth Hormone and insulin-like growth factor-1, oestrogen, testosterone and androgen. There is an increase in the quantity of these hormones during the growth spurt of puberty which contributes to the increase in bone mineral density seen at this age.

Optimum bone health also relies on an appropriate intake of calcium and adequate exposure to sunlight to enable the synthesis of vitamin D for the absorption of calcium. The Department of Health recommended reference nutrient intake for

calcium is 800 milligrammes.day⁻¹ for adolescent females and 1000 milligrammes.day⁻¹ for adolescent males. It has been demonstrated that children whose normal dietary intake of calcium is supplemented have a higher bone mineral density than control children without supplementation ([LLOY1996], [CADO1997]). In the UK there is no reference nutrient intake level for vitamin D for healthy individuals between 4 and 64 years of age.

It is evident from the literature that it is also beneficial to bone health to develop healthy, weight bearing exercise habits, which places a strain on bone such as the vertebrae via the quadratus lumborum and psoas muscles stimulating osteoblastic activity and enhancing the bone modelling/remodelling cycle [HERG1995]. This ultimately leads to an increase in bone mineral density.

The long term effects of the increase in BMD caused by exercise intervention studies and/or calcium supplementation have not yet been proven. It can be postulated from cross-sectional comparative studies of retired athletes that there is some positive latent effect of pre-pubertal exercise on BMD, although few researchers have evaluated the impact of exercise on the bones of pre-pubertal children before peak bone mass has been achieved. Yet, this age group may be where the most profound effects of exercise on bone may be seen. In addition even fewer authors have evaluated the impact of exercise on BMD in pre-pubertal males, presumably, because unlike females they are not perceived to be at high risk for osteoporosis later in life. No studies have followed children all the way through to late adulthood which would obviously be the ideal scenario. Extensive longitudinal follow up data on children is therefore needed to evaluate the effect of exercise programmes and calcium supplementation more fully and establish whether the increase in BMD is transient or permanent.

The incidence of osteoporosis and associated hip fracture in the adult population is rising and looks to continue to do so. The figures for this rise are primarily based on the increase in size of the ageing population and are therefore likely to be underestimated. This will have a significant impact on the financial resources of the health service, predominantly caused by the hospitalisation and treatment of these patients. More importantly it is recognised that much of the suffering related to osteoporosis is

preventable. However this is only possible if there is early diagnosis of the disease allowing prophylactic treatment to be prescribed. Such diagnosis and treatment is targeted at high risk populations, particularly post-menopausal women. There is, however, another course of action that could be taken which may have an impact on reducing the incidence of osteoporosis in adulthood. That is, to focus more attention on diagnosing the early stages of the disease during growth and development when appropriate intervention can be made to minimise the long-term risks of osteoporosis.

Chapter 3 Anorexia nervosa and related eating disorders

This chapter is divided into two themes. The first is a brief review of the diagnosis of anorexia nervosa and related eating disorders (ANRED) in children and the second section is a review of the literature on osteoporosis in patients with anorexia nervosa.

3.1 *The diagnosis of ANRED*

In children there is a range of eating disorders which share the common feature of a refusal to ingest adequate quantities of food appropriately. They include anorexia nervosa, food avoidance emotional disorder, selective eating, appetite loss secondary to depression, food refusal, pervasive refusal and bulimia nervosa. Each of these is briefly outlined in this section.

Anorexia nervosa

The essential feature of anorexia nervosa is a determined refusal to maintain a normal body weight [APA1994]. The term anorexia means a loss of appetite. However, ironically, patients with anorexia nervosa rarely suffer from a loss of appetite [IBID].

Anorexia nervosa is perceived as a disease afflicting mainly adolescent and adult females however the disease also afflicts children of both genders. Early diagnostic criteria for anorexia nervosa such as those by Feighner et al [FEIG1972] have been criticised in the paediatric literature as being too narrow thus excluding children from being categorised as having anorexia nervosa. In particular Feighner included a criterion specifying a loss of 25% or more of original body weight. This is considered inappropriate for children because as Irwin [IRWI1981] argues a girl of for example nine years of age and of normal weight has a much smaller percentage of total body fat than her sixteen year old counter-part. Fosson et al [FOSS1993] estimate the size of the physical insult to a child's body of inadequate nutrition as being inversely proportional to age. So that as a child develops and increases in height her/his weight should demonstrate a concurrent increase. If a child remains the same weight but increases five centimetres in height, it is clear that in real terms that child has lost weight. The younger child has a smaller percentage of body fat than the older adolescent and so the physical insult is significantly greater for a

smaller percentage of loss of body weight. It is also difficult to know what the child's pre-morbid weight was, which makes the criterion of a percentage weight loss difficult to enforce. Feighner's criteria stifled research into the diagnosis and treatment of affected children and adolescents until 1987 when The American Psychiatric Association [APA1987] agreed DSM III R criteria for the diagnosis of anorexia nervosa in pubertal and post-pubertal adolescents (table 4). It is of note that the criterion relating to 25% weight loss was amended to 15% and in addition was related to normal weight for age.

Table 4 DSM-III-R Criteria for Anorexia Nervosa, 1987

-
- Refusal to maintain body weight over minimal normal weight for age and height (e.g. weight loss leading to body weight below 15% expected) or failure to make expected weight gain during growth period leading to body weight 15% below expected
 - Intense fear of gaining weight or becoming fat, even though underweight
 - Disturbance in the experience of body weight, size or shape (e.g. claiming to feel fat when emaciated, believing that one area of body is too fat when underweight)
 - The absence of at least three consecutive menstrual cycles when otherwise expected to occur (primary or secondary amenorrhoea)
-

The DSM-III-R criteria have helped in the diagnosis of childhood onset anorexia nervosa but are still limiting as they include a reference to the absence of menstrual cycles. Primary amenorrhoea is difficult to assess as the age of onset of menarche is so variable. However the more recent criteria (DSM-IV) specify clearly this criterion as "in post-menarcheal females". Table 5 shows the criteria for the diagnosis of anorexia nervosa which are published by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Those published in the international classification of diseases (ICD 10) are also used.

Table 5 DSM-IV Criteria for Anorexia Nervosa, 1994

-
- Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g. weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
 - Intense fear of gaining weight or becoming fat, even though underweight.
 - Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
 - In post-menarcheal females, amenorrhoea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhoea if her periods occur only following hormone, e.g. oestrogen administration).

Specify type

Restricting type: during the current episode of anorexia nervosa the person has not regularly engaged in binge-eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics or enemas)

Binge-eating/purging type: during the current episode of anorexia nervosa the person has regularly engaged in binge-eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics or enemas).

At Great Ormond Street Hospital for Children, Fosson et al [FOSS1987] developed criteria specifically to diagnose pre-pubertal children with anorexia nervosa (table 6). The primary criteria are that the child should exhibit determined food avoidance and that there should be either weight loss or a failure to gain weight during the pre-adolescent growth spurt.

Table 6 Fosson's Criteria for Anorexia Nervosa, 1987

-
- Determined food avoidance
 - Weight loss or failure to gain weight during the period of pre-adolescent accelerated growth (10-14 years) in the absence of any physical or mental illness
 - Any two or more of the following
 - preoccupation with body weight
 - preoccupation with energy intake
 - distorted body image
 - fear of fatness
 - self-induced vomiting
 - extensive exercising
 - purging (laxative abuse)
-

Food avoidance emotional disorder

This disorder was originally reported in 1989 by Higgs et al [HIGG1989] who examined the case notes of 8051 children presenting to a child psychiatry unit over a 26 year period. Of these children, 27 fulfilled the criteria for anorexia nervosa and a further 23 had food avoidance and emotional disorders (FAED). The age range of the group with FAED was from 8 to 14 years and only a third of these children were less than 80% weight for height, compared to three quarters of the anorexia nervosa group. Higgs et al reported that patients with this disorder have a better prognosis than anorexia nervosa. They concluded that FAED was a mild form of anorexia nervosa combined with childhood emotional disorder. Children with FAED do not have a distorted body image or a preoccupation with weight and shape; they do however have weight loss, mood disturbance and avoid food [BRYA2000]. The differential diagnosis between FAED and anorexia nervosa is therefore difficult.

Selective eating

These children may be described as “fussy eaters”; their diet mainly composed of two or three different foods, typically those high in carbohydrates such as potatoes, crisps or biscuits [BRYA1993]. They tend to have a long history of selective food intake but often begin to relax these eating habits during adolescence as they try to conform with peers [BRYA2000]. Their weight may be low, normal or high and they do not have abnormal cognitions about their weight or shape and therefore do not meet the criteria for anorexia nervosa.

Appetite loss secondary to depression

The differentiating factor between this and anorexia nervosa is the absence of a pervasive refusal of food and a lack of pre-occupation with body image [BRYA1993].

Food refusal

This is a fairly common disorder in young children and it is distinguished from anorexia nervosa in a number of ways. The most obvious is that the child does not have a distorted body image or an intense fear of fatness. Rather s/he will refuse food when it suits her/him to do so and s/he rarely refuses her/his favourite foods. In addition the child will often eat at a friend’s house with relatively little problem [BRYA1993].

Pervasive refusal

Lask and Bryant-Waugh [LASK1991] describe this condition in children as a “profound and pervasive refusal to eat, drink, walk, talk or engage in self-care”. It is postulated as a variant of post-traumatic stress disorder. Although the child is typically underweight and adamantly refuses food and water s/he does not fit any other criteria for anorexia nervosa.

Bulimia nervosa

This eating disorder is characterised by uncontrollable behaviour regarding food consumption and evacuation. Periods of starvation are often preceded by periods of over-eating and bingeing followed by laxative abuse and self-induced vomiting. As in anorexia nervosa there remains an exaggerated fear of fatness. This is an uncommon diagnosis in children, particularly those who are pre-pubertal. Table 7 shows the American Psychiatric Association [APA1994] diagnostic criteria for bulimia nervosa.

Table 7 DSM-IV Criteria for Bulimia Nervosa, 1994

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- Recurrent episodes of binge-eating. An episode of binge-eating is characterised by both of the following:
 1. eating, in a discrete period of time (e.g. within any 2 hour period) an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances
 2. a sense of lack of control over eating during the episode (e.g. a feeling that one cannot stop eating or control what or how much one is eating).
 - Recurrent inappropriate compensatory behaviour in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
 - The binge-eating and inappropriate compensatory behaviours both occur, on average, at least twice a week for 3 months.
 - Self-evaluation is unduly influenced by body shape and weight.
 - The disturbance does not occur exclusively during episodes of anorexia nervosa.

Specify type

Purging type: during the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics or enemas

Nonpurging type: during the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviours, such as fasting or excessive exercise but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics or enemas.

The incidence of anorexia nervosa in children

There appears to have been an increase in the number of children with anorexia nervosa in recent years. There are at least two potential causes of this. The first is the development and use of criteria adapted specifically for children allowing the diagnosis of an eating disorder to be made more easily [NICH2000]. The second is the increasing awareness of the general public about the disease, brought about in part by the publication of cases in the media. It is now not uncommon to see a television documentary or a newspaper story about females with eating disorders ([SEAR1999], [WARD1999]). The tragic death of the singer Lena Zavaroni following brain surgery for her long standing problems with anorexia nervosa was reported as a national headline news-story [BBC1999]. The increase in the number of children receiving clinical attention may therefore be related to an increased sensitivity of both health care professionals and the general public to the disease or an increase in the prevalence of the disease. However it is more likely that the effect

is a combination of both increasing awareness and an increasing prevalence of the disease.

There is a dilemma when reviewing past literature relating to anorexia nervosa, since various institutions have used different criteria for the diagnosis of the condition. The effect of this difference in diagnosing criteria has been demonstrated by Nicholls et al [NICH2000] who compared the reliability of DSM-IV, ICD 10 and Great Ormond Street (GOS) eating disorders criteria for children and adolescents. They discovered that when two independent observers retrospectively classified the eating disorders of 81 children using these three sets of criteria the inter-observer reliability was highest using the GOS criteria. The lowest inter-observer reliability was found using the ICD 10 criteria. This they argue is because the GOS criteria were developed specifically for the childhood population, whereas the other two were of a more generic nature applying to a wider age range.

McSherry [MCSH1986] strongly believes that the true prevalence of anorexia nervosa has been underestimated as a result of many centres adhering too strictly to the diagnosing criteria. Criticising the adherence to such strict diagnosing criteria McSherry goes further saying that such practice prevents the recognition of early stages of the disorder where “effective intervention may more readily be accomplished.” Some institutions strictly adhere to the criteria in a manner that excludes those with early onset anorexia nervosa in childhood, whereas others adapt the criteria slightly for their institution.

Prevalence rates also depend on the population sample studied: whether it is from a primary, secondary or tertiary NHS centre or indeed a private hospital/clinic. Furthermore only those who have received medical attention for an eating disorder would be included in such prevalence studies. The diagnosis of a specific eating disorder is also dependent on the clinician who sees the patient and her/his primary specialty. The prevalence of early onset anorexia nervosa in childhood is therefore difficult to evaluate. However Higgs et al [HIGG1989] performed a retrospective study and reviewed the case notes of 8051 children seen as psychiatric patients over a twenty-six year period. They found that only 27, that is 0.3% of this population,

met their childhood criteria for anorexia nervosa. These children ranged in age between 8 and 16 years and an unusually high proportion of them were male (30%).

Predisposing factors and features of anorexia nervosa

Anorexia nervosa is a complicated, heterogeneous disorder and although predisposing factors have been identified there is still more research required to fully understand its aetiology. Gillberg & Rastam [GILL1998] explain that since anorexia nervosa is multifactorial it should be interpreted in a “biopsychosocial perspective”. They argue that considering it in isolation as biological, psychological or family-based will limit understanding of the disorder.

It is reported in the literature that there are particular personality traits that predispose individuals to anorexia nervosa. Fairburn et al [FAIR1999] reported that negative self-evaluation and perfectionism were substantially more common in patients with anorexia nervosa. Casper et al [CASP1992] demonstrated that females with anorexia nervosa showed “greater than normal self-discipline, emotional caution and conscientiousness”. Higgs et al [HIGG1989] reported a high incidence of a past history of obsessionality and Fosson et al [FOSS1987] noted depressive symptoms in 56% of their anorexia nervosa group. Bryant-Waugh et al [BRYA1988] found that children with depressive symptoms before and during treatment for anorexia nervosa had a poor outcome in terms of recovery from the disease. Other features quoted in the literature as predisposing to anorexia nervosa are:

- a disturbed mother-daughter relationship [JACO1986];
- sensitivity to the onset of puberty ([GOWE1991], [BRYA1988], [CRIS1983]);
- traumatic life events, such as the death of a close family member or parental conflict ([HIGG1989], [MARG1985], [WARR1968]);
- pre-morbid obesity and resultant teasing ([MARG1985], [IRWI1984], [WARR1968]).

Although these features have been associated with anorexia nervosa they may also be seen in other conditions.

The metabolic impact of anorexia nervosa

The risk of osteoporosis in anorexia nervosa arises from an interlinked series of physiological disturbances including reduced oestrogen levels and increased cortisol secretion [APA1994].

Oestrogen

As has already been explained osteoblasts contain oestrogen receptors which stimulate the production of insulin-like growth factor-1 which in turn stimulates bone modelling/remodelling. Oestrogen also has a rate limiting effect on the function of osteoclasts and so in its presence less bone is removed and in its absence there is enhanced absorption of bone [MUND1996]. As the weight of a female patient with anorexia nervosa reduces she is likely to suffer from hypogonadism, demonstrable on pelvic ultrasonography, [LAI1994] with associated reduced levels of circulating oestrogen [KIRI1992]. In Golden's study [GOLD1994] 28 female patients with anorexia nervosa (mean age 15.1 years) had significantly reduced levels of oestrogen when compared to age matched healthy controls. As early as 1985 the administration of oestrogen was suggested to minimise the risk of reduced bone mineral density in women with anorexia nervosa [SZMU1985]. Seeman et al [SEEM1992] measured bone mineral density in 65 women with anorexia nervosa and he noted that bone mineral density was higher in 16 patients who had taken oral contraceptives than in 49 patients who had not. However of the 49 patients who had not received oral contraceptives 37 had secondary and 12 had primary amenorrhoea whereas all of the patients receiving oral contraceptives had previously attained menarche. In addition those with primary amenorrhoea were on average 20 years of age which is seven years younger than those with secondary amenorrhoea who were receiving oral contraceptives. They were also several centimetres shorter and six kilogrammes lighter. As well as the effect of exposure to oral oestrogen in some patients these additional factors may therefore have contributed to the differential in bone density seen between the groups. Both Bachrach et al [BACH1991] and Klibanski et al [KLIB1995] investigated the effect on bone density of administering oestrogen to females with anorexia nervosa. Bachrach reported only one female who received oestrogen for six months and she gained 10% in spinal bone mineral density despite losing weight over the same period. Klibanski et al found no improvement in

bone mineral density in a cohort of 19 female anorexia nervosa patients who received oestrogen and progestin supplementation compared to 25 controls who did not. However when the data were re-analysed excluding 6 patients in the control group with spontaneous resumption of menses a significant positive effect on bone density was seen in the group who received oestrogen and progestin.

Cortisol

Cortisol levels are significantly higher in patients with anorexia nervosa compared to normal controls. This hypercortisolaemia is associated with both increased bone resorption and impaired bone formation [KIRI1992]. These levels return to normal once the disease is in remission and weight is restored. However, as increased cortisol levels are also seen in depression there may be a more fundamental neurophysiological cause of hypercortisolism in patients with anorexia nervosa independent of that caused by weight loss [GOLD1986]. In Gold's paper [IBID] cortisol levels had returned to normal in those anorexia nervosa patients who had stabilized their weight gain for a period of six months or more.

Growth Hormone and insulin-like growth factor-1

Argente et al [ARGE1997] reported two patterns of growth hormone secretion in 50 female patients with anorexia nervosa. Those who secreted excess GH: hypersecretors (n=19) and those who secreted insufficient GH: hyposecretors (n=31). In this study there were no significant differences between the two groups in terms of height, weight, BMI, duration of disease or age. In addition after achieving increases of between 6 and 8% of their original body weight GH levels in both groups returned to control values.

In a small study of eight female patients with anorexia nervosa compared to eleven controls Stoving et al demonstrated that GH was raised in patients with anorexia nervosa and was significantly correlated to the BMI [STOV1999].

Insulin-like growth factor-1 (IGF-1) levels are reduced in anorexia nervosa patients which may account for the reduced linear growth which is often seen [GRIN1996]. In the study by Grinspoon et al [IBID] a cohort of 23 women with anorexia nervosa and reduced basal levels of IGF-1 received either a placebo or a dose of recombinant

IGF-1 varying between 30 and 100 microgrammes.kilogramme⁻¹ twice daily for a six day period. Over this six day period markers of bone formation (osteocalcin and type I procollagen carboxyl-terminal propeptide) increased implying an increase in bone modelling leading to an increase in bone density.

Golden hypothesises that the reduction in IGF-1 and increase in GH which is seen in patients with anorexia nervosa may arise as a result of inadequate GH secretion or resistance to GH or both combined [GOLD1994]. When weight gain was resumed in his cohort of 28 patients, levels of GH and IGF-1 returned to normal. The reduction of IGF-1, which has been associated with a rise in basal levels of Growth Hormone (GH), is most marked in emaciated anorexia nervosa patients [FOSS1993].

It is difficult to reconcile these contradictory data about GH. The variations seen in GH levels in different studies are reflective of the heterogeneity of anorexia nervosa and the inherent difficulties investigating the disorder. However, importantly, it appears that GH levels do return to normal on recovery from the eating disorder.

Calcium

Insufficient nutrition reduces the amount of calcium that is available to be resorbed from the intestine for use in the body. There is also some evidence to suggest that there is an increased amount of calcium excreted in the urine of patients with anorexia nervosa [ABRA1993]. So not only is less calcium taken in to the body via the diet, but also the amount of calcium which is removed from bone stores and excreted via urine appears to be increased in anorexia nervosa.

3.2 ANRED and osteoporosis

Most studies to investigate the risk of reduced bone mineral density in patients with ANRED have involved studies on small cohorts of women with anorexia nervosa. Adolescent and adult females with anorexia nervosa are at significant risk of osteoporosis ([RIGO1984], [TREA1987], [BILL1989], [BACH1990], [DAVI1990a], [DAVI1990b], [PRIO1990], [BACH1991], [HAY1992], [ABRA1993], [YOUN1994], [GRIN1999]). There are few long-term longitudinal studies of patients with anorexia nervosa who have osteoporosis however it has been demonstrated that bone mineral density does improve with recovery from the eating disorder but it remains lower than controls though often not in the osteoporotic range ([HERZ1993], [BROO1998]). A recent study by Lucas et al [LUCA1999] demonstrated that women with a past history of anorexia nervosa had a 2.9 fold increased risk of fracture. In the Lucas cohort of 208, there were 88 fractures in total and, interestingly, 20 were of the feet or toes. A further 26 were of long bones including the clavicle, ribs and distal forearm. There is however minimal evaluation of the bone status of pre-pubertal children with ANRED, particularly pre-pubertal males. Further, no studies have evaluated the bone status of children and adolescents with ANRED using broadband ultrasound attenuation. Pre-pubertal children with ANRED may be most at risk of osteoporosis, because as has been demonstrated in the section on the effect of exercise on peak bone mass, the greatest opportunity to make an impact on bone density is in the period prior to puberty. It is therefore reasonable to assume that if the largest positive impact on bone density occurs in the pre-pubertal years then also the largest negative impact may occur at this stage.

Dual energy X-ray absorptiometry is frequently used to measure bone mineral density. Unfortunately one of the problems when using this technique in a malnourished population such as those with anorexia nervosa is the difficulty in differentiating bone edge from soft tissue [MOLG1997]. This raises a risk of inaccuracy in bone mineral density measurements. Furthermore this problem is exacerbated when examining children with ANRED because of their small stature and low weight.

The following section is divided into 6 key factors which impact on osteoporosis in patients with anorexia nervosa: exercise, weight, oestrogen, duration of anorexia nervosa, age and previous history of anorexia nervosa. It is however recognised that reduced bone mineral density in patients with anorexia nervosa depends on the varying interactions between these contributing factors.

The impact of exercise

There is a lack of consensus on the effects of exercise in patients with anorexia nervosa. In Rigotti's study [RIGO1984] of 18 women with anorexia nervosa and amenorrhoea for one year there were 5 who undertook intense physical activity at least three times a week and they demonstrated a higher bone mineral density than the rest of the group. Interestingly Bachrach [BACH1990] also measured bone mineral density and exercise patterns in adolescents with anorexia nervosa. She studied 18 patients aged between 12 and 20 years. In this group she noted a significantly reduced bone mineral density in patients with anorexia nervosa when compared to a control group but found that the degree of exercise undertaken by the patients had no influence on bone density. Young [YOUN1994] examined the BMD of 18 female anorexia nervosa patients with a mean age of 18.1 years and compared it to that of 44 female ballerinas. The BMD of the ballerinas was higher than that of the anorexia nervosa patients and they hypothesised that the BMD of the ballerinas had been preserved by weight-bearing exercise. In a study by Siemers et al [SIEM1996] a greater proportion of women with anorexia nervosa than control women reported undertaking high levels of physical activity (40% versus 10%). In the anorexia nervosa group the proportion undertaking high levels of physical activity increased to 70% after the onset of the eating disorder. However the bone density of the anorexia nervosa group remained significantly lower than controls ($p < 0.001$).

The impact of weight

Weight loss is a key factor in anorexia nervosa and many studies have demonstrated a correlation between weight loss and reduced bone mineral density. All 18 women in Rigotti's study [RIGO1984] had at least 25% weight loss and their cortical bone density was significantly lower than a normal control group. In addition 2 patients suffered vertebral fractures. Weight reduction in excess of 15% for age and size was

associated with a marked reduction in lumbar spine bone mineral density in Herzog's study [HERZ1993] giving a mean Z score of -2.18 for a group of 51 patients with a past history of anorexia nervosa. Brookes et al [BROO1998] scanned 36 women an average of 11.4 years after the initial diagnosis of anorexia nervosa and demonstrated that 50% of the women with a past history of anorexia nervosa had osteopenia and 35% had osteoporosis despite regaining weight to within 90% of ideal body weight. Siemers et al [SIEM1996] found that total body bone mineral density decreased with decreasing body mass index in their cohort of anorexia nervosa patients, however the same relationship was not seen when only lumbar spine bone mineral density was examined. This may be an anomalous result caused, as suggested by Molgaard et al [MOLG1997], by the difficulty in differentiating the edge of bone in lumbar spine DXA examinations and obtaining accurate bone mineral density measurements in such malnourished patients.

The impact of oestrogen

The effect of a reduction in circulating oestrogens on bone mineral density in females with anorexia nervosa has been examined in a number of studies. All but one of the 26 females in the study by Biller et al [BILL1989] had secondary amenorrhoea and spinal BMD was noted to be 2 standard deviations below age-matched normals in 50% of these women. They also noted that the longer the duration of amenorrhoea the more severe was the osteoporosis. Treasure et al [TREA1987] measured the bone mineral density in 45 patients with active anorexia nervosa aged between 14 and 54 years and in 25 patients aged between 23 and 52 years who had recovered from anorexia nervosa. They noted that 20 females with 6 years or more of amenorrhoea had a BMD of the femoral neck which was 2 standard deviations below the mean. Prior [PRIO1990] performed two bone density scans one year apart and reported that a reduced bone mineral density was associated with irregular menstrual cycles. In the study of 18 women with anorexia nervosa by Poet et al [POET1993] they found that there was a significant negative correlation between BMD and duration of amenorrhoea.

Young [YOUN1994] examined 18 female anorexia nervosa patients with a mean age of 18.1 years and compared them to 44 female ballerinas. Patients in both groups either had a complete absence of menstrual cycles or had intermittent menses. The

BMD of the patients with anorexia nervosa was lower than that of the ballerinas. Brooks et al [BROO1998] also noted that the lumbar spine bone mineral content was significantly correlated with the total years of oestrogen exposure calculated as [(current age of subject – age at menarche) – years of amenorrhoea]. This correlation, although statistically significant at $p < 0.002$ has a relatively poor correlation of only $r = 0.50$, giving an r^2 coefficient of determination of 0.25 which indicates that 75% of the variance in bone mineral content is due to factors other than years of oestrogen exposure. Compared to this the correlation of bone mineral density with years of oestrogen exposure was even lower at 0.34 ($p < 0.05$).

One of the most interesting studies on the relationship between oestrogen exposure and bone mineral density was published by Grinspoon et al [GRIN1999]. They compared the bone mineral density of 30 women with anorexia nervosa with 19 women with hypothalamic amenorrhoea to evaluate the impact of oestrogen withdrawal and nutrition. The groups were well matched both having a mean age of 24 years and there was no significant difference in terms of lifetime duration of amenorrhoea, age of menarche, prior use of oestrogen or exercise. There was however a significant difference in bone mineral density between the two groups ($p < 0.0001$) with 40% of anorexia nervosa patients having bone mineral density t-scores of the spine of -2 or lower compared to 16% of those with hypothalamic amenorrhoea. The authors conclude that this suggests that bone mineral density is crucially dependent on both nutritional factors and oestrogen deficiency in women with anorexia nervosa.

The impact of the duration of the eating disorder

Treasure et al [TREA1987] demonstrated that the severity of the reduction in BMD was related to the length of the eating disorder and notably that 20 females with 6 years or more of amenorrhoea had a BMD 2 standard deviations below the mean. In comparison Poet et al [POET1993] could not identify a significant relationship between the duration of the eating disorder and reduced bone mineral density, although a significant relationship was noted between years of amenorrhoea and reduced bone mineral density. Hergenroeder [HERG1995] concluded that the longer the anorexia nervosa persisted the less likely it was that the bone mineral density would return to normal.

The impact of age at onset of the eating disorder

Few studies have examined the relationship between reduced bone mineral density and whether it is affected by the age at which the eating disorder commenced. Bachrach et al [BACH1991] measured total body BMD in 15 adolescents with anorexia nervosa and noted that 8 of those 15 had BMD Z scores of -2 or lower after one year of treatment for the eating disorder. Only 7 adolescent females aged between 13 and 20 years were examined in Abram's [ABRA1993] study and they all had a reduced bone mineral density for their age. Biller et al [BILL1989] could not find a statistically significant reduction in BMD in seven adolescents with anorexia nervosa. The authors concluded that this was probably related to the short duration of amenorrhoea in the group along with the small number of females studied. It may however be related to the post-pubertal onset of the eating disorder for the majority of the group and the short duration of the eating disorder. As has already been discussed peak bone mass tends to be attained approximately two years post menarche. Therefore the females in Biller's study [IBID] may have already been approaching their peak bone mass when they developed the eating disorder thus the impact of the eating disorder is minimised. However when the onset of anorexia nervosa is prior to the attainment of menarche there may be a more significant reduction in BMD as was seen in the one female in Biller's group with primary amenorrhoea.

Previous history of anorexia nervosa

Treasure et al [TREA1987] demonstrated that bone mineral density was within normal limits in 25 women who had recovered from anorexia nervosa. Brookes et al [BROO1998] demonstrated that 50% of their cohort of 36 women with a past history of anorexia nervosa in adolescence had osteopenia and 35% had osteoporosis when they were scanned an average of 11.4 years after the initial diagnosis of the eating disorder. Herzog [HERZ1993] measured bone mineral density using dual photon absorptiometry on 51 female patients with a past history of anorexia nervosa. After an average of 11.7 years following the first admission for anorexia nervosa, five patients with a poor outcome (pathologic menstrual state and a weight reduction in excess of 15% less than that for age and size) showed a marked reduction in lumbar bone mineral density (mean Z score -2.18). Eighteen showed a mild reduction in bone mineral density (mean Z score -0.54) and 28 with a good outcome (regular

menstruation and a weight within 15% of that expected for age and size) demonstrated a minimal deviation of bone mineral density (mean Z score -0.26). From these results one can conclude that those patients who suffer the worst long-term outcome from anorexia nervosa in terms of body weight and abnormal menstruation are likely to have the lowest bone mineral density.

3.3 Summary

There are a variety of eating disorders in childhood which are associated with a refusal to adhere to normal eating patterns. The diagnosis of the eating disorder is dependent upon which of a variety of criteria are used although the DSM-IV criteria from the American Psychiatric Association are the most widely accepted internationally, particularly for anorexia nervosa and bulimia nervosa.

The incidence of anorexia nervosa in children is variable and prevalence rates of less than 0.5% have been reported.

The causes of childhood-onset ANRED are multi-factorial and inter-related, but predisposing features include traumatic life events; pre-morbid teasing for obesity; sensitivity to the onset of puberty and a disturbed mother-daughter relationship. However, since these eating disorders are multi-factorial in origin they should be considered from the wider biopsychosocial perspective.

Likewise the causes of osteopenia and osteoporosis in patients with anorexia nervosa are multi-factorial and inter-related. However the key factors seen in patients with anorexia nervosa which puts them at risk of osteopenia or osteoporosis are:

- deficiencies of oestrogen, insulin-like growth factor-1 and calcium;
- increases in cortisol;
- increases and occasionally decreases in Growth Hormone;
- reduced weight and therefore reduced muscular strain on bone;
- longevity of the eating disorder.

Patients with anorexia nervosa who report undertaking increased amounts of exercise have a higher bone mineral density than those who undertake less exercise, although they have a lower bone mineral density than controls who undertake less exercise.

Therefore, for anorexia nervosa patients, exercise appears to have a positive effect on bone mineral density. However it must be recognised that exercise is only one of a range of factors as discussed in this chapter which have an effect on bone mineral density.

Precise measures of the incidence of osteopenia and osteoporosis in patients with anorexia nervosa is difficult because of the problem in accurately differentiating bone edge from soft tissue using DXA techniques in malnourished patients. This is particularly interesting because the reduced weight seen in patients with anorexia nervosa is reported to have a significant correlation with reduced bone mineral density in many studies.

Although some studies mention one or two examples of pre-pubertal females with anorexia nervosa who have a reduced bone mineral density the incidence of osteopenia and osteoporosis in pre-pubertal children with anorexia nervosa and related eating disorders has not been fully evaluated.

Chapter 4 Diagnosing osteoporosis

Most methods of assessing osteoporosis use ionising radiation: radiographic absorptiometry (RA); single photon absorptiometry (SPA); single X-ray absorptiometry (SXA); dual photon absorptiometry (DPA); dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT). In all of these techniques the higher the proportion of calcium and phosphate (hydroxyapatite) within the matrix of bone in the area under investigation the more the beam of ionising radiation will be attenuated [KOVA1996]. Conversely when the inorganic component of bone is reduced there is a reduction in attenuation of the beam of ionising radiation traversing that tissue which results in the characteristic radiographic appearances of osteopenia and osteoporosis.

Two non-ionising diagnostic methods of assessing bone strength and integrity are quantitative magnetic resonance (QMR) and quantitative ultrasound (QUS). These two techniques are not in widespread clinical use in the adult population and little work has been done on their use in paediatrics. As they obviate the need for ionising radiation they offer a particularly useful technique for clinicians to use in paediatric populations provided their efficacy can be evaluated and confirmed. QUS is preferable to QMR because the capital cost is significantly less, in addition, ultrasound machines are portable and relatively easy to use, with markedly lower running costs. It is clear that as the techniques available to diagnose reduced bone mineral density are many they will vary between centres and some centres will have more than one device to assess osteoporosis. This proliferation of diagnostic devices to detect and monitor osteoporosis was part of the impetus that led the Department of Health to set up an Advisory Group on Osteoporosis (AGO) in 1993. The remit of the group included an evaluation of the then “current” imaging techniques and treatments available for osteoporosis in the United Kingdom to culminate in a set of recommendations of best practice. AGO published their final report in 1994 [DOH1994] and one of the recommendations was that osteoporosis services should be made available to high risk groups of patients. AGO also called on the need for further research into the utility of QUS.

4.1 ***Ionising radiation techniques***

Radiographic Absorptiometry

In 1939 Mack et al [MACK1939] described a reproducible method of measuring the density of bones and teeth from radiographs. Radiographic absorptiometry is a technique based on a comparison of the difference in attenuation of a beam of ionising radiation by bone and an aluminum step wedge when irradiated simultaneously. The aluminium step wedge, calibrated to known density values, allows the observer to calculate the relative phalangeal bone mineral density [MORG1967]. Although the technique is relatively inexpensive and easy to use it does rely on the subjective placement of a densitometer over the region of interest which limits its diagnostic reproducibility. It could also be argued that the assessment of phalangeal bone is not adequately representative of the bone mineral density at skeletal locations which are composed of predominantly trabecular bone, for example the lumbar spine. The vertebrae are reported by some to contain between 60% and 70% trabecular bone [VOGE1987], whilst others are more generous suggesting that they contain between 66% and 90% trabecular bone [EINH1996]. This is an important consideration when the aim of the radiological examination is to assess the fracture risk of a bone high in trabeculae.

Single Photon Absorptiometry (SPA)

Initially described in 1963 [CAME1963], SPA depends on the attenuation by bone of a beam of gamma rays emitted by a radioisotope source. The most common source of the gamma rays is Iodine¹²⁵ which has a photon energy of 27.5 kilo electron Volts (keV) and a half life ($T_{1/2}$) of 60 days [GUGL1995]. As the time taken for half of the nuclei present in the Iodine¹²⁵ source to disintegrate is only 60 days it is clear that maintaining the Iodine¹²⁵ source incurs considerable continuous costs as it will need to be replaced several times each year if the efficiency of the SPA examination is to be regulated. The pencil beam of gamma photons produced by the radioisotope and attenuated by bone is detected by a sodium iodide detector with a narrow beam collimator. SPA provides a measure of bone mineral content (BMC) in grammes.centimetre⁻¹ length unit at appendicular skeletal sites such as the distal radius and ulna [LAND1981] and the calcaneum [KLEM1976]. Only appendicular skeletal sites can be examined with SPA as the area under investigation needs to be

immersed in water or wrapped in an alternative tissue equivalent substance to obtain a constant soft tissue thickness overlying the bone. Water may be used as a tissue equivalent material since its density, average atomic number and electron density are almost identical to those of muscle tissue and blood and close to those of fat [MERE1974]. Differential photon absorption of the bone mineral in the path of the beam can then be calculated. The effective dose equivalent (EDE) of an SPA scan is estimated at approximately 1 microSievert (μSv).

Single X-Ray Absorptiometry (SXA)

The physical principles behind the SXA technique are the same as those for SPA, however the use of an X-ray tube to produce the ionising radiation allows faster scanning times. In SXA an X-ray tube with a low voltage generator (40kV) produces an X-ray beam which is filtered using tin to give a beam with a lower energy spectrum comparable to SPA [BORG1995]. The improvement in scanning speed in turn improves the precision of the technique as it alleviates the problem of patient movement during a scan. The technique is also more cost effective as the permanent source of ionising radiation obviates the need to replace a radioisotope source throughout the year.

Dual Photon Absorptiometry (DPA)

The low energy levels of the ionising radiation used in the SPA and SXA techniques limits their ability to examine anything other than superficial skeletal sites such as the appendicular skeleton. In addition the techniques necessitate the use of a water bath or other tissue equivalent material. The development of a dual energy method of assessing bone mineral content obviates the need for a water bath and allows measurement of axial skeletal sites, such as the lumbar spine and proximal femur. A radioisotope with a dual energy, such as Gadolinium¹⁵³ which has two energy peaks of 44 and 100keV is typically used [GUGL1995]. The lower energy peak of 44keV is attenuated more by soft tissue than the higher energy peak of 100keV, but both are attenuated more by mineral in bone than by soft tissue. Thus a calculation of the relative difference in attenuation values between soft tissue and mineral and between the low and the high energy peaks allows the mineral content of the bone under examination to be assessed.

Dual Energy X-ray Absorptiometry (DXA)

In 1987 the first commercial X-ray system was developed which could produce X-ray beams of two energy peaks able to replace DPA [STEI1987]. The method of production of the two energy peaks of the X-ray beam depends on the manufacturer of the equipment but values of 70kVp and 140kVp may be produced with alternating pulses or filtered from an X-ray spectrum.

The effective dose equivalent often quoted for a DXA scan is between 1 and 3 microSieverts, which is considerably lower than the 60 microSieverts cited [GUGL1997] for a typical quantitative computed tomography (QCT) spinal scan. Recently however, as a result of the development of new generation fan beam DXA devices, the inherent radiation dose of a DXA scan has increased and values similar to those received during a QCT scan are now being quoted. In a recent study [STEE1998] the effective dose equivalent for an AP lumbar spine scan on a Lunar Expert-XL fan beam densitometer was reported as 59 microSieverts, whilst for a total body scan it was higher at 75 microSieverts. In the recent past to a certain extent advocates of the DXA technique used the lower radiation dose as a marketing tool. This met with considerable success, as dual energy X-ray absorptiometry is the most common osteoporosis assessment device used in the United Kingdom with approximately four available per million population [EURO1998]. The risk of increased exposure by the fan beam is balanced against the benefit of the improved resolution it allows.

The bone mineral content is expressed in grammes but DXA software programmes also calculate a measurement of bone density described as **areal** bone mineral density in grammes.centrimetre⁻². As true density is based on a volumetric measurement the value of the two dimensional density measurement calculated by DXA needs to be assessed carefully. The two dimensional measurement is calculated because the true volume of bone cannot be accurately measured using the DXA technique. A volume based on the bone's area is therefore predicted and the density calculated using this value.

