

Bone Mineral Density Determination in Children

Evaluation of a Novel Method and
Application to Duchenne Muscular Dystrophy

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Göteborg 2009

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Layout by Roland Thomeé
Language revision by Jeanette Kliger
Illustrations by Annette Dahlström
Printed by Intellecta Infolog, Göteborg, Sweden, 2009

ISBN: 978-91-628-7884-9

To My Family

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Abstract

Abstract

Aims: The overall aims of this thesis were to evaluate the dual-energy X-ray and laser (DXL) method for bone densitometry measurements of the calcaneus in children, to provide reference data for bone mineral density (BMD) in the heel bone in young children and to apply the DXL technique to patients with Duchenne muscular dystrophy (DMD) and conduct a survey about bone health of DMD patients.

Study populations and methods: The DXL Calscan method was modified and adapted for measurements in children and applied to bone densitometry in all subsequent studies. To provide percentile reference data for the DXL measurements, a total of 334 healthy children aged 2, 4 and 7 years were measured (Study I). Measurement data were collected from 112 individuals, aged 2-21 years, to evaluate the relationship between the heel DXL measurements and whole-body dual-energy X-ray absorptiometry (DXA) measurements (Study II). In a cross-sectional study, 24 DMD patients, aged 2-20 years, were compared with 24 healthy age- and gender-matched controls with special emphasis on bone mass assessed at different skeletal sites and bone turnover (Study III). In a longitudinal study, 18 DMD patients from Study III and 6 patients with Becker muscular dystrophy (MD) were followed for 4 years with the emphasis on bone mass development, body composition, muscle strength and motor function (Study IV).

Results: The DXL method was readily applied, both in the very young children and in the children with various disabilities. Reference data for BMD were provided as percentile values for children aged 2, 4 and 7. Additional data (a total of 645 DXL Calscan measurements (328 girls/317 boys)) from a follow-up study enabled the presentation of BMD reference curves (mean \pm 2 SD) for children (girls and boys respectively) between 2 and 10 years of age. A high correlation was found between the heel DXL measurements and DXA measurements in the hip, in the spine and in the total body. The DXL measurements predicted the lowest DXA-determined BMD values at these sites with high sensitivity (0.9-1.0) and high specificity (0.86-0.95). In the DMD patients, the BMD levels were generally lower compared with the healthy controls. These differences increased with increasing age and were particularly evident in the hip and the heel. Biochemical markers of bone turnover demonstrated reduced bone formation as well as reduced bone resorption in the DMD patients. The fracture rate was no higher in the DMD group compared with the control group, but the fractures were more frequently located in the lower extremities in the patient group. The BMD values were significantly reduced in the DMD patients, even when compared with Becker MD patients. The Becker MD patients, in turn, showed significantly reduced BMD levels compared with healthy controls at most sites. A significant association was found between the changes in lean mass (muscle mass) and bone mass with time and there was also a strong association between BMD measurements and muscle function parameters.

Conclusions: It is feasible to perform DXL bone densitometry measurements of the calcaneus in very young children as well as in children with disabilities. The DXL measurements can predict low BMD values as measured by whole-body DXA. DMD patients had both reduced bone turnover and reduced BMD values compared with healthy controls. The impaired muscle strength and reduced motor function, as observed in the DMD patients, were associated with reduced bone mass during growth. The level of disability appeared to have a major effect on skeletal development, which was, for example, demonstrated as a decrease in hip BMD in the DMD patients with time.

Keywords: adolescents, age- and gender-matched, Becker, bone densitometry, bone markers, bone mineral density, calciotropic hormones, children, DXA, DXL, Duchenne, glucocorticoids, muscular dystrophy, normative, reference values, skeleton

List of papers

List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals:

- I Söderpalm A-C, Kullenberg R, Albertsson Wikland K, Swolin-Eide D. **Pediatric Reference Data for Bone Mineral Density in the Calcaneus for Healthy Children 2, 4 and 7 Years of Age by Dual-Energy X-Ray Absorptiometry and Laser.** J Clin Densitom. 2005; 8(3):303-313.
- II Söderpalm A-C, Kullenberg R, Swolin-Eide D. **The Relationship Between Dual-Energy X-Ray Absorptiometry (DXA) and DXA With Laser (DXL) Measurements in Children.** J Clin Densitom. 2008; 11(4):555-560.
- III Söderpalm A-C, Magnusson P, Åhlander A-C, Karlsson J, Kroksmark A-K, Tulinius M, Swolin-Eide D. **Low bone mineral density and decreased bone turnover in Duchenne muscular dystrophy.** Neuromuscul Disord. 2007; 17(11-12):919-928.
- IV Söderpalm A-C, Magnusson P, Åhlander A-C, Karlsson J, Kroksmark A-K, Tulinius M, Swolin-Eide D. **Bone mass in Duchenne and Becker muscular dystrophies: A four-year longitudinal study.** Submitted, Ms.No.NMD-09-00181, under revision.

Abbreviations & Definitions

Abbreviations

aBMD	Areal bone mineral density (g/cm^2)
ALP	Alkaline phosphatase
AUC	Area under the ROC curve
BA	Bone area (cm^2)
BALP	Bone specific alkaline phosphatase, a bone formation marker
BMAD	Bone mineral apparent density (g/cm^3), in this thesis (mg/cm^3)
BMC	Bone mineral content (g)
BMD	Bone mineral density (g/cm^2), equal to aBMD
BMI	Body mass index (kg/m^2)
BMU	Basic multicellular unit of bone remodelling
BUA	Broadband ultrasound attenuation (dB/MHz)
CTX	Carboxy-terminal cross-linking telopeptide of type I collagen (cathepsin K generated), a bone resorption marker
CV	Coefficient of variation (%)
DMD	Duchenne muscular dystrophy
DXA	Dual-energy X-ray absorptiometry
DXL	Dual-energy X-ray absorptiometry and laser
FN	Femoral neck
GC	Glucocorticosteroids
GH	Growth hormone
ICTP	Carboxy-terminal cross-linking telopeptide of type I collagen (matrix-metalloprotease generated), a bone resorption marker
IGF-I	Insulin-like growth factor-I
IISD	Intra-individual standard deviation
MES_m	Minimum effective strain for modelling
MES_r	Minimum effective strain for remodelling
MD	Muscular dystrophy
OC	Osteocalcin, a bone formation marker
PBM	Peak bone mass
PINP	Type I procollagen intact amino-terminal propeptide, a bone formation marker
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RDI	Recommended daily intake

Abbreviations & Definitions

ROC	Receiver operating characteristic
ROI	Region of interest
SD	Standard deviation
SEM	Standard error of mean
SOS	Speed of sound (m/s)
TB	Total body
TB_{HE}	Total body, head excluded
TRACP-5b	Tartrate-resistant, acid phosphatase isoform 5b, a bone resorption marker
vBMD	Volumetric bone mineral density (g/cm ³)

Definitions in brief

Accuracy	How close the BMD measured by densitometry is to the actual calcium content of the bone (ash weight)
Precision	Measures the reproducibility of a bone densitometry technique, usually expressed as the coefficient of variation (CV%)
Sensitivity	True positive rate – how well the DXL can truly predict low whole-body DXA values
Specificity	True negative rate – how well the DXL can truly predict whole-body DXA values, which are not regarded as low values
T-score	Refers to SD differences in BMD values measured by DXA in an individual compared with mean values in young women, who have achieved their PBM
Z-score	Refers to age-related reference values

Preface

Preface

The word “orthopaedics” originates from the Greek “orthos”, which means straight or correct, and “paideia”, which means rearing or upbringing, or “pais”, which means child. Orthopaedics is therefore often referred to as “the art of correcting and preventing deformities in children”.

With a healthy skeleton and normal growth, a child most frequently has straight bones adapted to withstand loads imposed upon them without fracturing. However, already in ancient times different kinds of skeletal deformity were recognized, together with fractures, and over the years scientists have tried to find the causes and the optimal treatment.

Orthopaedics was not organised in more detail until the middle of the 19th century when industrialisation and urbanisation brought many children with disabilities and deformities together in the larger cities. Children with disease such as rickets, tuberculosis, poliomyelitis or congenital deformities were recognized and specific hospitals for children were opened in Central of Europe, Great Britain, Scandinavia and the United States of America ⁴⁹. Knowledge of different skeletal disorders and diseases grew and it was realised that rickets could be cured through sun-light, and the importance of vitamin D was recognised ⁹¹.

Mechanical influences on bone architecture were also discussed during this period by both Hueter and Volkmann, 1862 and Wolff, 1892, but the techniques now available for understanding the modelling or remodelling of bone tissue had not yet been developed.

The advent of radiographic technology in 1895 made it possible to obtain a picture of skeletal density. During the early 20th century, it was believed that trauma produced osteoporosis, since the skeleton appeared less dense on radiograms taken following a fracture ⁴².

During the last few decades, a large-scale increase in fracture incidence, especially in the elderly, has been observed and this has led to an increasing call for the development of techniques for measuring bone mass. Bone mineral density, one of several factors with an important impact on bone strength, is related to the risk of fracture and there is evidence to suggest that the most

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important time for creating strong bone is during the growth period. This is one reason for monitoring skeletal development in children and adolescents. Chronic diseases or medical treatment could hamper children from developing an optimal skeleton. Moreover, changes in life-style factors, such as physical activity and nutrition among young people today, could possibly have an impact on skeletal development, which would imply an increased risk of fractures later in life. The way to measure bone mass or bone strength, interpret bone measurement data in children and decide when and which children that should be measured are controversial issues.