The technique of DPA is now less common having been superseded by DXA. As with SXA and SPA the physical principles behind the X-ray technique of DXA are based on those of the radioisotope technique.

Quantitative Computed Tomography (QCT)

QCT can be performed in single energy or dual energy mode. Each QCT scan examines both the patient and a reference phantom concurrently. The reference phantom is composed of various concentrations of solid hydroxyapatite providing a series of linear attenuation coefficients comparable to a range of values between normal and osteoporotic bone. The region of interest (ROI) in the central portion of the trabecular rich vertebral body is selected and a CT number is allocated to it. CT numbers are measured in Hounsfield units (HU) where water represents zero HU and air -1000 HU [GUGL1997]. A comparison between the HU of the phantom and bone enables bone mineral density in $\text{grammes.centimetre}^{-3}$ to be calculated. This simultaneous scanning of phantom and body tissue allows for the instabilities of the scanner and the effect of variable beam hardening and patient body habitus [GUGL1997].

Since QCT evolved in the late 1970s [GENA1977] it has been used in many centres in preference to other densitometry techniques. This is for three key reasons, the first and possibly most pragmatic reason is logistical, namely that many centres already possess a Computed Tomography (CT) scanner for imaging and diagnostic purposes and so the technique is deemed more cost effective. The second and clinically more important reason is the inherent ability of the CT technique to be able to differentiate between trabecular and cortical bone. This is an important consideration as trabecular bone is metabolically more active and is therefore most likely to show the earliest signs of the impact of osteoporosis. The third reason is also clinical in that QCT is reported to be the only technique that can measure a true bone mineral density as it is capable of accurately measuring, rather than merely estimating bone volume. One of the disadvantages of QCT is that it carries a moderately higher radiation burden than most of the other ionising radiation methods of assessing bone mineral density. An EDE of approximately $60 \mu\text{Sv}$ including $30 \mu\text{Sv}$ for the location image is typical [GUGL1997].

Peripheral QCT (pQCT)

Ruegsegger et al [RUEG1976] developed the first computed tomography system to determine the bone mineral density of parts of the peripheral skeleton specifically the radius and ulna and from this development the term pQCT was derived. The original system used a radioisotope source of Iodine¹²⁵ and a sodium iodide crystal detector. Subsequent developments included replacing the radioisotope source with an X-ray tube and using a fanbeam of radiation, allowing the procedure time to be reduced minimising movement artifact. The EDE of pQCT is 0.03 μ Sv.

4.2 Non-ionising radiation techniques

Although great importance is placed on the quantity of mineral in bone as a predictor of fracture risk it is essential to appreciate that such a risk, if it is to be accurate, must be calculated by assessing other contributing factors, such as the propensity to fall and the strength of bone. The strength of bone may be evaluated by measuring its mineral density, modulus of elasticity and trabecular structure. Advocates of quantitative ultrasound and quantitative magnetic resonance would argue that elasticity and trabecular anisotropy are the most important contributing factors as they structurally effect the strength of a bone to resist fracture. The example from Einhorn [EINH1992] supports this statement as it clearly demonstrates the difficulties inherent in extrapolating predictors of fracture risk from bone mineral content/density values. He cites the case of a patient with osteopetrosis who having a bone mineral density eleven standard deviations higher than age matched controls nevertheless had brittle bones. Osteopetrotic bones show little or no elastic properties when loaded and so fracture easily. Clearly it is of little value to know the bone mineral density in this instance as it tells nothing of the structure of the bone itself and therefore its propensity to fracture.

Quantitative Magnetic Resonance (QMR)

QMR is a comparatively recent development, being applied only since the early 1990s and then limited to isolated centres where it tends to be used predominantly as a research tool. This is possibly related to the high capital cost of magnetic resonance scanners along with their relatively low prevalence per head of the population particularly in the United Kingdom. Bone strength is predicted with QMR by evaluating the various aspects of trabecular structure, viz., trabecular

thickness, orientation and distribution. Spin echo and gradient echo techniques are used. Spin echo sequences use a 180° radiofrequency pulse to obtain an echo signal whereas in a gradient echo sequence the magnetic field gradient is reversed [MAJU1997]. The production of the image relies on the significant difference in quantity of hydrogen protons in marrow and bone.

4.3 Quantitative ultrasound (QUS)

In addition to QMR quantitative ultrasound is a diagnostic technique which does not use ionising radiation and can be used to assess osteoporosis. Quantitative ultrasound is a particularly useful technique to use on children because it has no deleterious effects on human tissue at the intensities and frequencies used. It therefore has no risk in terms of radiation dose to either the patient or the health care professional performing the scan. The first report of the application of ultrasound techniques to assess bone structure and integrity was in 1958 by researchers who were able to quantitatively evaluate the porosity of bone [ANAS1958] by measuring the speed of 20 kiloHertz longitudinal ultrasound waves across a fracture site and comparing it to normal bone. Further research into the utility of ultrasound as a technique for assessing the quality of bone was superceded by interest in single photon absorptiometry as a method of measuring bone mineral content until, in 1984, Langton [LANG1984] developed the technique of broadband ultrasound attenuation (BUA). In the relatively short time since 1984 the number of commercial QUS machines now available has reached double figures and table 8 demonstrates just a selection of them.

QUS machines are relatively cheap to purchase with a typical cost in the region of £10,000 - £15,000. In comparison whole body DXA machines vary between approximately £50,000 and £70,000 and peripheral QCT and DXA devices between £15,000 and £30,000. Most of the ultrasound machines are also portable, and for example the McCue CUBA Clinical used in this research study weighs just 10 kg. An ultrasound scan is typically very quick to perform varying from approximately 2 seconds (Norland Paris Bone Densitometer) to several minutes.

Table 8 Quantitative ultrasound bone analysis machines

Machine	Coupling medium	Anatomical region measured	Parameter	Imaging system
Achilles+ Ultrasound Densitometer Lunar	Water	Calcaneum	BUA, SOS, stiffness index	No
CUBA Clinical McCue PLC	Gel	Calcaneum	BUA, VOS	No
DBM Sonic 1200 IGEA	Gel	Phalanges	SOS	No
DTU-one Osteometer	Water	Calcaneum	BUA, SOS	Yes
Norland Paris Ultrasound Bone Densitometer	Gel	Calcaneum	BUA, VOS	No
Omnisense Sunlight ultrasound technologies	Gel	Multiple sites, including patella, tibia, calcaneum, radius, ulna etc	SOS	No
QUS-2 Ultrasound Bone Densitometer	Gel	Calcaneum	BUA, ultrasound bone index	No
Sahara Clinical Bone Sonometer Hologic	Gel	Calcaneum	BUA, SOS	No
Soundscan Compact Myriad	Gel	Tibia	SOS	No
UBA 575X Walker Sonix	Water	Calcaneum	BUA, VOS	No
UBIS 3000 Diagnostic Medical Services	Water	Calcaneum	BUA, SOS	Yes

As can be seen in table 8 QUS machines differ in five main ways.

- The type of coupling agent used - i.e. whether gel or water is used to allow transmission of the ultrasound beam into the region of interest.
- The ultrasound parameter measured, i.e., BUA, SOS, VOS, or a combination of the two to provide an additional parameter such as stiffness index.
- The choice of anatomical area under investigation. Although as can be seen from the table the most common region investigated is the calcaneum.
- The manner in which the ultrasound measurement site is located. The majority of QUS scans are performed using a blind* technique, although at least two machines produce an ultrasound image of the area under investigation to facilitate the selection of a reproducible region of interest on the calcaneum.
- The method of obtaining the ultrasound parameters i.e. whether by transmission, reflection or refraction of the ultrasound beam through the bone under investigation.

There are two recent innovations in quantitative ultrasound equipment. The first is the development of a method of identifying the region of interest on the bone under examination by using a crude ultrasound image, for example in the UBIS 3000 and the DTU-one osteometer. This development should improve the precision of the quantitative ultrasound procedure, as a blind technique is not required. The second development is the DBM Sonic by IGEA, which measures amplitude dependent speed of sound through the phalanges. Both of these techniques are reported to be particularly useful in paediatrics [GLUE1999], because they allow more accurate measurement of the region of interest with improved precision.

The nature of ultrasound

An ultrasound wave is a mechanical pressure wave caused by the movement of particles which constitute a medium. The ultrasound wave is the medium itself in a state of motion. That motion leads to the transfer of energy through the medium and is caused by the particles in the medium moving either parallel (longitudinal waves) or perpendicular (transverse/shear waves) to the direction of the wave and transferring energy from one particle to the next as they move. This movement of

* A "blind" technique is one whereby the location of the region of interest is selected by positioning the area under investigation according to external landmarks on the surface of the limb.

energy from particle to particle happens at a microscopic level so the material through which the beam traverses remains static to the naked eye. The QUS techniques rely on the use of longitudinal waves, although bone is one of the few organic materials that can support transverse waves because of its low compressibility.

The piezoelectric effect

A substance that acts as a transducer by converting a voltage into a mechanical vibration and vice versa is exhibiting the piezoelectric effect. If a piezoelectric substance is in contact with a medium when the voltage is applied it will cause a change of pressure in that medium initiating a pressure wave within it. This also works in the reverse, so that if a pressure wave from the medium strikes the piezoelectric element it will cause the element to deform causing a change in the potential difference across the element, which can then be displayed on a cathode ray oscilloscope. The ability of a substance to exhibit this piezoelectric effect makes it an ideal choice as a transducer for ultrasound purposes.

In QUS the piezoelectric crystal undergoes a process known as shock-excitation. This involves the administration of a short (several nanoseconds), high voltage (300-400 volts) pulse to the crystal which causes it to vibrate for several microseconds. The resultant pulse of ultrasound which contains a range of frequencies is then transmitted through the medium under investigation.

Continuous and pulsed waves

In ideal circumstances a continuous sound wave has a single frequency whereas a pulsed wave contains a range of frequencies. In QUS the ultrasound transducer has a low nominal frequency of typically 1MHz which produces a short pulse of ultrasound with a wide bandwidth. The bandwidth of the transducer is calculated by evaluating the full width of the frequency spectrum at half the maximum amplitude of the frequency.

Broadband ultrasound attenuation (BUA)

Langton et al [LANG1984] were the first group to report the value of BUA as a measure of the state of osteoporotic bone, and to recommend it as a suitable replacement for bone mineral content assessment. The technique is based on scientific empirical observations of the difference in attenuation of a broadband beam of ultrasound when traversing the bone of healthy women compared to those with a history of fracture. However there is little evidence of theoretical physics to underpin the technique.

BUA is calculated by measuring the reduction in the intensity of a pulse of ultrasound after it has traversed a sample of bone such as the calcaneum. Although ultrasound intensity is usually measured in mW.cm^{-2} , the reduction in intensity is measured in decibels (dB).

$$\text{Relative Intensity} = 10 \log_{10}(I_o / I_t) \text{ dB} \qquad \text{Equation 1}$$

where I_o = original intensity and I_t = transmitted intensity

In diagnostic clinical ultrasound it is conventional to define the attenuation coefficient in terms of signal amplitude. Equation 1 can therefore be amended to reflect this.

$$\text{Intensity} = \text{Amplitude}^2 \qquad \text{Equation 2}$$

Therefore

$$\text{Relative Intensity} = 20 \log_{10}(A_o / A_t) \text{ dB} \qquad \text{Equation 3}$$

where A_o = original amplitude and A_t = transmitted amplitude

BUA is calculated by comparing the variation in amplitude of the frequency spectrum through a reference material (normally water) with that received when the pulse is transmitted through a specific tissue (for example the calcaneum) [LANG1990]. The difference in attenuation between bone and the reference sample is calculated logarithmically as shown in equation 4.

$$\text{Attenuation} = 20 \log_{10}(A_w/A_b) \text{ dB} \quad \text{Equation 4}$$

where A_w = amplitude through water and A_b = amplitude through bone

In the CUBA system used in this study the variation in amplitude is measured temporally and a fast Fourier transform is used to convert this to a frequency spectrum of the transmitted received pulse [LANG1996]. Spectral analysis of the difference between the known and unknown materials allows a logarithmic assessment of the difference between the two ranges of values. This will provide a linear regression slope of attenuation in decibels (dB) on frequency when values between 200 and 600 kHz are considered. In healthy bone attenuation of the ultrasound beam will increase with increasing ultrasound frequency. The gradient of the resultant plot (the difference in decibels divided by the difference in frequency) provides a measure of BUA in dB.MHz^{-1} . The region between 200 and 600kHz tends to be used in clinical practice [TRUS1998] because in young, healthy adults non-linear effects are seen in the ultrasound wave beyond approximately 600kHz [LANG1994].

BUA is based on a measurement of the reduction in amplitude of a pulse of ultrasound as it traverses a medium such as bone. The reduction in amplitude of the pulse of ultrasound is caused by scattering (reflection and refraction) and absorption of the pulse by the medium through which it traverses. In vitro research has demonstrated that BUA is a good indicator of mineral loss in bone [TAVA1991]. It has been suggested that absorption is dependent upon the calcium content of bone and scattering is dependent on trabecular structure [DALY1997]. Ideally a wide rather than a narrow ultrasound beam is preferable to use so that more trabeculae are examined. BUA therefore provides information about density as well as structure of bone, both of which contribute to the strength of a bone and its resistance to fracture.

McCue contact ultrasound bone analyser (CUBA)

In the CUBA two 12.5mm unfocussed ultrasound transducers are mounted co-axially and placed on either side of the tissue under investigation. One transducer transmits the ultrasound wave and the other receives it. Not all quantitative ultrasound machines take account of variations in foot size which is a particularly important

consideration for children. Rather, as in the Lunar Achilles+ a standard heel width is assumed. However, the CUBA measures the width of the foot for every scan and includes this in the algorithm to measure BUA. Although the CUBA system does not use a water bath as a coupling medium for measurements of the calcaneum the reference trace is obtained through a water bath during manufacture of the equipment and stored in the software so that future calculations of BUA in vivo are based on it. The received signal through the calcaneum is compared to the signal received through water and values for BUA are calculated as described previously.

Velocity and speed of ultrasound (VOS & SOS)

The velocity of sound (VOS) or speed of sound (SOS) through bone is related to the elasticity of the bone, and therefore its strength. Both are measured in units of metres.second⁻¹.

Commercial QUS machines measure either VOS or SOS through tissue and the terms are often used interchangeably. The speed of sound through a tissue can be calculated if the distance through which the ultrasound pulse has travelled and the time taken for the pulse to travel that distance are both known.

$$c = d/t \qquad \text{Equation 5}$$

where c= speed of sound, d = distance and t = time

To calculate SOS accurately it is necessary to measure the propagated wave at the same crossing point through both water and tissue. Some manufacturers use the first zero crossing point of the negative slope of the received signal whereas others use the second or third [STRE1996].

The speed of sound (c), wavelength (λ) and frequency (f) are fundamentally related as shown in equation 6.

$$c = f\lambda \qquad \text{Equation 6}$$

In 1981 Greenfield [GREE1981] commented that many of the densitometric techniques used at that time were limiting in the utility of the information which they

were able to provide about osteoporosis. He stressed the importance of measuring organic as well as inorganic components of bone as together they contribute to bone strength and fracture resistance. The risk of bone fracturing depends on both its density as well as its elasticity (equation 7). In his study of the radial cortex, Greenfield advocated the use of BMC and speed of sound combined to calculate the modulus of elasticity in Newtons.metre⁻². Elasticity can then be used to calculate the relative fracture risk. Fifteen years later Njeh et al [NJEH1996] came to the same conclusion. They measured bovine cancellous bone in vitro. Density was assessed by QCT and combined with the speed of sound to provide a measure of the elasticity of bone. This they opined would be a better indicator of “bone fragility” than density alone.

$$E=\rho c^2$$

Equation 7

Where E=modulus of elasticity, c= speed of sound and ρ =the density of bone.

Selecting the measurement site

Normally the bone most at risk of fracture would be considered the most appropriate to measure. In adults the femoral neck is the site most likely to fracture whereas in children and adolescents the long bones are more likely to fracture. However, a more important consideration with children is to use a technique which is not harmful to developing bone and tissue, particularly if they are likely to have repeated examinations throughout their life. Quantitative ultrasound (QUS) of the calcaneum is an appropriate technique to use as a preliminary diagnostic tool in paediatrics because of its non-invasive nature and because it has equivalent predictive capabilities for hip fracture as femoral neck bone mineral density [HANS1996].

The nature of ultrasound is such that the source of the ultrasound pulse must either be in direct contact with the tissue to be examined or there must be an appropriate coupling medium such as water or ultrasonic gel to provide the contact. Ultrasound waves are strongly attenuated by air so for example after traversing the gas-filled bowel in the abdomen insufficient energy remains in the ultrasound wave to provide useful diagnostic information about the vertebrae. Examining the vertebrae from the posterior aspect would also be difficult because of attenuation of the beam by overlying tissues viz., quadratus lumborum, psoas muscle, skin, fat, vertebral spinous

processes and the spinal cord. The calcaneum however is an acceptable alternative as it has comparable trabecular content to vertebrae and in addition is a weight bearing site and is readily accessible with ultrasound.

To date there have been many studies discussing the appropriateness of QUS of the calcaneum as a measure of osteoporotic fracture of the vertebrae or femoral neck and correlation coefficients between QUS and other densitometric parameters have been calculated. The paediatric studies are shown in table 9.

Table 9 Paediatric correlation coefficients between QUS and DXA

Reference	Ultrasound machine		Region	BMD machine	Region	BUA r	SOS r	n
JAWO1995	Achilles	W	Heel	Lunar DPX	Heel	0.83	0.67	28
JAWO1995	Achilles	W	Heel	Lunar DPX	L2-L4	0.83	0.67	40
JAWO1995	Achilles	W	Heel	Lunar DPX	TBBMD	0.8	0.67	89
MUGH1996	CUBA	C	Heel	Hologic QDR-1000	TBBMD	0.74	NM	58
SUND1998	Achilles+	W	Heel	Lunar DPX-L	TBBMD	0.73	0.64	280
SUND1998	Achilles+	W	Heel	Lunar DPX-L	L1-L4	0.68	0.56	280
SUND1998	Achilles+	W	Heel	Lunar DPX-L	FN	0.56	0.55	280

C – contact technique; W – waterbath technique; NM – not measured;
 FN – femoral neck; TBBMD – total body bone mineral density;
 L1/L2-L4 – lumbar vertebra 1 or 2 to lumbar vertebra 4;
 BUAr and SOSr – BUA or SOS correlation coefficient; n – number in the study

Reported correlation values for DXA and BUA techniques typically extend from 0.32 - 0.87 ([BARA1988], [BARA1991], [AGRE1991], [TRUS1992], [LEES1993], [MASS1993], [SCHO1993], [YOUN1993], [FAUL1994] [MORI1995], [ARDE1996], [CUNN1996], [MART1996], [ROSE1996]). In these studies a variety of ultrasound machines using either direct contact or water bath techniques have been used and have measured a variety of anatomical regions including the tibia, phalanges and calcaneum. Correlation plots have been used to compare these ultrasound measurements with bone mineral density measured at a variety of different anatomical locations and using a variety of machines. Researchers have also published correlation coefficients for speed of sound and bone mineral density and values range from 0.31 [ROSE1996], 0.53 [AGUA1996] to 0.73 [CUNN1996].

Few studies have directly compared the same anatomical region with both BUA and DXA. Correlations of the calcaneum using these two techniques range from 0.44 [CUNN1996], 0.73 [WAUD1992], 0.79 [LANG2000], 0.81 [DIES2000] to 0.83

[JAWO1995]. In 1992 Gluer et al [GLUE1992] performed site matched correlation studies on the calcaneum comparing BUA and DXA measurements. They observed a correlation of 0.7 giving a coefficient of determination of 0.49 which leaves 51% of the variability between the two measurements unaccounted for and they argue that this unexplained variability may be related to additional information on bone strength and structure which the ultrasound technique provides. Langton & Langton [LANG2000] also performed site matched calcaneal BUA and BMD measurements and reported a correlation of 0.79 and a coefficient of determination of 0.63. They explain that this high correlation arises from the “exacting protocol for site-matched region of interest positioning” which was used in their study.

Correlation plots to assess the extent of agreement between two methods of clinical measurement can be misleading in some circumstances and it may be more appropriate to use the Bland-Altman method [BLAN1986]. Using this statistical technique the mean difference between two sets of measurements and the standard deviation of these differences is calculated. If the range of differences approximates a normal distribution 95% of the differences will lie within 2 standard deviations either side of the mean difference (the limits of agreement). If the differences within the limits of agreement are not clinically significant the two measures can be used interchangeably.

Broadband ultrasound attenuation studies in paediatrics

There are four key paediatric normative data studies of broadband ultrasound attenuation. Jaworski [JAWO1995] measured BUA in 89 Polish children; Mughal [MUGH1996] measured BUA in 58 British children and in a subsequent paper reported normal values in 367 children [MUGH1997]. Sundberg [SUND1998] measured BUA and speed of sound in 280 Swedish children.

The first study by Jaworski et al [JAWO1995] was on a sample of Polish children aged between 6 and 13 years using a water bath ultrasound technique (Lunar Achilles). The authors found a good correlation ($r=0.83$) between BUA and DXA measurements at the same location on the calcaneum, and a reasonable correlation of 0.67 between the speed of sound and DXA at the calcaneum in 28 children. The precision of BUA was measured using the coefficient of variation and was 1.5%.

The BUA measurements ranged from a mean of 83 dB.MHz^{-1} at 6 years of age to a mean of 104 dB.MHz^{-1} at 13 years of age. They combined the data of males and females up to 12 years of age because they found no difference in BUA measurements between the sexes in this young age group ($p>0.05$).

Mughal et al [MUGH1996] studied 58 children aged 7-17 years with a dry, contact technique (McCue CUBA) and demonstrated a correlation of 0.74 with DXA. They reported a coefficient of variation precision error of 5% for BUA. In 1997 Mughal et al [MUGH1997] published a second paper based on BUA measurements of a larger paediatric sample of 367 healthy white children aged 6 to 15 years. The precision remained unchanged at 5%. The normative BUA data was calculated separately for boys and girls and was reported in one year age-bands. They found that BUA was significantly correlated with age ($r=0.67$), weight ($r=0.73$) and height ($r=0.71$). The only significant difference between BUA measurements was seen at age 10-11 years and age 12-13 years, when BUA in the male group was significantly higher than that in the female group.

Sundberg [SUND1998] compared BUA using a water-bath technique (Lunar Achilles+) to DXA measurements on 280 children aged between 11 and 16 years. They reported correlations of 0.44 to 0.73 between the two techniques. Interestingly they noted only slight differences in BUA parameters between boys and girls after adjusting for age and BMI and concluded that gender was therefore unimportant in this age group. The precision of the technique was not reported in this study.

4.4 Precision

It is essential to know the precision of a technique to be able to accurately assess the significance of observed changes in repeated measurements on a patient. Precision can simply be measured using the standard deviation (s) of repeated measurements (x) as shown in equation 8, where n is the number of measurements, $n-1$ is the degrees of freedom, x_{ij} is the i th measurement on the j th subject.

$$s = \sqrt{\left(\frac{\sum_i (\chi_{ij} - \bar{\chi}_j)^2}{n-1} \right)} \quad \text{Equation 8}$$

$$\text{where } \bar{\chi}_j = \frac{\sum_i \chi_{ij}}{n}$$

When using standard deviation as a measure of precision error it is important to use the correct number of degrees of freedom. Most computer programmes offer the option of using n or $n-1$ as the denominator in equation 8 and it is therefore easy to underestimate the precision error by calculating the standard deviation using n rather than $n-1$. This has the greatest impact in small sample sizes. Standard deviation can also be measured using the square root of the arithmetic mean of the variances of the subjects in a sample. This is known as the root mean square standard deviation (RMS SD). The root mean square standard deviation takes into consideration the standard deviation of repeated measurements on a number of subjects and is therefore more accurate to use when measuring combined precision error in a variety of subjects. Equation 9 demonstrates the equation for the calculation of RMS SD where j is the j th measurement on n subjects and d is the difference between paired measurements.

$$RMS\ SD = \sqrt{\frac{\sum_j d_j^2}{2n}} \quad \text{Equation 9}$$

Since measures of standard deviation do not inform one of the range of values of the measuring variables being used it is useful to calculate a relative precision error which does take this into account.

Relative precision error

The relative precision error can be assessed using the coefficient of variation (the ratio of the standard deviation of repeated measures to the mean of those measures expressed as a percentage) which provides some indication of the measuring range available with the equipment being used. The coefficient of variation (CV) is the most frequently used measure of relative precision in DXA and QUS techniques although the standardised coefficient of variation is also used. The standardised coefficient of variation (SCV) is a ratio of the standard deviation of repeated measures to the dynamic range of measured values in the system being used, expressed as a percentage.

Gluer et al [GLUE1995] recommend the use of RMS SD rather than the arithmetic mean SD as a measure of precision error. They have demonstrated that using the arithmetic mean of a number of standard deviation measurements underestimates the true error of the measurement. Nevertheless this underestimation will decrease as the number of repeat examinations per subject increases. As can be seen in table 10 where the true precision is known to be 1 the minimum number of degrees of freedom needed to obtain a precision close to 1 with the RMS SD is 27. The degrees of freedom are calculated by subtracting one from the n repeat measurements and multiplying this by the m number of subjects.

Table 10 Assessing precision error using standard deviation

Precision estimate based on			Arithmetic average		RMS average	
m subjects	n repeat examinations	Degrees of freedom	SD	CV	SD	CV
27	2	27	0.797	0.799	0.987	0.990
14	3	28	0.889	0.894	0.995	1.000
9	4	27	0.925	0.929	1.003	0.997

Adapted from Gluer et al, 1995 [GLUE1995]

Ironically, unlike the name, there is no agreed standard method of measuring the standardised coefficient of variation (SCV). The numerator of the equation should be the root mean square standard deviation for the reasons outlined already, although some researchers use the arithmetic mean of several standard deviations. The SCV uses the dynamic range of measurement values rather than their arithmetic mean as the denominator of the equation. Ideally the range used as the denominator when calculating the CV should be from a discrete patient group, for example young

normal, osteoporotic female or osteoporotic male; the SCV is then a fair reflection of the relative precision error of the equipment for that particular patient group.

Relative precision error is used to assess the validity of the difference between two subsequent BUA measurements on a subject, particularly if the subject has a disease or is receiving medication which may alter her/his BUA values.

One of the most common methods of measuring the relative precision error is to calculate the coefficient of variation of the difference between paired measurements using equation 10. Where d is the difference between each pair, n is the number of subjects measured and \bar{x} is the mean of all of the measurements.

$$RMSCV = \left(\frac{\sqrt{\sum_j (d_j)^2 / 2n}}{\bar{x}} \right) \times 100 \quad \text{Equation 10}$$

Equation 10 can be adapted to measure the standardised coefficient of variation by using the range of measurements as the denominator of the equation rather than the mean value.

The minimum change which can be detected and can be recorded as valid can be calculated based on statistical formulae adapted by Gluer et al 1995 [GLUE1995]. The effects of both the significance and the power of the significance should be incorporated into the equation. In clinical work this refers to the significance level which is acceptable for treatment and the power of that significance. The use of appropriate significance levels limits the risk of attaining Type I errors as described at the beginning of chapter 5. If a 10% significance level ($Z_\alpha = 1.28$) is selected at 80% power ($Z_\beta = 0.84$) equation 9 can be adapted to calculate the minimum statistically significant difference necessary between two BUA measures as shown in equation 11 (from Blake et al, page 154 [BLAK1999]).

$$Z_\alpha + Z_\beta = \frac{\Delta BUA\%}{\sqrt{2} \cdot CV} \quad \text{Equation 11}$$

$$\begin{aligned} \text{So } \Delta BUA\% &= (1.28 + 0.84) \cdot \sqrt{2} \cdot CV\% \\ &= 2.12 \cdot \sqrt{2} \cdot CV\% \\ \Delta BUA\% &= 3 \cdot CV\% \end{aligned}$$

From this calculation we can see that it is necessary to have a difference of at least 3 multiples of the relative precision error between scans. Therefore the calculation of precision error is crucial for the use of BUA as a clinical longitudinal monitoring device. In the case of the CUBA Clinical, where the precision error, measured as CV for BUA, in paediatrics is quoted as 5% [MUGH1997], this would suggest that there must be a difference of at least 15% between scans before any clinical significance can be placed on the validity of the difference between two measurements on the same patient. It has been argued that it is more appropriate to use the standard deviation rather than the coefficient of variation as the marker for significant clinical change when BMD measurements are used [RAVA1999]. This is because precision error is independent of the original measurement, therefore an absolute value (standard deviation) rather than a percentage value is a more valid measure to use. The same argument is likely to be true for BUA measurements and this will be examined in chapter 7.

Poor precision can be attributed to a number of causes. The scale which is read by the operator may be liable to inaccuracies; there may be random variability in the measuring device or there may be inaccuracies in reproducibly locating the region of interest to be measured. Poor precision in quantitative ultrasound is mainly attributed to one of two causes. The first is the effect of the interaction of an ultrasound wave with heterogeneous tissue such as trabecular bone which is anisotropic and the second is the effect of positioning problems when selecting a reproducible region of interest to be measured. Strelitzki and Truscott [STRE1998] identified four potential causes of imprecision related to the interaction of an ultrasound wave with heterogeneous tissue.

- Averaging – when parts of the ultrasound wave are attenuated differently in the propagation path.
- Phase cancellation – when parts of the ultrasound wave arrive at different times at the receiver caused either by path length or sound speed fluctuations.
- Diffraction – spreading of the ultrasound wave after it interacts with a boundary such as a trabecular strut.
- Scattering – the interaction of the ultrasound wave with the small trabecular struts causing wave energy to be dispersed in many directions.

This thesis will examine the effects on precision of locating a reproducible region of interest for longitudinal studies in children.

Precision can be measured and the error possibly improved however there is no guarantee that what is being measured is appropriate. That is good precision, particularly in non-imaging QUS techniques, does not ensure validity. Good precision in such techniques does not prove that the correct portion of the calcaneum is being measured or that the same portion of the calcaneum will be measured after a year or even two years of growth. Therefore the precision may be good but the accuracy unknown. This can be explained by using the analogy of a dartboard and a set of three darts. If a darts player aims for the bull's eye of the dartboard and does not hit it, but the three darts thrown come close to it, s/he has good accuracy but poor precision. In comparison a darts player who is also aiming for the bull's eye and misses it completely with each of three darts but consistently hits treble 20 would be described as having good precision, but poor accuracy.

4.5 Summary

There are a wide variety of techniques used to diagnose and assess osteoporosis, the majority of which use ionising radiation. Osteoporosis is assessed using these techniques by calculating how much hydroxyapatite is in the bone under examination and the most widely accepted method of measuring this is dual energy X-ray absorptiometry. There are also two other diagnostic tools which are used to assess osteoporosis and which obviate the need for ionising radiation. They are quantitative magnetic resonance and quantitative ultrasound. Quantitative ultrasound is a relatively inexpensive technique to use and its non-invasive nature makes it a particularly suitable examination for children. These instruments are used to refine and revise treatment strategies for patients and it is therefore important for the clinician to be able to differentiate significant clinical change from inherent errors in the measuring device. Therefore one of the main considerations when using any of these techniques in clinical practice is the precision of the measuring device and its ability to detect significant clinical changes. The recognised standard for significant clinical change is accepted as three multiples of the precision error or relative precision error.

There are a limited number of studies which have reported the utility of measuring broadband ultrasound attenuation through bone in children and all have been cross-sectional rather than longitudinal. The majority however have used the McCue CUBA device, the machine which was selected for this research. The relative precision error of BUA measurements for these ultrasound devices is reported as varying between 1.5% and 5%.

Chapter 5 Research methods

Scientific researchers make inductions or inferences about how things generally behave based on a series of observations. Such scientific quantitative research is evaluated by assuming the null hypothesis and then testing it. That is, the researcher makes a hypothetical assumption that no difference exists between experimental data groups and then tests this assumption. A Type I error occurs when the null hypothesis is falsely rejected when it should be accepted (false positive), whereas a Type II error occurs when the null hypothesis is falsely accepted when it should be rejected (false negative). The significance level of a test is the probability of a Type I error; that is, the probability of falsely rejecting the null hypothesis [SIEG1996].

Descriptive statistics are used to summarise data from a sample and to draw conclusions from the data. A more advanced analysis of a data sample is to make statistical inferences about the population from which a sample is derived. The skill of the researcher lies in quantifying the probability of error in the data analysis so that statistical inferences can be made which are valid and reliable.

5.1 *Ethics*

The ethics of this research study were considered in accordance with the policy of the Local Research Ethics Committee, The Children Act [DOH1989] and the recommendations from the World Medical Association Declaration of Helsinki [WMA1964]. Ethical approval to measure broadband ultrasound attenuation through the calcaneum in children and adolescents aged 5-18 was sought and obtained from the Local Research Ethics Committee.

There were three main ethical issues to consider for the children and adolescents participating in this research study. Firstly the subjects should be free from psychological or physical harm; secondly they should voluntarily participate in the study and finally they should be assured of the anonymity or confidentiality of the results [POLI1985].

Freedom from psychological or physical harm and discomfort

The potential physical as well as psychological trauma to subjects in a research study should be anticipated and avoided. The potential physical harm in this study refers to the use of ultrasound and since there is no evidence to support the notion that quantitative ultrasound of the calcaneum is unsafe to use in human subjects, this risk is eliminated.

Due consideration was also given to the risk of psychological harm in this research study caused by the lifestyle interview. In this interview subjects are questioned about their exercise habits, menstrual status and milk intake. Since some subjects with eating disorders use exercise as a method of controlling weight it was anticipated that valid answers about exercise habits would be difficult to elicit and so due care and attention was paid to this section of the interview. Appropriate consideration was also given to providing adequate privacy for all subjects when removing outer clothing to be weighed. The only people present in the room with the research subject during the investigations and interviews were the researcher and anyone invited by the subject her/himself.

Voluntary participation in the study

All subjects and/or parents were informed of the aims, methods, anticipated benefits and potential hazards of the research study prior to their inclusion in the study. In this research study both children and parents received an information sheet about the research with their appointment letter for the scan. This information was posted to arrive at least one week prior to the examination so that children and parents could familiarise themselves with the research study before arriving at the hospital. On arrival in the X-ray department the examination was explained to the child and parent allowing sufficient time for questions. In line with the Department of Health recommendations from the Children Act, 1989 [DOH1991] the researcher made a judgement on an individual basis about the level of understanding of each child and parent. This ensured that the procedure was explained in non-technical language appropriate to the understanding of both. If both child and parent were happy with the explanation of the procedure they were asked if they were prepared to proceed with the research. The child was invited to give written and/or verbal consent/assent

to the research and written consent was also obtained from parents/guardians where appropriate. All children gave their verbal consent or assent as appropriate to their age and those able to understand the written instructions also gave written consent. No child was coerced into participating in the study and it was made clear at the outset of the examination and repeated throughout that at any time the child could withdraw from the study. It was the child's own choice whether to proceed at each stage of the research.

The privacy of the subjects relating to publication of the results

Whatever information a researcher discovers about her/his subjects must be kept confidential. If the results are made available for other people to read there should be no method of being able to identify the subjects, for example each subject may be given a number in lieu of their name, as was the practice in this study.

5.2 Selection of eating disorders sample

The sample size for a study is usually calculated using one of two methods. The first is based on the requirement of a specified precision error and the second is an estimation of sample size based on demonstrating a statistically significant difference between two or more groups. The latter method was chosen for the estimation of sample size in this research for the following reasons. A one standard deviation difference between subjects with ANRED and control subjects was deemed clinically significant because a standard deviation reduction of one in bone mineral density is consistent with a diagnosis of osteopenia [DOH1994]. In addition evidence in the literature review demonstrated that in adult studies of osteoporosis in anorexia nervosa a reduction of between one and two standard deviations has been seen compared to matched control subjects. Therefore a broadband ultrasound attenuation standard deviation Z score of -1 or lower was deemed clinically significant for this paediatric sample.

Power calculations

The sample size necessary to demonstrate a specified significant difference between two groups can be determined using a power calculation. Most researchers quote the power of a study using it as a method of assuring the validity of the difference seen between samples. The power calculation used to assess the necessary sample size for the eating disorders sample is based on equation 12 as follows.

$$n > 2F (\sigma/d)^2 \quad \text{Equation 12}$$

where,

n is the sample size

F is taken from a standard power table [WADE1995]

σ is the standard deviation of the paired difference between normal and diseased children

d is the smallest difference considered to be of clinical or scientific significance

Following a review of the literature on osteoporosis in anorexia nervosa and as a result of the pilot study a standard deviation reduction in broadband ultrasound attenuation of one was anticipated to be significant in this sample of children and adolescents.

Therefore using equation 12, $n > 2F (1)^2$

From power tables [WADE1995] for a power lever of 95% and a significance level of 0.025, $F = 15.10$, therefore $n > 30.2$

Therefore this power calculation indicates that a minimum of thirty children with ANRED aged between 8 and 18 years will need to be recruited. This figure is based on detecting a minimum difference of one standard deviation between the healthy cohort and the ANRED cohort that is significant at 2.5% (2 sided) with a power of 95%. That is we can be 95% certain that the difference is a true difference and we would expect to obtain a false positive result 2.5% of the time. To allow for some degree of attrition in the longitudinal study more patients were recruited than predicted necessary from the power calculations.

Recruitment

All patients with ANRED who were newly referred to the hospital were scanned within two weeks of their initial consultation, and long standing female patients were scanned when they attended for one of their three-monthly pelvic ultrasound scans.

5.3 Selection of control cohort

The subjects in the control sample were recruited to take part in this research by a variety of methods as described below.

Control sample

A target of 60 was set for the control sample to allow at least 2 controls per case, however to allow for attrition over the 2 year period of the study the recruitment target was revised to 100. Particular emphasis was placed on the recruitment of peri-pubertal controls because at this age one would expect to see the largest difference in broadband ultrasound attenuation as a result of the growth spurt of puberty. Also the peri-pubertal period is a peak time for the onset of an eating disorder.

Exclusion criteria

Recruits to the control group were excluded if they had endocrine or chromosomal disorders, delayed or precocious puberty, eating disorders, were receiving endocrine therapy, glucocorticoids or had other risk factors for osteoporosis including rickets.

Recruitment

One hundred control subjects were eventually recruited using three methods. The first was a written invitation to 150 parents/guardians of children selected at random from the database of a local school. This elicited a poor response rate of only 12% (18 children). The second method was by direct verbal invitation from the researcher and this led to the recruitment of an additional 12 children. The third and final recruitment strategy was the most successful and this involved sending a recruitment poster to all X-ray departments in the South-East of England. This final strategy led to the successful recruitment of a further 70 children.

5.4 Validity of the measuring variables

“A valid measure is one which measures what it is intended to measure” [DEVA1996]. The caveat to this statement is that it may not be the measure which is valid or invalid rather the use to which the measure is put which is valid or invalid. Therefore validity is dependent on the measure being used appropriately.

There are three ways to assess validity: criterion, content and construct [IBID].

Criterion validity

The validity of a new technique can be assessed by comparing it to a well-established one. In the case of this research the new technique is broadband ultrasound attenuation (BUA) in paediatrics compared to the more traditional technique of dual energy X-ray absorptiometry (DXA). If the two measures are highly correlated the new approach (BUA) can be considered valid.

There is a problem associated with this criterion assessment of validity. Namely that the validity of the old (DXA) technique is taken as the gold standard and therefore assumed to be true. If the new (BUA) technique does not correlate with it the logical conclusion is that the new technique is invalid, whereas it may actually be the old technique which is invalid.

Content validity

The content validity of a technique depends on the definition of what the technique is intended to measure. In the case of densitometry measurements such as DXA the technique does not directly measure the risk of osteoporosis and the propensity of a bone to fracture, rather it measures the areal mineral density of a bone. If the measure required is merely a measure of the mineral density of a bone then such a technique is valid. In osteoporosis assessment however the aim is to assess the propensity of a bone to succumb to fracture. Therefore since densitometry techniques do not take into account all aspects of bone strength contributing to osteoporosis it can be argued that they lack **content** validity.

Construct validity

This approach evaluates a measure by how well it conforms to theoretical expectations. For example BUA is a relatively new technique to use to assess osteoporosis in paediatrics, however it has been used in the post-menopausal population for over a decade. On the basis of theory, broadband ultrasound attenuation measurements are inversely proportional to the osteoporotic status of the bone examined. Therefore if evidence demonstrates that using broadband ultrasound attenuation the lower the values the higher the risk of osteoporosis then the ultrasound measure can be said to have construct validity. This approach is, however, only acceptable if the theory is well established.

5.5 Precision

The precision of a piece of equipment is the ability of that device to produce the same measurement under the same conditions time after time. Precision measurements of densitometric and ultrasound techniques in the assessment of osteoporosis are essential to be able to assess whether changes observed are true changes in a patient or merely an effect of random fluctuations in the measuring equipment being used.

Precision of the BUA technique

The precision of the technique in this research study was evaluated by measuring the root mean square standard deviation, the coefficient of variation (CV) and the standardised coefficient of variation. This is discussed in detail in Chapter 7.

Precision of the stadiometer and weighing scales

The height of each subject was measured on either a wall-mounted stadiometer or a free-standing portable stadiometer (Marsdens). Each child was measured repeatedly until at least three measurements were obtained within 1 millimeter of each other, and the mean measurement recorded. The head was positioned with the orbito-meatal line parallel to the floor to allow reproducibility between measurements.

Each child was measured without shoes or socks and wearing light outer garments only. Such light clothing included T-shirts or blouses and skirt, trousers or shorts. Digital scales, (Marsdens) were used to measure the child's weight to the nearest 50 grammes.

5.6 Z scores

Commercially available QUS machines, in line with bone densitometry equipment, use software designed to calculate standard deviation scores of the ultrasound parameters measured. Such standard deviation scores are based on the bone mineral density of a normal population, and in the case of osteoporosis assessment, this population is normally a young pre-menopausal sample of Caucasian women. This standard deviation score, described as a t score, allows clinicians to utilise the World Health Organisation [WHO1994] criteria for the diagnosis of osteoporosis described in Chapter 2. Although these WHO criteria are based on bone mineral density measurements they have been adopted for QUS measurements. Most software will also calculate a standard deviation score of BUA compared to an age as well as a sex matched Caucasian sample of women, the Z score. The t score is most frequently used in the assessment of osteoporosis in adulthood. Since children have not normally attained a peak bone mass the Z score is used. Interpretation of QUS data using standard deviation scores also enables a broad comparison of measurements obtained with other osteoporosis assessment equipment.

The Z score for each patient in the eating disorders cohort was calculated as shown in equation 13.

Equation 13

$$\text{Z score} = \frac{(\text{BUA of patient} - \text{mean BUA for matched controls})}{\text{Standard deviation of matched controls}}$$

Therefore, for each patient the BUA Z score specifies how many standard deviations below or above healthy controls s/he is. Equation 13 is used in this research to measure the Z score of eating disorders patients compared to age matched controls by using the predicted mean BUA at each year of age and the standard deviation of the residuals of the least squares linear regression line for BUA on age. The Z scores for weight for eating disorders patients have also been compared to healthy controls using the mean and standard deviation of BUA at each kilogramme of weight.

5.7 Standardisation of examinations

A standard procedure was developed as a result of the pilot study (chapter 6) and the precision experiments detailed in chapter 7. Following informed consent there were two stages to the examination.

Stage one

A structured interview was conducted recording the subject's exercise habits, milk intake, menarcheal status (where appropriate), medication and family history of osteoporosis. A standard proforma was used during the interview to act both as a prompt and to record the details (appendix 1). Information was primarily supplied by the subjects themselves but was supplemented with data by parents where appropriate.

Stage two

A new centring point was developed and identified on each child's foot as shown in figure 1. A perpendicular line is drawn from the tip of the lateral malleolus of the fibula to the sole of the foot and bisected. A second perpendicular line is drawn from this bisector to the skin at the back of the heel. The bisector of this second line is the centring point.

Figure 1 Lateral image of a child's foot to identify the centring point



5.8 Statistical analysis

All statistical analyses were performed and graphs produced using SPSS 8.0 and Excel 7 for Windows 95. Exploratory data analyses were performed using scatterplots, boxplots, histograms, descriptive statistics, correlation coefficients and coefficients of determination. Simple linear regression was performed using age, height, weight and BMI as predictor variables. Least squares linear regression was used to predict BUA values from the weight and height of the control cohort. The standard deviations of the residuals of these lines were used in the calculation of the Z scores of the eating disorders children. For sample sizes greater than 20 confidence intervals were calculated using a t-value of 2. For smaller sample sizes t-values were obtained from a standard t-table. The standard error of the mean (SEM) was calculated as shown in equation 14, where s is the standard deviation of the sample and n is the sample size.

$$\text{SEM} = s/\sqrt{n} \qquad \text{Equation 14}$$

The 95% confidence intervals were calculated by multiplying the SEM by 2 (if the sample size was greater than 20) and adding this multiple to the mean value for the upper limit and subtracting it from the mean value for the lower limit.

One way analysis of variance (ANOVA) was used to identify differences in the mean values of 3 or more groups, provided they met the following criteria:

- they were approximately normally distributed
- they were collected by random sampling
- they had approximately equal variances.

Chi square tables were used to test the strength of relationships between variables.

Where differences between two groups of non-parametric data were tested the Mann-Whitney U-test was used. Where differences between two groups of parametric data were tested the student t test was used.

5.9 Summary

In this chapter the ethics of good research practice have been discussed and a process has been described to ensure that the researcher adhered to such ethical practice.

The sample size for the anorexia nervosa and related eating disorders cohort was calculated as 30 using power calculations based on the minimum predicted standardised difference between experimental and control cohort.

The validity of measuring variables used and the assessment of precision has been discussed and a standardised method of examining the subjects has been developed using an interview proforma and a new positioning technique on the heel.

The main statistical tests used and the software to perform them is also outlined.

Chapter 6 The pilot study

The key aim of this experiment was to identify potential problems which could be solved prior to the final study and used to inform its design. There were two objectives:

- to observe and evaluate the standard BUA technique during clinical paediatric practice;
- to establish quantitatively whether BUA could identify children and adolescents with ANRED who are at risk of osteoporosis.

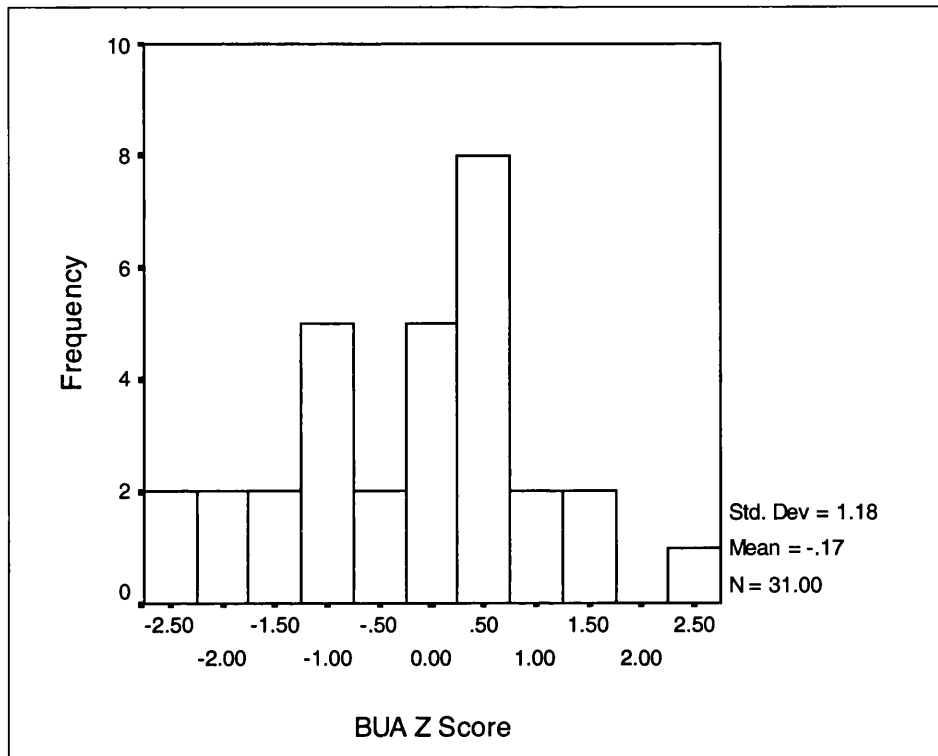
6.1 *Methods*

A convenience sample of thirty-one female patients with ANRED, who, over a three month period presented to the ultrasound department for a pelvic ultrasound scan, were invited to participate in this pilot study. The procedure for the BUA scan was explained to each patient and verbal consent obtained prior to the scan. One operator performed the scan on the left foot of each patient using the standard positioning protocol as recommended by the manufacturer [MCCU1995]. This technique involves using a measurement of the patient's foot length as a guide for the choice of plastic foot insert to be placed in the ultrasound machine to position the foot for the scan. The aim of this positioning technique is to measure the posterior most parallel portion of the calcaneum. Standard DXA scans of the lumbar spine were also performed on 24 of these patients.

At the time of the scan BUA Z score was recorded as well as the date of birth of the patient. Data on weight and height were obtained retrospectively from medical records and were used to calculate body mass index and percentage body mass index for age ($\% \text{ BMI.age}^{-1}$) for each patient.

6.2 *Results*

It was observed during the study that using plastic foot inserts to position the foot in relation to the ultrasound transducers allowed for variations in foot length. The inserts did not however allow for variations in foot width or shape.

Figure 2 Histogram of BUA Z scores

Exploratory data analysis was performed by plotting histograms of BUA Z score, age and BMI and also calculating the mean and standard deviation of each of these parameters. Each of the histograms (figures 2-4) demonstrate an approximately normal or Gaussian distribution.

Surprisingly despite all patients in the sample having eating disorders there is a wide range of Z scores from -2.5 to $+2.5$ with a mean of -0.17 . To test the probability that the sample mean is representative of the true population mean the standard error of the mean (SEM) and 95% confidence intervals were calculated. The SEM, calculated as the standard deviation divided by the square root of the sample size is 0.21. It is therefore 95% certain that the true population mean Z score lies within the confidence interval: sample mean \pm 2SEM, i.e. between $+0.25$ and -0.60 . Therefore since the confidence intervals include the value 0, the null hypothesis that there is no difference in BUA Z scores between eating disorders patients and control subjects is accepted.

Figure 3 Histogram of BMI

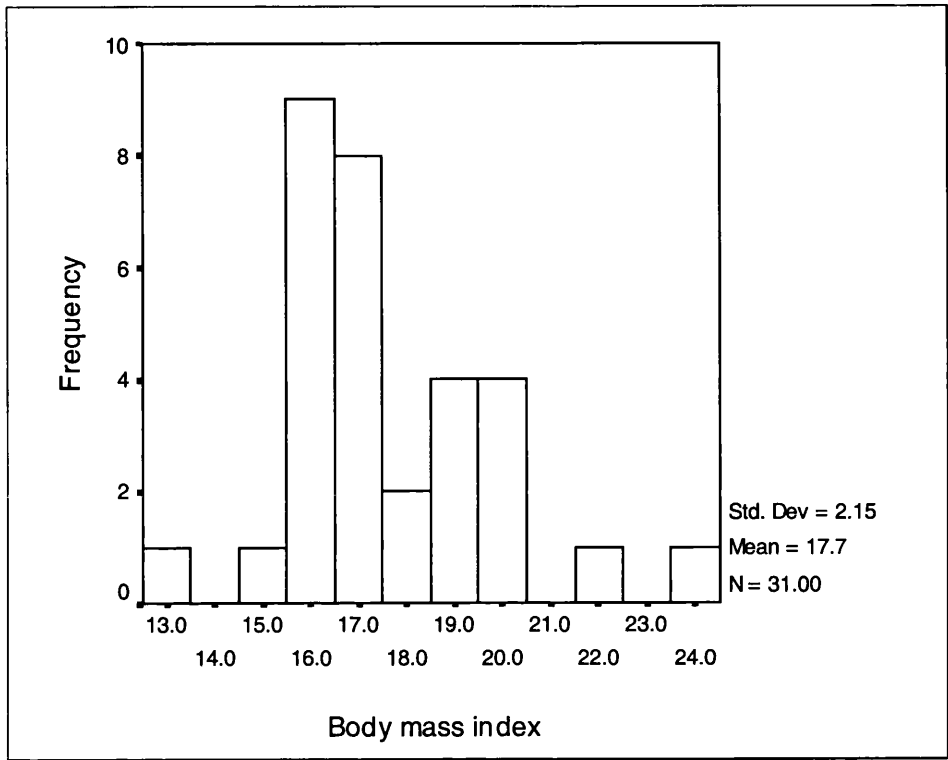


Figure 4 Histogram of age

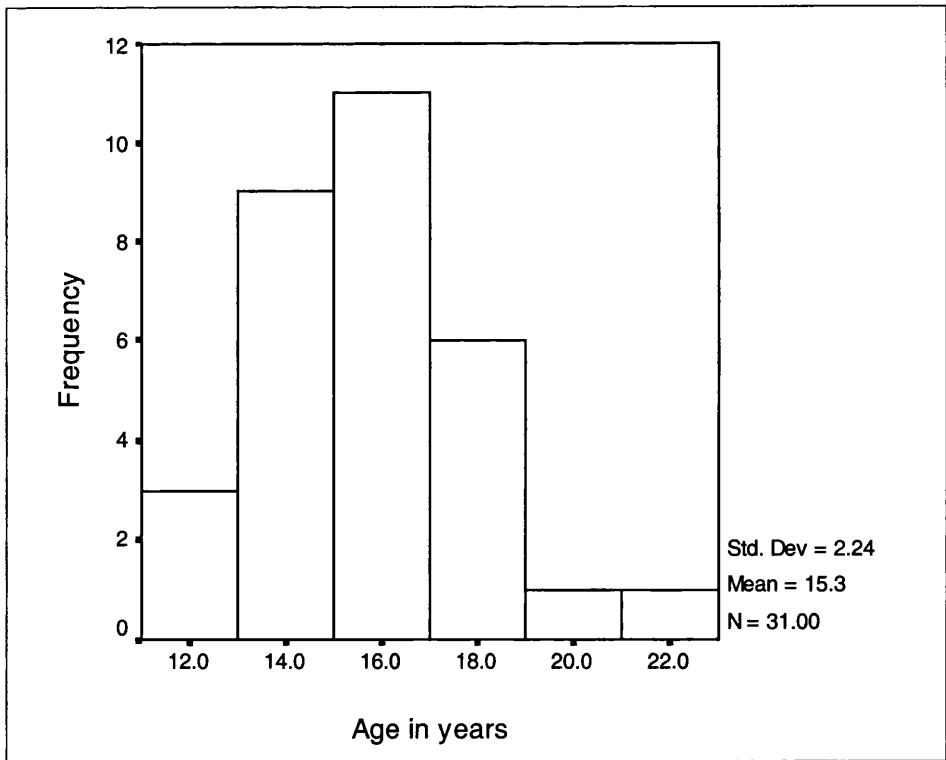
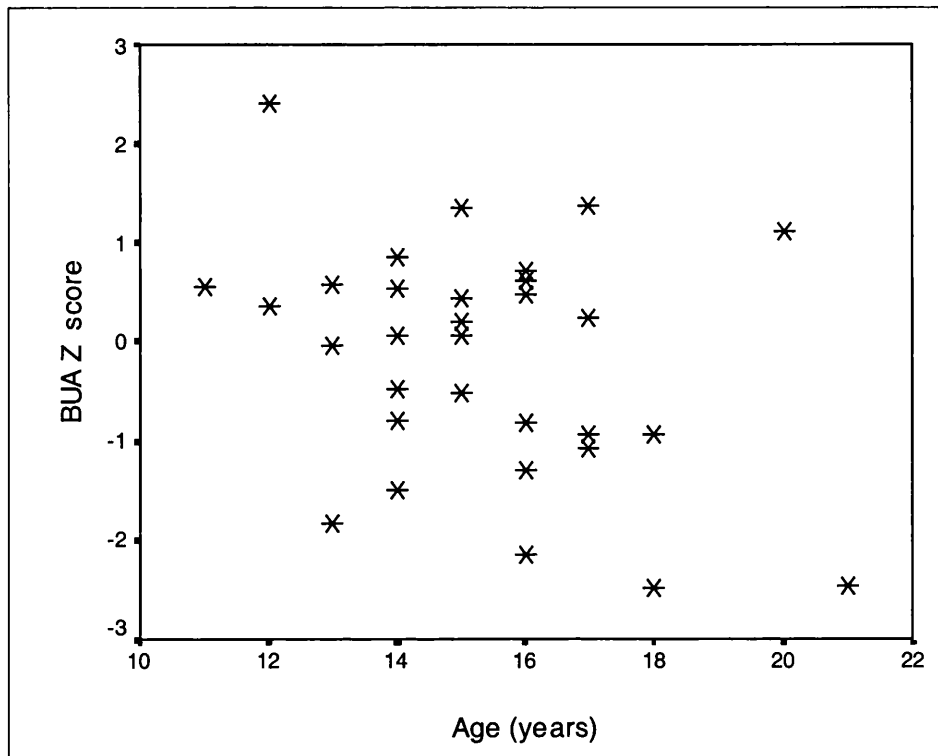
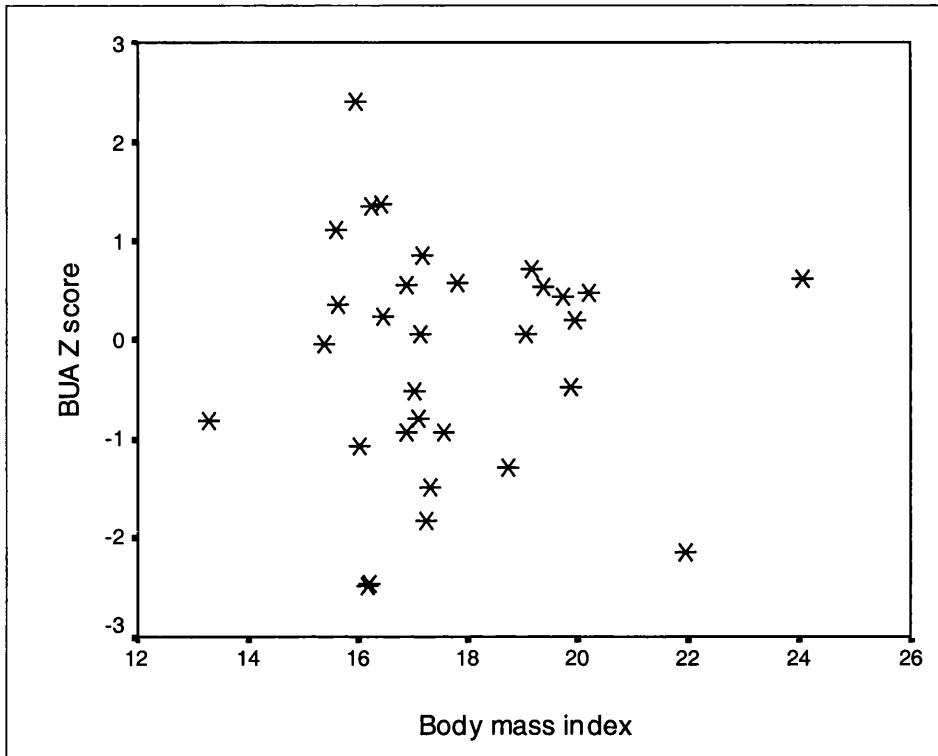


Figure 5 Scatterplot of BUA Z score and age

BUA Z scores were plotted against age and BMI to examine these relationships (figures 5-6). Examining the scatterplot of age and BUA Z score carefully a weak relationship between decreasing BUA Z score and increasing age can be seen. The null hypothesis that BUA Z scores in patients with ANRED are on average not correlated with age was tested using a two tailed Pearsons correlation coefficient and was statistically significant ($r = -0.35$, $p < 0.05$). Pearsons correlation coefficient rather than the Bland-Altman statistical method was used to compare BUA and DXA to enable useful comparison with other publications.

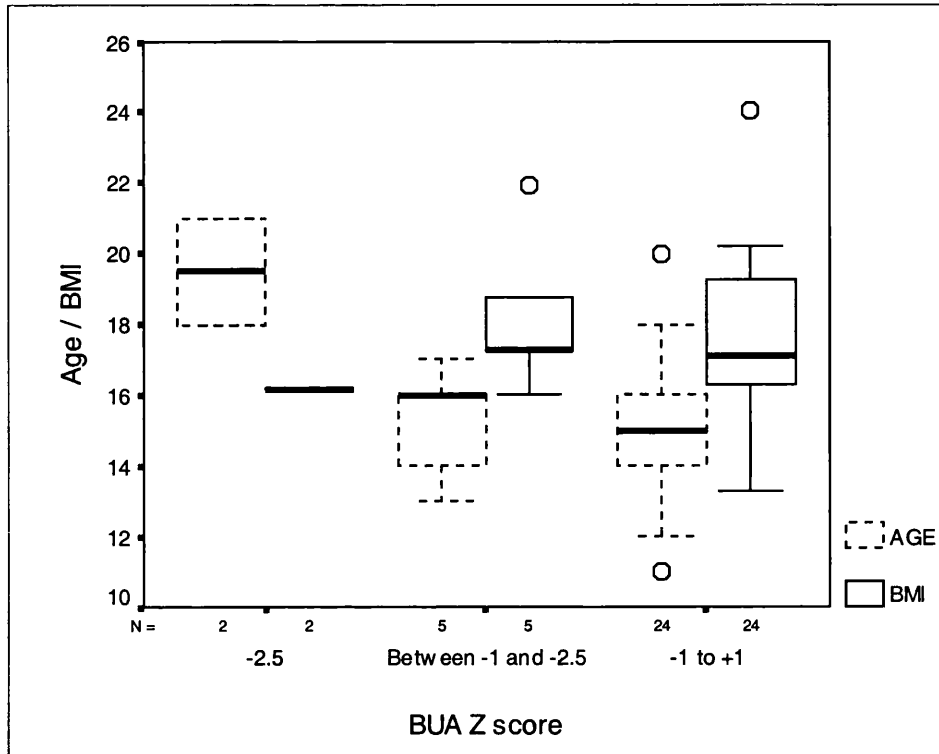
Figure 6 Scatterplot of BUA Z score and BMI



Viewing the scatterplot for BUA Z score and BMI no obvious relationship is seen. This is supported by the lack of significance shown in Pearsons correlation coefficient ($r=0.009$, $p=0.96$).

Pearsons correlation coefficient was also used to evaluate the relationship between DXA of the lumbar spine and BUA in 24 of these patients. The correlation was 0.88 and was statistically significant ($p<0.001$).

Figure 7 Pilot study boxplots



The values for median, interquartile range and minimum and maximum values of the age and BMI of three sub-groups of the eating disorders sample are shown in figure 7. An individual with a BMI equal to or below 17.5 kg.m^{-2} is classified as underweight [APA1994]. The subgroups are categorised in line with the WHO criteria for osteoporosis [WHO1994]. Outliers are identified if their value lies more than 1.5 box lengths away from the upper or lower quartile. The box length is the interquartile range, representing 50% of the sub-sample.

Only seven patients (23%) had a BUA Z score in the osteopenic or osteoporotic range (-1 to -2.5). Of these patients, five had a BUA Z score of between -1 and -2.2 (osteopenic) and two had a BUA Z score of -2.5 (osteoporotic). A further six patients had BUA Z scores between -0.5 and -0.9. All thirteen patients with a BUA Z score of -0.5 or less are shown in table 11. The individual measurements on each of these thirteen patients demonstrate that the two patients with a BUA Z score of -2.5 were adult, aged 18 and 21 years and both had a BMI of 16.2. Interestingly of the only other two adults in this pilot study one was aged 20 years and she also had a low BMI of 16 although her BUA Z score was much higher at +1.1. The other was aged 18 with a BMI of 16.9 and a BUA Z score of -0.9.

Table 11 Patients in the pilot study with Z scores < -0.5

Z score	Age	BMI	% BMI.age ⁻¹
-2.5	18	16.2	77
-2.5	21	16.2	75
-2.2	16	21.9	105
-1.8	13	17.2	90
-1.5	14	17.3	86
-1.3	16	18.7	90
-1.1	17	16.0	74
-.9	17	17.6	80
-.9	18	16.9	80
-.8	16	13.3	63
-.8	14	17.1	81
-.5	15	17.0	82
-.5	14	19.9	99

6.3 Discussion

Observing the BUA scan procedure during the pilot study it became clear there were a variety of foot sizes, in terms of length, width and shape in the age group examined. It is likely that this variation in foot size will be even wider within a younger age group, having a noticeable effect in longitudinal studies on children. Thus the current BUA positioning technique may be reasonably precise (cv of 5% quoted by the manufacturer) but its accuracy is unknown.

When the BUA Z scores of the patients with eating disorders were evaluated a weak inverse relationship was noted with age, indicating that the older patients had lower Z scores (some with osteopenia and osteoporosis) than the younger ones. However since only children over 12 years of age were examined in this study the pattern of BUA Z scores for pre-pubertal children cannot be predicted. The BUA Z scores in this sample are comparable with other studies where the bone mineral density Z scores of patients with anorexia nervosa have been measured. Grinspoon et al [GRIN1996] quoted a mean BMD Z score of -1.7 in a sample of women with anorexia nervosa and in Biller's sample [BILL1989] 50% of women with anorexia nervosa had a BMD which was 2 standard deviations below the age-matched normal mean. Furthermore the inverse relationship of BUA Z score with age is also

reflective of adult studies where the duration of anorexia nervosa is inversely related to BMD Z score ([TREA1987], [HERG1995]).

The significant correlation between DXA of the lumbar spine and BUA through the left heel was unsurprisingly significant ($r=0.88$, $p<0.001$) as this confirms the data presented in many other research studies. However the inclusion of the correlation coefficient for DXA and BUA was considered appropriate to the pilot study and to act as a baseline for comparison with other studies.

There were insufficient data about the eating disorders of the patients, consequently the patients in this sample may have had different eating disorders and been at varying stages of treatment for their eating disorder at the time of the BUA scan. Other data which could easily have been collected at the time of the scan and which affect BUA Z scores are the menarcheal status, physical activity levels and milk intake.

6.4 Conclusion

The pilot study of 31 females aged between 12 and 20 years demonstrated that 7 patients (23%) had a reduced BUA Z score (between -1 and -2.5). Furthermore there was a weak negative association of BUA Z score and increasing age, so that older patients had lower BUA Z scores. However when the entire group of 31 female patients with ANRED were compared to a control sample they demonstrated no statistically significant difference in mean BUA Z score (corrected for age).

The foot size of the patients was variable in terms of length, width and shape.

The pilot study highlighted four specific issues which will be considered and revised in the design of the main study. This will lead to improvements in the quality of the final data analysis and enable useful statistical inferences to be made.

- Children with ANRED who are younger than 12 years of age will be included in the main study to provide information on the effect of eating disorders on the BUA values of pre-pubertal children.

- The following additional data will be collected: the specific eating disorder of the patient and the duration of the disease; the menarcheal status of the females; exercise habits and milk intake.
- The precision error of the BUA technique will be evaluated thoroughly using the root mean square standard deviation.
- A new centring point will be developed with the aim of improving the accuracy of the BUA technique in longitudinal studies on children. To reduce the confounding effects of using a different centring point for the measurement of BUA compared to that used for the manufacturer's original normative dataset BUA scans on healthy control children will also be performed and used to create a new normative database.

Chapter 7 The precision and accuracy of CUBA

There are two broad sections to this chapter. The first section is an evaluation of the precision of a new paediatric positioning technique, developed by the author, for the measurement of BUA through the heel in children. The precision of this new technique is tested against the precision of a paediatric technique already published in the literature [JAWO1995]. The second section is an experiment to test whether an improvement in BUA precision is observed when the McCue CUBA is adapted to facilitate the location of a new positioning point.

7.1 *The development of a new centring point technique*

Burston et al [BUR1998] demonstrated that using the McCue CUBA the posterior region of the calcaneum allowed the most precise, reproducible measurements of BUA. They identified this region using a centring point described as follows: “5/9 of the way along a line, 45⁰ to the vertical, from the tip of the lateral malleolus to the most posterior part of the heel”. For this research a new technique was developed by the author to increase the probability of measuring the same posterior region of the calcaneum in growing children during longitudinal studies over a period of years. The precision of this new technique was compared to the precision of the paediatric technique developed by Jaworski [JAWO1995].

The key aim of this experiment was to evaluate and compare the precision error of two centring point techniques for the measurement of BUA through the heel in children and adolescents. The precision error was assessed by comparing the precision of each centring point technique on a set of paediatric radiographs of the feet. The null hypothesis is that on average there will be no difference in the precision of the new centring point and Jaworski's centring point.

Method

This experiment was performed on lateral radiographs of children's feet. To obtain a wide variety of foot sizes the radiographs of 30 patients aged between 4 and 10 years who had attended the radiology department over a three year period for radiography of the foot were chosen at random from the radiology patient information database. Of these 30 patients there were 15 with radiographs showing the region from the soft tissue of the heel to the head of the fifth metatarsal and the tip of the lateral malleolus

to the sole of the foot. Some patients had more than one examination over a period of time and some also had both feet radiographed which eventually provided 29 different images. All radiographs were copied twice and the pairs were coded alphabetically to facilitate later analysis. Although radiographs of both left and right feet were used, centring points were marked on the radiographs as though it were the left foot, i.e. radiographs of all right feet were inverted.

The new centring point as illustrated in figure 1 on page 84 is located as follows. A perpendicular line is drawn from the tip of the lateral malleolus of the fibula to the sole of the foot and bisected. A second perpendicular line is drawn from this bisector to the skin at the back of the heel. The bisector of this second line is the centring point.

The Jaworski centring point is identified as follows. A line is drawn from the back of the heel to the tuberosity of the fifth metatarsal and a point at one third of the distance from the heel is marked. A second line is drawn from this point perpendicular to the first line. The centring point is 1cm along this second line.

The films were randomly and independently marked twice by one observer. The new centring point was marked on the first day and the Jaworski centring point was marked two days later. The observer repeated the experiment on the same day to examine the effect of self-training. The observer was an experienced radiographer (WN), who was not familiar with either the new technique or the Jaworski technique.

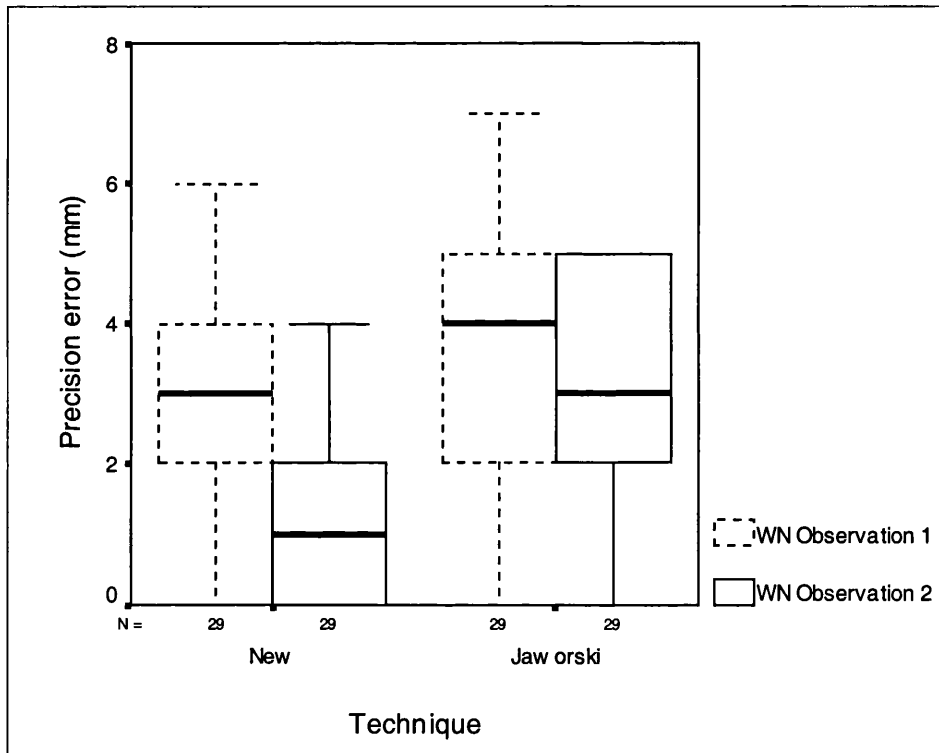
After the centring point was marked on all the films they were matched into 29 pairs. Each of the pairs of films was placed together over a light box and manipulated until they were superimposed over each other as closely as possible. A small ruler (precision 1mm) was used to measure how far apart in millimetres the superimposed centring points were from each other. The centring points on each film were identified using a water-soluble marker, so that after the precision had been measured the films could be wiped clean and the experiment repeated.

Results

The mean age of the fifteen patients was 7.4 years with a standard deviation of 2.0 years (range 3.8 to 10.3 years).

The boxplots in figure 8 demonstrate the median, range, upper and lower quartiles of the precision error measurements for each technique. The median precision improved from the first series of observations to the second for both techniques. This reflects the effect of self-training by the observer.

Figure 8 Precision boxplots



The median precision for the new technique was 1mm, compared to 3mm for the Jaworski technique. The precision error for both techniques (table 12) was calculated to the nearest millimetre using equation 9 (page 70) to measure the root mean square standard deviation (RMS SD).

Table 12 Precision errors

	RMS SD (new technique)	RMS SD (Jaworski technique)
Observation 1	2mm	3mm
Observation 2	1mm	2mm

Statistical analyses were performed on the precision data obtained from the second series of observations for each technique. This is to allow for the effect of self-training of the observer for the position of the centring point. As the data are not normally distributed non-parametric tests were performed to test the null hypothesis stated at the beginning of this section. Using the Mann-Whitney U-test, statistically significant differences were confirmed in the precision of the two techniques ($p < 0.001$). Therefore the precision of the new technique is on average statistically significantly better than that of the Jaworski technique.

Discussion

The use of radiographs of feet to assess the precision error of two centring point techniques is not an ideal surrogate for performing the examination in clinical practice. It does however provide a useful comparison of the reproducibility of two techniques when performed repeatedly under the same conditions by an independent observer.

The observer found the Jaworski technique more difficult to use than the new technique. In the Jaworski technique it was difficult to confidently identify the tuberosity of the fifth metatarsal, whereas in the new technique the location of the tip of the lateral malleolus of the fibula was clearly visible on all radiographs. This is likely to be a more significant problem in vivo as the tuberosity of the fifth metatarsal is not easily palpable or visible, particularly in the feet of children. However the tip of the lateral malleolus of the fibula is a well-recognised palpable landmark which is frequently used in radiographic positioning techniques (figure 1). Furthermore the tip of the lateral malleolus is readily identifiable at all ages.

The coefficient of variation of the measurements was not used as a measure of the relative precision error of the two techniques. This is because the aim of the experiment was to be able to locate the same centring point on each pair of

radiographs. When this is performed perfectly the precision in millimetres is zero; that is, there is no visual difference between the location of the two centring points when the radiographs are superimposed. Thus the mean value (which is the denominator of the CV formula) for a reproducible technique will be closer to zero than that for a technique which is less reproducible. Therefore using a smaller denominator in the CV formula would raise the value of the CV giving a false impression of poor precision. It is therefore reasonable to quote the precision of each technique to the nearest millimetre. Thus the new technique with a median precision of 1mm (RMS SD 1mm) was more precise than the Jaworski technique with a median precision of 3mm (RMS SD 2mm). The range of precision measurements for the Jaworski technique was greater than that for the new technique which supports the notion that the new technique is on average more precise than the Jaworski technique as demonstrated in the Mann-Whitney U-tests.

The ultrasound transducers which are used for the measurement of BUA in the McCue CUBA are 12.5 mm in diameter and the location of the correct centring point in the study was proved to be precise to +/- 3mm for Jaworski's technique and +/- 1mm for the new technique. The size of the transducer relative to the precision of the centring point suggests that the difference in precision of the two techniques would not be significant in clinical practice. However, the precision error for the Jaworski technique may be under-estimated since the technique was performed on radiographs which allow identification of the tuberosity of the fifth metatarsal whereas this landmark is more difficult to locate in clinical practice on a child's foot.

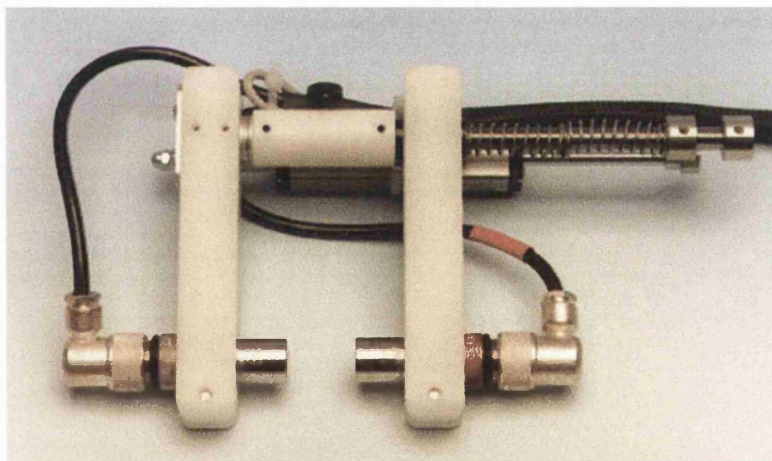
Conclusion

A new precise method of locating the posterior most parallel portion of the calcaneum in children was developed and validated under controlled experimental conditions. The effect on BUA measurements of using this new centring point in clinical practice will be assessed in the next section. This experiment demonstrates that not only is the new technique a more precise method to use than the Jaworski technique but it is also easier to perform. The limiting precision of this new technique is 1mm. However the difference in precision between the two techniques may actually be under-estimated. This is because the experiment was performed on radiographs with identifiable bony landmarks whereas in clinical practice it is more difficult to locate the necessary bony landmarks, particularly the tuberosity of the fifth metatarsal necessary for the Jaworski technique.