This thesis presents a novel method for measuring bone mass in the calcaneus in children and its application in patients with Duchenne muscular dystrophy, which could serve as a model demonstrating the importance of muscular strength and function for bone formation.

Introduction

Introduction

Osteoporosis

The term osteoporosis means porous bone and was at first introduced in France in the early 1820s as a description of a pathological state of the bone¹³⁶. The term initially implied a histological diagnosis, but the definition has been changed over time.

In 1991, osteoporosis was defined as “a disease characterized by low bone mass, microarchitectural deterioration of bone tissue, and a consequent increase in fracture risk”¹³⁵.

In 1994, the World Health Organisation (WHO) defined osteoporosis in adults as a bone mineral density (BMD) value of 2.5 standard deviations (SD) or more below the peak BMD in young women⁸². The definition T-score, usually used in adults, refers to SD differences in BMD values measured by dual-energy X-ray absorptiometry (DXA) in an individual compared with mean values in grown-up young women (Figure 1).

In 2000, osteoporosis was redefined as “a skeletal disorder characterized by compromised bone strength, predisposing to an increased risk of fracture”¹³⁵, and it was proposed that the risk of fracture and osteoporosis, as reflected by low bone mass, overlaps, but is not identical. This definition points out that not only bone mass but also other factors contribute to bone strength.

In the Utah Paradigm, Frost suggests that physiological osteopenia is present when “reduced bone ‘mass’ and whole-bone strength properly fit a subject’s reduced voluntary physical activities and muscle strength”. In pathological osteopenia, also called “true osteoporosis”, the bone strength is less than that needed for the daily mechanical use⁴⁶.

It has been shown that osteoporosis indicates a greater fracture risk in adults⁷⁷ and, during the last few decades, osteoporosis has become a worldwide health problem, with an increasing incidence of fragility fractures. More than 200 million people worldwide are considered to be affected by osteoporosis⁹³, but the northern parts of Europe in particular are worst hit. The increasing percentage of elderly people in the population appears to be an important reason for this trend, even if recent reports from Finland suggest that the age-

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adjusted incidence of hip fracture has declined ⁸³. In Sweden there are about 70,000 fractures related to osteoporosis every year ¹ with an average one-year cost of SEK 4.6 billion ²².

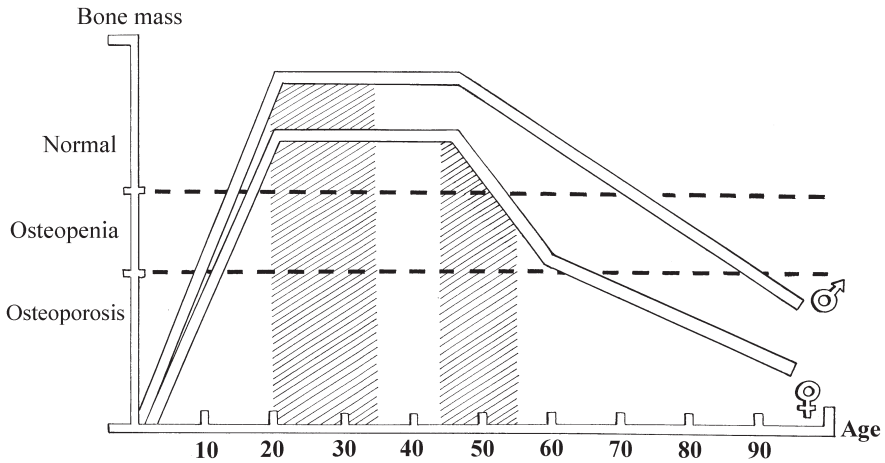


Figure 1 - Bone mass life line in men (♂) and women (♀) who achieve their full genetic potential peak bone mass (first shaded area). Second shaded area indicates menopause. T-score definition according to the WHO in 1994: Normal bone mass ≥ -1 SD, Osteopenia -1 to -2.5 SD, Osteoporosis ≤ -2.5 SD.

In children, osteoporosis has not yet been clearly defined, but it has been shown that, even in children, a lower BMD indicates an increased fracture risk ⁵⁶. Since children and adolescents have not yet reached their peak bone mass, it is not possible to use T-scores in this growing population; instead, Z-scores are used. Z-scores refer to age-matched reference values. For a diagnosis of osteoporosis in children and adolescents (5-19 years of age), the ISCD 2007 Pediatric Official Positions of the International Society for Clinical Densitometry ¹²³ has recently recommended the presence of both low bone mineral content (BMC) and low BMD, defined as a BMC or areal BMD (aBMD) Z-score less than or equal to -2.0 adjusted for age, gender and body size and a clinically verified fracture history as follows: one long bone fracture of the lower extremities; one vertebral compression fracture; two or more long bone fractures of the upper extremities.

Introduction

The bone

Bone is one of the hardest tissues in the human body. It is unique in that it should be light enough to allow locomotive activity but at the same time flexible and strong enough to withstand bending, torsion and compressive forces without fracturing. The skeleton also protects vulnerable inner organs, contains the red bone marrow where new blood cells are produced and plays an important endocrine role and serves as a reservoir for minerals, mainly calcium and phosphorus. To fulfil these requirements, bone continually adapts to the mechanical and physiological demands placed upon it.

The composition of bone

The skeleton can be divided into the axial (e.g. vertebrae and the pelvis) and the appendicular skeleton (long bones). As an organ, bone is made up of the cartilaginous joints, the calcified cartilage in the growth plate in growing individuals, the marrow space, and the mineralised structures i.e. the bone. The cortical bone is a densely compacted tissue, which forms the outer layer predominantly of long bones. The cancellous bone is characterised by a network of trabeculae resulting in a large surface area, which makes this bone more sensitive to metabolic changes¹²⁹. Bone as a tissue, is composed of intercellular calcified material, the bone matrix, and bone cells. The matrix, organic (~20%) and inorganic (~70%), is formed and maintained by the osteoblasts. The unique combination of organic and inorganic material results in the characteristic property of bone; the hardness and the elasticity⁸⁰ (Figure 2).

Bone cells

There are three cell types in bone: 1) the bone-forming osteoblasts which, when engulfed in mineral, become 2) osteocytes and 3) the bone-resorbing osteoclasts⁹.

The osteoblast

Osteoblasts are differentiated from mesenchymal stem cells, a process that is controlled by a multitude of cytokines and can be divided into several stages, including proliferation, extracellular matrix deposition, matrix maturation and mineralisation. The osteoblasts synthesise and secrete collagen and non-collagenous proteins that comprise the organic matrix of bone and subsequently mineralise the organic bone matrix. They express high levels of alkaline phosphatase (ALP), a serum marker of bone formation. Subsequently, some osteoblasts disappear through programmed cell death (apoptosis), whereas

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others differentiate into flat cells lining the bone surface (lining cells) or become cells surrounded by the bone matrix in small lacunae (osteocytes) ¹¹⁶.

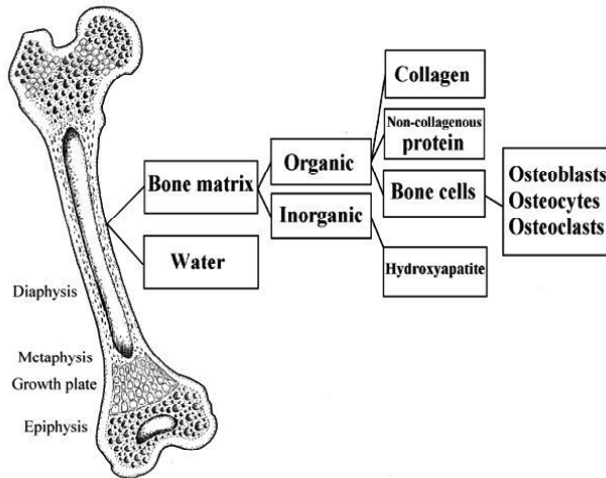


Figure 2 – Long bone.

The osteocyte

It is well known that bone tissue is sensitive to the mechanical demands imposed on it and that inactivity or abnormally low mechanical stress results in reduced bone mass and disease osteoporosis. The osteocytes, embedded deep within the lacunae, express a variety of molecules, which make them respond to both mechanical and hormonal stimuli. They are characterised by long cell processes, which form a complex network through-out the bone matrix, providing intercellular communication, which plays an important role in the mechanosensitivity of the bone. This network also connects with osteoblasts and lining cells on the bone surface ⁷³.

The osteoclast

The osteoclasts, the exclusive bone-resorbing cells, are large multinucleated cells of hematopoietic origin. They are usually found in close association with bone surface and they are characterised by their ruffled border. Attached to the bone surface, the osteoclasts create an acid environment and bone tissue is dissolved by proteinase and enzyme activity. As with osteoblasts, osteoclast differentiation is regulated by cytokines and osteoclasts are also influenced by hormones and other factors.