7.2 **Adapting the CUBA to use the new technique**

The new technique was used to identify the region of interest on the heel on which to position the transducers. The transducers in the CUBA are normally in a fixed position housed within a hard plastic positioning device. To place the transducers on the new centring point previously developed the foot is positioned and immobilised with foam pads. The aim of this experiment is to measure the precision of the new technique in clinical practice and to assess whether it can be improved by removing the transducers from the plastic surrounding of the machine onto a handheld calliper device. The ultrasound transducers (12.5mm diameter) are shown on the handheld callipers in figure 9. The null hypothesis is that the precision of the BUA measurements with the transducers in the fixed position within the plastic positioning device will on average be the same as the precision of the BUA measurements with the transducers placed on a handheld calliper device.

Figure 9 **Ultrasound transducers placed on handheld callipers**



Methods

Paired measurements of BUA on 50 children, obtained with the transducers in the standard fixed location in the CUBA were analysed and compared to paired measurements of BUA on 50 children, obtained with the transducers placed on a handheld calliper device. The children were randomly assigned to each group and all paired measurements for each child were performed on the same day with re-positioning between.

The precision was calculated using equation 9 on page 70 to measure the root mean square standard deviation (RMS SD) of these two sets of paired measurements. The coefficient of variation was used as a measure of relative precision error and was calculated using equation 10 (page 71). The standardised coefficient of variation was also measured by replacing the mean with the range of values as the denominator in equation 10. The null hypothesis was tested using the independent samples t test on the BUA values.

Results

The results of the mean and standard deviation of age, mean and range of BUA and precision measurements of the two groups of children are shown in table 13.

Table 13 Assessment of precision error in clinical practice

Parameter measured	Transducers fixed within plastic casing of CUBA	Transducers on a handheld calliper device
Mean age of group (years)	12.83 (SD 2.94)	11.37 (SD 3.05)
RMS SD (dB.MHz ⁻¹)	6.05	6.02
BUA range (dB.MHz ⁻¹) 1 st scan	87.00	82.60
BUA range (dB.MHz ⁻¹) 2 nd scan	85.00	88.55
Mean range (dB.MHz ⁻¹)	86.00	85.58
Mean BUA (dB.MHz ⁻¹) 1 st & 2 nd scans together	36.23	47.21
Coefficient of variation (%)	16.70	12.75
Standardised coefficient of variation (%)	7.03	7.03

As shown in the table the RMS SD precision error was not significantly different for paired BUA measurements whether in the fixed position within the CUBA or on the handheld callipers. The relative precision error calculated using the coefficient of variation improved from 16.70% for paired measurements obtained using fixed callipers to 12.75% for paired measurements obtained using the handheld callipers. However the relative precision error calculated using the standardised coefficient of variation was 7.03% for BUA measurements obtained with transducers either fixed in position or on handheld callipers.

The mean difference of paired BUA measurements obtained with the transducers in the plastic housing was $-0.29 \text{ dB.MHz}^{-1}$ and when the transducers were on handheld callipers it was $-2.66 \text{ dB.MHz}^{-1}$. The 95% confidence interval (-0.96 to +5.71) for the mean difference in dB.MHz^{-1} of the two sets of paired BUA measurements contained zero confirming the null hypothesis ($p < 0.16$), that there is no statistically significant difference in precision when the transducers are moved onto the handheld callipers.

Discussion

The precision of BUA when measured on the CUBA in children aged between 6 and 18 years is reported as 5% ([MUGH1996], [WILM1996], [MUGH1997]). In comparison Jaworski et al quote a precision of 1.5% for BUA in children when scanned with the Lunar Achilles [JAWO1995]. Sundberg et al [SUND1998] do not quote the coefficient of variation for BUA used in their paediatric study using the Lunar Achilles. Schonau et al [SCHO1994] and Lappe et al [LAPP1995] measured the speed of sound rather than the BUA in healthy children.

In this study the relative precision error of the new technique at 12.75% for CV and 7.03% for standardised CV is poor compared to previous paediatric publications using the same equipment. It was however measured systematically and precisely using the root mean square standard deviation of 50 randomly assigned paired measurements. In comparison it is not clear in each of the three CUBA papers referenced above exactly how the researchers measured the coefficient of variation in children. The authors merely state what the CV is for children aged between 6 and 18 years but do not outline the methodology used. One would assume that if the root mean square standard deviation of paired measurements had been used to calculate the coefficient of variation this would have been stated. Nor do Jaworski et al state how CV was measured in their study but they do mention that 27 children were scanned twice on one day. However the 27 children were then divided into three groups to assess the effect on CV of three different ultrasound measuring techniques. If the arithmetic mean of several CV values was used as the relative precision error in these paediatric papers this may have led to the lower CV values than are reported in this study. It is recognised that the arithmetic mean of several values of CV to calculate the overall CV of a sample will underestimate the true precision error by up

to 25% for duplicate measurements [GLUE1995]. Also the BUA scale used in the Lunar Achilles is such that it is likely to produce a higher mean value for children, though a lower range than that obtained with the McCue CUBA. For example the dynamic range of BUA measured on the CUBA Clinical used in this research is 86 dB.MHz⁻¹ compared to the Lunar Achilles which in the paediatric study by Jaworski et al [JAWO1995] has a dynamic range of only 21 dB.MHz⁻¹. The corollary being that the larger the BUA range used as the denominator in the standardised CV calculation the lower the value of standardised CV. This larger measurement range for CUBA also suggests that it has increased sensitivity compared to the Achilles because for the same time-span between scans BUA measured by CUBA will increase by a greater amount than that measured by the Achilles.

As demonstrated in the results in table 13 when the CV was calculated for the two techniques (root mean square standard deviation divided by the mean and converted to a percentage) the numerator of the CV equation was the same but the denominator was different. This made a significant impact on the resultant CV measurement. In these data this led to a higher CV for BUA measurements with the transducers fixed into the plastic housing of the CUBA leading to the erroneous conclusion that BUA is more precise when the transducers are mounted onto a handheld calliper. However when the standardised CV was measured the results demonstrate that there is no improvement in relative precision error when the callipers are removed from the plastic housing of the CUBA machine, which is confirmed with the independent samples t test.

When the RMS SD is used as a measure of the precision error the values are in absolute units rather than percentages. The RMS SD calculated in this chapter was 6.02dB.MHz⁻¹ and it can be used as a tool to identify significant changes in BUA when the children are scanned after one year. It is particularly useful because it is not calculated as a proportion of the original BUA value which, in children, may be in single figures and therefore lead to a poor precision error measured as CV or SCV.

It is also noteworthy that the mean differences of the two sets of measurements in these data were negative, suggesting that the second scans produced higher BUA values than the first scans. This effect was amplified when the transducers were

placed on handheld callipers. On examination of these children during the procedure it was noted that the ultrasound transducers left a small imprint on both sides of the heel after the scan because of compression of overlying tissue. This effect was investigated further by performing ten sequential scans *in vivo* on ten volunteers. It was noted that in each subject sequential BUA values increased in magnitude, and this was associated with a reduction in heel width as overlying skin was compressed during the scan. It was therefore assumed that the greater difference in BUA measurements seen with the transducers in the handheld callipers arises because the transducers can more accurately be located over exactly the same region of interest for the second scan and therefore over the previously compressed area of subcutaneous tissue. In comparison this is more difficult to do when the transducers are located within the plastic housing of the CUBA.

It was postulated that the ability of the new technique to reproducibly position the transducers over the region of interest in the calcaneum was limited by the difficulty in precisely positioning children's feet in relation to fixed transducers within the plastic housing of the CUBA. Therefore it was hypothesised that more precise positioning of the callipers over the region of interest on the heel would be possible by placing the transducers onto handheld callipers. Although it was physically easier for the operator to position the foot using the handheld calliper device the precision results confirm that there was no statistically significant improvement in precision error when the machine was adapted to locate the new centring point.

Conclusion

In summary a change in technique improved the accuracy of measuring the correct region of the calcaneum. Adapting the equipment to allow easier positioning of the transducers over the region of interest on the heel using the new technique appeared to improve the relative precision error from 16.70% to 12.75% when the coefficient of variation was calculated. However the relative precision error calculated using the standardised CV was 7.03% both before and after adaptation of the equipment.

These results demonstrate that if relative precision error is measured it is more appropriate to use the standardised coefficient of variation (7.03% in this study) rather than the coefficient of variation as this more accurately reflects the

reproducibility of the equipment and the technique. The root mean square standard deviation of paired measurements of BUA ($6.02\text{dB}\cdot\text{MHz}^{-1}$ in this study) is an alternative useful method of reporting the precision of the ultrasound technique.

The independent samples t test confirmed the null hypothesis that there was no improvement in precision by adapting the equipment, although the operator did find the handheld callipers easier to use.

Chapter 8 The normative data

The main aim of this chapter is to analyse the normative paediatric data so that the measurements can be used to predict BUA and calculate BUA Z scores for children and adolescents with ANRED. The control subjects were recruited to take part in this research by a variety of methods as outlined in section 5.3.

8.1 *Exploratory data analysis*

One hundred healthy subjects aged between 5 and 20 years consented to enter this research study. Four subjects were excluded: three females and one male. Two of the females (17 years and 14 years) failed to arrive for their research appointment and one (11 years) was receiving treatment for rickets. One male (5 years) refused the ultrasound scan. The final cohort comprised 41 males and 59 females. The majority of the cohort (89%) described their ethnicity as white; 2% as black caribbean; 1% as black african; 1% as indian; 1% as asian “other”; 1% as chinese and 5% of children described their ethnicity as “other”.

The scatterplots in figures 10 – 13 were used to evaluate the relationships between BUA and age, BUA and weight, BUA and height and BUA and BMI. In each scatterplot the males are identified separately to the females so that the effect of gender on the relationships can be visualised. Pearsons correlation coefficient for each of the relationships was also calculated and the r values with statistical significance levels are shown on the scatterplots. Age, weight, height and BMI all demonstrated significant correlations with BUA.

Figure 10 Scatterplot of age and BUA (controls)

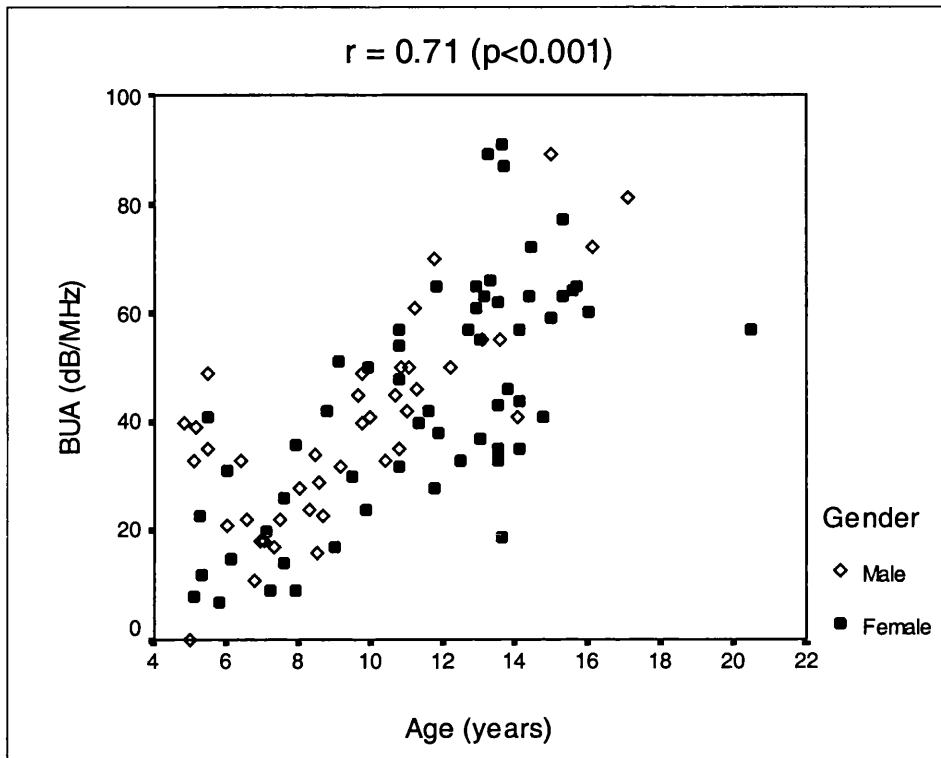


Figure 11 Scatterplot of weight and BUA (controls)

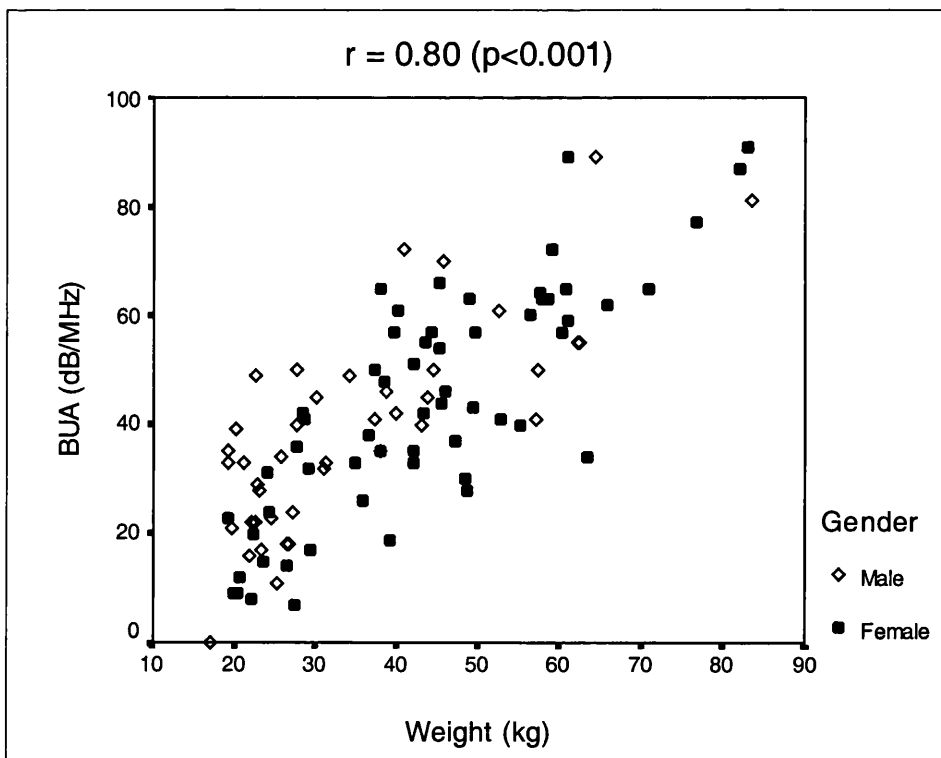


Figure 12 Scatterplot of height and BUA (controls)

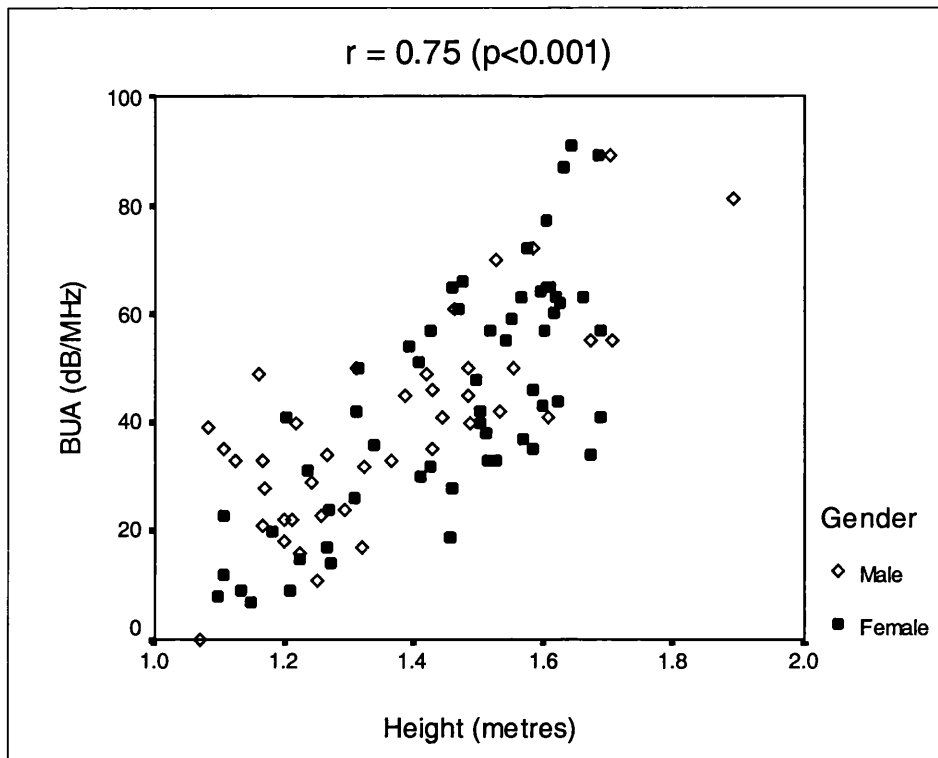
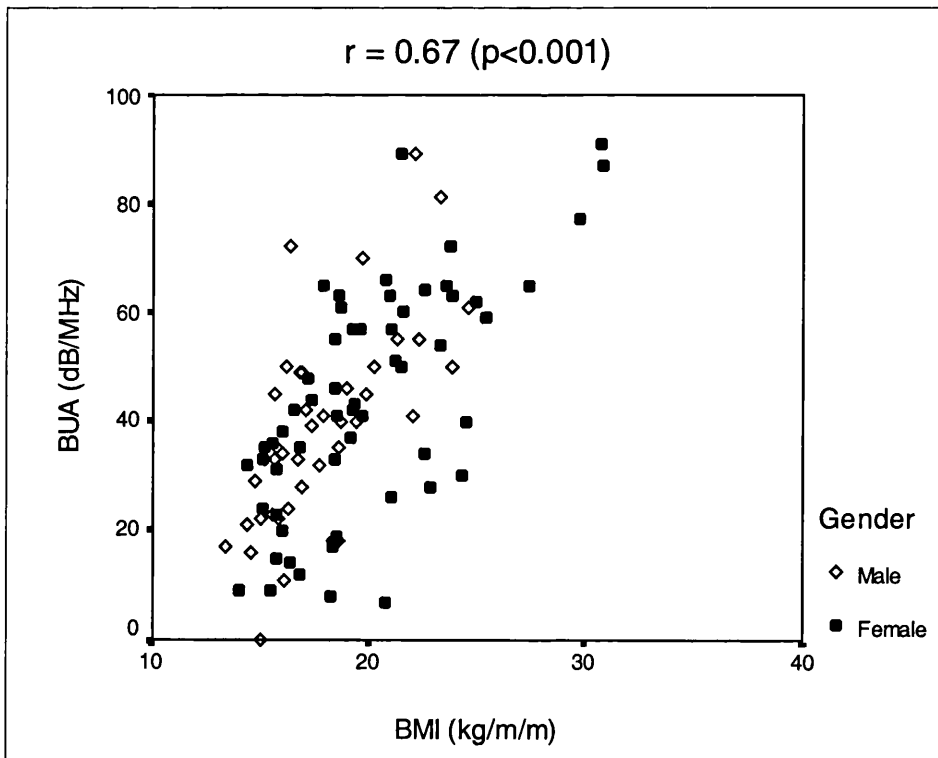


Figure 13 Scatterplot of BMI and BUA (controls)



The coefficients of determination calculated from the scatterplot correlations are shown in table 14 and demonstrate that for males and females combined, weight has the greatest predictive ability for BUA accounting for 64% of the variance.

Table 14 Coefficients of determination (controls)

Predictor variable	Coefficient of determination (r^2)
Age (years)	0.50
Weight (kg)	0.64
Height (m)	0.56
BMI ($\text{kg}\cdot\text{m}^{-2}$)	0.45

There were differences between males and females in the relationships between BUA and age and BUA and weight as shown by the gender specific correlation coefficients in table 15.

Table 15 Correlation coefficients by gender (controls)

	Correlation coefficient	
	BUA and age	BUA and weight
Males	0.76	0.77
Females	0.68	0.82

There was a stronger relationship between BUA and age for males than females. This is demonstrated in the scatterplot where there is a wider range of values for BUA for females than males. This is particularly marked for females at age 14 years.

Linear regression

Least squares linear regression analyses were performed for males and females separately to calculate the equation of the line of best fit specific to gender. BUA was the dependent variable and age the predictor variable for figures 14 and 15. Weight was used as the predictor variable for BUA in figures 16 and 17. The line of best fit is plotted on each of the scatterplots together with the 95% confidence intervals (innermost pair of lines) and the 95% prediction intervals (outermost pair of lines). In this sample of healthy subjects the confidence intervals for each of the regression lines will contain the true population mean for BUA on 95% of occasions. The gender specific coefficient of determination as an r^2 value is also shown on each scatterplot.

Figure 14 Linear regression of BUA on age (males)

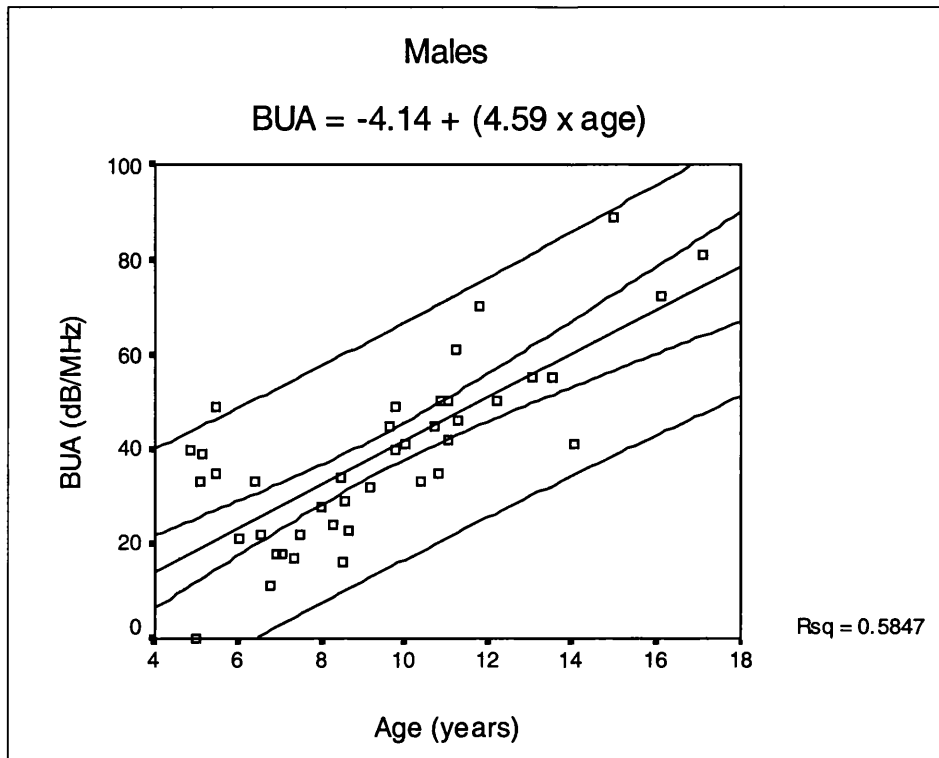


Figure 15 Linear regression of BUA on age (females)

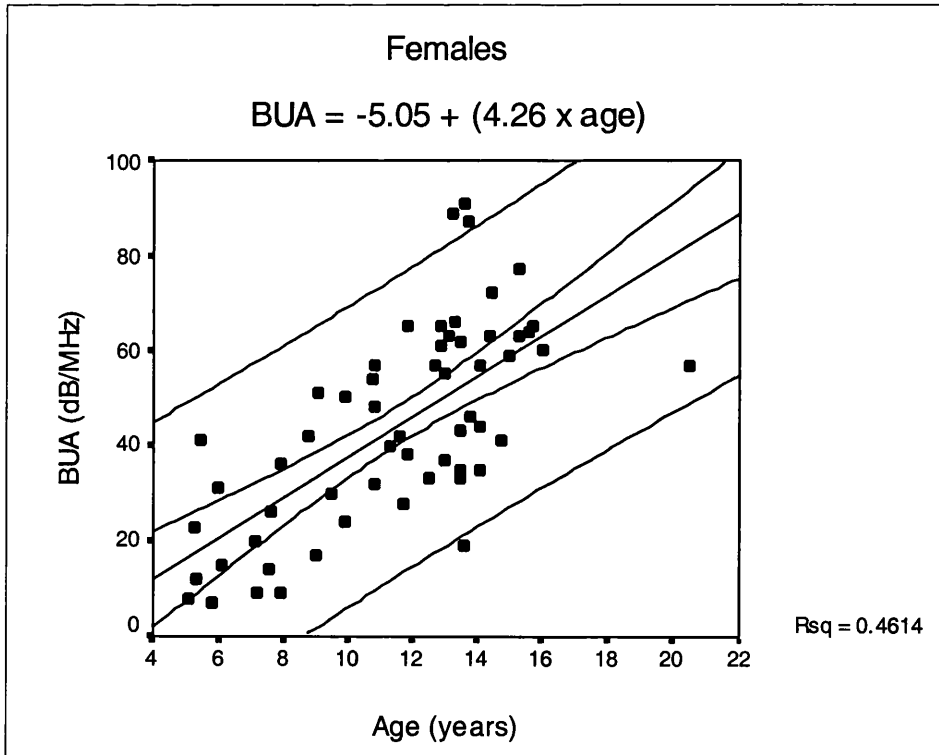


Figure 16 Linear regression of BUA on weight (males)

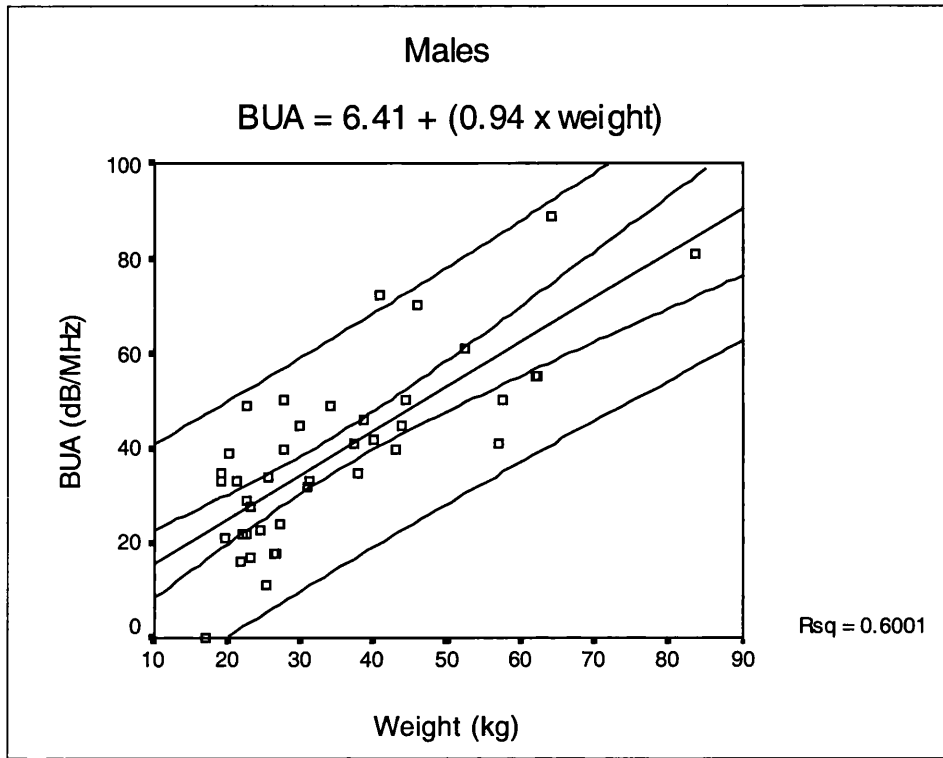
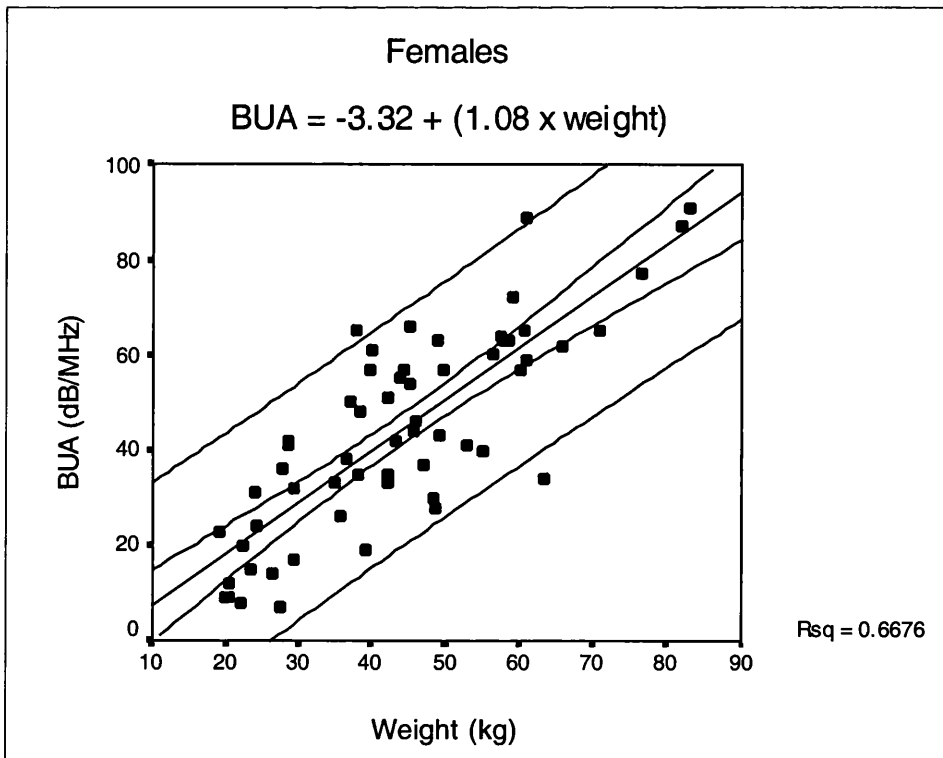


Figure 17 Linear regression of BUA on weight (females)



Using the linear regression equation for each predictor variable, specific for gender, the mean predicted value of BUA for each age between 5 and 20 years and for each kg of weight between 20 and 65 kg has been calculated as shown in tables 16 and 17.

Table 16 Age predicted BUA

Age (years)	Predicted BUA (dB.MHz ⁻¹)	
	Male	Females
5	18.81	16.25
6	23.40	20.51
7	27.99	24.77
8	32.58	29.03
9	37.17	33.29
10	41.76	37.55
11	46.35	41.81
12	50.94	46.07
13	55.53	50.33
14	60.12	54.59
15	64.71	58.85
16	69.30	63.11
17	73.89	67.37
18	78.48	71.63
19	83.07	75.89
20	87.66	80.15

Table 17 Weight predicted BUA

Weight (kg)	Predicted BUA (dB.MHz ⁻¹)				
	Male	Female	Weight (kg)	Male	Female
18	23.33	16.12	41	44.95	40.96
19	24.27	17.20	42	45.89	42.04
20	25.21	18.28	43	46.83	43.12
21	26.15	19.36	44	47.77	44.20
22	27.09	20.44	45	48.71	45.28
23	28.03	21.52	46	49.65	46.36
24	28.97	22.60	47	50.59	47.44
25	29.91	23.68	48	51.53	48.52
26	30.85	24.76	49	52.47	49.60
27	31.79	25.84	50	53.41	50.68
28	32.73	26.92	51	54.35	51.76
29	33.67	28.00	52	55.29	52.84
30	34.61	29.08	53	56.23	53.92
31	35.55	30.16	54	57.17	55.00
32	36.49	31.24	55	58.11	56.08
33	35.55	32.32	56	59.05	57.16
34	38.37	33.40	57	59.99	58.24
35	39.31	34.48	58	60.93	59.32
36	40.25	35.56	59	61.87	60.40
37	41.19	36.64	60	62.81	61.48
38	42.13	37.72	61	63.75	62.56
39	43.07	38.80	62	64.69	63.64
40	44.01	39.88	63	65.63	64.72
			64	66.57	65.80
			65	67.51	66.88

When calculating Z scores the standard deviations of the residuals for age and weight are used as the denominator in equation 13 (page 83). The standard deviations of the residuals of the regression lines for age are 12.07 for males and 15.47 for females. The standard deviations of the residuals for the regression lines for weight are lower at 11.84 for males and 12.15 for females.

8.2 Discussion

The scatterplots, correlations and coefficients of determination helped to identify weight as the most useful predictor of BUA in this sample. As weight increases so does BUA; the relationship is linear and is statistically significant. The height and age of the subjects also demonstrate a statistically significant linear relationship with BUA. Despite the relatively low value of r^2 for age as a predictor variable it has been retained and is used in subsequent analyses. This is to allow useful

comparisons with other studies because normative quantitative ultrasound charts in current use are based on age and gender-matched healthy control populations ([MUGH1997], [JAWO1995]). Weight is also retained for use in subsequent analyses and this is for two reasons. Firstly, weight is often used in paediatric growth charts for example those developed by Han and Babcock which provide normative graphs for ultrasound measurements of paediatric kidney length based on weight as well as age [HAN1985]. More importantly, weight is used as a predictor for BUA in subsequent analyses because it has been demonstrated herein that weight is the strongest predictor of BUA in healthy children in this sample.

The initial scatterplots demonstrated gender differences in the relationships between BUA and weight and BUA and age. These were confirmed by the subsequent calculation of gender specific correlations. The data were therefore categorised by gender for further analyses and these differences are reflected in the linear regression equations and subsequent predicted BUA values. Further sub-categorisation by pubertal status was not performed because the strongest predictor of BUA was weight and gender specific graphs were used in subsequent analyses.

The 95% confidence intervals for predicted BUA for females (figures 15 and 17) are narrower when weight rather than age is used as the predictor variable. We can therefore be confident that for females the true population mean for BUA lies closer to the predicted mean for BUA at each kg of weight than at each year of age. In addition the 95% prediction lines for females are also narrower for weight than for age. This is particularly useful for this research since weight is a key factor in children and adolescents with anorexia nervosa and related eating disorders. These predicted BUA values will be used in the next chapter to calculate Z scores for the subjects in the ANRED cohort.

The predicted values for BUA based on an equation derived from weight are interestingly very close to the actual weight of the subject, particularly for the females. For example a female subject who weighs 35kg has a predicted BUA of 34.48 dB.MHz⁻¹, and one who weighs 50kg has a predicted BUA of 50.68dB.MHz⁻¹. This could therefore be a useful “rule of thumb” to be applied in the clinical situation when a female is initially referred for treatment for an eating disorder. Therefore

only those who are at high risk of reduced BUA as assessed by this “rule of thumb” need be referred for an ultrasound scan.

These normative data were derived from adolescents in the South East of England which may introduce a degree of bias. However as the ANRED patients were also recruited in the South East of England such bias is limited in these data analyses.

8.3 Conclusion

Broadband ultrasound attenuation data on 41 healthy males and 59 healthy females have been analysed. Weight, age and height all demonstrate statistically significant linear relationships with BUA, however only weight and age have been used in subsequent analyses. Weight has been used because it accounts for 64% of the variance in BUA in this cohort. Age has been used to allow comparisons with published data. Least squares linear regression has been used to calculate predicted BUA values for each kg of weight between 18 and 65 kg and for each year of age between 5 and 20 years. The analyses have been performed on males and females separately to allow for gender differences.

Chapter 9 The ANRED cohort

The main aim of this chapter is to test the clinical null hypothesis stated in chapter 1 which is that children and adolescents with ANRED will on average have the same broadband ultrasound attenuation values through the left heel as healthy children and adolescents.

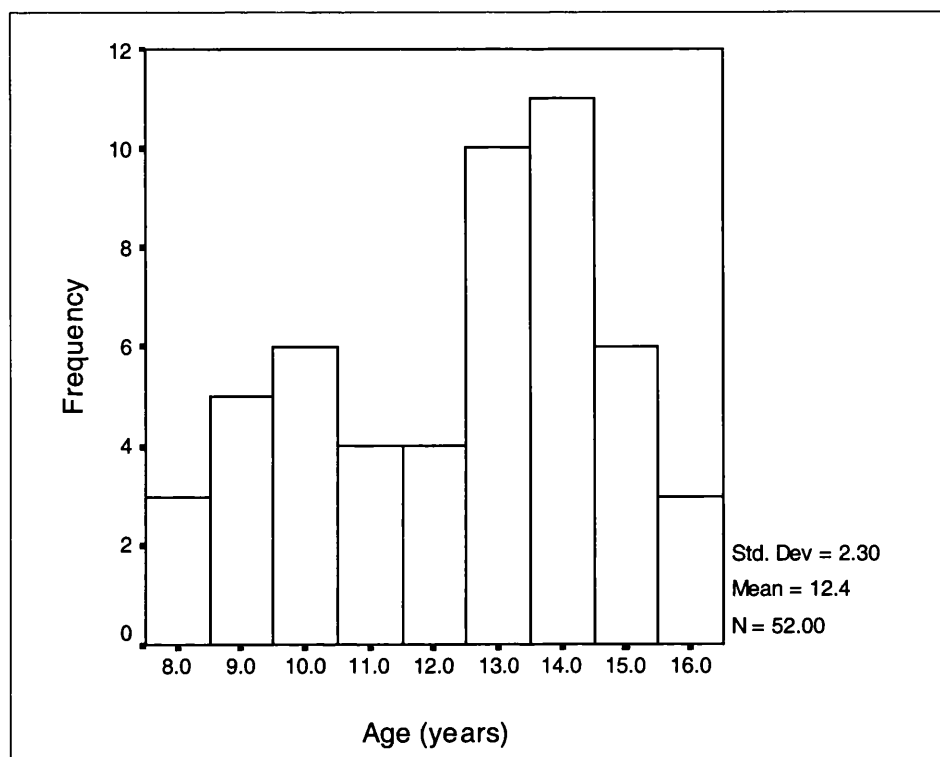
The secondary aim of the chapter is to investigate potential causes of any differences seen within and between the groups.

The clinical procedure for the examination is outlined in section 5.7 on page 84.

9.1 Exploratory data analysis

Fifty-two patients aged between 8 and 16 years consented to enter this research study. One 14 ½ year old female was excluded because she refused to be weighed. The final cohort comprised 11 males and 41 females. The majority of the cohort (47 patients) described their ethnicity as white; 2 described it as indian; 1 as asian “other” and 2 children described their ethnicity as “other”.

Initial evaluations were performed on these data using histograms and scatterplots of the 52 eating disorders patients as one group. The histogram in figure 18 illustrates the age distribution of this sample and highlights both a mean and a mode of patients of peri-pubertal age. This is unsurprising, as it is known that one of the contributing factors to anorexia nervosa is the onset of puberty (see chapter 3).

Figure 18 Histogram of age (eating disorders)

Data on the 52 eating disorder patients and 100 healthy subjects were plotted together to compare the relationships of BUA and age (figure 19) and BUA and weight (figure 20) between the two groups. A least square regression line is superimposed on the scatterplots for each of the two groups and the coefficients of determination are also included in the figure. The least squares regression line for weight as a predictor of BUA is of a steeper gradient for ANRED patients than healthy controls. A similar but less marked pattern is observed for the least squares regression line for age as a predictor of BUA.