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Skeletal morphogenesis

Skeletal development starts from mesenchymal condensation. Ossification is controlled by two major mechanisms: intramembranous ossification (long bone formation directly from mesenchymal cells) and endochondral ossification (bone formation mediated by a cartilage scaffold)¹⁶³. Histologically, there are two varieties of bone tissue: the immature, woven, primary bone; and the mature, lamellar, secondary bone, containing the same structural components but with differently organised collagen bundles. The immature bone that forms during embryonic life or after a fracture is eventually replaced by secondary bone tissue during growth and fracture healing⁸⁰.

Bone modelling and remodelling

There is a delicate balance between bone-resorbing and bone-forming activity, referred to as bone remodelling (Figure 3), which is the dominant process in the adult skeleton. In the “basic bone multicellular unit” (BMU), osteoclastic bone resorption initiates osteoblastic bone formation⁴⁶. This continuously replaces damaged and old bone with new bone tissue in the healthy skeleton. In the ageing skeleton, the balance is shifted in favour of resorption, which results in weaker, thinner bone. In the growing skeleton, modelling is dominant, when bone mass is added and the periosteal and endocortical diameters of bone are expanded. Modelling also includes changes in bone shape throughout life and promotes bone strength. Macro-modelling, is a term that is used when geometric properties are changed and improved due to regionally added bone mass. Mini-modelling occurs within the cancellous bone when the orientation of the trabeculae is changed in response to loading⁴⁶.

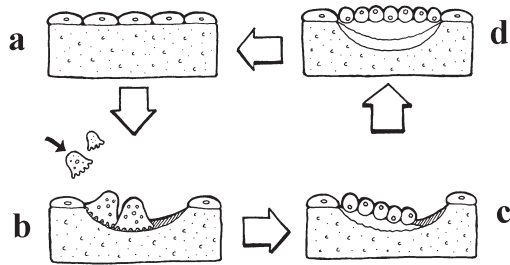


Figure 3 – Remodelling cycle in trabecular bone: a) inactive face, b) bone resorption by osteoclasts, c) bone formation by osteoblasts (osteoid) and d) bone formation by osteoblasts (osteoid-mineralisation).

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Bone mass development during childhood

During normal growth, bone mass continuously increases in concordance with skeletal growth in length and breadth and the total skeletal mass peaks a few years after growth arrest. This peak bone mass (PBM) is achieved at different ages depending on skeletal site and the way the measurement of bone mass is performed⁶⁷. Since it has been suggested that a higher PBM reduces the risk of osteoporotic fractures later in life¹¹⁴ it could be important to optimise PBM. This can only be done during childhood and adolescence. Bone mass accrual depends on hereditary factors, physical activity and muscle development, dietary factors, hormones and growth factors. All these factors, which are important for achieving optimal predisposed PBM during growth, are continuously important throughout life⁶⁷ (Figure 4).

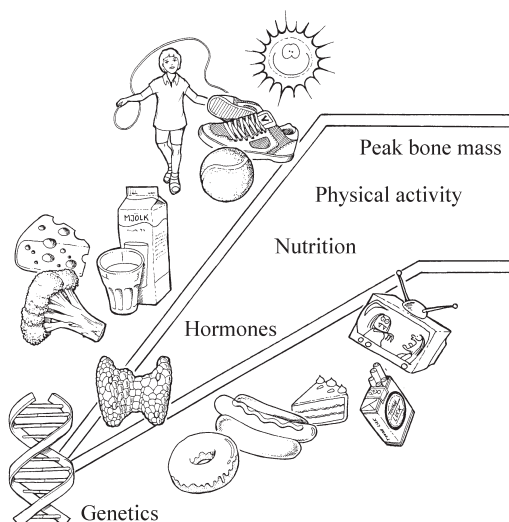


Figure 4 – Factors influencing the achievement of peak bone mass.

Hormones that have an established role in the complex regulation of postnatal growth include growth hormone (GH), insulin-like growth factor-I (IGF-I) and sex steroids. GH and IGF-I have different target cells in the epiphyseal growth-plate and they are both important for bone remodelling and bone mineral accrual. During pre-puberty, GH mainly promotes the growth of the long bones in terms of final height, but it also has a major effect on muscle mass

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development¹⁴¹. During puberty, sex steroids also have an important effect^{111,115,149,158}.

In 1892, Wolff published an early milestone for understanding mechanical influence on bone. He suggested that “Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture and equally definite alteration in their external conformation, in accordance with mathematical laws”. However, before him, more than 400 years ago, Galileo and Vesalius suspected that skeletal geometry might depend on usage⁴⁶. In 1862, Hueter and Volkmann separately postulated that: “There is an inverse relationship between compressive forces along the long axis of epiphyseal growth and the rate of epiphyseal growth”¹⁴³.

According to Frost, who introduced the modern mechanostat theory, “Wolff refers to lamellar bone deposition and not to bone development; indeed, he felt that early bone formation from cartilage models was independent of mechanical stress! “. The mechanostat theory suggests that all skeletal organs (spongiosa, cortical bone, growth plate, articular cartilage, tendons, ligaments and muscle) adapt their structure, stiffness and strength to their voluntary mechanical usage. The mechanostat of the bone acts through a feed back system with two threshold ranges for strain (bone deformation during loading). Below the lower range, minimum effective strain for remodelling (MESr), there is an inadequate stimulus or disuse resulting in bone loss. Above the upper range, minimum effective strain for modelling (MESm), modelling will result in more bone being added. Between MESr and MESm, there is the physiological loading zone, where bone is held in a steady state. The thresholds can be altered during life due to for instance puberty or menopause or because of medical treatment, when a steady state mode will be replaced by either a disuse mode or a modelling mode⁴⁶ (Figure 5).

Several intervention studies of the effect and benefit of increased physical activity in children have been published^{89,90,96,101}. In adults, studies of bed-rest-induced bone losses have shown a recovery of bone mass and a remarkably high initial re-accrual rate of bone when the individuals start to bear weight again. The accrual of bone mass was comparable to that during the pubertal growth spurt and it followed the neuromuscular recovery, which clearly indicates, that the adult skeleton also has the capability to adapt to mechanical stimuli¹²⁷.

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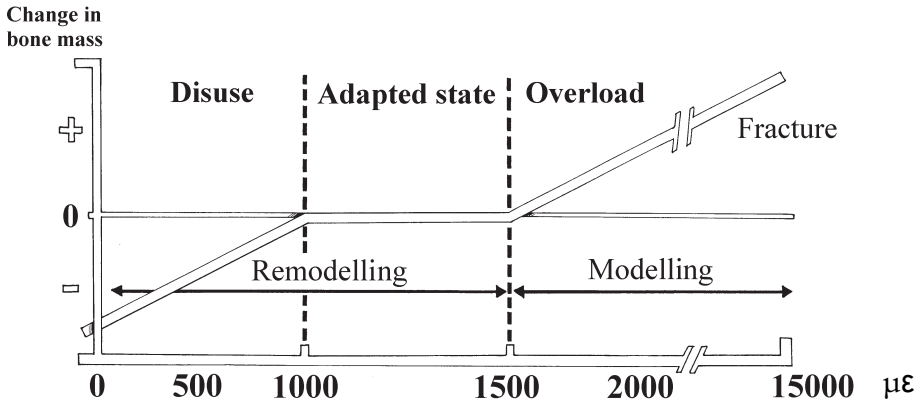


Figure 5 – Skeletal adaptation to mechanical loading according to the mechanostat theory by Frost ⁴⁶. X-axis shows microstrain ($\mu\epsilon$).

To be able to follow bone mass development during growth in health and disease, normative values for different skeletal sites and for different bone measurement techniques have been established ^{17,105,106,144,164}. Studies have shown that peak height velocity during puberty precedes the highest velocity of bone mineral accrual by one to two years in both girls and boys ¹³ and that the metaphyseal bone strength at the distal radius lags behind the longitudinal growth ¹²²; this could be a reason for the increased fracture incidence seen during this period of life ⁸⁷. It has also been shown that muscle development precedes bone development during the pubertal growth spurt ¹²¹.

Biochemical markers of bone metabolism

Components are released into the circulation when bone matrix is formed and degraded during the continuous process of bone modelling and remodelling. These components can be assessed and monitored in serum and urine and are referred to as biochemical markers of bone turnover. Bone markers change rapidly in response to changes in bone formation and resorption, in contrast to the more slowly occurring changes detectable by any radiographic method ³⁵. Markers that specifically characterise either bone formation or resorption have been recognised, but most markers are also present in tissues other than bone. Moreover, these specific markers reflect bone turnover changes independently of underlying cause or skeletal site.

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Markers of bone turnover can estimate fracture risk in postmenopausal women and older men ^{50,102} but the dominating advantage of these markers appears to be in the monitoring of anti-osteoporotic therapy (Table 1).

Table 1 – Biochemical markers of bone metabolism.

Bone formation markers	Bone resorption markers
Bone-specific alkaline phosphatase (BALP)	Carboxy-terminal cross-linking telopeptide of type I collagen (CTX) (cathepsin K generated)
Osteocalcin (OC)	Tartrate-resistant, acid phosphatase isoform 5b (TRACP-5b)
Type I procollagen intact amino-terminal propeptide (PINP)	Carboxy-terminal cross-linking telopeptide of type I collagen (ICTP) (matrix-metalloprotease generated)

Bone formation markers

The most frequently used marker of bone formation is the enzyme alkaline phosphatase (ALP). There are several ALP isoforms of which four are bone specific (BALP) (B/I, B1, B2, and B1x). BALP, primarily expressed on the osteoblast cell surface, is cleaved from the cell surface and found within mineralised matrix. Studies of hypophosphatasia, a rare inherited disorder with impaired skeletal mineralisation, suggest that BALP plays a role as a pyrophosphatase (i.e. cleaving inorganic pyrophosphate, a potent inhibitor of mineralisation), thus promoting mineral deposition in vivo ⁶⁹. BALP is used as an early marker of bone formation; it is stable when sampled, has a relatively long half-life in serum (1-2 days) and its activity is highly correlated with longitudinal growth ³³.

Osteocalcin (OC), also known as bone Gla protein (γ -carboxyglutamic acid-containing), is a small protein (5.8 kDa) that accounts for approximately 10% of the non-collagenous protein of bone. OC is regarded as a late marker of osteoblast differentiation and it is exclusively synthesised by osteoblasts, except for a very small fraction synthesised by odontoblasts. Theoretically, OC should be the most accurate marker of osteoblast activity; however, OC is relatively unstable with a short half-life in serum (5 min), which limits the diagnostic use

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of this marker. Moreover, OC is cleared by the kidneys, and is thereby affected by changes in kidney function.

When type I collagen is synthesised by the osteoblasts, a larger precursor molecule, procollagen type I, is first formed. The large globular ends of the procollagen molecule are enzymatically cleaved and secreted into the circulation as the carboxy- and amino-terminal propeptides of type I procollagen, PICP and PINP, respectively ¹⁴⁰.

Bone resorption markers

Initiating bone resorption, the osteoclast adheres to the bone surface and creates a closed zone between itself and the bone surface, where an acid environment is created and the mineralised bone is degraded. The osteoclasts release tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), which can be used as a marker of bone resorption and it has also been suggested that TRACP-5b reflects the number of active osteoclasts ¹²⁵. In the acidified setting, the organic matrix is exposed, and type I collagen can be degraded by enzymes, which cleave cross-links within the molecule. Products of degradation can be detected in serum; they include the carboxy-terminal telopeptides of type I collagen, CTX and ICTP. It has been suggested that CTX reflects osteoclast activity ¹²⁵ (Figure 6).

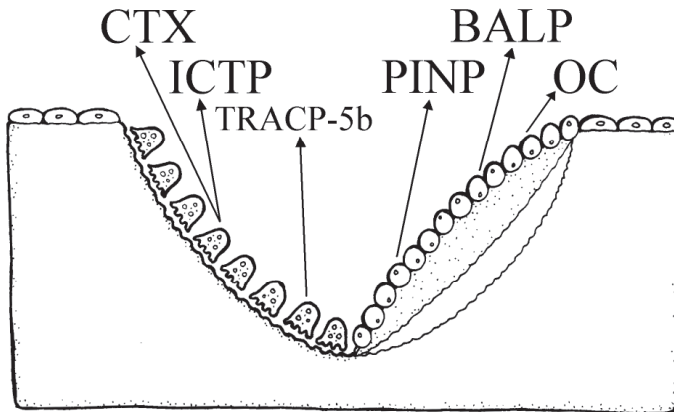


Figure 6 – Illustration of bone remodelling with bone markers released into the circulation during bone resorption (TRACP-5b, CTX, ICTP) and during bone formation (PINP, BALP, OC).

Introduction

Vitamin D

By maintaining physiological serum calcium and phosphorus levels, vitamin D supports metabolic functions, neuromuscular transmission and skeletal mineralisation. Vitamin D is provided indirectly through sunlight exposure and to a lesser degree directly from food intake. It is hydroxylated in the liver to 25-hydroxyvitamin D (25(OH) D), which is usually the metabolite measured in serum to evaluate the individual vitamin D status. Further hydroxylation occurs in the kidneys to 1,25-dihydroxyvitamin D (1,25(OH)₂ D), the active metabolite. Vitamin D mediates the mineralisation of newly synthesised osteoid tissue within bone, but, if dietary calcium is inadequate, vitamin D interacts with the osteoblast-osteoclast coupling to dissolve bone for calcium release into the circulation ⁷¹.

Vitamin D deficiency causes rickets in children and osteomalacia in adults, but there is a growing conviction that less severe degrees of deficiency may also cause skeletal disease ⁶⁶. It is known that vitamin D deficiency results in muscle weakness and it has been observed that 1,25(OH)₂ D improves muscle function. Living in the northern part of the world, above 35° of latitude, implies a major risk of vitamin D deficiency ⁷¹. In Sweden, the recommended daily intake (RDI) of vitamin D for children is 10 µg/day ¹³². However, the definition of 25(OH) D-insufficiency is unclear, as is the optimal RDI of vitamin D. Biggar and co-workers ¹⁶ recommended that supplementation is appropriate if serum 25(OH) D is < 20 µg/L, without making a distinction between vitamin D insufficiency and deficiency. Parathyroid hormone (PTH), a peptide hormone that regulates the minute-to-minute level of ionised calcium, stimulates bone resorption if the extracellular calcium levels are depleted, and, as suggested by Heaney ⁶⁶, 25(OH) D levels of < 30 µg/L should be regarded as vitamin D insufficiency based on the physiological response/elevation of PTH. However, as reviewed by Holick ⁷¹, most reports concur that serum 25(OH) D levels of < 20 µg/L should be regarded as vitamin D deficiency.

Bone mass measurement

The importance of the amount of mineral in the skeleton for bone strength was recognised increasingly during the 1960s ^{14,109}. In further biomechanical research, the ultimate compressive strength of vertebral bodies was found to be positively correlated with the BMC ⁶³. It was also indicated that the bone mineral level, measured in vivo, could be used as a criterion of fracture risk in elderly women ³⁴. For the analysis of bone, biopsies can be used to determine the

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histomorphometry, the exact bone mineral density and the microarchitecture of the bone. It is not realistic, however, in view of the prevalence of osteoporosis, to perform bone biopsies in every suspected case of this kind. Instead there has been an increasing call for indirect measurement techniques.

History

With the discovery of the radiographic technique by Wilhelm Conrad Röntgen in 1895, an opportunity to identify osteoporosis was introduced. During the planning of a Workshop on Bone Densitometry in 1959 a survey was made of the literature published at that time. The committee was “amazed at the volume of references and the number of techniques” that had been lost and then re-invented over the years. A bibliography of 125 items from 1935 till 1960 was presented in 1962⁴⁸. Some of these first attempts to measure bone density were performed using conventional radiograms and a visual comparison of the known density of specific phantoms.

The photon absorptiometry techniques improved bone densitometry with the invention of single-photon absorptiometry (SPA) by Cameron and Sorensen in 1963³² and Nilsson in 1966¹¹². This technique enabled the calculation of the amount of bone tissue; however, it was limited to a peripheral site only. It also required the subject’s arm to be placed in a water bath to provide a uniform path length through which gamma rays would pass.

In dual-photon absorptiometry (DPA), gamma rays of two different energies were used, making it possible to distinguish soft tissue from bone and axial sites as the spine and the hip could be measured and bone mass estimated.

In the late 1980s, the SPA and DPA techniques were superseded by X-ray techniques, first single X-ray absorptiometry (SXA) and subsequently dual-energy X-ray absorptiometry (DXA), which resulted in improved accuracy and precision and also reduced the radiation dose compared with the SPA and DPA methods³².

Dual-energy X-ray absorptiometry

DXA measures the transmission of X-rays with high- and low-energy photons through the body. The X-ray sources can be of the pencil beam, fan beam or narrow fan beam type, which has an impact on magnification and the speed with which a scan is performed. The technique is capable of measuring two different tissue components, bone and soft tissue, and it is assumed that the

Introduction

relationship between lean soft tissue and adipose tissue is constant. This may lead to measurement errors, with an impact on accuracy as well as precision^{20,43}. The DXA measurements reveal the areal bone mineral density (aBMD, g/cm^2) and the bone mineral content (BMC, g) at the site on the body that is being measured. Since DXA is a projectional technique, a three-dimensional object is described as two-dimensional and it is not possible to reveal a volumetric density in g/cm^3 . This affects the interpretation of DXA scans obtained from bones of different sizes, in growing children, for example¹³⁹. In adults a decrease in BMD measured using DXA is associated with an increased risk of fragility fractures⁷⁷; this risk is also age-dependent⁹³. Also in children, low bone mass has been shown to be associated with an increased fracture risk²⁸.

Dual-energy X-ray absorptiometry and laser

The dual-energy X-ray absorptiometry and laser (DXL) Calscan technique measures bone mass in the heel bone, using the principle of the DXA technique (fan beam) in combination with a laser measurement of total heel thickness. This combined measurement makes it possible to determine the fat-to-lean tissue ratio at the measurement site and the bone mass can be measured with greater accuracy⁷⁸. This technology therefore reduces the uncertainty related to the variable composition of soft tissue in adults⁶². In both elderly women and men, BMD impairment assessed using DXL is associated with a higher odds ratio for forearm fracture⁸ (Figure 7).