Figure 19 Linear regression of BUA on age

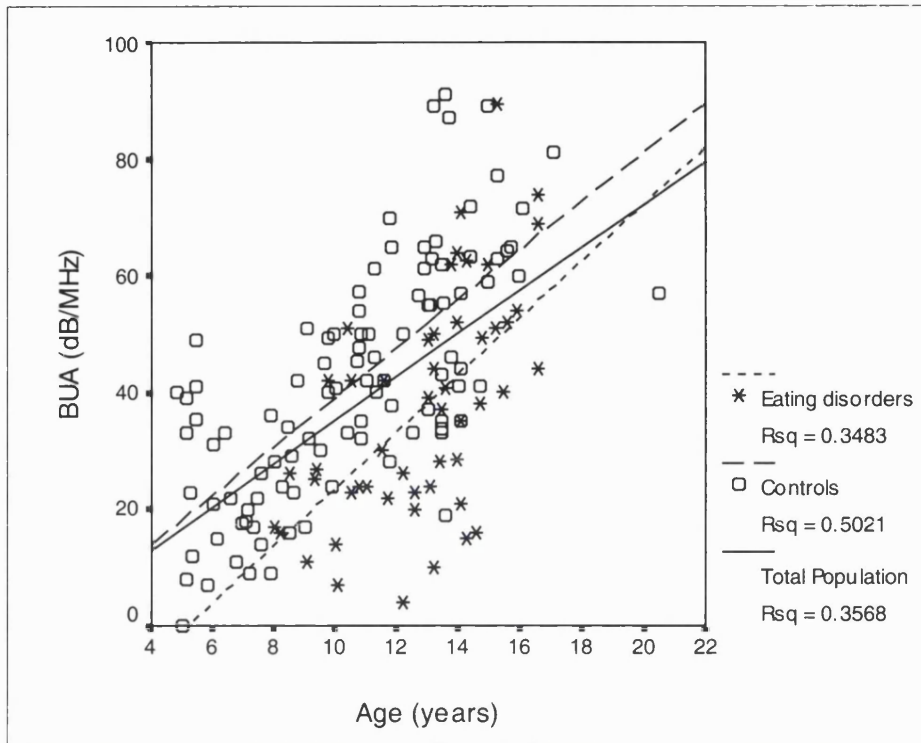
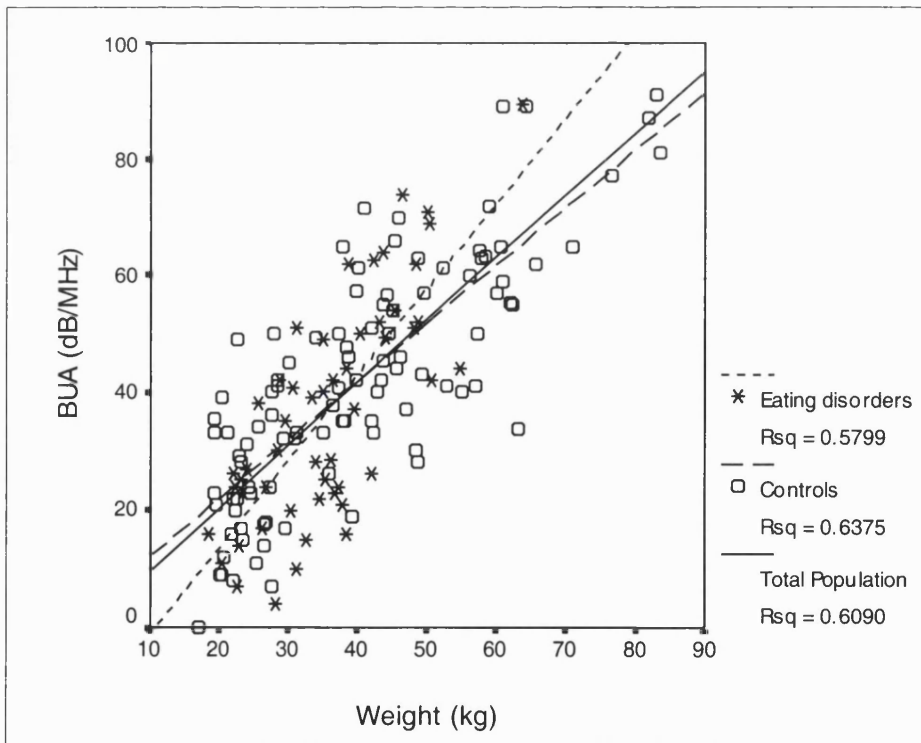


Figure 20 Linear regression of BUA on weight



BUA Z scores were calculated for all patients in this cohort using equation 13 and based on the mean and standard deviation values derived from the control sample as described in chapter 8. There are three sets of BUA Z scores for each patient as shown in tables 18 and 19. The first set is based on the mean BUA and associated standard deviation which is predicted by age and gender from the paediatric normative database provided with the CUBA machine by the manufacturer [MCCU1995]. The second and third sets of BUA Z scores are based on the new data from the control sample who were scanned for this research study. These new data BUA Z scores are based on the mean predicted BUA for age and gender, and for weight and gender. Tables 18 and 19 also demonstrate: the age of each patient at the time of the scan; the age at menarche for the females (pm = pre-menarcheal); the pubertal status of the boys (pre – pre-pubertal; peri – peri-pubertal) and the eating disorder of each patient. The eating disorders are abbreviated as follows: AN - anorexia nervosa; BN - bulimia nervosa; FAED - food avoidance emotional disorder and SE - selective eating.

Table 18 Female patients with eating disorders

Patient	Age	Age at menarche	Eating disorder	Z Score based on		
				Age (CUBA)	Age (New data)	Weight (New data)
670117	8	PM	FAED	-2.17	-0.84	0
701631	9	PM	FAED	-2.79	-1.44	-1.33
711813	9	PM	AN	-1.68	-0.41	0.54
703784	10	PM	Other	-3.35	-1.97	-0.93
669851	10	PM	FAED	-2.86	-1.52	-1.33
591664	10	PM	FAED	-2.63	-0.88	-0.42
671043	10	PM	FAED	-0.91	0.29	0.62
706190	10	PM	AN	-0.3	0.87	1.18
661145	11	PM	FAED	-2.58	-1.28	-0.05
8437	11	PM	AN	-2.43	-1.15	-0.77
637570	11	PM	FAED	-1.23	0.01	0.97
648131	12	PM	FAED	-4.1	-2.72	-2.78
652413	12	PM	AN	-3	-1.69	-1.37
701626	12	PM	FAED	-2.78	-1.49	0.12
670120	12	PM	AN	-2.56	-1.30	0.37
603740	13	PM	FAED	-3.97	-2.61	-1.12
656680	13	PM	FAED	-3	-1.70	-2.28
703198	13	PM	AN	-2.7	-1.44	0.18
707496	13	11.8 years	Other	-2.1	-0.86	-0.24
671046	13	PM	AN	-1.99	-0.73	0.46
8332	13	PM	FAED	-1.3	-0.09	1.20
T8953	13	11.5 years	AN	-1.22	-0.02	0.74
670444	13	12 years	BN	-0.35	0.75	1.02
669862	14	PM	AN	-3.82	-2.49	-0.99
666587	14	PM	FAED	-3.32	-2.17	-1.73
709053	14	PM	AN	-2.96	-1.65	-1.25
T8721	14	PM	AN	-2.33	-1.07	0.20
708607	14	PM	AN	-1.53	-0.36	0.40
T8776	14	11 years	AN	-1.31	-0.17	0.20
709283	14	12 years	AN	-0.61	0.54	1.73
654027	14	PM	SE	-0.61	0.61	2.16
710743	14	12 years	AN	-0.01	1.06	1.67
709176	15	11 years	AN	-2.43	-1.22	0.45
T8732	15	12 years	AN	-1.66	-0.51	0.20
707497	15	11 years	AN	-1.59	-0.44	0.73
8489	15	11 years	AN	-1.45	-0.31	0.63
T8775	15	10 years	AN	-1.58	0.20	1.91
616426	15	11 years	BN	0.97	2.01	1.99
656679	16	12.5 years	AN	-2.52	-1.24	-0.99
705283	16	11 years	AN	-0.78	0.38	1.51
706914	16	13 years	AN	-0.45	0.70	2.19

Table 19 Male patients with eating disorders

Patient	Age	Pubertal stage	Eating disorder	Z score based on:		
				Age (CUBA)	Age (New data)	Weight (New data)
T8740	8	Pre	SE	-2.19	-1.29	-1.17
701013	8	Pre	SE	-1.66	-0.55	-0.09
704490	9	Pre	SE	-2.04	-1.01	-1.21
708424	9	Pre	FAED	-2.06	-1.01	-0.26
710781	9	Pre	SE	-0.99	0.40	0.07
709171	10	Pre	Other	-2.51	-1.55	-0.50
700615	11	Pre	FAED	-2.46	-1.35	-0.23
658939	13	Pre	FAED	-2.47	-1.20	0.46
702326	13	Peri	SE	-2.23	-0.96	0.08
635453	14	Peri	FAED	-4.4	-3.74	-1.89
645792	14	Peri	FAED	-3.18	-2.08	0.03

The boxplots in figure 21 demonstrate the median, interquartile range and upper and lower limits of the BUA Z scores for all ANRED children. The BUA Z scores calculated using normative data from the manufacturer are lower than those calculated using the control data gathered during this research study.

Figure 21 Boxplots of BUA Z scores

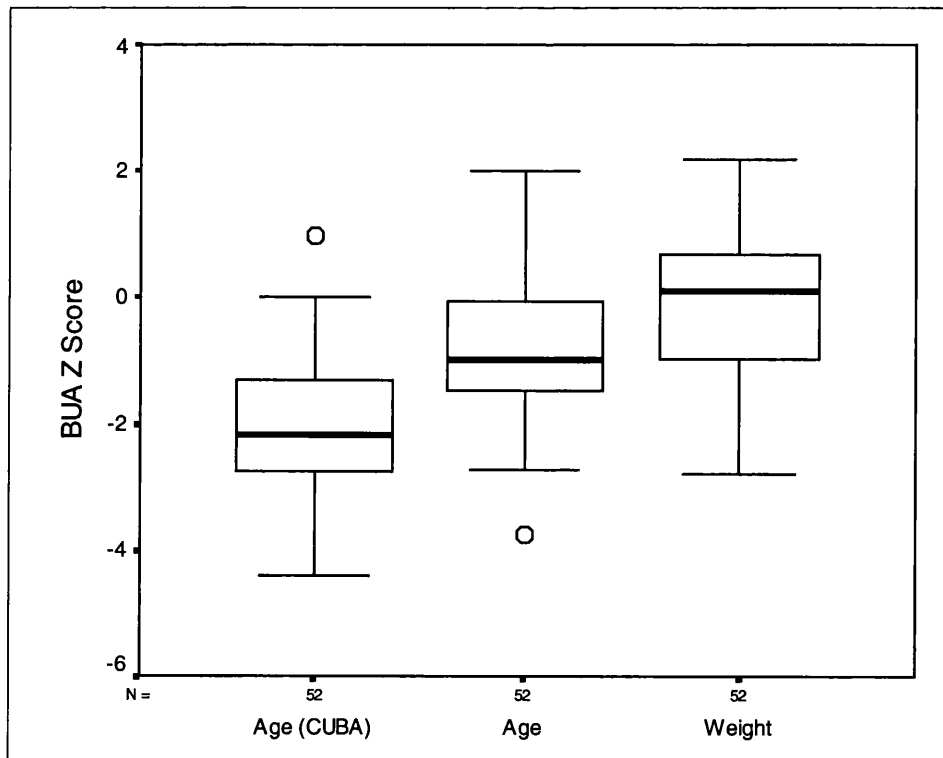


Figure 22 depicting a histogram of the distribution of BUA Z scores based on age and gender demonstrates a mean Z score of -0.82 and a mode Z score of -1.50 . In comparison the distribution of BUA Z scores based on weight and gender shown in the histogram in figure 23 demonstrates a much higher mean Z score of 0.02 and a mode Z score of 0 .

Testing the clinical null hypothesis

To test the probability that the mean of this sample of children and adolescents with ANRED is representative of the true population mean the standard error of the mean (SEM) and 95% confidence limits were calculated.

Table 20 Confidence intervals of BUA Z scores

	BUA Z Score (age and gender)	BUA Z Score (weight and gender)
Upper confidence limit	-0.52	0.33
Lower confidence limit	-1.12	-0.29
Mean	-0.8	0.02

These confidence intervals demonstrate that in 95% of cases the true population mean for BUA Z scores corrected for age and gender lies between -0.52 and -1.12 , whereas the true population mean for BUA Z scores corrected for weight and gender lies between 0.33 and -0.29 . Therefore since the confidence intervals for BUA Z scores corrected for age and gender do not contain the value 0 we can reject the null hypothesis that there is no difference between BUA values in children and adolescents with ANRED compared to the normal age and sex matched population. However the 95% confidence intervals for the BUA Z scores corrected for weight and gender do include the value 0 . In this situation we cannot reject the null hypothesis and instead we accept that there is no difference in BUA values between the two populations when they are normalised for weight and gender.

Figure 22 Histogram of BUA Z scores based on age and gender

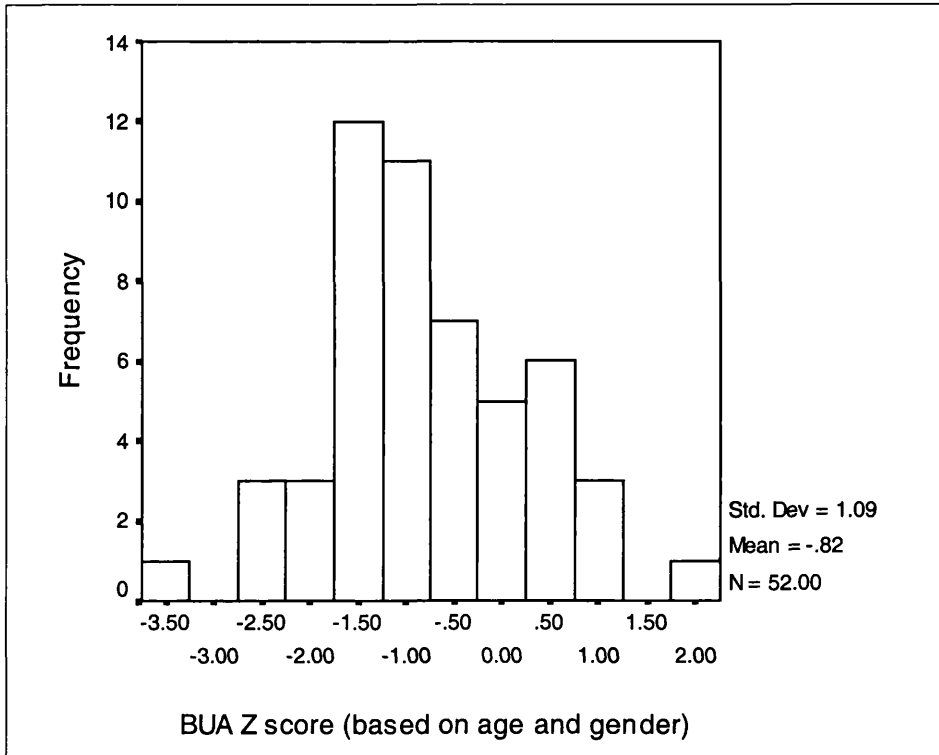
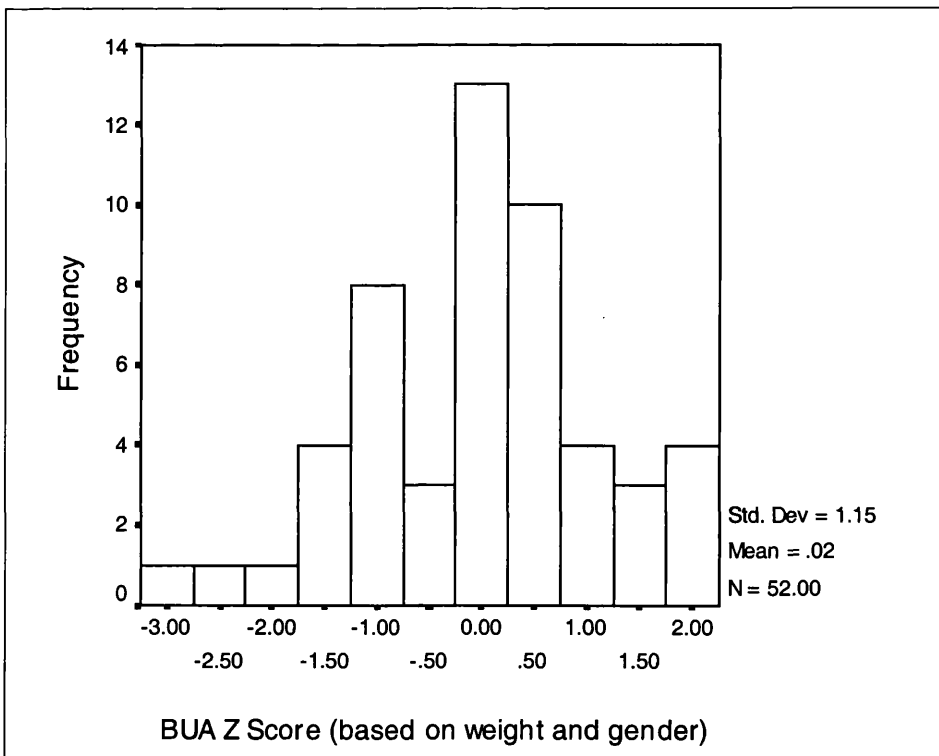


Figure 23 Histogram of BUA Z scores based on weight and gender



9.2 Sub-group analyses

The ANRED patients were analysed in a variety of sub-groups. Although this led to groups containing less than 30 patients, violating the conditions of the power calculation performed in chapter 5, these sub-analyses provide useful additional information about the impact of specific eating disorders in childhood on BUA measurements. The first evaluation was performed according to their psychiatric diagnosis. The males and females were then separated into different groups and the females were subdivided into those who were pre-menarcheal and those who were post-menarcheal at the time of the BUA scan. Additional analyses were performed to evaluate the effect of the longevity of the eating disorder on the BUA Z score of the patient.

Analysis by eating disorder

At the time of the examinations the operator was blinded to the psychiatric diagnosis of the patients. These diagnoses were obtained retrospectively from medical records and are shown in table 21.

Table 21 Psychiatric diagnoses of the eating disorder cohort

Eating disorder	N
Anorexia nervosa	23
Food avoidance emotional disorder (FAED)	18
Selective eating	6
Bulimia nervosa	2
Other eating disorder not specified	3

Box-plots demonstrating the median, interquartile range and minimum and maximum values were used for the exploratory analysis of these sub-groups in comparison to the 100 healthy subjects. All 23 children with anorexia nervosa were female and just over half of this group were post-menarcheal (n=12). There were 18 children with FAED and of these the majority were female (n=13), and they were all pre-menarcheal. There were 11 males in the entire eating disorders cohort and 5 were diagnosed as having FAED, 5 as being selective eaters and only one was diagnosed as having an “other” unclassified eating disorder. Figures 24 and 25 illustrate the distribution of age and weight in each of these sub-groups compared to the control sample.

Figure 24 Boxplots of age (eating disorders and controls)

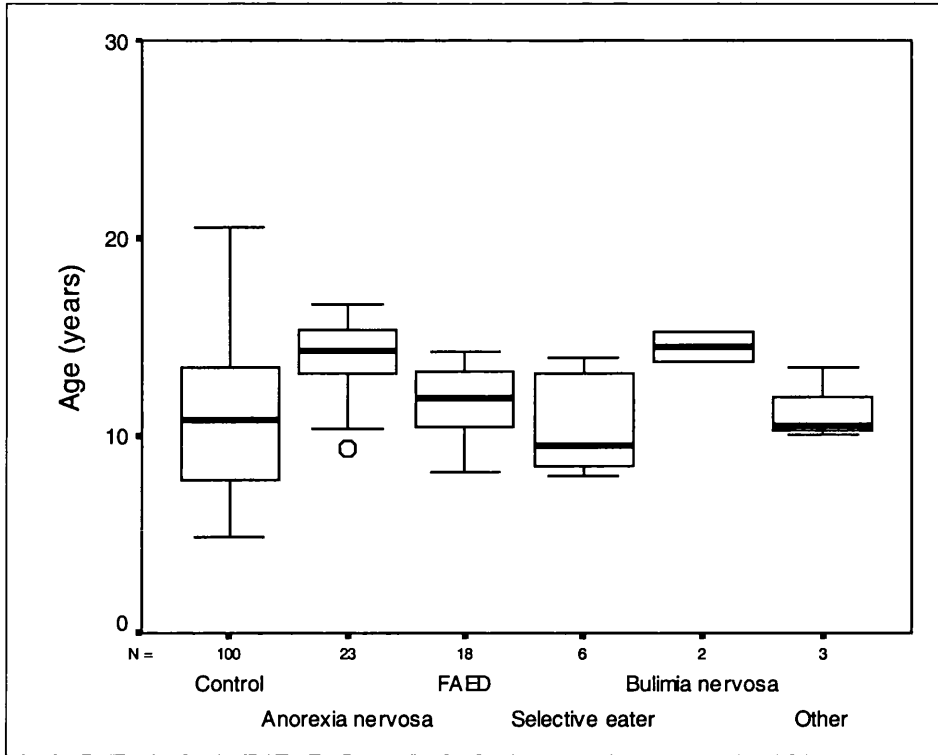


Figure 25 Boxplots of weight (eating disorders and controls)

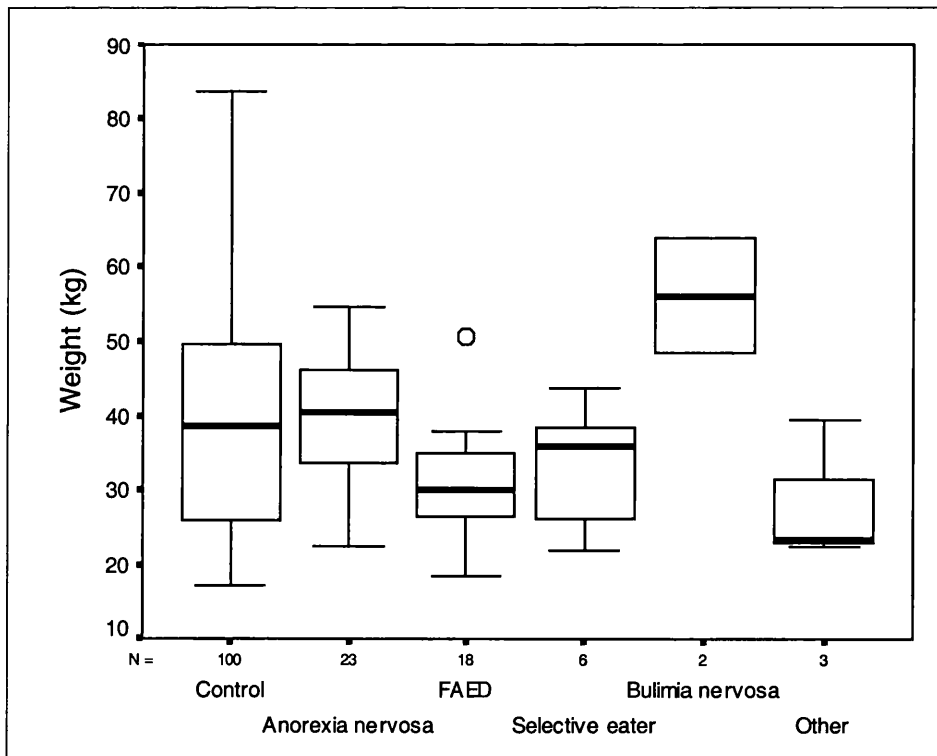


Figure 26 Boxplots of BMI (age-matched subjects)

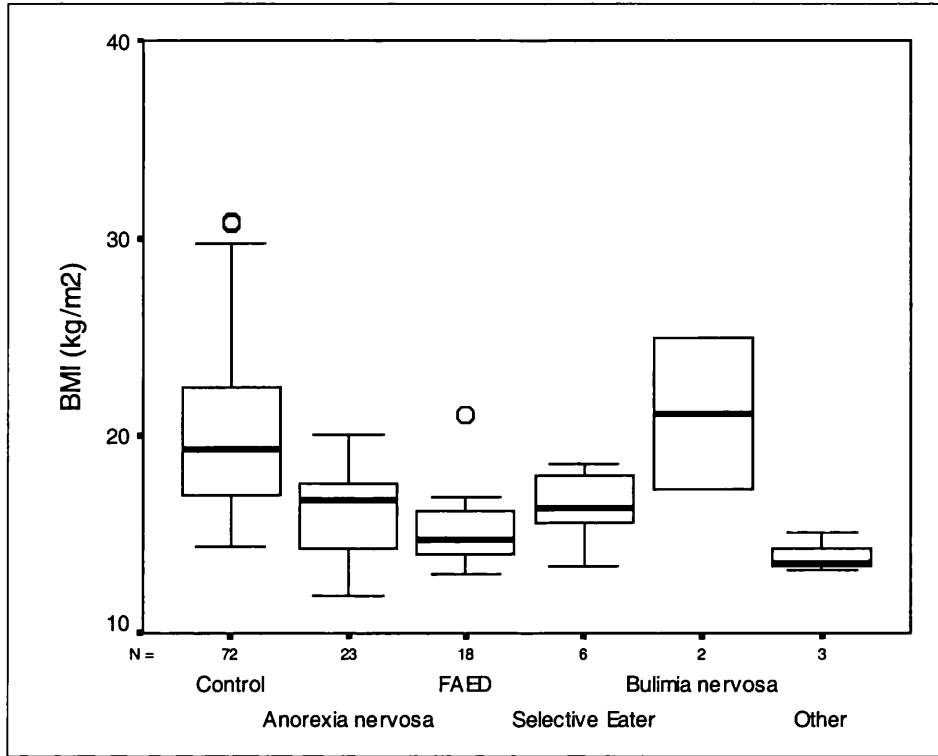
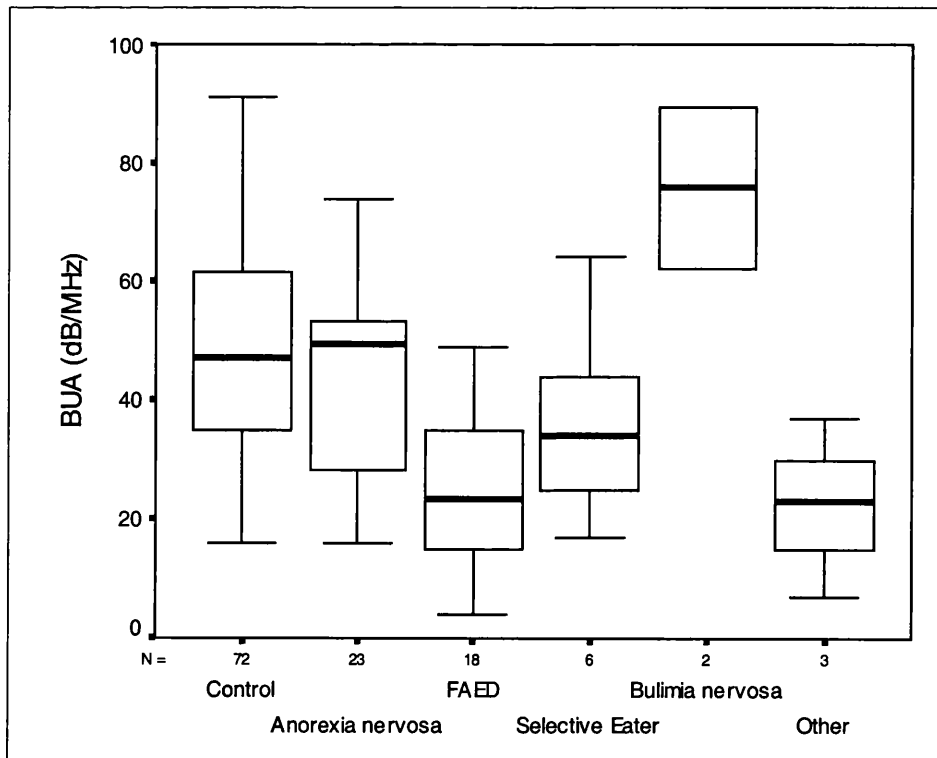


Figure 27 Boxplots of BUA (age-matched subjects)



Patients with anorexia nervosa are of a higher median age than those with FAED, and they also have higher median values for weight, BMI and BUA. The patients with FAED have the lowest median values for weight, BMI and BUA. The two post-menarcheal females with bulimia nervosa have the highest values for weight, BMI and BUA, in addition they are older than the other eating disorders patients.

The scatterplots in figures 28 and 29 illustrate the relationships between BUA Z score (corrected for gender and age/weight) and the age of the patient. The three sub-groups on each scatterplot demonstrate the difference between BUA Z scores for males and pre and post menarcheal females. When BUA Z scores are corrected for age and gender there are 16 pre-menarcheal females, 2 post-menarcheal females and 8 males (6 pre-pubertal and 2 peri-pubertal) with BUA Z scores between approximately -1 and -4. In comparison when BUA Z scores are corrected for both weight and gender there are only 8 pre-menarcheal females and 3 males (2 pre-pubertal and one peri-pubertal) with BUA Z scores between -1 and -3.

When BUA Z scores are corrected for age and gender only two post-menarcheal females have values less than -1. Whereas when BUA Z scores are corrected for weight and gender all post-menarcheal females have values within the normal range, i.e. greater than -1.

Figure 28 Scatterplot of BUA Z score (corrected for age and gender)

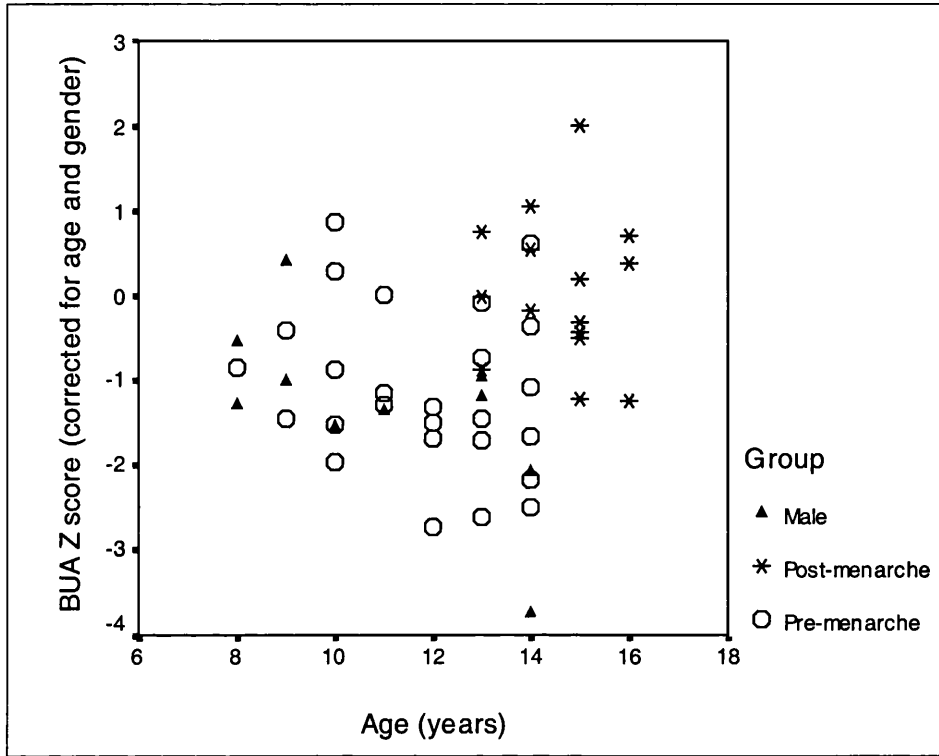
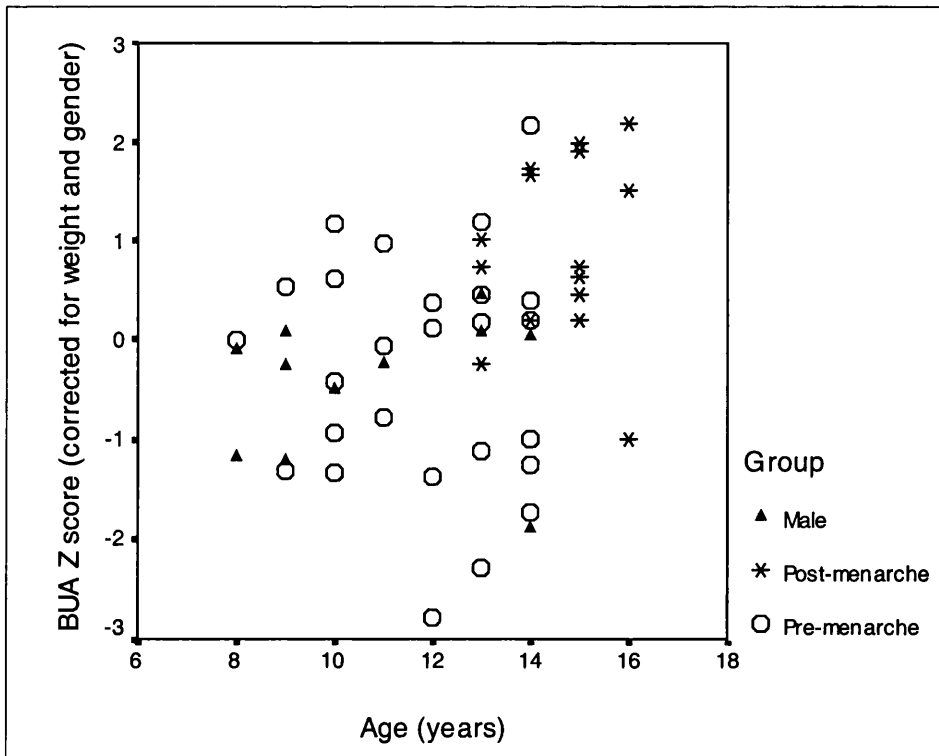


Figure 29 Scatterplot of BUA Z score (corrected for weight and gender)



Statistical testing

The data from the three largest sub-groups (AN, FAED and SE) fulfilled the criteria for the one way analysis of variance (ANOVA) as outlined in section 5.8 so this statistical test was used to identify any differences between the mean BUA Z scores of those with anorexia nervosa, FAED and selective eating. Two sets of BUA Z scores were used:

- BUA Z scores corrected for age and gender;
- BUA Z scores corrected for weight and gender.

Five patients were excluded because of the small size of their sub-groups: two females with bulimia nervosa and two females and one male with “other” undiagnosed eating disorders.

Comparing the three largest groups: anorexia nervosa, FAED and selective eating using ANOVA there is a statistically significant difference between them for gender-matched BUA Z scores corrected for age ($p < 0.009$) and for gender matched BUA Z scores corrected for weight ($p < 0.022$).

Table 22 ANOVA test for differences between AN, FAED and SE

		Sum of Squares	df	Mean Square	F	Sig.
BUA Z score (age and gender)	Between Groups	9.195	2	4.598	5.204	0.009
	Within Groups	38.870	44	0.883		
	Total	48.065	46			
BUA Z score (weight and gender)	Between Groups	9.837	2	4.919	4.184	0.022
	Within Groups	51.726	44	1.176		
	Total	61.563	46			

NB df = degrees of freedom

To ascertain which groups are different from each other the means and 95% confidence intervals were calculated as shown in figure 30.

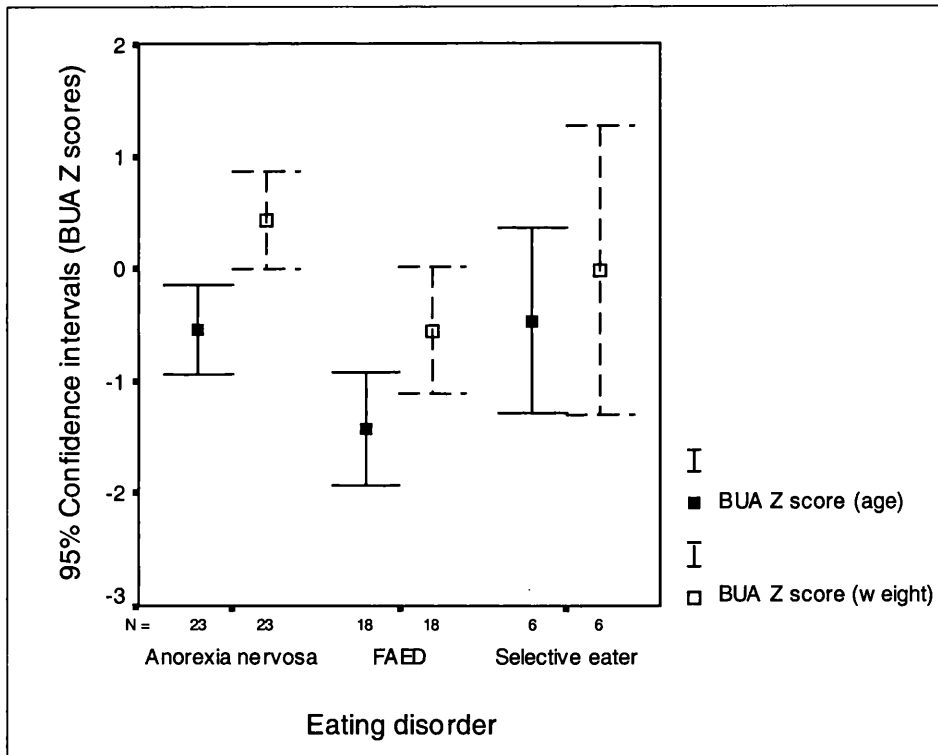
The means and 95% confidence intervals for BUA Z scores corrected for gender and age are significantly different from zero for patients in two of the three groups:

- anorexia nervosa (mean, -0.54, 95% CI -0.17, -0.91);
- FAED (mean, -1.43, 95% CI -0.96, -1.90).

The mean and 95% confidence intervals for BUA Z scores corrected for gender and weight are significantly less than zero for patients in only one group:

- FAED (mean, -0.56, 95% CI -0.03, -1.09).

Figure 30 Confidence intervals for BUA Z scores



The independent 2 sample t test was used to identify any statistically significant differences between the mean BUA Z scores of the pre-menarcheal (n=25) and post-menarcheal (n=12) females with AN, FAED and SE. Significantly lower BUA Z scores were seen in the pre-menarcheal group when corrected for both gender and age ($p < 0.003$) and gender and weight ($p < 0.004$).

Further analysis of patients with anorexia nervosa or FAED

Statistically significant differences between pre-menarcheal and post-menarcheal females with anorexia nervosa, FAED or selective eating were therefore confirmed using the t test, but the specific effect of each eating disorder was not evaluated.

Data analysis in the previous section demonstrated that children with anorexia nervosa or FAED have lower BUA Z scores than those with selective eating. Moreover although FAED is reported as being a mild variant of anorexia nervosa, these children have lower BUA Z scores than those with anorexia nervosa. These latter two groups were therefore examined further to identify potential predisposing features for the lower BUA Z scores. To establish whether the difference in BUA Z scores is reflective of eating disorder or menarcheal status, the females with anorexia nervosa and FAED were sub-divided according to eating disorder and menarcheal status. There were no post-menarcheal females with FAED leaving three sub-groups:

- Pre AN: pre-menarcheal females with anorexia nervosa (n=11);
- Post AN: post-menarcheal females with anorexia nervosa (n=12);
- Pre FAED: pre-menarcheal females with FAED (n=13).

ANOVA was used to test for differences in mean BUA Z scores between the three sub-groups of females to evaluate the effect of menarcheal status on BUA Z score over and above that of eating disorder.

Table 23 ANOVA test for differences in pre AN, post AN and pre FAED

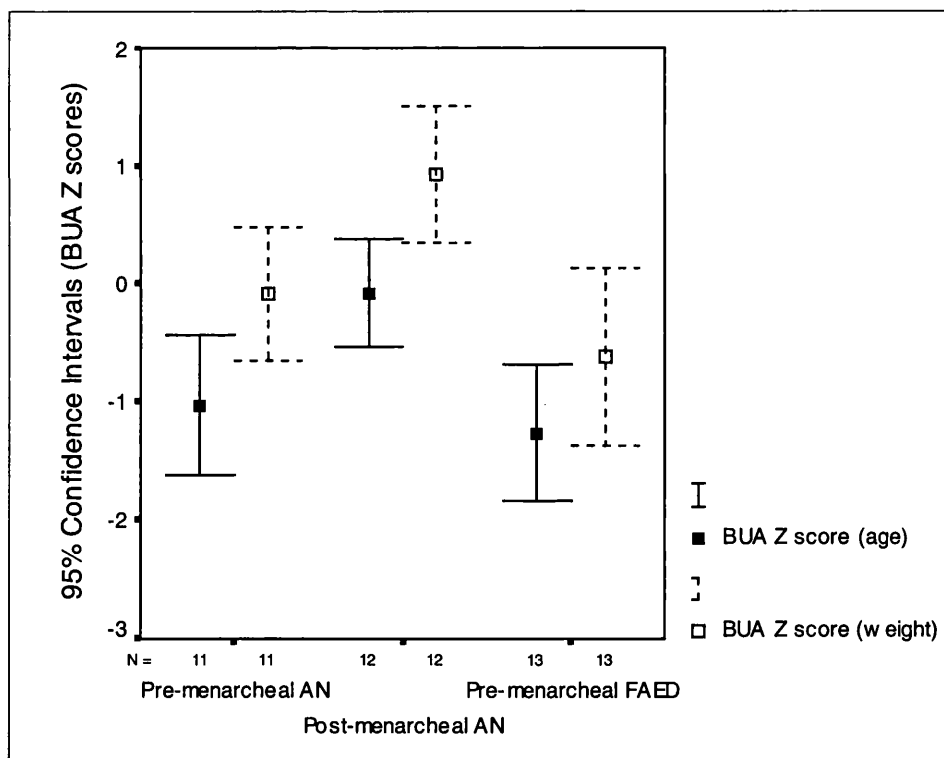
		Sum of Squares	df	Mean Square	F	Sig.
BUA Z score (age and gender)	Between Groups	9.550	2	4.775	6.517	0.004
	Within Groups	24.181	33	0.733		
	Total	33.732	35			
BUA Z score (weight and gender)	Between Groups	15.097	2	7.549	7.127	0.003
	Within Groups	34.953	33	1.059		
	Total	50.050	35			

NB df = degrees of freedom

Table 23 illustrates that there were statistically significant differences in the BUA Z scores when corrected for age and gender ($p < 0.004$) and when corrected for weight and gender ($p < 0.003$).

The means and 95% confidence intervals were once more used to identify which particular groups had BUA Z scores that were significantly different from zero and therefore were different from the control sample. Figure 31 demonstrates the 95% confidence intervals for each of these sub-groups and it is clearly visible that only pre-menarcheal females with anorexia nervosa and pre-menarcheal females with FAED have mean BUA Z scores (corrected for age and gender) which are significantly less than zero.

Figure 31 Confidence intervals based on menarcheal status



Although on figure 31 the 95% confidence intervals and mean BUA Z scores when corrected for weight and gender are not significantly different from zero the precise values have been calculated. The values for the pre-menarcheal subjects are shown in table 24. When calculating the confidence intervals a t-value of 2 was not assumed because the 3 groups are relatively small, containing less than 20 subjects. Standard t tables were used and calculating the degrees of freedom for each group as

n-1, the t-value for the pre-menarcheal anorexia nervosa sub-group is 2.23 based on 10 degrees of freedom. The t-value for the post-menarcheal anorexia nervosa sub-group is 2.20 based on 11 degrees of freedom. The t-value for the pre-menarcheal FAED sub-group is 2.18 based on 12 degrees of freedom. The mean BUA Z score (corrected for age and gender) for the 12 post-menarcheal anorexia nervosa patients was -0.09 (95%CI $+0.36, -0.54$) and the mean BUA Z score (corrected for weight and gender) was 0.91 (95%CI $+1.50, +0.33$).

Table 24 Confidence intervals (pre-menarcheal subjects)

	BUA Z Score (based on age and gender)		BUA Z Score (based on weight and gender)	
	AN	FAED	AN	FAED
Upper confidence limit	-0.45	-0.70	+0.47	+0.12
Lower confidence limit	-1.63	-1.83	-0.66	-1.36
Mean	-1.04	-1.26	-0.10	-0.62

These confidence intervals demonstrate that the BUA Z scores corrected for age and gender are significantly lower than zero (and therefore less than healthy controls) for pre-menarcheal females whether their eating disorder is anorexia nervosa or FAED. Furthermore, although the BUA Z scores based on weight and gender are not significantly different from zero for either group, the upper confidence interval for pre-menarcheal females with FAED is lower than that of pre-menarcheal females with anorexia nervosa and only just contains zero.

The effect of longevity of the eating disorder

Data on the duration of the eating disorder were available for 37 of the 41 children with anorexia nervosa or FAED. Three pre-menarcheal females with FAED and one post-menarcheal female with anorexia nervosa were unable to confirm the duration of their eating disorder. The scatterplots in figures 32 and 33 demonstrate the effect of longevity of anorexia nervosa and FAED, gender and for females menarcheal status on BUA Z score. Apart from one female with anorexia nervosa, children with FAED report suffering the longest duration of an eating disorder. BUA Z scores when corrected for age and gender vary from approximately -2.5 to $+0.5$ and -1 to $+0.5$ when corrected for weight and gender. The patients with the lowest BUA Z scores are those with FAED, the majority of whom are pre-menarcheal females.

Figure 32 Duration of eating disorder and BUA Z score (age and gender)

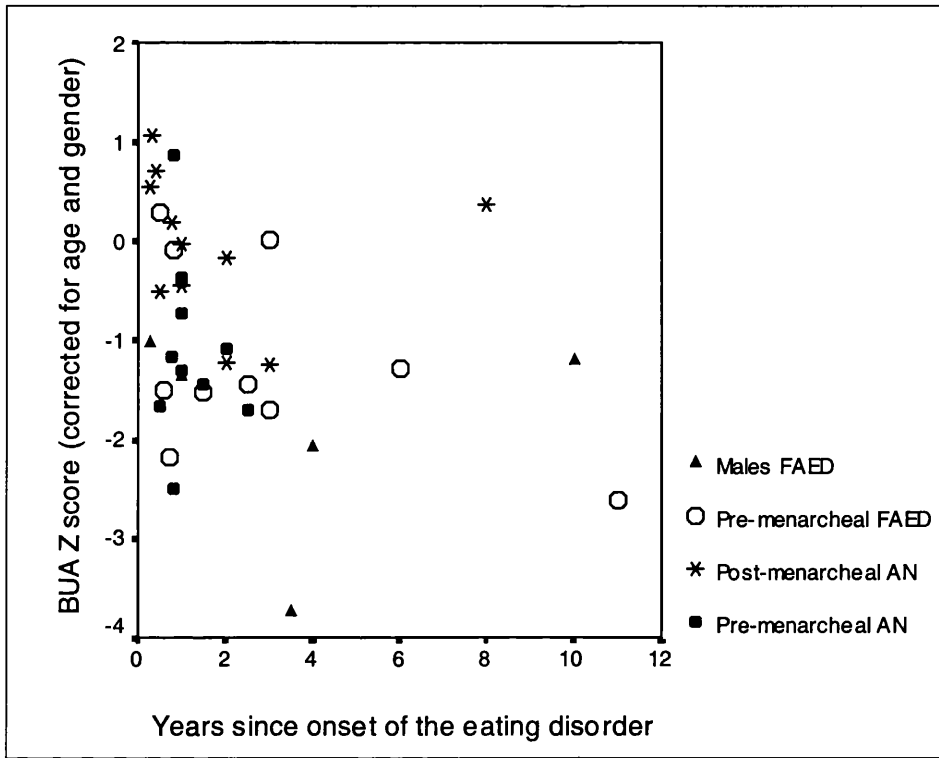


Figure 33 Duration of eating disorder and BUA Z score (weight and gender)



9.3 Discussion

As demonstrated in the previous chapter age is not as useful a predictor of BUA for children as is weight. It could be reasonably assumed that because of the nature of ANRED the effect of weight on BUA would be emphasised in these children. This is demonstrated visually on the least squares regression lines in figure 20, where for each kilogramme increase in weight the ANRED patients are predicted to have a greater increase in BUA than healthy subjects, i.e. the slope of the least squares regression line is steeper. However the coefficient of determination for weight as a predictor variable demonstrated that it accounted for approximately 58% of the variance in BUA in patients with ANRED compared to 64% in healthy subjects. The coefficient of determination for age as a predictor of BUA was only 35% in ANRED patients compared to 50% in control subjects. So although it is accepted that weight does not account for all of the variance seen in BUA in paediatrics it accounts for more of the variance than age. This concurs with BUA paediatric papers already published [MUGH1997] yet the most commonly used normative charts for BUA during growth are still based on age rather than weight.

All 52 ANRED children were initially analysed together and three sets of BUA Z scores were calculated (tables 18 and 19) and evaluated. The lowest series of BUA Z scores were those based on age and gender calculated from control data provided by the manufacturer of the equipment [MCCU1995]. These data were collected in a different region in England and using a different positioning technique for the measurements which may account for the differences seen in BUA Z scores.

The second and third sets of BUA Z scores were based on control data collected during this study and were normalised for age and gender for comparison with other studies and also weight and gender as weight was the strongest predictor of BUA. Statistical analyses performed on both the BUA Z scores provided by the manufacturer and those gathered during this research demonstrated that the 52 children in the eating disorders cohort had a significantly reduced mean BUA for their age when compared to healthy controls. However, surprisingly, when the mean BUA Z score was normalised for weight and 95% confidence intervals were calculated, there was no statistically significant reduction in BUA Z score. Although the BUA Z score normalised for weight is not significantly different from zero there

is a mean overall reduction in weight in these children as a result of their eating disorder when compared to healthy controls. Therefore the BUA Z score normalised for weight appropriately reflects the status of the bones as being equivalent to a group of children of lower weight. Consequently the ANRED children as a group are of a reduced mean weight for their age causing a reduction in their age corrected BUA Z score, however the BUA Z score is appropriate for their weight. BUA is therefore reduced for age in children with ANRED, but this is related to their reduction in weight with age.

When the children were sub-divided into groups with different eating disorders useful information was obtained about the impact of specific eating disorders on BUA during childhood. In particular the negative effect of anorexia nervosa on BUA was surprisingly not as marked as the negative effect of FAED. However further data analysis demonstrated that the poor results for those with FAED is primarily because the children with FAED were pre-menarcheal (if female) and pre or peri-pubertal if male.

The two patients with bulimia nervosa were of a normal weight for their age and both were post-menarcheal. It is therefore unsurprising that their BUA Z scores were appropriate for both age and weight. Of the three children with “other” eating disorders two were female and one was male. One of the females was pre-menarcheal and one was post-menarcheal and both had reduced BUA Z scores for age and weight. The male was pre-pubertal and also had a reduced BUA Z score for both age and weight.

The main analysis was on the remaining children who were divided into three sub-groups: anorexia nervosa (n=23), food avoidance emotional disorder (n=18) and selective eating (n=6). The mean BUA Z scores normalised for age were significantly different from zero (reduced) in children with FAED and those with anorexia nervosa, whereas those with selective eating had normal BUA Z scores. Surprisingly however, when the BUA Z scores for these three groups of children were corrected for weight only those with FAED were significantly reduced below zero. The BUA Z scores for those with anorexia nervosa and selective eating when normalised for weight were both within normal limits. These results are unexpected

because FAED is reported to be a mild form of anorexia nervosa with a better prognosis [HIGG1989], yet the children in this study with the lowest BUA Z scores were those with FAED. However of the 18 children with FAED 13 were female and all were pre-menarcheal at the time of the scan and five were male; three pre-pubertal and two peri-pubertal (aged between 9 and 14 years). In comparison there were 23 children with anorexia nervosa all of whom were female: 12 were post-menarcheal and 11 were pre-menarcheal. It is therefore likely that since over 50% of the sub-group of children with anorexia nervosa were post-menarcheal they were closer to attaining their genetically pre-determined peak bone mass than those in the FAED group who were all pre-menarcheal. As explained in chapter 2 a genetically pre-determined peak bone mass can be attained as early as two years post-menarche for females. When pre-menarcheal and post-menarcheal females with anorexia nervosa and pre-menarcheal females with FAED were compared the two pre-menarcheal groups had significantly reduced mean BUA Z scores corrected for age and gender but normal mean BUA Z scores corrected for weight and gender. This suggests that although the two groups of females had different eating disorders the predisposing feature for low BUA Z scores was actually their menarcheal status at the time of the scan, rather than the eating disorder itself.

The data in this research support the notion that the onset of menarche has a positive impact on BUA and that weight is a more useful predictor of BUA than age. If for example a female adolescent of 14 years of age has an eating disorder and is post-menarcheal she may have a BUA equivalent to a child of only 12 years of age and yet still have a normal BUA for her weight. However if that female has delayed puberty and is the same age and weight but is pre-menarcheal it is likely that her BUA would be even lower than that of someone of the same age and weight who was post-menarcheal, regardless of the psychiatric diagnosis of the eating disorder. The age, weight and pubertal status of a child have a significant impact on the BUA Z score and in children with ANRED the pubertal status of the child at the onset of the eating disorder is critical to bone growth and development as measured with BUA.

Several authors ([HERG1995], [HERZ1993], [BROO1998]) have reported osteoporosis or osteopenia as being amongst the long-term effects of anorexia

nervosa. It is of note that in these papers the osteoporosis or osteopenia is most marked in those women who have had oligomenorrhoea and/or amenorrhoea and the bone mineral density of these women has been negatively correlated with the chronicity of the eating disorder. Data on pre-menarcheal children in this research demonstrate that the severity of the BUA values is related to the pubertal status and weight at the age of onset of the eating disorder as well as the duration of the eating disorder. Those children with a BUA Z score below -1 and who reported having an eating disorder for between 4 and 12 years were all pre-menarcheal or male. The most severe BUA Z scores were seen in pre-menarcheal females and males in the first 4 years of the onset of an eating disorder. When BUA Z scores were corrected for age and gender there were 23 children with values below -1 and of those: 30% were male; 61% were pre-menarcheal female and only 9% were post-menarcheal female. These data therefore indicate that the menarcheal status of females and the menarcheal status at the age of onset of an eating disorder are both important factors to consider when assessing the risk of osteoporosis or osteopenia using BUA measurements during growth and development. The effect of menarcheal status would account for the unexpected high BUA Z scores seen in the anorexia nervosa group where 50% of the group were post-menarcheal females and BUA Z scores corrected for weight were not significantly reduced from zero.

Pre-menarcheal females with ANRED reported undertaking significantly less exercise per week than controls (Mann-Whitney U test, $p < 0.04$), averaging approximately 3.7 hours a week compared to 7.9 hours a week for healthy pre-menarcheal control females of the same age. In comparison there was no significant difference in hours of exercise undertaken each week between post-menarcheal females and post-menarcheal controls. The literature review in section 2.2 demonstrated that when exercise intervention programmes are introduced for as little as 10 months during the pre-pubertal years there is a surprisingly significant positive impact on bone mineral density. The corollary being that if the greatest positive effect on bone development can be made in the pre-pubertal years, so the greatest negative effect can also be made at this age. Just as the exercise intervention programme which lasted only 10 months duration made a significant positive impact on bone mineral density [BRAD1998] so an eating disorder of short duration can have a significant negative impact on BUA.

The combined effect of reduced weight, delayed pubertal status and reduced exercise places pre-pubertal children with anorexia nervosa or FAED at the greatest risk of reduced BUA and therefore potentially future osteoporosis and bone fractures. The power calculations in chapter 5 demonstrated that at least 30 children with ANRED were needed to be confident of seeing a difference of 1 standard deviation that was significant at 2.5% with a power of 95%. When BUA Z score data (corrected for age and gender) on the entire group of 52 ANRED children were compared to that of healthy control children those with ANRED had a mean BUA Z score of -0.8 which was significantly different from zero (95% CI -0.52, -1.12). This reduction in BUA Z score is particularly relevant if extrapolations are made from data in post-menopausal populations where it has been demonstrated that a standard deviation reduction of 1 in quantitative ultrasound or bone mineral density doubles the patient's fracture risk [HANS1996].

9.4 Conclusion

Weight has the greatest predictive ability for BUA in both healthy children and those with ANRED and accounts for up to 64% of the variance seen. The mean BUA Z score (corrected for age and gender) of the 52 children with ANRED was significantly lower than zero however the BUA Z scores (corrected for weight and gender) were not significantly different from zero. Therefore, since BUA Z scores corrected for age and gender are the accepted standard method of measuring BUA, the clinical null hypothesis outlined in chapter 1 can be rejected, i.e. there is a statistically significant difference in BUA between ANRED children and controls. Reduced weight for age is the main cause of reduced BUA for age in children with ANRED.

Although BUA normalised for age is the standard method of assessing bone status in children these data suggest that it is more appropriate to measure BUA Z scores normalised for weight. This more accurately identifies those children at the greatest risk of osteoporosis as represented by reduced BUA values in this study.

These data demonstrate that children with FAED have significantly reduced Z scores when BUA is corrected for both age and weight, whilst children with anorexia nervosa have significantly reduced BUA Z scores corrected for age but normal BUA Z scores corrected for weight. However when female children with FAED and anorexia nervosa were sub-divided into groups according to menarcheal status, BUA Z scores were significantly reduced below zero for the two groups who were pre-menarcheal, regardless of their primary eating disorder. Therefore pre-menarcheal status at the onset of the disease rather than specific eating disorder is more predictive of low BUA values.

The surprisingly marked reduction in BUA Z scores for children with FAED and pre-menarcheal children with anorexia nervosa may be related to the synergistic effects of reduced weight, delayed pubertal status, reduced exercise and the young age at the onset of the eating disorder.

Although the number of children with selective eating and bulimia nervosa were very small and therefore not statistically significant, their results are worthy of comment. The BUA Z scores of these children were either not significantly different from zero or were greater than zero. This reflects the positive relationship between weight and BUA.

Children and adolescents who have had an eating disorder for up to 12 years have similar BUA Z scores as pre-menarcheal children who have had an eating disorder for less than 4 years. This indicates that low BUA Z scores are related to pubertal status at the onset of an eating disorder

Chapter 10 The longitudinal data

The main aim of this chapter is to examine and evaluate the magnitude of annual changes in BUA in children with ANRED compared to healthy control children. This will enable useful statistical inferences about the populations from which the data are derived which will contribute to the body of knowledge of research in this field. The null hypothesis as shown below and outlined in chapter 1 will be tested using the longitudinal data recorded on children and the precision error data derived in chapter 7. The null hypothesis is that the precision error of the broadband ultrasound attenuation technique in the McCue CUBA Clinical in children and adolescents will on average be the same as the annual increase in broadband ultrasound attenuation in children and adolescents. The corollary being that the CUBA will not be sensitive enough to detect significant annual changes in BUA in children and adolescents.

There are two main themes to this chapter. The first is an evaluation of the utility of broadband ultrasound attenuation as measured by CUBA as a longitudinal monitoring device for examinations of the heel during growth and development. The second theme is to examine the causes of the changes seen in BUA during growth and development in children with ANRED compared to healthy control children.

10.1 Exploratory data analysis

Methods

The initial data analyses and evaluations were performed with children grouped into one of two categories: those with or without an eating disorder. There were 23 children (44% of the original sample) with ANRED who responded positively to the invitation for the follow up scan. In comparison, 62 healthy control children (62% of the original sample) agreed to participate in the follow up study. In the power calculation in chapter 5, section 2 it was calculated that 30 children with ANRED would need to be recruited to detect a significant difference of 1 standard deviation between the ANRED cohort and the control cohort. This recruitment target was intentionally exceeded for the cross-sectional study (chapter 9) because of an anticipated high attrition rate. However, although 52 children with ANRED were initially recruited it was not possible to retain the target figure of 30 for the longitudinal study. The power of the longitudinal study was therefore re-assessed retrospectively using equation 12 (page 79) and standard power tables as in chapter 5. Reducing the power from 95% to 90% and reducing the significance level to 5% gives a revised F value of 10.51. Replacing this in equation 12 allows a reduced recruitment target of 21 patients for the longitudinal analysis to detect a standardised difference of one standard deviation between the ANRED and the control sample.

The baseline data of those children who did and did not participate in the follow up study were categorised separately and evaluated to examine any effects of selection bias in both the ANRED sample and the healthy control sample. The results of these baseline data (means and standard deviations) are shown in tables 26 and 27.

Attrition from the anorexia nervosa and related eating disorders sample

The 23 children with ANRED who participated in both parts of the study are detailed by specific eating disorder in table 25.

Table 25 ANRED patients attending for follow-up scans

	N	Proportion of original sub-group sample (%)
Anorexia nervosa	10	43
FAED	9	50
Selective eater	3	50
Bulimia nervosa	0	0
Other	1	33

There were 24 females who did not take part in the follow up study and of those, 12 were pre-menarcheal and 12 were post-menarcheal at the time of the first scan. In comparison there were 17 females who attended for both scans and of these only 3 were post-menarcheal at the time of the first scan. There were 14 pre-menarcheal females who participated in the follow up study and of these, 4 attained menarche in the year between the scans.

Four of the six boys who attended for both scans were pre-pubertal and 2 were peri-pubertal. Four of the five boys who did not take part in the follow up study were pre-pubertal and one was peri-pubertal.

Table 26 ANRED baseline and follow up data

	Participation in follow up study	
	No (n=29)	Yes (n=23)
Male:female	5:24	6:17
Age (years)	12.24 (2.52)	12.52 (2.04)
Weight (kg)	36.63 (11.05)	36.64 (8.68)
Height (m)	1.49 (0.15)	1.47 (0.13)
BMI (kg.m ⁻²)	16.03 (2.70)	15.76 (2.13)
BUA (dB.MHz ⁻¹)	37.38 (21.55)	36.07 (16.46)
1 st scan		
BUA Z score (age and gender)	-0.45 (1.01)	-1.29 (1.01)*
BUA Z score (weight and gender)	0.34 (0.99)	-0.38 (1.23)**

** p<0.022, * p<0.005

The independent student t test confirmed significant differences in BUA Z scores corrected for both age and gender (p<0.005) and weight and gender (p<0.022)

between the two groups: the mean BUA Z scores of the group who attended for follow up scans were significantly lower at baseline. The absolute BUA value in dB.MHz^{-1} was not however significantly different between the two groups ($p=0.811$). Apart from the difference in baseline BUA Z scores there were no significant differences in the baseline parameters between those who did and did not attend for the second part of the study.

Attrition from the healthy control sample

There were 38 children who did not participate in the follow up study and their details are shown in table 27 together with the details of the 62 children who did take part in the follow up study.

Table 27 Control baseline and follow up data

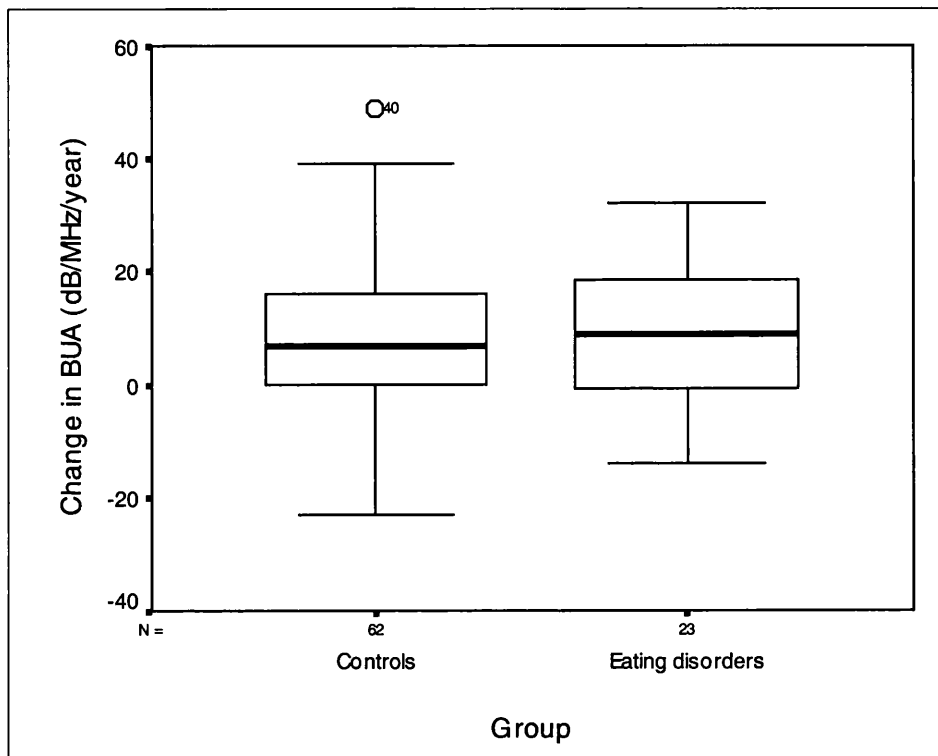
	Participation in follow up study	
	No (n=38)	Yes (n=62)
Male:female	14:24	27:35
Age (years)	11.09 (3.58)	10.38 (3.32)
Weight (kg)	40.57 (16.92)	39.80 (16.06)
Height (m)	1.43 (0.18)	1.41 (0.04)
BMI (kg.m^{-2})	19.00 (4.02)	19.10 (3.51)
BUA (dB.MHz^{-1})	43.32 (21.72)	41.13 (19.38)
1 st scan		

As in the ANRED sample there were 24 female controls who did not take part in the follow up study. Thirteen of these 24 females were post-menarcheal and 11 were pre-menarcheal at the time of the first scan. In comparison there were 35 females who participated in both scans and of these 13 were post-menarcheal at the time of the first scan and 22 were pre-menarcheal. Three of these pre-menarcheal females attained menarche during the year between scans. Independent sample student t tests demonstrated that there were no statistically significant differences in the baseline parameters between those who did and did not participate in the follow-up study. The pubertal status of the boys in this control sample was not assessed.

10.2 Evaluating longitudinal BUA changes

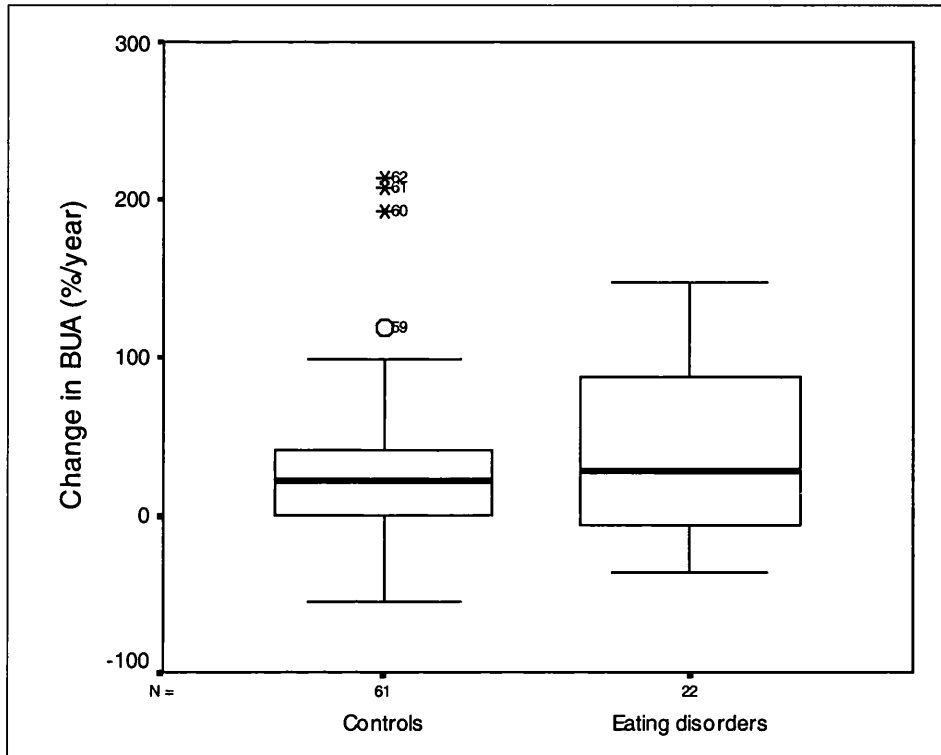
In this study the precision error of the CUBA, calculated as the root mean square standard deviation in section 7.2, was 6.02 dB.MHz^{-1} . The relative precision error calculated as the standardised coefficient of variation in section 7.2 was 7.05%. Therefore, as explained in section 4.4, to be reasonably certain that BUA differences seen between scans are as a result of true in vivo developments there should be changes of at least 3 multiples of the precision error (18dB.MHz^{-1}) or relative precision error ($21\%\text{dB.MHz}^{-1}$) between two scans on the same child. This enables true clinical changes to be seen with 80% certainty and 10% significance.

Boxplots showing the annual changes in BUA (dB.MHz^{-1}) and percentage BUA for the healthy control children compared to those with ANRED are shown in figures 34 and 35. Two children were excluded from the calculation of percentage annual increase. One child from the control sample increased in BUA from 0 dB.MHz^{-1} at the first scan to 5 dB.MHz^{-1} , making it inappropriate to calculate a percentage increase and one child with FAED had an annual increase in BUA of 32dB.MHz^{-1} from 4 dB.MHz^{-1} at the first scan which is equivalent to an increase of 800% making it an extreme outlier, well beyond the range of the rest of the group.

Figure 34 Longitudinal changes in BUA

The boxplots of annual BUA changes in dB.MHz^{-1} (figure 34) demonstrate a similar pattern for both cohorts of children. There is a median annual increase in BUA of 9dB.MHz^{-1} with an upper quartile value of 18.5dB.MHz^{-1} for ANRED patients compared to a median annual increase of 7dB.MHz^{-1} and an upper quartile value of 16dB.MHz^{-1} for the control children. The interquartile range for both cohorts is also similar: 19dB.MHz^{-1} for the ANRED cohort and 16dB.MHz^{-1} for the control cohort. In comparison the annual percentage changes in BUA are different for the two cohorts. The boxplots in figure 35 demonstrate a median annual increase of 28.5% and an upper quartile value of 87% for children with ANRED compared to a median annual increase of 22% and an upper quartile value of only 41% for the control children. Therefore one quarter of children in the ANRED cohort had annual percentage improvements in BUA of between 28.5% and 87% and a further quarter had increases in excess of 87% between scans.

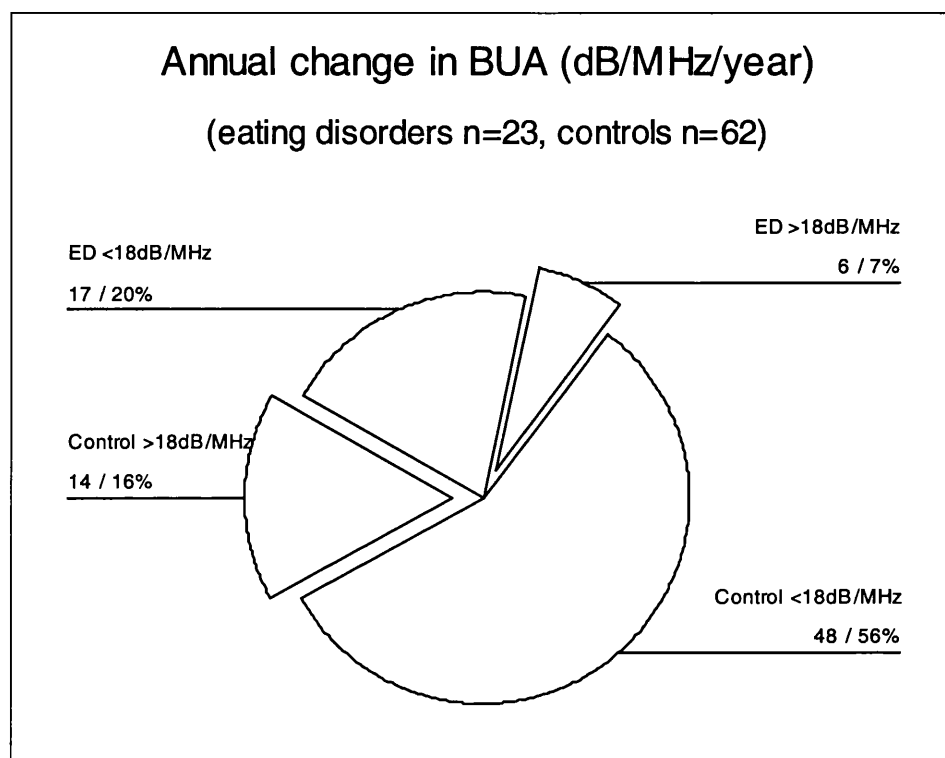
Figure 35 Longitudinal changes in percentage BUA



Clinically significant differences

Pie charts were used to identify those children who had clinically significant changes in BUA, i.e. changes which were greater than 3 multiples of the precision error or the relative precision error. In each pie chart children have been categorised according to whether they have an eating disorder or are controls and then sub-categorised by whether the annual changes in BUA were more or less than 3 multiples of the precision error or relative precision error.

The pie chart in figure 36 demonstrates annual BUA changes in children in relation to three multiples of the precision error (18 dB.MHz⁻¹). Only 24% of the total follow up sample (14 control children and 6 with ANRED) had annual BUA scans which exceeded 18dB.MHz⁻¹.

Figure 36 Annual changes in BUA

A chi-square test for a 2 way table was performed on these data to test the null hypothesis that there is no significant difference between the expected number of ANRED children and control children who achieve annual increases in BUA in excess of three multiples of the precision error.

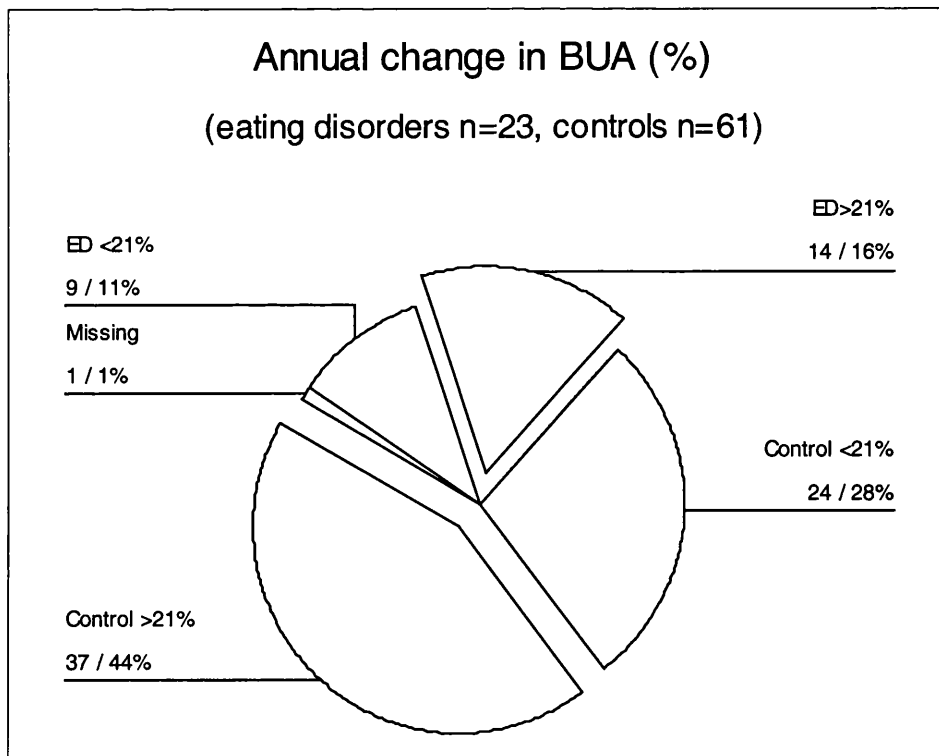
Table 28 Chi square table for controls and ANRED (dB.MHz⁻¹.yr⁻¹)

Observed	Less than 18 dB.MHz ⁻¹ .yr ⁻¹	More than 18 dB.MHz ⁻¹ .yr ⁻¹	Total
Controls	48	14	62
ANRED	17	6	23
Total	65	20	85
Expected			
Controls	47.41	14.59	62
ANRED	17.59	5.41	23
Total	65	20	85
(Observed-expected)²/expected			
Controls	0.007	0.024	0.031
ANRED	0.020	0.065	0.085
Total and chi square statistic			0.116

The chi square critical value at the 5% significance level for one degree of freedom is 3.84 and the chi statistic calculated on these data was 0.12 (2dp). Therefore there is no significant difference in the proportion of children receiving treatment for ANRED and control children who have annual BUA increases in excess of three multiples of the precision error of 18dB.MHz⁻¹.

The analysis was repeated using three multiples of the relative precision error (21% dB.MHz⁻¹.year⁻¹). The pie chart in figure 37 demonstrates that 60% of the total follow up sample (14 children with ANRED and 37 control children) had changes in BUA which were equal to or in excess of three multiples of the relative precision error. One child was excluded from this analysis because it was not possible to calculate a percentage increase in his BUA values (0dB/MHz at the first scan and 5dB/MHz at the second).

Figure 37 Annual changes in percentage BUA



Once again a chi-square test for a 2 way table was performed on these data to test the null hypothesis that there is no significant difference between the expected number of ANRED children and control children who achieve annual percentage increases in BUA in excess of three multiples of the relative precision error.

Table 29 Chi square for controls and ANRED (%dB.MHz⁻¹.yr⁻¹)

Observed	Less than 21% dB.MHz ⁻¹ .yr ⁻¹	More than 21% dB.MHz ⁻¹ .yr ⁻¹	Total
Controls	24	37	61
ANRED	9	14	23
Total	33	51	84
Expected			
Controls	23.96	37.04	61
ANRED	9.04	13.96	23
Total	33	51	84
(Observed-expected)²/expected			
Controls	8.0x10 ⁻⁵	5.0x10 ⁻⁵	1.3x10 ⁻⁴
ANRED	2.0x10 ⁻⁴	1.0x10 ⁻⁴	3.0x10 ⁻⁴
Total and chi square statistic			4.3x10⁻⁴

The chi square critical value at the 5% significance level for one degree of freedom is 3.84 and the chi statistic calculated on these data was 4.3×10^{-4} . Therefore the null hypothesis was accepted.