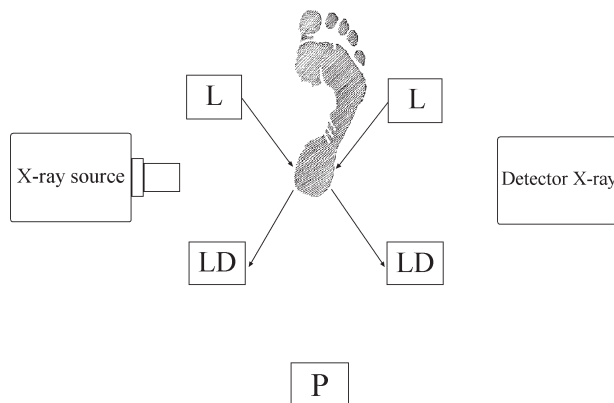


Figure 7 – Schematic diagram of the DXL Calscan method. L = Laser diode, LD = Laser Detector, P = Internal aluminium phantom for calibration.

Introduction

Quantitative computed tomography

To measure bone geometry and volumetric bone density with the ability to separately analyse cortical and trabecular bone, the method of choice would be quantitative computed tomography (QCT). Size-independent measurements are particularly useful in growing children. The major disadvantage of this technique, however, is the high radiation dose, which should be limited especially in children. The peripheral QCT (pQCT) technique gives a lower radiation dose and its usefulness has for example been shown in exercise intervention studies, where an increase in the cross-sectional area of cortical bone due to exercise could be demonstrated². So far, however, QCT has not been shown to be superior to DXA in predicting the risk of fragility fractures¹⁸.

Quantitative ultrasound

In the quantitative ultrasound (QUS) technique, broadband ultrasound attenuation (BUA, dB/MHZ) and the speed of sound (SOS, m/s) are used to reflect properties of bone related to density and architecture, respectively. The major advantages of this technique are that it is non-ionising and portable. In adults, QUS can predict fracture risk independent of bone mass determination¹⁰⁴. However, ultrasound values reflect not fully defined structural parameters and so far it has been difficult to use this information in children⁵².

MRI

Skeletal assessment using the magnetic resonance imaging (MRI) technique is based on varying amounts of water and lipids in different tissues, which enables the differentiation of various anatomic structures. The major advantage of this technique is that it provides volumetric measurements of bone without using ionising radiation¹⁵⁹. However, MRI has so far only been used in research and its applicability in clinical practice has still to be evaluated.

All available bone measurement techniques have their advantages and limitations. A summary is given in Table 2.

Introduction

Table 2 – Summary of different bone measurement techniques.

Technique	Site	Radiation dose (μSv)	Precision (CV%)	Advantages	Limitations
DXA	Lumbar spine	0.4-4	< 1	1. Rapid scan times 2. High precision 3. Availability of pediatric reference data 4. Low radiation dose 5. Can assess body composition	1. Size-dependent measurements 2. Integral measurement of trabecular and cortical bone 3. Sensitive to body composition changes
	Total body	0.02-5	1-2		
	Proximal femur	0.15-5.4	0.15-5.4		
DXL	Calcaneus	< 0.2 adult < 0.12 children	1.2 2.4-9.8 ages 2-7	1. See DXA 1-4 2. Portable equipment	1. See DXA 1-2 2. Only applicable to the heel bone
Axial QCT	Spine	3-D 55 2-D 50-60	0.8-1.5	1. Size-independent 2. Separate measure cortical + trabecular bone 3. Measures bone geometry, muscle and fat 4. Axial + peripheral sites	1. Relatively high radiation dose 2. Access to equipment can be problematic 3. Operation requires skilled staff 4. Long scan time
	Femur	3-D 10-20	< 1		
Peripheral QCT	Radius	<1.5-4 per scan	0.8-1.5	1. See Axial QCT 1-3 2. Low radiation dose	1. See 3-4 Axial QCT 2. Only applicable to peripheral sites
	Tibia		3.6-7.8 ages 3-5 1.3-1.8 age 12		
QUS	Calcaneus Radius	None	BUA 1.6-5 SOS 0.5-1.2	1. Nonionizing 2. Portable equipment	1. Relatively low precision 2. Sensitive to scan environment
MRI	Humerus Femur	None	0.12-1.02 0.55-3.63	1. Nonionizing 2. See 1-4 Axial QCT	1. Noisy 2. See 2-4 Axial QCT 3. Claustrophobia in some patients & parents cannot be in room with children
Radio-gram-metry	Meta-carpal	0.17	< 1	1. Retrospect. analysis 2. Low radiation dose 3. Widely available	1. Applicable to hand radiographs only 2. Cortical measurements only

Compiled from tables in “Bone Densitometry in Growing Patients, Guidelines for Clinical Practice” and references therein¹⁵⁹ and from Study I. **DXA** = dual-energy X-ray absorptiometry, **DXL** = dual-energy X-ray absorptiometry and laser, **QCT** = quantitative computed tomography, **QUS** = quantitative ultrasound, **BUA** = broadband ultrasonic attenuation, **SOS** = speed of sound, **MRI** = magnetic resonance imaging.

Introduction

Measuring children

The best technique for determining bone mass in growing children is the subject of debate. The interpretation of bone mineral measurements is more complex in children than in adults irrespective of the technique that is used, as children are continuously growing. Several bone measurement techniques are relatively time consuming and they require that the subject does not move for quite a long period of time, which may be difficult for many children. For paediatric patients with different disorders, this could be even more difficult or even impossible and in most cases sedation is regarded as an unnecessary medical risk. Moreover, children with physical deformities can frequently not be whole-body-scanned. The same thing applies to patients who have undergone surgical procedures with metallic implants, where screws or plates interfere with the measurement. As a result, some patients cannot be adequately studied using regular whole-body, spine or hip DXA techniques, QCT or MRI techniques.

A method that measures bone mass with high precision and that is easy, rapid and well tolerated by children and, in addition, gives a low absorbed radiation dose would be preferable. Our desire to create a device suitable for young children and children with disabilities, who would be complicated to measure in the whole-body DXA devices, led to the further development of the DXL Calscan for this purpose. This will be discussed in more detail in this thesis.

Duchenne and Becker muscular dystrophy

There are descriptions of Duchenne muscular dystrophy (DMD) as early as 1836 (Conte and Gioja)³⁷ and, in 1851, Meryon, a physician in London, gave a description of a family with four affected boys³⁸.

Guillaume Benjamin Duchenne de Boulogne (1806-1875), the father of the application of electricity to medicine and the inventor of a biopsy needle for muscle biopsies, described the disease in more detail during the 1860s. At that time, it was believed that the condition might have a cerebral origin because of frequently occurring intellectual impairment in the affected children. In 1879, Gowers illustrated how these boys often behave when getting up from the floor by pushing themselves up using their legs – the Gowers' manoeuvre (Figure 8).

DMD is a genetic disorder caused by a mutation in the dystrophin gene on the X-chromosome, and it is also the most common type of muscular dystrophy (MD) in childhood³⁷, with an incidence of 1:4,500 male births. Becker MD, is

Introduction

caused by a deletion in the same gene as DMD, but it has a milder clinical course and is far more uncommon, with an incidence of 1:30,000 male births¹¹⁰. Both disorders have a recessive inheritance, but not uncommonly they appear due to a new mutation located on the Xp21 dystrophin gene. This gene is one of the largest known genes and encodes for a correspondingly large protein, dystrophin, which is found in association with the sarcolemma in the skeletal muscle and it has been suggested that it plays an important part in stabilising the muscle cell membrane¹⁶⁶. In DMD, virtually no dystrophin is produced, which causes progressive muscle wasting with necrosis and degeneration of muscle fibres and the muscle tissue is gradually replaced by adipose and connective tissue. In Becker MD, a smaller, but yet partially functional dystrophin is maintained, which makes a significant contribution to the milder course of this disease. The diagnosis of DMD or Becker MD is based on typical clinical signs and is moreover confirmed by muscular biopsies, which demonstrate dystrophin abnormality and DNA analysis⁷⁶.

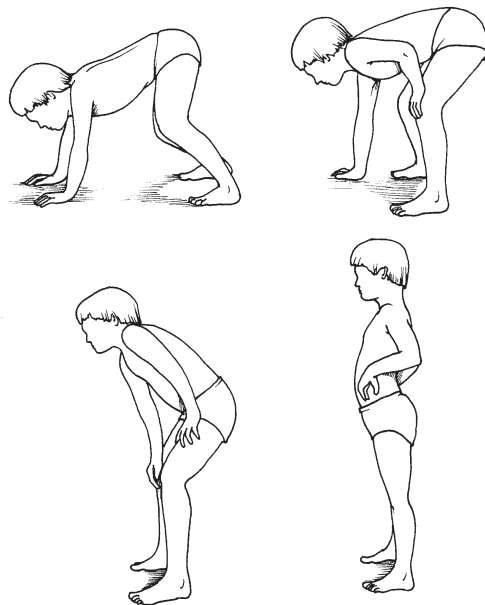


Figure 8 – Gowers' manoeuvre.

Introduction

Clinical features

A waddling gait is the most generally applied description. The early appearance of weakness of the gluteus medius and minimus muscles, makes it difficult for the child to support the weight of his body when raising one leg and hip extensor weakness leads to a forward tilt of the pelvis and a compensatory lumbar lordosis to maintain an upright posture. Standing on the toes makes it easier to maintain a vertical posture and toe-walking often occurs before any fixed shortening of the Achilles tendon³⁷ (Figure 9).