Sub-group analysis by eating disorder

The mean annual changes in BUA and percentage BUA for each sub-group by eating disorder are shown in table 30. The mean annual increase in BUA was within the limit of $\pm 18 \text{dB.MHz}^{-1}$ for each of the sub-groups and was therefore not clinically significant. In comparison there was a mean annual increase in BUA of at least 21% for three of the four groups (control, anorexia nervosa and FAED). There were only 3 patients with selective eating and they were all male (2 pre-pubertal and 1 peri-pubertal). The mean BUA of the selective eating group stayed virtually unchanged between the two scans.

Table 30 Annual changes in BUA by sub-group

Group	N	Mean annual change in BUA in dB.MHz^{-1} (SD)	Mean annual percentage change in BUA
Control	62	+8.3 (13.20)	+30
Anorexia nervosa	10	+11.1 (16.90)	+49
FAED	9	+10.6 (10.41)	+126
Selective eating	3	-0.3 (3.79)	-4

As there was only one child with an eating disorder described as “other” she was excluded from sub-group analyses. The BUA for this female increased from 7dB.MHz^{-1} at the first scan to 19dB.MHz^{-1} at the second.

Sub-group analysis by gender and menarcheal status

The control and eating disorders children were sub-categorised according to gender, which unfortunately led to small sample sizes, violating the conditions of the revised power calculation so whilst the results are informative they should be treated with caution. Pre-pubertal control children who were younger than those with eating disorders were excluded from the analyses to reduce the confounding effect of differences in age. Thus only control females older than 11 years of age and control males older than 9 years of age were included in the following analyses. Females were also categorised according to menarcheal status. The mean annual changes in BUA and %BUA for these three sub-groups (male, pre-menarcheal and post-menarcheal) are shown in figures 38 to 40. The absolute mean value for each group has been rounded up to the nearest whole number for inclusion on the bar charts.

Pre-menarcheal females

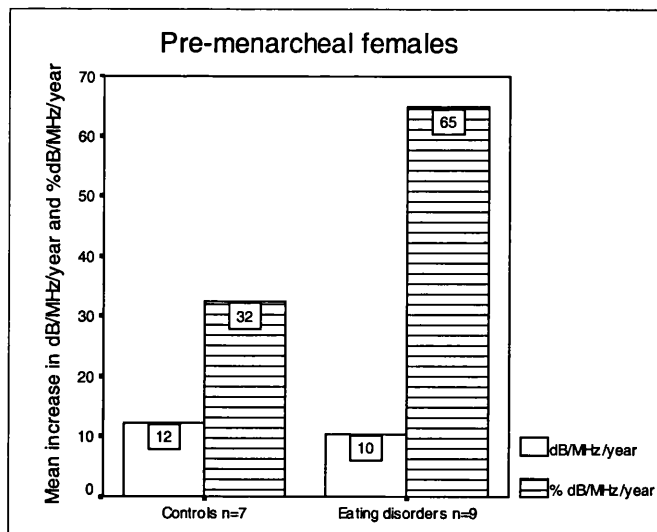
The changes in variables from baseline to follow up in the 16 pre-menarcheal age matched females are shown in table 31. The changes in BUA are shown in figure 38. For both groups the mean changes in $\text{dB.MHz}^{-1}.\text{year}^{-1}$ are less than three multiples of the precision error, however changes in the mean annual percentage increase in BUA were greater than 3 multiples of the relative precision error. The independent student t test was used to test for the significance of annual changes in age, weight, height, $\text{dB.MHz}^{-1}.\text{year}^{-1}$ and $\% \text{dB.MHz}^{-1}.\text{year}^{-1}$ between the two groups of children. The only statistically significant difference was that the BUA in dB.MHz^{-1} at the second scan was significantly higher for the control females than those with ANRED ($p < 0.032$).

Table 31 Change in parameters after one year (pre-menarcheal)

Mean (SD)	ANRED (n=9)	Controls (n=7)
Age (yrs)	13.87 (1.63)	12.72 (0.92)
Age range (yrs)	11.20 – 15.84	11.63 – 13.98
Wt.yr ⁻¹ (kg)	5.74 (4.48)	7.64 (3.33)
Ht.yr ⁻¹ (cm)	4.44 (2.24)	5.71 (1.70)
Ht range (cm)	1.00 – 7.00	3.00 – 7.00
$\text{dB.MHz}^{-1}.\text{yr}^{-1}$	10.44 (13.86)	12.29 (10.45)
$\% \text{dB.MHz}^{-1}.\text{yr}^{-1}$	64.89 (65.58)	32.43 (29.84)
BUA at 2 nd scan (dB.MHz^{-1})	38.00 (16.39)	55.71 (12.38)*

* $p < 0.032$

Figure 38 Annual changes in BUA (pre-menarcheal females)

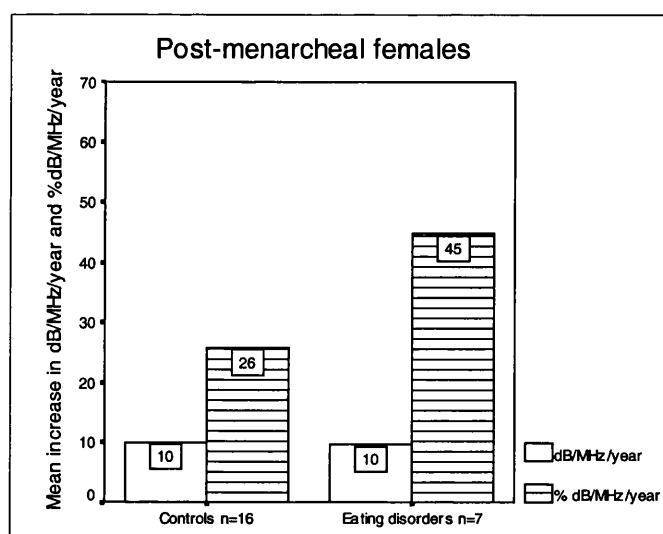


Post-menarcheal females

The changes in variables from baseline to follow up in the 23 post-menarcheal age matched females are shown in table 32. The changes in BUA are shown in figure 39. For both groups the mean changes in $\text{dB.MHz}^{-1}.\text{year}^{-1}$ are less than three multiples of the precision error, however changes in the mean annual percentage increase in BUA was greater than 3 multiples of the relative precision error. The independent student t test was used to test for the significance of annual changes in age, weight, height, $\text{dB.MHz}^{-1}.\text{year}^{-1}$ and $\% \text{dB.MHz}^{-1}.\text{year}^{-1}$ between the two groups of children and none were demonstrated. However the ANRED females had significantly less menstrual cycles per year than controls ($p < 0.001$, Mann-Whitney U test). The post-menarcheal control females reported having a median of 10 menstrual cycles per year compared to 6 per year for the ANRED group. However, the mean age of menarche of the two groups was not significantly different ($p = 0.22$).

Table 32 Change in parameters after one year (post-menarcheal)

Mean (SD)	ANRED (n=7)	Controls (n=16)
Age (yrs)	15.34 (1.56)	15.05 (0.86)
Age range (yrs)	13.23 – 18.21	13.70 – 16.59
Wt.yr ⁻¹ (kg)	5.58 (3.48)	3.00 (2.87)
Ht.yr ⁻¹ (cm)	2.43 (1.81)	2.44 (1.26)
Ht range (cm)	0 – 5.00	1.00 – 5.00
$\text{dB.MHz}^{-1}.\text{yr}^{-1}$	9.57 (14.98)	10.00 (13.03)
$\% \text{dB.MHz}^{-1}.\text{yr}^{-1}$	44.86 (62.32)	25.75 (36.87)
BUA at 2 nd scan (dB.MHz^{-1})	51.29 (12.89)	66.13 (20.75)

Figure 39 Changes in BUA (post-menarcheal females)

Males

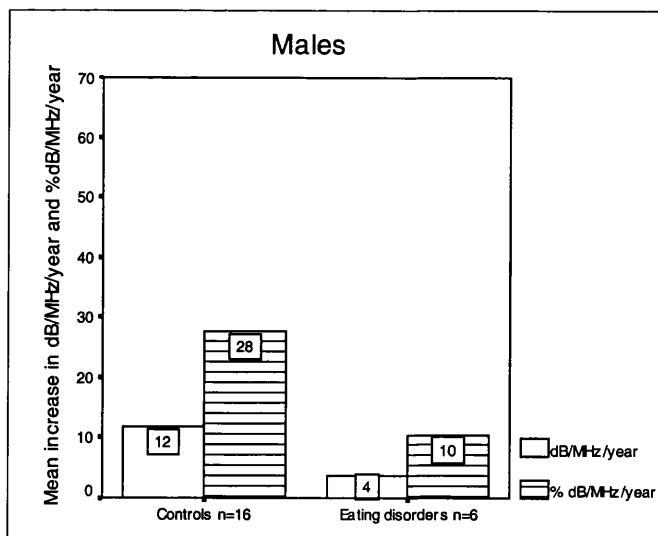
The changes in variables from baseline to follow up in the 22 age matched males are shown in table 33. The changes in BUA are shown in figure 40. For both groups the changes in $\text{dB.MHz}^{-1}.\text{year}^{-1}$ are less than three multiples of the precision error, however changes in the mean annual percentage increase in BUA was greater than 3 multiples of the relative precision error for the control males but not those with eating disorders. The independent student t test was used to test for the significance of annual changes in age, weight, height, $\text{dB.MHz}^{-1}.\text{year}^{-1}$ and $\% \text{dB.MHz}^{-1}.\text{year}^{-1}$ between the two groups of males and a significant difference in BUA at the second scan was demonstrated ($p < 0.046$).

Table 33 Change in parameters after one year (males)

Mean (SD)	ANRED (n=6)	Controls (n=16)
Age (yrs)	12.87 (2.80)	12.03 (1.62)
Age range (yrs)	9.19 – 16.11	9.45 – 14.95
Wt.yr ⁻¹ (kg)	5.93 (4.65)	5.17 (4.09)
Ht.yr ⁻¹ (cm)	5.33 (2.07)	6.38 (2.06)
Ht range (cm)	3.00 – 9.00	4.00 – 11.00
$\text{dB.MHz}^{-1}.\text{yr}^{-1}$	3.67 (5.96)	11.69 (14.80)
$\% \text{dB.MHz}^{-1}.\text{yr}^{-1}$	10.33 (19.55)	27.56 (36.27)
BUA at 2 nd scan (dB.MHz^{-1})	33.50 (16.84)	55.81 (23.32)*

* $p < 0.046$

Figure 40 Changes in BUA (males)



The effect of menarcheal status on BUA

It is recognised that the onset of puberty has a significant positive impact on increases in bone mineral accretion (see chapter 2). This effect was examined more thoroughly using 2 way chi-square tables on the data of 51 females (controls and ANRED) who had longitudinal follow up scans. The males were not evaluated in a similar manner because the pubertal status of the control males was not known.

It was hypothesised that a higher proportion of pre than post-menarcheal females would have changes in excess of $18\text{dB.MHz}^{-1}.\text{year}^{-1}$ or $21\%\text{dB.MHz}^{-1}.\text{year}^{-1}$. In the previous section the chi-square test was used to examine the effect of eating disorder on annual changes in BUA and none was seen, so data from the ANRED and control females have been combined for this analysis. The chi-square 2 way table is used to test the hypothesis that there is a relationship between menarcheal status and annual increases in BUA in dB.MHz^{-1} and $\%\text{dB.MHz}^{-1}$. For both tests the critical chi-statistic at the 5% level is 3.84.

Table 34 Chi square table for pre and post menarcheal ($\text{dB.MHz}^{-1}.\text{yr}^{-1}$)

Observed	Less than 18 $\text{dB.MHz}^{-1}.\text{yr}^{-1}$	More than 18 $\text{dB.MHz}^{-1}.\text{yr}^{-1}$	Total
Pre-menarcheal	22	6	28
Post-menarcheal	16	7	23
Total	38	13	51
Expected			
Pre-menarcheal	20.86	7.14	28
Post-menarcheal	17.14	5.86	23
Total	38	13	51
(Observed-expected)²/expected			
Pre-menarcheal	0.06	0.18	0.24
Post-menarcheal	0.08	0.22	0.30
Total and chi square statistic			0.54

Table 35 Chi square table for pre and post menarcheal (%dB.MHz⁻¹.yr⁻¹)

Observed	Less than 21% dB.MHz ⁻¹ .yr ⁻¹	More than 21% dB.MHz ⁻¹ .yr ⁻¹	Total
Pre-menarcheal	9	19	28
Post-menarcheal	13	10	23
Total	22	29	51
Expected			
Pre-menarcheal	12.08	15.92	28
Post-menarcheal	9.92	13.08	23
Total	22	29	51
(Observed-expected)²/expected			
Pre-menarcheal	0.79	0.60	1.39
Post-menarcheal	0.96	0.73	1.69
Total and chi square statistic			3.08

Both calculated chi-square statistics (0.54 and 3.08) are lower than 3.84 suggesting that there is no statistically significant relationship between menarcheal status and the proportion of females having increases of more than 18dB.MHz⁻¹.year⁻¹ or 21%dB.MHz⁻¹.year⁻¹.

Impact of menarche

There were only 3 post-menarcheal females from the ANRED group who had both scans one year apart. However an additional 4 females attained menarche between the first and the second scans. Two of these four had anorexia nervosa and two had FAED.

Scatterplots were used to examine the effect of menarche on the annual increase in BUA seen in post-menarcheal females. Figure 41 demonstrates that the annual increase in BUA in dB.MHz⁻¹ diminishes with years since the onset of menarche. A similar effect is seen when the percentage annual BUA is plotted against years since menarche (figure 42). The statistical test in section 10.2 on page 156 demonstrated that upon attainment of menarche post-menarcheal females with ANRED have significantly less menstrual cycles per year than controls (p<0.001). The effect of this reduction in frequency of menses is demonstrated in the scatterplots in figures 41 and 42 where annual changes in BUA and %BUA with years post-menarche are slightly lower for females with ANRED than controls.

Figure 41 Change in BUA with menarche

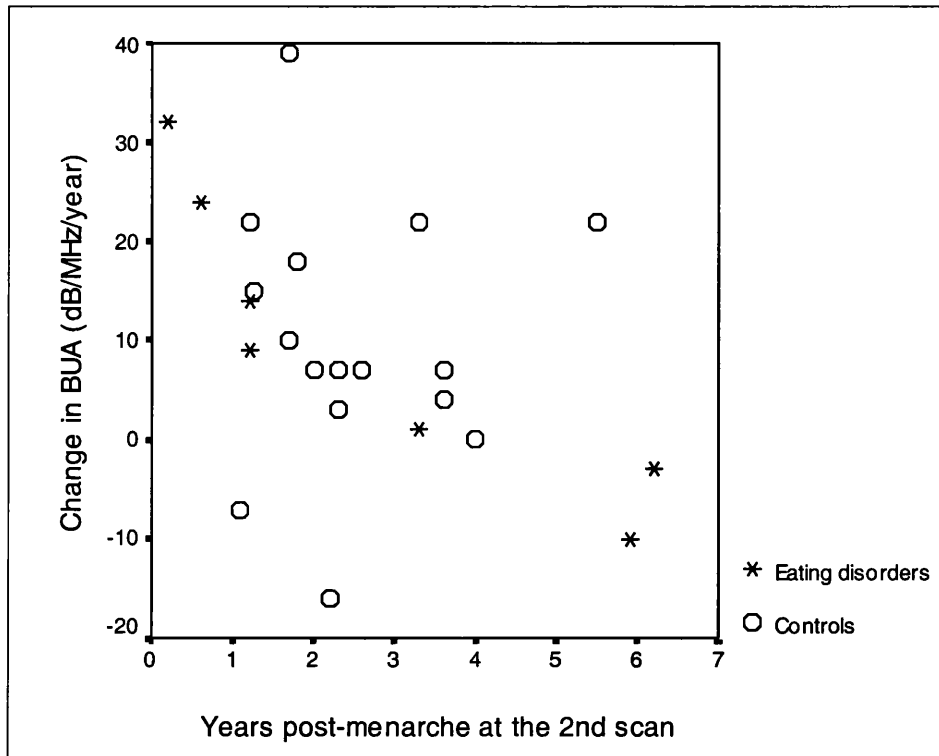
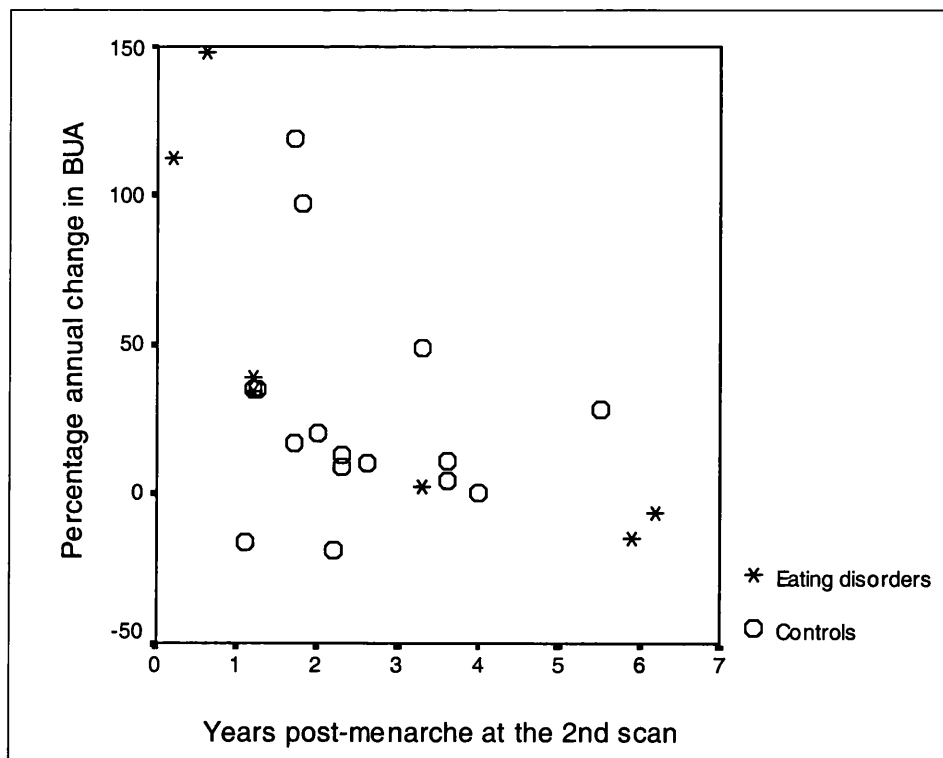


Figure 42 Percentage BUA change with menarche



10.3 Evaluating longitudinal BUA Z score changes

The magnitudes of the changes in BUA were also examined using Z scores as in the previous chapter. BUA Z scores were calculated using the cross sectional normative data and predicted BUA values for age and weight specific to gender which were determined in chapter 8. To test the probability that the mean BUA Z scores of this longitudinal sample of children and adolescents with ANRED are representative of the true population means the standard error of the mean and 95% confidence limits were calculated and are illustrated in figure 43. The mean and 95% confidence intervals for the BUA Z scores corrected for age and gender for both the first and the second scans for the 23 ANRED children do not contain the value zero, so the BUA Z scores are significantly lower than zero. There is therefore a 95% probability that the true population mean for BUA Z scores corrected for age and gender for children with ANRED lies between -0.87 and -1.71 (mean, -1.29) for the first scan and remains below zero after a year of treatment (mean, -1.06, 95%CI -0.60, -1.52). In comparison the mean BUA Z scores corrected for weight and gender are greater than zero at both the first and the second scans.

Figure 43 Error bars for follow-up BUA Z scores for 23 ANRED children



Sub-group analysis of BUA Z score by eating disorder

The ANRED children were sub-categorised according to specific eating disorder and gender then menarcheal status. This once more caused small sample sizes, violating the conditions of the revised power calculation so whilst the results are informative they should be treated with caution. Bar charts and error bars of the 95% confidence intervals of the BUA Z scores sub-grouped by eating disorder, gender and then menarcheal status demonstrate the changes in BUA Z score between scans.

The mean BUA Z scores corrected for age and gender for each of the eating disorders sub-groups are shown in the bar chart in figure 44. Each sub-group demonstrated annual improvements in BUA Z score apart from the three males with selective eating, whose BUA Z score deteriorated between scans. The 95% confidence intervals demonstrated that the mean BUA Z scores are significantly lower than zero for both scans for all sub-groups of children. The BUA Z scores (corrected for weight and gender) demonstrate similar changes from the first scan to the second and once again the three males with selective eating have the lowest BUA Z scores with a mean decrease rather than increase between scans.

There were only four children who had BUA Z scores corrected for weight and gender which were below -1 at the second scan. Two were females with anorexia nervosa and two were males, one with FAED and the other with selective eating. The younger of the females was aged 14 years and remained pre-menarcheal with a BUA Z score corrected for weight and gender of -1.13 at the second scan. The other female was aged 16 and was post-menarcheal with a BUA Z score corrected for weight and gender of -1.15 at the second scan. The younger of the two boys was pre-pubertal, aged 8 years and had a BUA Z score corrected for weight and gender of -1.66 . The older boy was post-pubertal, aged 14 and had a particularly low BUA Z score corrected for weight and gender of -3.31 . This latter boy had taken oral steroid therapy of 10mg and 4mg of prednisolone on alternate days for five years as treatment for inflammatory bowel disease which would clearly have had a significant impact on his reduced BUA values.

Figure 44 Longitudinal changes in BUA Z scores (age and gender)

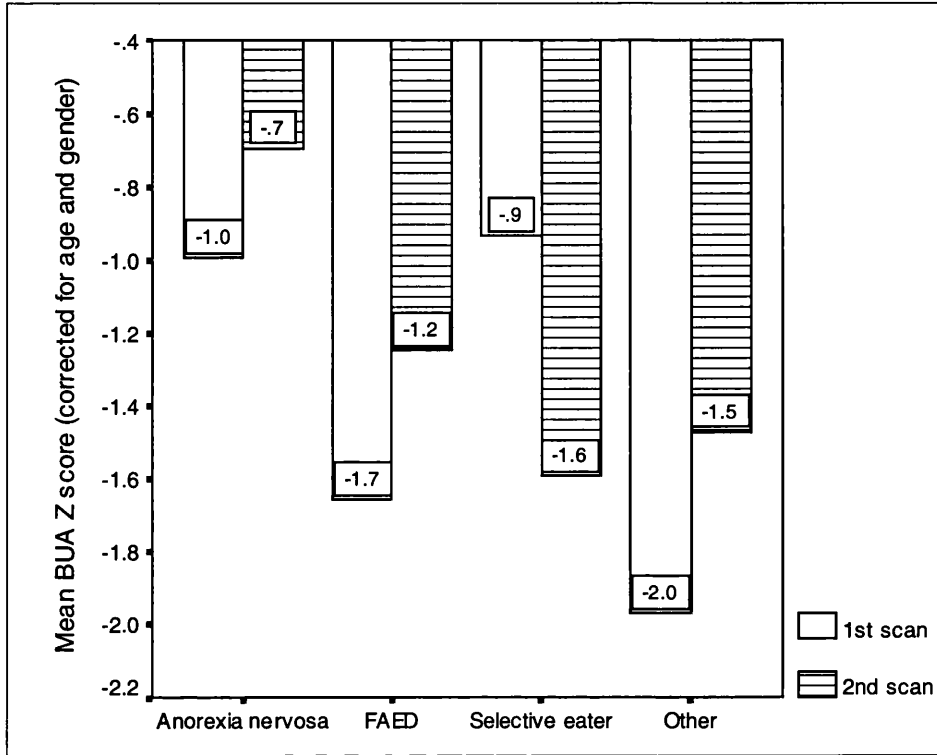


Figure 45 95% confidence intervals (BUA Z score age and gender)

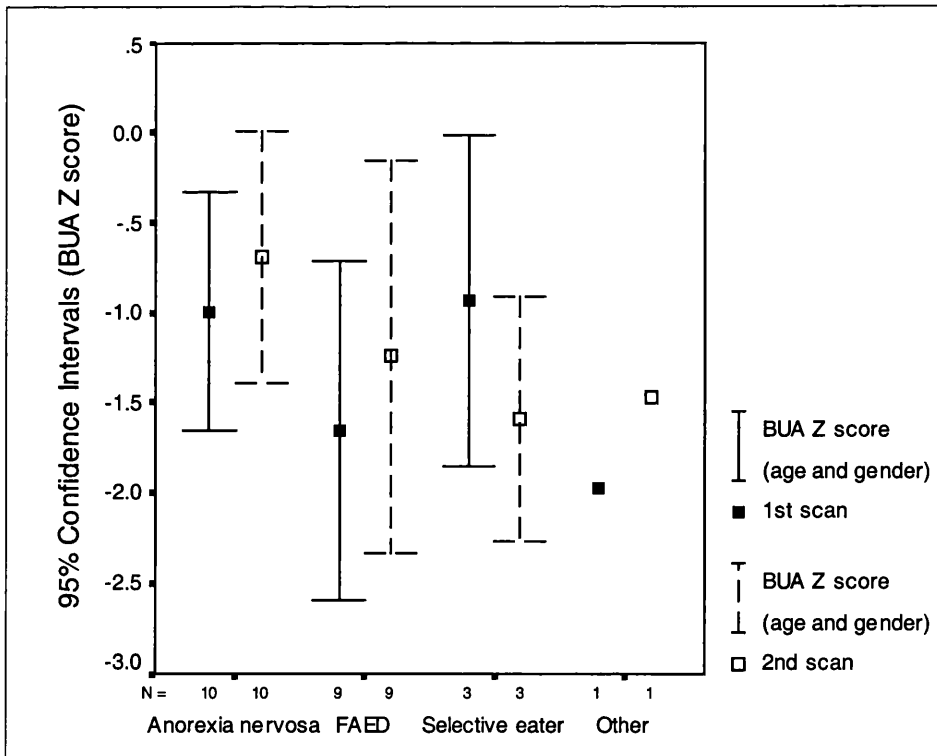


Figure 46 Longitudinal changes in BUA Z scores (weight and gender)

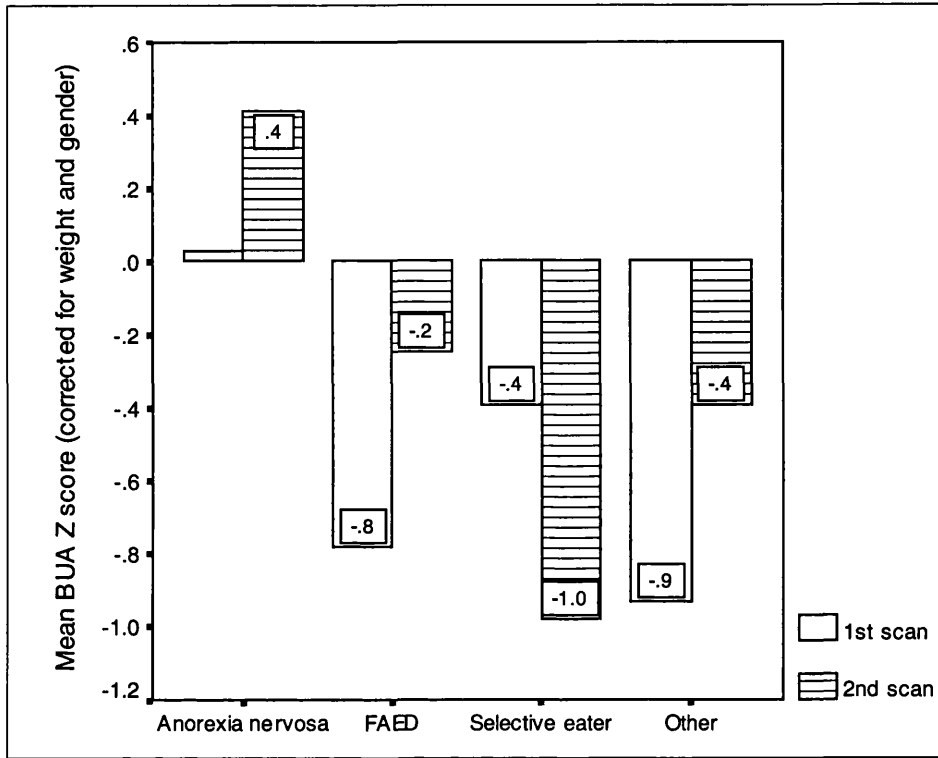
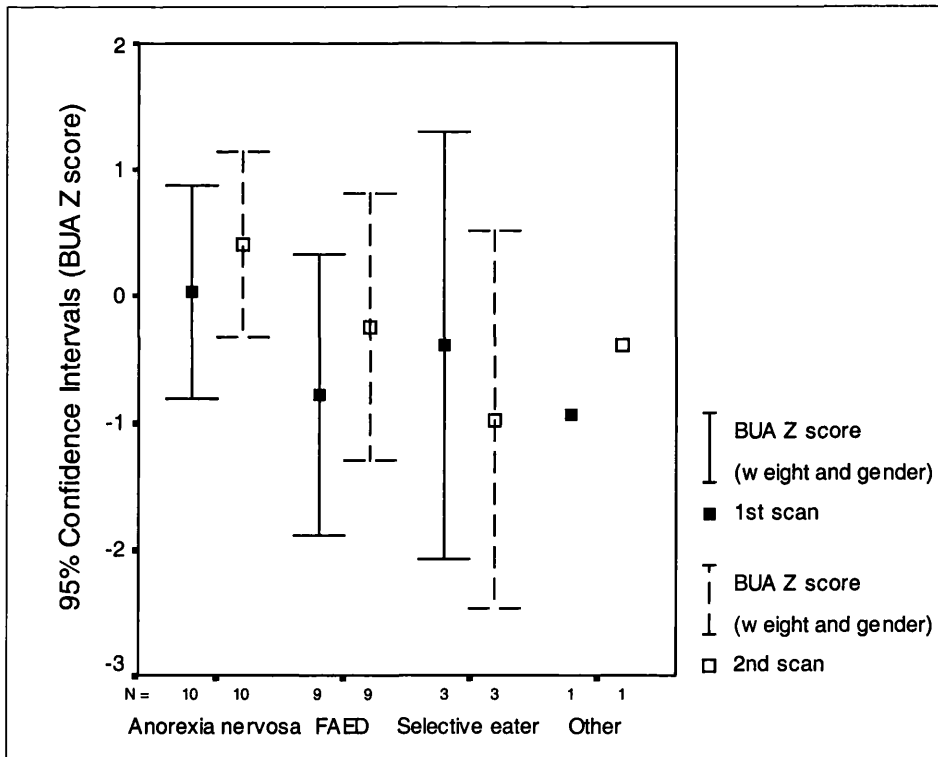


Figure 47 95% confidence intervals (BUA Z score weight and gender)



Sub-group analysis by gender and menarcheal status

Improvements in the mean BUA Z scores (corrected for age and gender) were seen between the first and second scans for the pre and post-menarcheal females but not the males, whose mean BUA Z score reduced from -1.5 to -1.8 (figure 48). The mean BUA Z score of each sub-group together with the 95% confidence intervals are shown in figure 49. The mean BUA Z scores for the males and the pre-menarcheal females are significantly different from zero and importantly are lower than -1 for both the first and the second scans. The post-menarcheal females have BUA Z scores which are not significantly different from zero for either the first or the second scan.

As above, there are small though not significant improvements in the mean BUA Z scores (corrected for weight and gender) between the first and second scans for both pre and post-menarcheal females but not males (figure 50). The improvements, although not statistically significant, are most marked in the pre-menarcheal females increasing from a mean of -0.5 to a mean of $+0.2$ between scans. In contrast the mean BUA Z score for the males deteriorated from -0.5 to -0.8 between the two scans. However the 95% confidence intervals in figure 51 demonstrate that none of these three sub-groups have mean BUA Z scores corrected for weight and gender which were significantly different from zero at either the first or the second scan.

Figure 48 Longitudinal changes in BUA Z scores (age and gender)

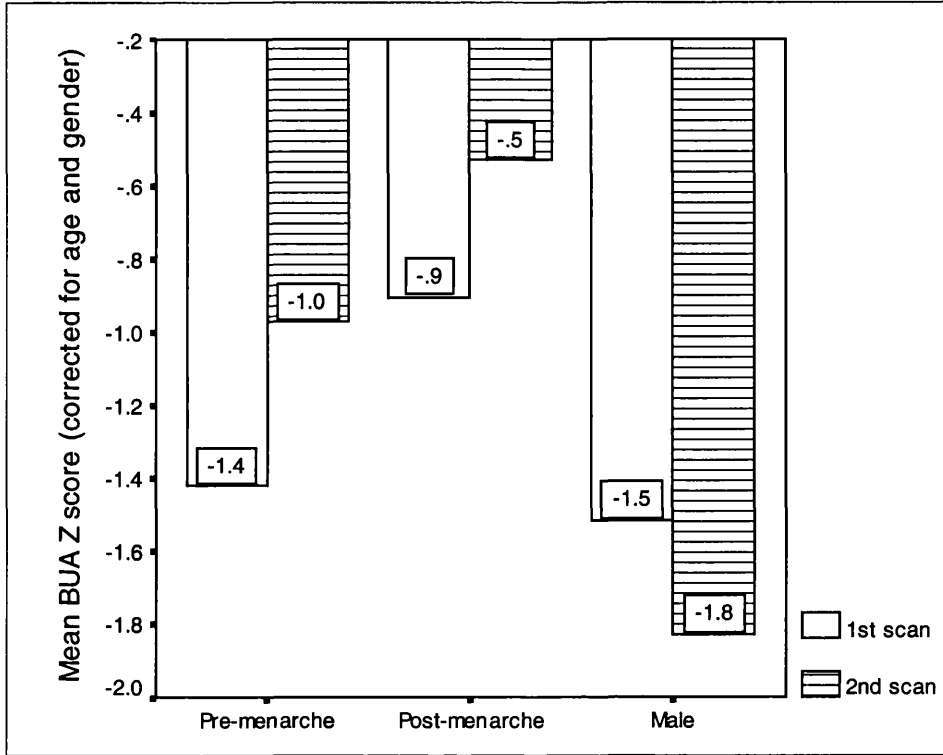


Figure 49 95% confidence intervals (BUA Z scores age and gender)

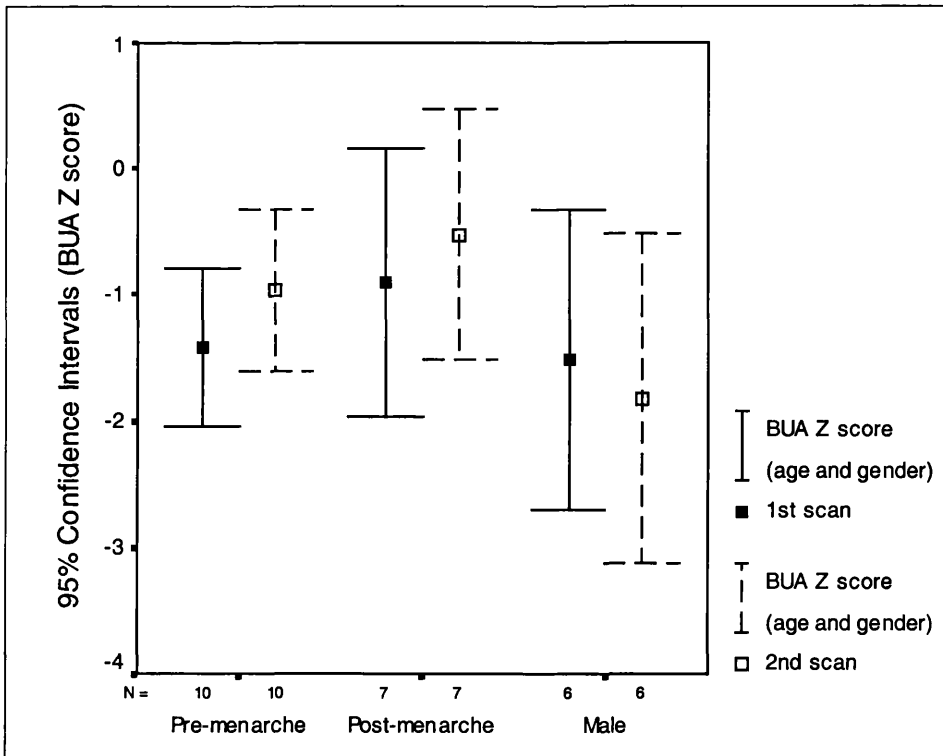


Figure 50 Longitudinal changes in BUA Z scores (weight and gender)

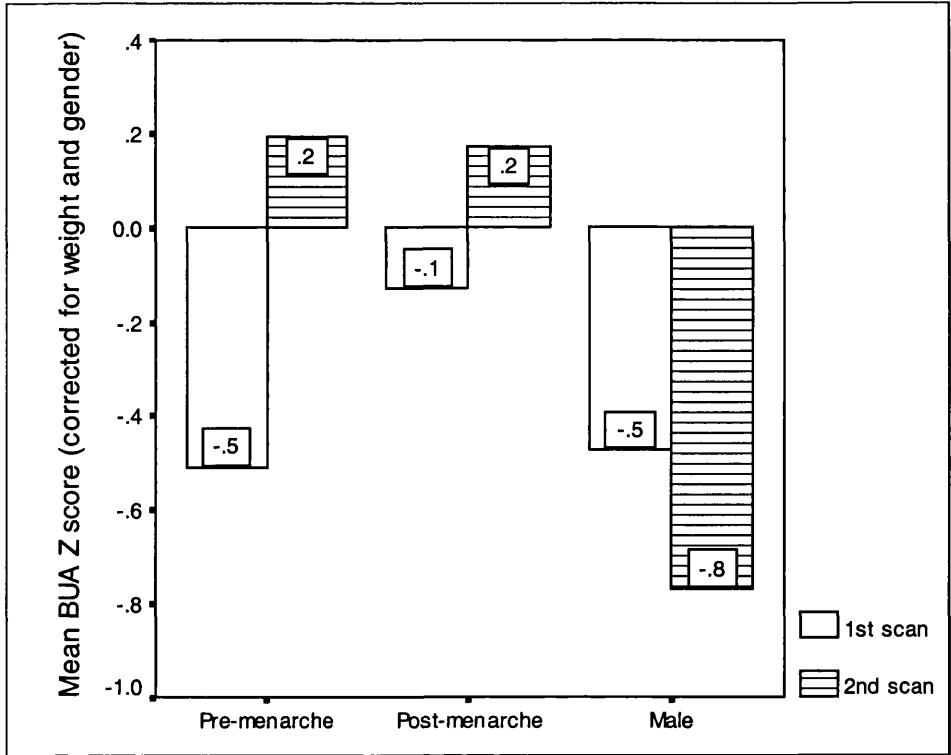
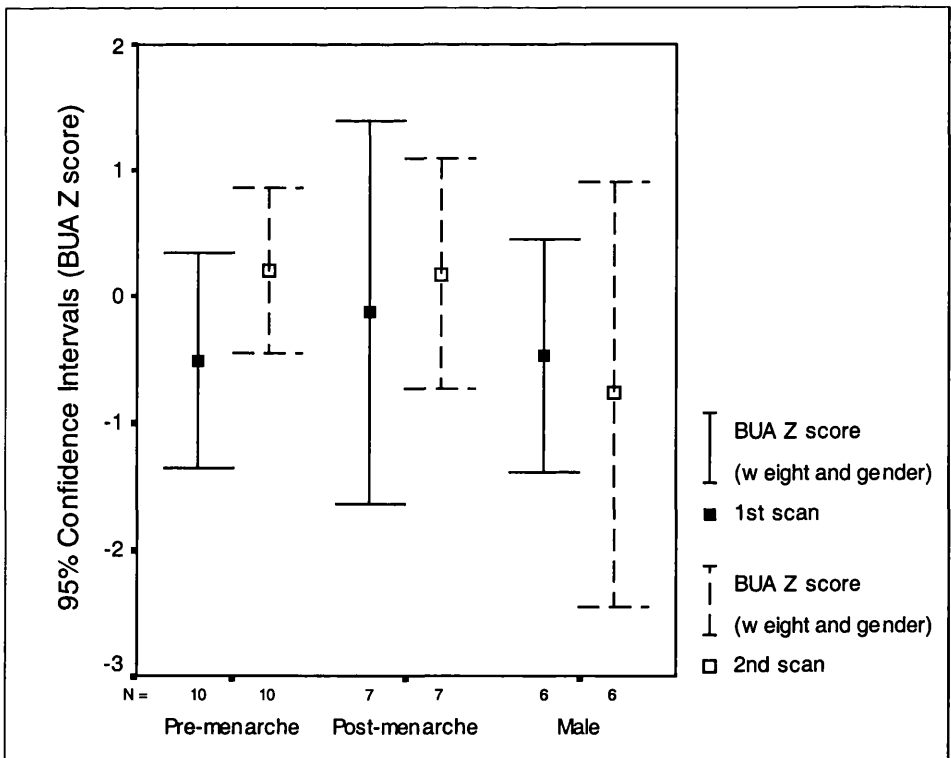


Figure 51 95% confidence intervals (BUA Z scores weight and gender)



10.4 Discussion

This observational longitudinal study was intended to examine the relationship between BUA and growth during childhood. A secondary aim was to compare and evaluate the differences in BUA seen between healthy control children and those with ANRED during growth. The discussion is divided into two sections according to the initial hypotheses proffered in chapter 1.

The attrition rates were high for both cohorts despite the parents of all children and adolescents being contacted by telephone on at least one occasion to arrange the follow up scans. However it is unsurprising that the rate of attrition for the cohort with ANRED at 56% was higher than that of the control cohort (38%). It is difficult to maintain high retention rates for longitudinal studies involving patients with ANRED, which is one of the reasons why studies with as few as 18 subjects ([RIGO1984], [YOUN1994]) are published. Consequently although anything more than zero attrition in a follow up study is undesirable the significance of attrition rates will vary with the aims of a study. The only significant difference seen in the baseline data between those who attended for one scan and those who attended for both was in the BUA Z scores for the ANRED cohort. This difference was however useful to the aims of the research since those children with the highest BUA Z scores were the ones that tended to self-select themselves out of the follow up study. The analysis therefore concentrated on those with the lowest BUA Z scores who would therefore be clinically more at risk of osteoporotic fracture. There are no data available on why some ANRED children did not attend for the second ultrasound scan and in the context of this study it was considered unethical to contact the children or their parents to establish why they did not attend for their follow-up scans.

Evaluating the technical null hypothesis

The data in this chapter were used to evaluate whether the CUBA Clinical, a commercially available ultrasound machine for measuring BUA, could be used to monitor bone growth and development on an annual basis in children with ANRED. The data analysis in chapter 7 demonstrated that using the CUBA Clinical on children the minimum change in BUA between scans in vivo should be at least 3

multiples of the precision error. The precision error calculated in chapter 7 as the root mean square standard deviation, was 6.02dB.MHz^{-1} and the relative precision error, calculated as the percentage ratio of the precision error to the measurement range, was 7.03%. Therefore these longitudinal data must demonstrate changes in excess of $\pm 18\text{dB.MHz}^{-1}$ or $\pm 21\% \text{dB.MHz}^{-1}$ for them to be considered clinically significant.

The 85 children were initially divided into two groups according to whether they had ANRED or were healthy controls. The median values of annual BUA changes for the 23 children with ANRED was 9dB.MHz^{-1} and for the 62 controls was 7dB.MHz^{-1} . These values just exceed the precision error value of 6.02dB.MHz^{-1} , but are clearly much smaller than three multiples of the precision error. In comparison the median annual percentage increase in BUA was 28.5% for children with ANRED and 22% for control children, which for both groups is in excess of three multiples of the relative precision error. Further analysis of the children involved dividing them into specific eating disorders sub-groups and not one of these had a mean BUA increase of more than 18dB.MHz^{-1} , however three of the four eating disorders sub-groups as well as the control cohort had increases in excess of $21\% \text{dB.MHz}^{-1}$. The same relationships between annual increases in dB.MHz^{-1} and percentage annual increases in dB.MHz^{-1} were seen when the females were divided into those who were pre-menarcheal or post-menarcheal. Only the percentage BUA increases exceeded the threshold of three multiples of the relative precision error. When the ANRED and control males were compared both groups had mean annual increases of less than 18dB.MHz^{-1} but only the control group had a mean percentage BUA increase that was in excess of 21%.

In the pre-menarcheal groups the annual increase in %BUA for females with ANRED although not significant was almost double that of the controls: 64.89% versus 32.43%. However the absolute BUA values between the two groups was similar (10.44dB.MHz^{-1} versus 12.29dB.MHz^{-1}). In the post-menarcheal group the %BUA increase was also higher for those with ANRED (44.86% versus 25.75%) but the BUA was virtually the same for both groups (9.57dB.MHz^{-1} versus 10.00dB.MHz^{-1}).

The chi-square test was used to evaluate the impact of menarcheal status on annual changes in BUA. This test demonstrated that there was no significant difference between pre and post-menarcheal children in terms of the proportion of each group having annual increases of more than three multiples of the precision error or relative precision error ($p < 0.05$).

It is important to be able to diagnose true differences between two BUA scans and for both post-menarcheal and pre-menarcheal females the increase in BUA is not sufficient to be certain that the changes seen are not merely an effect of random machine fluctuations. This is particularly emphasised in the post-menarcheal cohorts where both healthy control children and those with ANRED had the same annual BUA increase. If only the %BUA were examined it would appear that all groups of children had a clinically significant increase in BUA.

The annual changes in BUA between healthy control children and those with ANRED also differed according to menarcheal status and years since the onset of menarche. Data in chapter 9 led to the conclusion that children with reduced values of BUA for their age and weight would exhibit a “catch-up” phenomenon caused by the synergistic effects of increasing weight and age. However when this effect was examined using the chi-square test there was no statistically significant effect of eating disorder on the annual increase in BUA at the 5% level. When the females were re-examined after one year the groups with ANRED had higher mean increases in weight than the control groups although the differences did not reach statistical significance. This coincided with a greater annual increase in %BUA for the females with ANRED, again not reaching statistical significance. The changes in BUA were most pronounced when children were analysed by gender and for females menarcheal status. For the two female sub-groups the percentage increases in BUA were greater for those with eating disorders than controls, although the absolute increases in BUA were similar.

These data demonstrate that if a child has a low BUA at the first scan then the percentage increase will be greater than a child who has a higher BUA at the first scan but has a similar or greater improvement in absolute BUA in dB.MHz^{-1} . It is therefore important to note that for children, measuring the percentage change in

BUA using the CUBA is not as clinically informative as measuring the absolute change in BUA in dB.MHz^{-1} , or calculating the BUA Z score. Another example demonstrates this. The post-menarcheal controls who had a mean BUA at the second scan of 66.13dB.MHz^{-1} demonstrated a mean annual increase in BUA of 10.00dB.MHz^{-1} compared to post-menarcheal ANRED patients (mean BUA at the second scan: 51.29dB.MHz^{-1}) who also demonstrated a mean annual increase of 10.00dB.MHz^{-1} . However when the percentage increases were compared, the post-menarcheal controls increased in BUA by 25.75% whereas the post-menarcheal ANRED females increased by almost double this at 44.86%. This is because percentage increases are based on the absolute BUA value at the first scan, so the lower the initial BUA value the greater the percentage increase for the same absolute increase in BUA. If it is more important to determine whether the increase in BUA is caused by in vivo change rather than machine fluctuation then calculating the %BUA can be misleading. Clearly there is likely to be a higher proportional percentage increase in BUA the lower the absolute BUA value. This situation is particularly likely to occur where the dynamic range of BUA measurements is wide extending for example from 0 to 100dB.MHz^{-1} as in the CUBA Clinical. Whereas the Lunar Achilles ultrasound machine used in the paediatric research by Jaworski et al [JAWO1995] has a narrower dynamic range extending from 83dB.MHz^{-1} to 104dB.MHz^{-1} . In this latter situation an increase of, for example, 2dB.MHz^{-1} (the difference seen between 6 and 7 years of age) from 83dB.MHz^{-1} would lead to a 2.41% increase in BUA compared to a 2dB.MHz^{-1} increase at 104dB.MHz^{-1} which would lead to a 1.92% increase. In this situation with the Lunar Achilles it would be appropriate to use the percentage standardised coefficient of variation as the higher absolute BUA values allow a sensible measure of relative precision and percentage increase. What is most important is to be able to determine for each particular patient whether the changes seen are clinically significant. However where the aim of measuring precision, whether absolute or relative, is to make useful comparisons with other pieces of equipment the relative precision error measured as the standardised coefficient of variation is more useful than the root mean square standard deviation of repeated measurements.

These examples substantiate the argument that the longitudinal annual changes in absolute BUA are not statistically significantly different from the precision error of

the CUBA Clinical. However the annual changes in %BUA are statistically significantly different from the relative precision error of the CUBA Clinical. Therefore if the root mean square standard deviation of repeated measurements is used as the precision error these results demonstrate that the CUBA Clinical is not precise enough to measure clinically significant annual changes in BUA in healthy children or those with ANRED. There is an 80% probability that the differences seen are not significantly different from the precision error of the CUBA Clinical and the null hypothesis is therefore accepted. However if the standardised coefficient of variation is used as a measure of relative precision error the CUBA Clinical can be considered precise enough to measure clinically significant percentage BUA changes from one year to the next in healthy children and those with ANRED. When the relative precision error is used the null hypothesis can therefore be rejected. It is however pertinent to consider that using these significant differences of at least three multiples of the relative precision error as outlined in chapter 4, section 4.4 there is a 10% possibility of making a Type I error, i.e. rejecting the null hypothesis when it should be accepted. This may indeed be the case in the %BUA situation.

Evaluating the clinical null hypothesis

Longitudinal changes in BUA Z scores were also evaluated in this chapter. The power calculation was revised because of the high attrition rate of the ANRED sample. The new calculation demonstrated that longitudinal data on 21 children with ANRED would be required to demonstrate a standardised difference of one standard deviation between ANRED children and control children, which was significant at the 5% level and with 90% power. The mean BUA Z score of the 23 ANRED children when corrected for age and gender was -1.29 for the first scan which was significantly lower than zero (95% CI $-0.87, -1.71$). It remained significantly lower than zero for second scan performed after one year of treatment (mean -1.06 , 95%CI $-0.60, -1.52$). Therefore the null hypothesis for BUA Z scores (corrected for age and gender) on these longitudinal data is rejected and there remains a statistically significant difference between ANRED children and control children after one year of treatment for their eating disorder. The mean BUA Z score corrected for weight and gender increased between scans and, as in the cross-sectional analysis in chapter 9, it was not significantly different from zero. The corollary to this is that children with ANRED have reduced BUA for their age, but since it is normal for their weight,

the low BUA Z scores are most likely to be related to reduced weight for age. However there were four interesting children (two males and two females) who had BUA Z scores both for age and for weight which were reduced below -1. On review of their notes each had an additional factor as well as their reduced weight which placed them at a higher risk of reduced BUA. One male with selective eating was aged 8 years and had suffered with dairy allergies for five years and consumed just 1 pint of soya milk each week, which is well below the lower reference nutrient intake for calcium for children of his age. The other male had FAED and had received oral steroid therapy (4 milligrammes and 10 milligrammes of prednisolone on alternate days) for irritable bowel syndrome for 5 years. Both of the females had anorexia nervosa, the youngest of whom had delayed puberty; she was aged 14 and remained pre-menarcheal at the time of the second scan. The second female was aged 16 and was post-menarcheal. She had however been receiving inhaled steroid therapy for asthma (200 microgrammes.day⁻¹ beclomethasone) for 15 years. Therefore these additional risk factors and the low BUA Z scores corrected for weight and gender identify these children as the ones most at risk of potential osteoporotic fracture. It may therefore be possible to identify those children with ANRED who are not likely to attain a high peak bone mass and who have the greatest risk of osteoporotic fracture, by using measurements of BUA Z score corrected for weight and gender rather than age and gender.

These data also demonstrated that pre-menarcheal ANRED females had a lower height velocity than pre-menarcheal controls and also had lower BUA values. Interestingly in the paper by Cadogan et al [CADO1998] they noted that in females height velocity peaked more than 2 years prior to menarche, whereas bone mineral density peaked at menarche. If this is the case then those pre-menarcheal females with ANRED who have a lower peak height velocity than matched controls are likely to be closer to attaining their peak bone mass than the controls. The corollary being that these pre-menarcheal females with ANRED and low height velocities are likely to attain a lower peak bone mass than matched controls.

The post-menarcheal ANRED females all had anorexia nervosa and they had significantly fewer menstrual cycles per year than post-menarcheal controls. As would be expected there were diminishing increases in BUA and %BUA when BUA

was plotted against years since menarche, however this effect was more pronounced for the anorexia nervosa post-menarcheal group than the control post-menarcheal group. This is likely to be related to the reduced frequency of menstrual cycles per year for those in the anorexia nervosa group.

Mean increases in BUA Z scores corrected for age and gender were seen in the subgroups of female children with ANRED, however the males demonstrated a net overall decrease in mean BUA Z score. The mean changes in BUA Z scores corrected for weight and gender were also examined and similar changes were seen, i.e. mean increases for the females and decreases for the males. The results for the males are however skewed reflecting the particularly low value of the male who was receiving oral steroid therapy.

These results confirm that, clinically, weight is a useful predictor of BUA in children with ANRED and BUA Z scores corrected for weight and gender identify those at the greatest risk of osteoporosis. Therefore these results fit well with the results of Chapters 8 and 9 where weight was shown to be the strongest predictor of BUA.

10.5 Conclusion

The use of the CUBA Clinical to detect longitudinal changes in BUA in healthy control children and those with ANRED was thoroughly evaluated in this chapter. The analyses demonstrated that it was clinically more relevant and useful to compare longitudinal changes in bone growth and development by measuring absolute changes in BUA in dB.MHz^{-1} rather than $\% \text{dB.MHz}^{-1}$. This is to enable changes in BUA to be evaluated independently from the original BUA values.

The analyses demonstrated that annual changes in BUA measured in dB.MHz^{-1} are not large enough to be able to differentiate machine fluctuations from valid in vivo changes in bone growth and development. The average increase in BUA for the 62 control children was 7dB.MHz^{-1} and the average increase in BUA for the 23 ANRED children was 9dB.MHz^{-1} compared to a precision error of 6.02dB.MHz^{-1} . Therefore there is an 80% probability that the annual changes in BUA are not statistically significantly different from the precision error of the CUBA Clinical.

The relative precision error measured as the standardised coefficient of variation demonstrates statistically significant annual changes in $\% \text{BUA}$ however using the CUBA Clinical, $\% \text{BUA}$ is not a useful method of measuring bone growth and development in children because the differences seen are biased towards low values of absolute BUA. If the second measurement increases by more than 21% of the first, one cannot be certain that the change is clinically significant unless the absolute BUA measured at the previous scan is known and considered. Therefore, using the CUBA Clinical, changes in BUA are most useful when considered in terms of dB.MHz^{-1} .

The standardised coefficient of variation is a useful method of comparing one machine to another both within and between manufacturers as it takes into account the dynamic range of the measuring equipment.

The high attrition rates of 56% for the ANRED cohort and 38% for the control cohort although undesirable were acceptable for two reasons. Firstly apart from the lower BUA Z scores seen in ANRED children who attended for two scans compared

to those who attended for just one, the baseline parameters between those who did and did not attend for follow-up scans were not significantly different. Secondly high attrition rates were difficult to avoid in the ANRED cohort because of the problems encountered in encouraging such children and parents to attend for follow up scans one year after initial clinical treatment for their disorder, particularly if they have been discharged from care.

The group of 23 children with ANRED had mean BUA Z scores for age and gender which were significantly less than zero at both the first and the second scans. Therefore, since BUA Z scores corrected for age and gender are the accepted standard method of measuring BUA, the clinical null hypothesis outlined in chapter 1 can be rejected with 90% power and 5% significance, i.e. there is a statistically significant difference in BUA between ANRED children and controls. However the mean BUA Z scores corrected for weight and gender were not significantly lower than zero for either the first or the second scan. Therefore reduced weight for age is the main cause of reduced BUA for age in children with ANRED.

The sub-groups of males and pre and post-menarcheal females were unfortunately small, reducing their statistical significance, however the analyses provided useful additional information about the BUA values of children with ANRED. The mean BUA Z scores, corrected for age and gender, of the children with ANRED were significantly lower than zero for males and pre-menarcheal females for both scans. In comparison the BUA Z scores were within normal limits for the post-menarcheal females, endorsing the positive impact of puberty on increasing BUA values in growth and development. The mean BUA Z scores corrected for weight and gender for the first and the second scans for the same three sub-groups were not significantly different from zero. However there were four children with BUA Z scores for weight and gender lower than -1 and these had additional risk factors for low BUA values including, the use of steroids, pubertal delay and reduced calcium intake due to a dairy allergy. Therefore BUA Z scores corrected for weight and gender are a useful predictor of bone status in children with ANRED and should be used in preference to BUA Z scores corrected for age and gender.

Annual increases in BUA are inversely related to years since menarche, and post-menarcheal ANRED females have significantly fewer menstrual cycles per year than

control females. Although this contributes to the lower values of BUA seen in post-menarcheal ANRED females compared to controls the differences do not reach statistical significance.

Chapter 11 Conclusions and recommendations

Two main hypotheses were initially proffered in the first chapter; one was technical and one was clinical. These hypotheses have been thoroughly tested in chapters 6 through to 10.

The technical null hypothesis was that the precision error of the broadband ultrasound attenuation technique in the McCue CUBA Clinical in children and adolescents would on average be the same as the annual increase in broadband ultrasound attenuation in children and adolescents. The experimental hypothesis being that the McCue CUBA Clinical would not be precise enough to detect annual changes in BUA during growth and development.

The clinical null hypothesis was that children and adolescents with anorexia nervosa and related eating disorders would on average have the same broadband ultrasound attenuation values through the left heel as healthy children and adolescents. The conclusions, which follow, are drawn from the analyses used to test these hypotheses and are grouped according to the hypothesis to which they relate.

11.1 The technical hypothesis

The evaluation of this hypothesis derives from the analyses of experimental data from chapters 6,7 and 10. The groundwork for the evaluation of precision error was undertaken in the pilot study by observing the standard BUA technique in clinical paediatric practice. The key observation was that the positioning technique developed by McCue for the CUBA Clinical did not adequately compensate for the wide variety of foot size and shape seen during growth; specifically in terms of variable foot length and heel width across the ages and between the sexes. It is clear that as a child grows the calcaneum in her/his foot will increase in size: length, width and depth. It is sensible to ensure that the same region of interest on the calcaneum is measured during growth, particularly if the scans are several years apart. This is to enable useful comparisons of BUA measurements within and between children. In support of this, Jaworski et al [JAWO1995] reported improved precision of a quantitative ultrasound technique using the Lunar Achilles when a new centring point was developed for children to identify the correct region of interest on the calcaneum. However although Jaworski's centring point appeared to improve precision it was technically difficult to use because it is founded on the identification of the tuberosity of the head of the fifth metatarsal, which is not a palpable anatomical landmark in children or adults.

Conclusion one

A reproducible method of locating the region of interest on the heel in children should be developed and used for quantitative ultrasound measurements.

The aim of the quantitative ultrasound technique is to measure the posterior most parallel portion of the calcaneum [LANG1984]. A new method of locating this region was therefore developed by examining this area of the calcaneum in relation to known radiographic anatomical landmarks on lateral radiographs of children's feet. The distal tip of the lateral malleolus of the fibula is an easily identifiable anatomical landmark on the surface of the skin which is used for accurate positioning of the ankles of children and adults for radiographic examinations. It was therefore used as the main anatomical landmark in the development of a new centring point on the lateral border of the heel. The precision and validity of this new centring point

was evaluated by an independent experienced observer (WN) and compared to that of the Jaworski technique using lateral radiographs of the feet of 15 children (29 radiographs in total). The root mean square standard deviation precision error of the new technique was 1mm (median 1mm) compared to 2mm (median 3mm) for the Jaworski technique ($p < 0.001$).

Conclusion two

The development and use of a new centring point caused a statistically significant improvement in precision error in children. The limiting precision of the new technique was 1mm.

This new centring point was used in clinical practice to evaluate whether the precision of the BUA technique could be improved in paediatrics by adapting the CUBA Clinical to allow precise positioning of the ultrasound transducers on the region of interest on the heel. This new centring point was used to identify the location for placing the ultrasound transducers on the heel of 100 children. Fifty of these children had two BUA scans using the McCue CUBA Clinical set up for standard use with the transmit and receive transducers located in a fixed position in a hard plastic casing. The second set of 50 children each had two BUA scans with the transmit and receive transducers mounted onto a handheld calliper device to allow more accurate positioning. The two scans on all 100 children were performed with re-positioning in between. The root mean square standard deviation precision error calculated on the two sets of 50 paired BUA measurements was 6.05 dB.MHz^{-1} when the transducers were fixed in the hard plastic casing compared to 6.02 dB.MHz^{-1} when they were positioned on the handheld calliper device. However when the relative precision error was calculated using the coefficient of variation there was a more pronounced improvement in precision with the transducers placed onto handheld callipers (16.70% improved to 12.75%). A further evaluation of the use of the coefficient of variation however demonstrated that the improvement in relative precision error, which was noted when the transducers were placed onto handheld callipers, was misleading. This was because the mean range of BUA values for the group of 50 children measured with fixed transducers was approximately 10 dB.MHz^{-1} lower than the mean of those measured using handheld callipers. Consequently even though the two groups had the same root mean square standard

deviation BUA the ratio of this BUA value to the mean BUA, expressed as a percentage, was worse for the group with the lower mean BUA value. The coefficient of variation is therefore not a useful measure of relative precision error on the McCue CUBA Clinical. However when the ratio of the root mean square standard deviation of BUA measurements to the range of BUA values converted into a percentage was used (standardised coefficient of variation) the measure of relative precision error became more realistic, being 7.03% for both series of scans.

Conclusion three

The coefficient of variation should not be used as a measure of relative precision error on the McCue CUBA Clinical for children. Instead the root mean square standard deviation should be used as a measure of precision error and if relative precision error is required the percentage ratio of the root mean square and the BUA range should be used to calculate the standardised coefficient of variation.

Conclusion four

Using the root mean square standard deviation as a measure of precision error the precision of the BUA technique was unchanged when the transducers were placed on handheld callipers.

The evaluation of the clinical longitudinal data in chapter 10 enabled the analysis of the utility of the root mean square as a measure of precision error and the standardised coefficient of variation as relative precision error in clinical paediatric practice. A between-scans difference of three multiples of the precision error or relative precision error [GLUE1995] was used as the benchmark for significant clinical change in ultrasound measurements on children scanned twice, one year apart. Using this method it is estimated that a true difference when it exists will be detected 80% of the time and a type I error (false positive) will be made 10% of the time. Therefore with the CUBA Clinical, using the new centring point on these samples of children there must be annual changes of at least 18dB.MHz^{-1} using the precision error. The results demonstrated that only 6 children with ANRED and 14 control children had annual BUA increases in excess of this threshold value. This represents only 24% of the total sample of children who attended for annual BUA

scans. The 23 children with ANRED demonstrated a median increase of 9dB.MHz^{-1} compared to a median increase of 7dB.MHz^{-1} for the 62 control children.

Conclusion five

The precision error calculated as the root mean square standard deviation in dB.MHz^{-1} for the McCue CUBA Clinical is not significantly different from the longitudinal changes in dB.MHz^{-1} seen in these samples of children with ANRED and control children. The technical null hypothesis is therefore accepted.

The significance of longitudinal clinical changes was also assessed using the relative precision error. Three multiples of the relative precision error is $21\%\text{dB.MHz}^{-1}$ and this was used as the benchmark to differentiate significant clinical differences in vivo in children from machine fluctuations. The results demonstrated that 14 children with ANRED and 37 control children had increases in excess of 21% representing 61% of the children who had follow up scans. Furthermore the 23 children with ANRED demonstrated a median increase of 29% and the control children a median increase of 22%. The corollary being that the relative precision error is sufficiently low enough to detect annual percentage changes in BUA. This is however misleading because of the use of percentage changes on small absolute numbers. Consequently a child with a BUA of 10dB.MHz^{-1} need only increase by 3dB.MHz^{-1} (half of the precision error of 6dB.MHz^{-1}) to demonstrate a significant clinical improvement in percentage BUA of 30%. In comparison a child with a BUA of 30dB.MHz^{-1} must demonstrate a change of at least 9dB.MHz^{-1} to demonstrate the same percentage clinical change of 30%. Therefore when the percentage clinical change is calculated using such small numbers the results are misleading to the clinician.

Conclusion six

The median increase in %BUA in children with ANRED and in control children is greater than three multiples of the relative precision error (the standardised coefficient of variation). However it is not recommended that the relative precision error be used as a measure of in vivo changes in bone during growth and development. This is because minor changes in BUA which are less than

the precision error i.e. less than 6dB.MHz^{-1} can be measured as percentage BUA changes which are greater than 21% and deemed clinically significant. This is therefore not a useful tool for clinical paediatric practice with the McCue CUBA Clinical.

11.2 The clinical hypothesis

The evaluation of this hypothesis pervades the analyses undertaken in all of the experimental chapters apart from chapter 7. The groundwork for the evaluation of the hypothesis was undertaken in the pilot study by analysing and evaluating the results of the standard BUA technique in clinical paediatric practice. A convenience sample of 31 females aged between 12 and 20 years with ANRED was examined. The standard BUA technique includes the measurement of foot length to calculate BUA Z scores. Seven females in this sample had low BUA Z scores between -1 and -2.5 , which would be deemed in the osteopenic range if the WHO criteria were adopted for quantitative ultrasound measurements. However the BUA Z scores for the total sample ranged in value from -2.5 to $+2.5$ and the mean of the sample was not significantly different from zero (95% confidence intervals $+0.25$ to -0.60).

Conclusion one

In the pilot study a convenience sample of females with ANRED aged between 12 and 20 years were examined and 7 females (23%) had reduced BUA Z scores between -1 and -2.5 . The mean BUA Z score of the group, however, was not significantly different from zero.

The control data were provided by the equipment manufacturer and were derived from a paediatric sample scanned in the North-West of England who were scanned with the same model of quantitative ultrasound machine as used for this research however the standard positioning technique recommended by the manufacturer was also used. In this research a new reproducible centring point was developed for use on longitudinal studies on children during growth and analysed and evaluated as shown in chapter 7. Therefore to reduce the confounding effects of using a new technique, a normative database was developed based on healthy control children scanned in this study. The new centring point was used to develop normative values on healthy control children for comparison with the ANRED cohort. Most normative data charts for BUA measurements on children are plotted with age as the predictor variable and for boys and girls separately. Consequently age was used as the predictor variable in this research to allow useful comparisons with other studies. However weight was found to be a more useful predictor of BUA in children

accounting for 64% of the variance seen in the control cohort. On review of the literature this finding has been noted previously [MUGH1997] yet normative BUA charts in clinical use for children are still based on age rather than weight. Least squares linear regression was performed with weight and age as predictor variables and linear regression equations were obtained. These equations were used to predict normal values of BUA specific for gender for each year of age between 5 and 20 years and for each kilogramme of weight between 18 and 65kg. These were used to calculate BUA Z scores on the ANRED cohort.

Conclusion two

In the children examined in this research weight had a greater predictive ability for BUA than age accounting for 64% of the variance seen in the control sample. Therefore BUA Z scores based on weight and gender should be used in clinical practice in preference to those based on age and gender.

Using the control data collected in this study BUA Z scores for age and weight specific to gender were calculated for the ANRED cohort. The mean BUA Z score (corrected for age and gender) of the 52 children with ANRED was significantly lower than zero, (mean, -0.8 and 95% confidence intervals -0.52, -1.12) whereas the mean BUA Z score (corrected for weight and gender) was not significantly different from zero (mean, 0.02 and 95% confidence intervals, +0.33, -0.29). However since BUA Z scores corrected for age and gender are the accepted standard method of measuring BUA, the clinical null hypothesis outlined in chapter 1 can be rejected with 95% power and 2.5% significance, i.e. there is a statistically significant difference in BUA between ANRED children and controls. Since BUA is normal for weight but reduced for age, then reduced weight for age is the main cause of reduced BUA for age in children with ANRED.

Conclusion three

As a group, children and adolescents with ANRED have a significantly reduced mean BUA Z score for age and gender, however their mean BUA Z score for weight and gender is not significantly different from controls. Therefore the reduction in BUA noted in children with ANRED is related to their reduced weight for age.

In both the cross-sectional analyses (chapter 9) and the longitudinal analyses (chapter 10) the pubertal status of the child at the onset of the eating disorder had a significant impact on the BUA Z scores. So that the BUA Z scores (age and gender) of pre-menarcheal females with anorexia nervosa or FAED were significantly less than zero, whereas BUA Z scores (age and gender) for post-menarcheal females were not significantly different from zero. In the cross-sectional analysis, children with FAED were all pre or peri-pubertal and they had significantly lower BUA values than children with anorexia nervosa which is surprising because FAED is considered to be a mild variant of anorexia nervosa. In chapter 9 data analysis was performed on 3 sub-groups of ANRED females: pre-menarcheal FAED, pre-menarcheal anorexia nervosa and post-menarcheal anorexia nervosa. A re-analysis of data in these three categories demonstrated that pre-menarcheal females had the lowest mean values of BUA Z score (age and gender), irrespective of whether they had FAED or anorexia nervosa. An analysis of the BUA Z scores corrected for weight and gender demonstrated that neither pre-menarcheal nor post-menarcheal females had BUA Z scores which were significantly less than zero. However when BUA Z scores (corrected for weight and gender) of the entire group of 18 children with FAED were compared with that of the 23 children with anorexia nervosa only the BUA Z scores of the children with FAED were significantly less than zero (mean, -0.56, 95% CI, -0.03, -1.09).

Conclusion four

Pre-menarcheal females who are underweight because of an eating disorder have significantly lower values of BUA Z score corrected for age and gender than controls. Post-menarcheal females who are also underweight because of an eating disorder do not have similar significant reductions in BUA Z scores. The reduction in BUA Z score for age and gender is therefore related to pubertal status at the time of the scan.

Conclusion five

Pre-pubertal males and females with FAED have mean BUA Z scores corrected for both age and gender and weight and gender which are significantly less than zero.

Conclusion six

The reduced BUA seen in pre-menarcheal females with ANRED is related to their reduced weight and pubertal status rather than the specific eating disorder.

The annual increases in BUA were inversely related to years since menarche, and post-menarcheal ANRED females had significantly fewer menstrual cycles per year than control females ($p < 0.001$). Although the differences do not reach statistical significance this may contribute to the lower values of BUA seen in post-menarcheal ANRED females compared to controls.

Conclusion seven

Post-menarcheal females with ANRED have significantly fewer menstrual cycles per year than control females which may contribute to the reduced BUA values seen in these females.

There were four children with BUA Z scores, corrected for weight and gender, which were less than -1 , all of whom had additional significant risk factors for reduced BUA, including delayed puberty, treatment with steroids and dairy allergies. Although this is a very small number and not a significant proportion of the total sample of children with ANRED, it suggests that low BUA Z scores, corrected for weight and gender may identify those children who are most at risk of potential osteoporotic fracture.

Conclusion eight

ANRED children with BUA Z scores corrected for weight and gender which are lower than -1 are not likely to attain a high peak bone mass and therefore have the greatest risk of future osteoporotic fracture. Measurements of BUA Z score corrected for weight and gender rather than age and gender should be used to identify children most at risk of osteoporotic fracture.

Children receiving treatment for ANRED have annual increases in BUA and percentage BUA, which are not significantly different from increases seen in control children.

Conclusion nine

Annual increases in BUA in children are independent of the primary eating disorder.

11.3 Recommendations

In this thesis the precision of a quantitative ultrasound technique has been evaluated, scrutinised and tested thoroughly. The results demonstrated a disappointing precision error of 6dB.MHz^{-1} and relative precision error of 7%. However, it is reasonable to assume that if the precision of other clinical investigative tools were subjected to the same degree of scrutiny the results could be similarly poor. In clinical practice it is acceptable to use a technique with relatively poor precision if it has good sensitivity. Quantitative ultrasound machines such as the McCue CUBA Clinical have good sensitivity but are not precise enough to monitor annual change. Good precision is used as a key argument for the use of DXA and values as low as 1% have been quoted in paediatrics ([KATZ1991], [SABA1999]). However this good precision is dependent on a number of variables including the DXA machine used for the scans (within and between manufacturers); the software used; the method of measuring precision (CV, SCV or RMSSD); the number of operators performing the scans and their experience in paediatric bone densitometry. In a best case scenario when precision is 1% and annual increases in BMD are at their greatest (peri-menarcheal increases of between 8% and 18% were noted by Sabatier et al [SABA1999]) DXA can be used to differentiate valid annual in vivo changes from machine fluctuations. The advantages of the ultrasound technique lie in its sensitivity, conservative cost and non-invasive nature compared to alternative diagnostic procedures such as DXA.

The broadband ultrasound attenuation technique is based on Langton's [LANG1984] scientific empirical observations of the attenuation of a broadband beam of ultrasound through bone. He observed the difference in attenuation through the bone of healthy women and those with a history of a fractured femoral neck. This led to the deduction that broadband ultrasound attenuation could differentiate between healthy and osteoporotic bone. There is however, little evidence of theoretical physics to underpin the technique and further research endeavours should include a broadening of the knowledge and understanding of the underlying physical principles.

The data analysis of the precision error and relative precision error of the McCue CUBA Clinical used in this study demonstrated an improvement in the precision and validity of the technique when a reproducible centring point was used to locate the region of interest on the heel. However, this technique along with most other quantitative ultrasound techniques remains limited because it is a “blind” technique, relying on the relationship between palpable bony landmarks to locate the region of interest in the calcaneum. Further research endeavours should be directed towards the continued development and refinement of ultrasound imaging techniques to locate a reproducible region of interest on the calcaneum. This is likely to improve not only the precision of the technique, but also the clinician’s confidence in the ultrasound results.

Analyses of these data have demonstrated that pre-pubertal children with ANRED and reduced weight are most at risk of reduced BUA and furthermore the nature of their primary eating disorder is not a significant factor in this reduction in BUA. The literature review in chapter 2 demonstrated that pre-pubertal children can increase their bone mineral density by following exercise intervention programmes for less than a year. The pre-menarcheal females with ANRED in this study did significantly less exercise per week than pre-menarcheal controls and this is therefore likely to have contributed to their reduced BUA values. Therefore, a prospective exercise intervention trial together with a standard clinical re-feeding programme should be undertaken in a pre-pubertal group of children with ANRED to establish whether the negative impact of ANRED can be reversed prior to the onset of puberty and the attainment of a peak bone mass. Such a group of children should be matched with a healthy group of children as a control data-set.

Evidence in the literature review and this research suggested that the pre-pubertal onset of an eating disorder has a negative impact on the measurement of bone mineral density and BUA. An observational study should be performed on women with a past history of pre-menarcheal onset ANRED to establish whether their BUA values are lower than a matched set of women with post-menarcheal onset ANRED. The hypothesis would be that females with a history of pre-menarcheal onset ANRED would have lower BUA values in adult life than those with post-menarcheal onset ANRED.

Data in this research demonstrated that weight was the greatest predictor of BUA in children and adolescents with ANRED. The reports published to date describe reduced bone mineral density Z-scores or t-scores when corrected for age in patients with ANRED. Normative data on BUA measurements corrected for weight and gender should be used for these adults and a cross-sectional study performed to evaluate whether adult patients with ANRED have reduced BUA Z scores when corrected for weight and gender. Standard life-style questionnaires from these patients should also be analysed to identify those who have additional risk factors for reduced BUA Z scores corrected for weight and gender, including pre-pubertal onset of the eating disorder. The hypothesis would be that those women with reduced BUA Z scores corrected for weight and gender would have additional risk factors and that these patients are therefore the ones most at risk of osteoporotic fracture. Appropriate consideration should be given to the size of the sample in such a study to allow a statistically significant difference to be demonstrated if one exists.

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Appendix 1 Lifestyle questionnaire

96RP03: The Use of Ultrasound to Measure Bone Density in Children

Date of scan.....

PERSONAL DETAILS

Name..... Sex Female Male

DOB.....Age.....yrs.....months..... Hospital number.....

Height.....Weight.....

BMI.....

ETHNICITY

Black Caribbean Black African Black other Indian

Bangladeshi Pakistani Asian other Chinese

White Other (please state) Prefer not to say

Comments.....

DIAGNOSIS AN FAED SE BN Other

DURATION.....

MEDICATION

1. What medications are currently being taken? eg steroids, hormones, vitamin supplements

MENSTRUAL HISTORY

2. Have menses commenced? Yes No

3. Age at menarche?.....4. Number of days between periods.....

5. Number of menstrual periods in the last year?.....

6. LMP.....

EXERCISE

7. Approximately how many hours each week are spent walking outdoors? For example to or from school/ friend's houses/clubs etc.

- 1 hour/week 1
- 2 - 6 hours/week 2
- More than 6 hours/week 3

8. What sort of exercise does the subject undertake?

- | | | | |
|------------|------------|----------|---------------|
| Aerobics | Cycling | Netball | Aqua-aerobics |
| Basketball | Football | Rugby | Badminton |
| Cricket | Tennis | Swimming | Squash |
| Running | Rowing | Hockey | Dancing |
| PE lessons | Gymnastics | Ballet | |

Other.....

9. How many hours of regular physical exercise would the subject estimate that s/he undertook each week?

- 1 hour/week 1
- 2 - 6 hours/week 2
- More than 6 hours/week 3

NUTRITION

10. How many pints of milk does the subject drink in a week? (Assume 1 yoghurt = 1/2 pint of milk)

.....pints

11. Does the subject's diet include any other dairy products? Yes 1 No 2

Comments.....

FAMILY HISTORY

12. Is there any family history of osteoporosis? Yes 1 No 2 Don't know 3

- | | | | | | | | | |
|------------|----------------------------|-------------|----------------------------|---------|----------------------------|-------|----------------------------|----------------------------------|
| 13. Mother | <input type="checkbox"/> 1 | Grandmother | <input type="checkbox"/> 2 | Sister | <input type="checkbox"/> 3 | Aunt | <input type="checkbox"/> 4 | |
| Father | <input type="checkbox"/> 5 | Grandfather | <input type="checkbox"/> 6 | Brother | <input type="checkbox"/> 7 | Uncle | <input type="checkbox"/> 8 | Other <input type="checkbox"/> 9 |