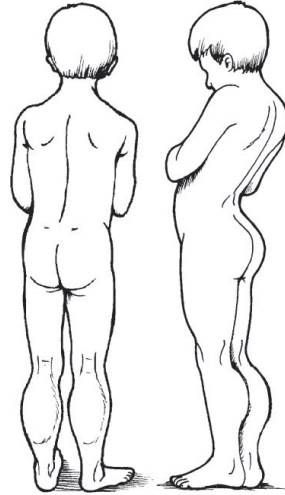


Figure 9 – Duchenne muscular dystrophy.

Characteristics:

- Onset at about 3 years of age for DMD and at about 11 years of age for Becker MD.
- Pain in the muscles, especially in the calves, often associated with exercise. Spasm in the calves is especially frequent in Becker MD. Toe-walking is a frequent habit in both DMD and Becker MD.
- Enlargement of the muscles, pseudohypertrophy, particularly the calves.
- Progressive symmetrical muscular weakness with increasing difficulty walking, climbing stairs and getting up from the floor (Gowers' manoeuvre, Figure 8).
- The age at which loss of independent walking occurs is between 7 and 13 years of age in DMD. Patients with Becker MD usually stay ambulant beyond the age of 16.
- When permanently in a wheel-chair, contractures and spinal deformities rapidly develop and respiratory problems occur in the later stages.
- Cardiac involvement is common. The skeletal muscle and the myocardium are primarily affected in these progressive disorders.
- Since dystrophin is also expressed in the brain, DMD and Becker MD are also associated with cognitive symptoms.

Introduction

Treatment

Gene-based therapies are currently being developed and hold great promise¹⁵², but as yet there is no cure for DMD or Becker MD. Glucocorticosteroids (GC) can, however, slow the progression and there is evidence from randomised controlled studies that GC therapy improves muscle strength and motor function in the short-term (six months to two years) in DMD⁹⁷. There are also indications that GC in a longer perspective (eight years) contribute to improved cardiac function, lesser scoliosis and a slower decline in vital capacity⁷². To maintain physical performance, physiotherapy is important at an early stage with stretching programmes and night splints to prevent ankle joint contractures⁷⁴. Whether strength training is beneficial or harmful remains controversial¹¹⁷. Endurance exercise with low resistance and high repetition has been shown to be more beneficial⁶⁵.

Glucocorticoid therapy and bone

In 1932, Harvey Cushing (1869-1939) described a syndrome caused by the excessive production of adrenal gland hormones, e.g. cortisol. Different symptoms could be observed, one of which was a marked osteoporosis of the skeleton. Cortisol/GC increase the glucose level in the blood and the metabolism of fat and protein is also influenced. Moreover, GC have an anti-inflammatory effect, which make them useful in the treatment of several diseases, such as asthma, rheumatoid arthritis and DMD. Pharmacological doses of oral GC suppress bone formation, due to reduced IGF-I expression in the osteoblasts¹⁴⁸ and increased osteoblastic apoptosis. GC also increase bone resorption, due to increased osteoclastogenesis²³. GC induce bone loss, which becomes evident after six to 12 months of chronic use⁹²; however, the effect of GC on the bone is dose and time dependent^{36,84,155}. It has been reported that children treated with oral GC have lower bone mass^{30,36,84}. The extent to which GC contribute to increased fracture risk in children is, however, unclear¹⁵⁴.

Duchenne muscular dystrophy and bone

In agreement with the mechanostat theory, which suggests that bone adaptation is driven primarily by changes in mechanical load⁴⁷, conditions that reduce mobility during childhood would be associated with a lower PBM compared with the situation if physical activity were at a normal level. Accordingly several authors have reported reduced BMD or BMC in children with disabilities^{3,10,68,120}. Moreover, low BMD has previously been reported in DMD

Introduction

patients^{5,15,88} and both Aparicio and co-workers⁵ and Larson and Henderson⁸⁸ demonstrated that DMD patients lose bone mass in the proximal femur while they still are ambulant.

It has recently been demonstrated that low BMD is associated with an increased risk of fragility fractures in children²⁸. For DMD patients it is most important not to sustain fractures, because this may result in the permanent loss of ambulation as a clinical consequence¹⁵⁶. In the study by Larson and Henderson⁸⁸, 44% of the DMD patients sustained a fracture and the majority of fractures were located in the lower extremities. Other previous reports demonstrate an increased fracture frequency in DMD patients^{100,156}. However, McDonald and co-workers¹⁰⁰ found that the question of fracture rate was still unanswered due to the diversity of data. Whether or not GC therapy contributes to fracture rate in children in general and in DMD patients in particular is also the subject of debate. Schara and co-workers were unable to find any differences in fracture rate between 13 DMD patients treated with deflazacort and 13 without steroid treatment¹³⁷. In a larger retrospective chart review (n=143) in 2007, King and co-workers found that patients with DMD on long-term GC treatment displayed a significantly reduced risk of scoliosis and an extended time of more than three years of independent ambulation. However, at the same time, they ran an increased risk of vertebral and lower limb fractures compared with steroid-naïve patients⁸⁵.

Aims of the thesis

General aims

To evaluate the DXL method for bone densitometry measurements in children, to apply the DXL technique to patients with DMD and further to make a survey of the bone health of DMD patients.

Specific aims

- Study I To evaluate the precision and utility of the DXL Calscan device in a paediatric environment, and to provide reference data for healthy two-, four- and seven- year-old children for BMD, BMC and BMAD.
- Study II To evaluate the relationship between conventional DXA measurements and DXL measurements in a young population and to explore the diagnostic capacity of the DXL Calscan device.
- Study III To investigate parameters for bone mass at different sites, biochemical markers of bone turnover and key regulators of bone mass in patients with DMD in comparison with healthy age- and gender-matched controls.
- Study IV To investigate and compare the longitudinal development of bone mass during a four-year period in patients with DMD and Becker MD. In addition, to investigate the impact of muscle strength and motor function on bone mass in these patients.

Subjects

Subjects

Study I

A total of 334 (172 girls/162 boys) Swedish children aged two (n=117, 60 girls/57 boys), four (n=110, 56 girls/54 boys) and seven (n=107, 54 girls/53 boys) were recruited in a random manner from schools and child health care units covering different socio-economic regions within and close to the city of Göteborg (approximately 500 000 inhabitants) in western Sweden. Only healthy, prepubertal children, born after 35 weeks of gestation and with no history of chronic disease or any medication, were included in the study. Sixteen children were excluded due to movement during the scanning procedure or because they did not meet the inclusion criteria. The term “two years of age” was defined as children aged between 2.0 and 3.0 years, “four years of age” as children aged between 4.0 and 5.0 years, and “seven years of age” as children aged between 7.0 and 8.0 years.

A few patients, one of each with the following diagnoses: Rett syndrome, osteogenesis imperfecta, cerebral palsy, Duchenne muscular dystrophy (DMD) and myelomeningocele, were also scanned in order to highlight the opportunity to use the DXL method in patient groups with disorders considered to affect the skeleton.

From Study I, at least 30% of the children in each age group were followed annually for another two years (Table 3 and Figure 10). These additional DXL measurement data (a total of 645 DXL Calscan measurements (328 girls/317 boys)) provided an opportunity to produce reference curves (mean \pm 2 SD) for calcaneal BMD for children aged two to 10 years, for girls and boys respectively.

Table 3 – Number of subjects in the DXL follow-up study for reference curves.

Age (years)	2	3	4	5	6	7	8	9
Subjects (n)	117	62	151	58	39	107	67	44
Girls/Boys (n)	60/57	34/28	77/74	29/29	20/19	54/53	32/35	22/22

Subjects

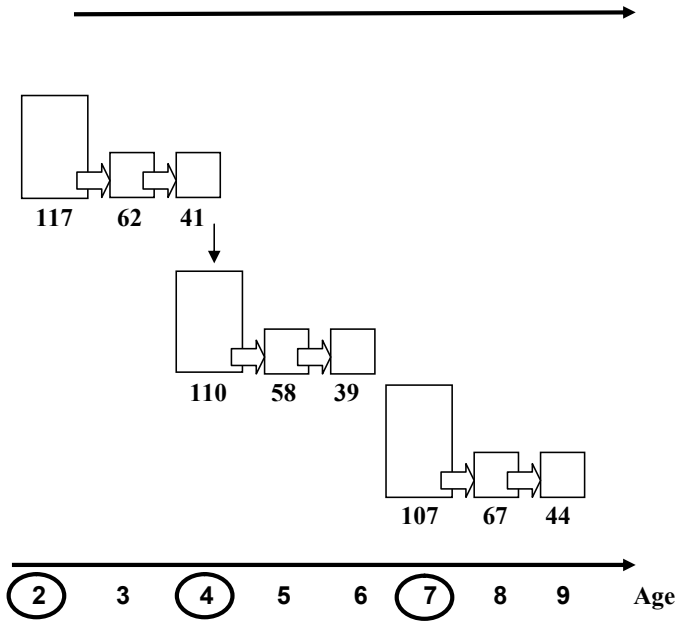


Figure 10 – Number of children evaluated in Study I (ages within circles). The number of children (ages not within circles) who provided the additional DXL measurement data for the reference curves presented in Figure 18.

Study II

The total number of measurement sets in this study was 128. The study group consisted of 112 individuals (38 girls/74 boys), aged 2.2-20.6 years. The patients were recruited from the Queen Silvia Children's Hospital, Göteborg, Sweden.

Subjects

The healthy individuals were known by the patients' families or by the hospital staff.

- 31 patients with DMD or Becker MD (all boys) (Figure 11)
- 34 patients with chronic juvenile idiopathic arthritis (28 girls/6 boys)
- 16 patients with kidney diseases (10 girls/6 boys)
- 31 healthy individuals (all boys)

The patients with kidney diseases participated twice, with a one-year interval between the measurements.

Study III

In this cross-sectional study, the patient group comprised 24 boys, 2.3-19.7 years of age (mean $11.9 \pm SD 5.2$), all with immunohistochemically verified DMD (Figure 11).

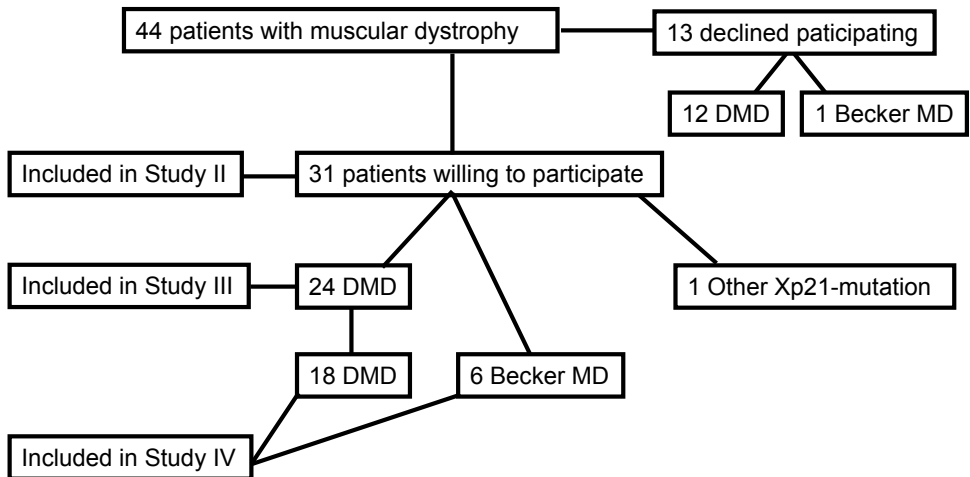


Figure 11 – DMD and Becker MD patients in Studies II-IV.

Subjects

The control group comprised 24 age-matched boys, 2.7-19.6 years of age (mean $11.8 \pm \text{SD } 5.1$), who were recruited randomly from healthy boys known by the patients' families or known by the hospital staff. Each DMD patient was matched as closely as possible with a control with respect to age (± 6.7 months).

Glucocorticoid (GC) treatment (Prednisolone 0.22 and 0.35 mg/kg/day):

Prior to this study (3.5-10.5 years before inclusion)

- 4 patients, treated for 1.3-3.2 years

At the time of inclusion

- 16 patients, treated for 0.1-11.5 years
- 4 patients, never treated with GC

Study IV

From Study III (baseline study), it was possible to include 18 patients with DMD (mean age $10.7 \pm \text{SD } 4.6$ (2.3–19.5)) for a follow-up study (mean age $14.6 \pm \text{SD } 4.7$ (6.6–23.5)) (Figure 11). At baseline, six patients with Becker MD (mean age $13.9 \pm \text{SD } 2.9$ (10.8–18.9)) were also included in this longitudinal study and they all also participated at follow-up (mean age $18.0 \pm \text{SD } 3.0$ (15.2–23.1)) (Figure 11). The study was performed between December 2003 and June 2008 and the mean follow-up time was 4.0 years for both the DMD and the Becker MD groups with a range of 3.5-4.5 years. For most of the statistical analyses, the DMD group (DMD_{tot}) was divided into two groups due to age differences between DMD and Becker MD patients; i.e. one DMD group < 10 years of age (DMD_{<10yr}), n=8 age at baseline mean $6.6 \pm \text{SD } 2.5$ and at follow-up mean $10.4 \pm \text{SD } 2.4$, and one DMD group > 10 years of age (DMD_{>10yr}), n=10, age at baseline mean $13.9 \pm \text{SD } 3.0$ and at follow-up mean $18.0 \pm \text{SD } 3.0$.

The diagnoses were immunohistochemically verified. From the baseline DMD patient group, two patients had died, one had moved to another part of Sweden and could not be reached for follow-up measurements, one patient was excluded because he had started treatment with bisphosphonates and two patients declined to participate in the follow-up study.

Subjects

Glucocorticoid (GC) treatment (Prednisolone 0.22 and 0.35 mg/kg/day):

DMD group

Prior to baseline (3.5-10.5 years before inclusion):

- 3 patients treated for 1.3-2.5 years

At baseline inclusion:

- 13 of the DMD patients, treated for 0.1-11 years
- 2 patients, never treated with GC

At follow-up inclusion:

- 12 of the 13 DMD patients, still treated (duration 4.2-15 years)
- 1 patient, treatment withdrawn after 4.5 years
- 2 newly diagnosed patients, treated for 3.3 years

Becker MD group

At baseline inclusion:

- 2 patients treated for 8 years
- 4 patients, never treated with GC

At follow-up inclusion:

- 2 patients still receiving treatment (duration 12 years).
- 1 patient treated for one year between baseline (initiated one year after) and follow-up.
- 3 patients, never treated with GC

Ethics

Ethics

All the studies were approved by the Human Ethics Committee at the Medical Faculty, Sahlgrenska Academy, at the University of Gothenburg.

Methods

Methods

Clinical assessments

In all the studies, height was measured using a wall-mounted stadiometer, weight was measured on analogue scales, and the left foot length was measured with a foot ruler. In Studies III and IV, it was not possible to measure standing height in some patients. In these patients the arm span, an accepted method for height assessment in disabled individuals, was measured ⁷⁰.

In all the studies, a questionnaire relating to general health and earlier fractures was designed for the participating patients and their parents to complete at inclusion and, in Study IV, a follow-up questionnaire was completed. In Studies III and IV, information on previous fractures was also collected from clinical files and spinal radiographs performed in some patients. In Study III, dietary records were kept for four days. Pubertal staging was performed according to the Tanner scale (Studies III and IV) ¹⁴⁹ and by a self-assessment of testis size made by the participants or by a parent (Study III) ¹¹⁸.

Bone mass measurements

Initially, a thorough survey was performed to adapt the DXL Calscan device for use in children. The tube current was lowered and the software was modified, so that the X-ray tube was operated at 68 and 35 kVp and the effective dose to the patient was less than 0.12 μ Sv. Moreover, after checking how the laser beam hit the heel, a foot support (17 x 11 x 2 cm) was constructed to position the foot correctly for scanning when the foot was smaller than 21.1 cm (French shoe size 35) (Figure 12).



Figure 12 – Foot and foot support in the DXL Calscan.

Methods

In Study I, both the right and the left foot were initially measured in 16 children of different ages (between 2 and 7 years of age) and no significant difference was found between the right and left foot (BMD, $p=0.33$; BMC, $p=0.61$, with 95% limits of agreement ranging from -0.083 to 0.063 g/cm^2 and -0.049 to 0.043 g/cm^2 respectively). Thereafter, only the left foot was scanned in all studies (Study I-IV) (Figure 13).

The region of interest (ROI), with a fixed area of 0.74 cm^2 , was placed in the trabecular portion guided by the two processes of the dorsal part of the calcaneus. The ROI was chosen to fit the relatively small calcaneal bone of the two-year-old children while being large enough to cover the dorsal portion of the calcaneal bone of the older children. The height of the calcaneus was measured with a soft ware ruler. In an attempt to calculate a value for BMAD (g/cm^3), the areal BMD (g/cm^2) value was divided by the height (cm) of the calcaneal bone at the location of the ROI (Figure 14).

$\text{BMAD} (\text{g/cm}^3) = \text{BMD} (\text{g/cm}^2) / \text{calcaneal height} (\text{cm})$. In this thesis, BMAD is expressed in mg/cm^3 in order more easily to distinguish BMAD from BMD in tables and figures.

In Studies II-IV, in addition to DXL measurements of the calcaneus, DXA (GE Lunar Prodigy, Madison, WI, USA) measurements of the total body (TB) were performed, as well as the $\text{TB}_{\text{Head Excluded (HE)}}$, the spine, the hip and the forearm. In these studies (II-IV), each individual was scanned using the two techniques (axial DXA and DXL Calscan) during the course of one day.



Figure 13 – DXL Calscan measurement.

Methods

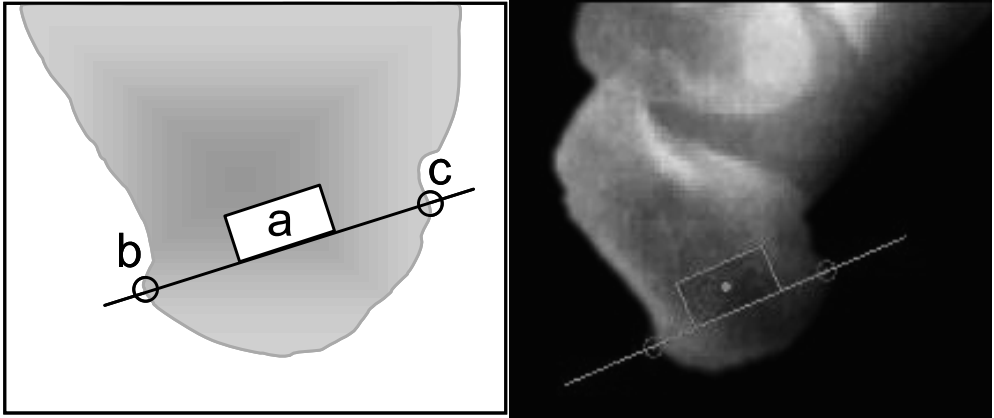


Figure 14 – The DXL scan of the calcaneus. The region of interest (a) is located in the dorsal third of the calcaneus. The calcaneal height (cm) is measured between the two processus tuberis calcanei (b and c).

Precision studies: The consistency of the DXL Calscan device was checked by measurements of hydroxyapatite in different concentrations incorporated in a solid, water-based, human-like phantom (Computerized Imaging Reference Systems, Inc., USA) by repeated measurements. The study duration was 26 months. The in-vitro precision of the device was determined from these phantom measurements according to the manufacturer's instructions. In-vivo precision was assessed by measurements on 23-27 children in each age group (2, 4, and 7 years) and the children were scanned twice in the DXL Calscan device by the same person and on the same day in order to determine the errors arising from both the repositioning of the foot and the selection of the ROI (Study I).

For the whole-body DXA measurements, twenty healthy persons (aged 6-37) were scanned twice in order to assess the in-vivo precision of the DXA Lunar Prodigy device (Study III). The in-vitro precision of the DXA Lunar Prodigy device was determined from phantom measurements according to the manufacturer's instructions.

Methods

Motor function and isometric muscle strength in the lower extremities

Motor function and muscle strength tests were performed in the patient groups in Studies III and IV. The classification of motor function was made according to the Vignos scale (grades 1-9) (Table 4) ¹⁵⁷. This grading basically reflects the strength of the lower extremities, trunk and pelvic musculature, represented by activities of ambulation and elevation.

Table 4 – Vignos scale.

Grade	Functional level
1	Walks unassisted and climbs stairs without assistance
2	Walks unassisted and climbs stairs with aid of railing
3	Walks unassisted and climbs stairs slowly with aid of railing (>25 seconds for eight standard steps)
4	Walks unassisted and rises from chair but cannot climb stairs
5	Walks unassisted but cannot rise from chair or climb stairs
6	Walks only with assistance or walks independently with long leg braces
7	Walks in long leg braces but requires assistance for balance
8	Stands in long leg braces but unable to walk even with assistance
9	Is in a wheelchair

Isometric muscle strength was measured using an electronic hand-held myometer. Abductors, extensors and flexors in the hip, extensors and flexors in the knee, ankle dorsiflexors, shoulder abductors and wrist extensors were investigated. In Studies III and IV, values from the knee extensor measurements (Figure 15) were used for further analysis. An isometric contraction of at least two to three seconds was required and the peak force in Newtons was recorded. The best of three values obtained on the non-dominant side was used for further analysis according to Scott and co-workers ¹⁴². In order to determine muscle strength in the knee extensors, regardless of the age of the patients, quotients were calculated between values obtained from the patients and reference values collected from healthy individuals of corresponding ages ^{11,12}.

Methods



Figure 15 – Myometry of the knee extensor strength.

Biochemical markers of bone metabolism

Blood samples were taken for analyses in Studies III and IV. Calcium, phosphate and total alkaline phosphatase (ALP) were determined by standardised and certified procedures. In Study III, bio-intact parathyroid hormone (PTH), i.e. the biologically active whole PTH molecule 1-84, was measured with an automated immunochemiluminometric assay using a Nichols Advantage® (Nichols Institute, San Clemente, CA, USA) ⁷⁵. At follow-up in Study IV, due to the discontinuation of the assay, serum intact PTH was analysed using an electrochemiluminescence immunoassay ¹³⁴.

Serum vitamin D 1,25(OH)₂ D and 25(OH) D were determined by ¹²⁵I RIAs (DiaSorin, Stillwater, MN, USA). Insulin-like growth factor-I (IGF-I) and testosterone were measured in the DMD group. An IGFBP-blocked RIA with a large excess of IGF-II was used to determine serum-IGF-I (Mediagnost GmbH, Tübingen, Germany) ¹⁹. Testosterone was determined by RIA (Spectra Testosterone RIA Kit, Orion Diagnostica, Espoo, Finland). Serum leptin concentrations were determined by RIA (Human Leptin RIA Kit, Linco Research, St Charles, MO, USA).

Methods

Markers of bone formation

Bone-specific alkaline phosphatase (BALP) activity was determined by an enzyme-linked immunosorbent assay (ELISA), (Quidel Corp., CA, USA) ⁵³. Serum osteocalcin (OC) was determined by a radioimmunoassay (RIA), OSTK-PR™ (CIS Bio International, Gif-sur-Yvette, Cedex, France) ⁹⁹. Serum type I procollagen intact amino-terminal propeptide (PINP) was determined by RIA (Orion Diagnostica, Oulunsalo, Finland) ¹⁰³.

Markers of bone resorption

Serum carboxy-terminal telopeptide of type I collagen (ICTP) was determined in serum using an ELISA (Orion Diagnostica, Oulunsalo, Finland). Type I collagen degradation was also assessed by the serum CrossLaps™ ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), which is reported to measure a cathepsin K degradation product of trivalently cross-linked type I collagen (CTX) ¹³³. The serum osteoclast-derived, tartrate-resistant, acid phosphatase isoform 5b (TRACP-5b) was determined by a solid-phase immunofixed enzyme activity assay (SBA Sciences, Oulu, Finland). A resorption quotient was calculated by dividing the values of CTX by TRACP-5b in order to illustrate the osteoclast activity in relation to the number of osteoclasts, i.e. CTX/TRACP-5b ¹²⁵. Paediatric age- and gender-specific reference intervals are reported for bioactive PTH ¹⁴⁷, intact PTH ²⁷, BALP and CTX ¹²⁴, testosterone ⁴, and IGF-I ⁹⁴.

Methods

Statistical methods

Continuous variables are presented as the mean \pm SD, mean \pm SEM, minimum, maximum and median values. Categorical variables are presented as n and percentages. The variation within individuals is given as intra-individual SD and CV and within operators as inter-operator SD and CV, together with limits of agreement. All the tests were two-tailed and conducted at a 5% significance level.

Study I

Fisher's non-parametric permutation test was used for comparisons between two groups. Wilcoxon's signed rank test was used for the analysis of inter-operator differences. Pitman's non-parametric permutation test was used for all correlation analyses. In addition, Pearson's correlation coefficient was calculated for descriptive purposes.

Tolerance intervals for heel BMD were calculated as a function of age and height respectively. The distribution of heel BMD as a function of heel BMD was not normally distributed. The ages were transformed to a normal distribution using the inverse of the normal cumulative density function as a function of the empirical cumulative density function for heel BMD.

A piecewise linear regression was estimated using the maximum likelihood principle, with transformed heel BMD values as a dependent variable and heel BMD, with a breakpoint at 0.2, as an independent variable. The SD of the residuals was estimated as a piecewise linear function of heel BMD with a breakpoint at 0.3. Given the estimated means and SD, it was possible to calculate the 95% tolerance intervals for the transformed heel BMD values.

Ninety-five per cent tolerance intervals could be directly given for the actual heel BMD values using the inverse function for normal transformation, described above. The mean for the actual heel BMD values at each heel BMD was calculated by taking the integral of 1-cumulative density function ($F(x)$).

Methods

Study II

For correlation analysis, Pearson's correlation coefficient was used in the article and Pitman's non-parametric permutation test was used in the summary chapter of a compilation dissertation. Wilcoxon's signed rank test was used for the analysis of inter-operator differences. In order to analyse whether there were differences in these correlations during early childhood compared with during adolescence, an analysis was also performed with the study group divided into two groups; one group consisting of children younger than 10 years of age ($n=32$) and one group consisting of children 10 years of age and older ($n=96$). Receiver operating characteristic (ROC) curves were produced with logistic regression to evaluate c-statistics (Area Under the ROC Curve (AUC)), the sensitivity and the specificity of the heel DXL measurements and to predict the lowest TB DXA quartile. Tolerance intervals for TB, TB_{HE} , spine and hip BMD respectively as a function of heel BMD were calculated and are described below for TB BMD. The distribution of TB BMD as a function of heel BMD was not normally distributed. The TB BMD values were transformed to a normal distribution using the inverse of the normal cumulative density function as a function of the empirical cumulative density function for TB BMD.

A piecewise linear regression was estimated using the maximum likelihood principle, with transformed TB BMD values as a dependent variable and heel BMD, with a breakpoint at 0.2, as an independent variable. The SD of the residuals was estimated as a piecewise linear function of heel BMD with a breakpoint at 0.3. Given the estimated means and SD, it was possible to calculate the 95% tolerance intervals for the transformed TB BMD values.

Ninety-five per cent tolerance intervals could be directly given for the actual TB BMD values using the inverse function for normal transformation, described above. The mean for the actual TB BMD values at each heel BMD was calculated by taking the integral of 1-cumulative density function ($F(x)$).

Methods

Study III

Tests between groups regarding continuous variables and number of fractures were performed using Wilcoxon's signed rank test in order to preserve the matched-pair design. For comparisons with reported reference values within the patient group Wilcoxon's signed rank test was used. Correlation analyses were performed with Spearman's non-parametric rank correlation.

Study IV

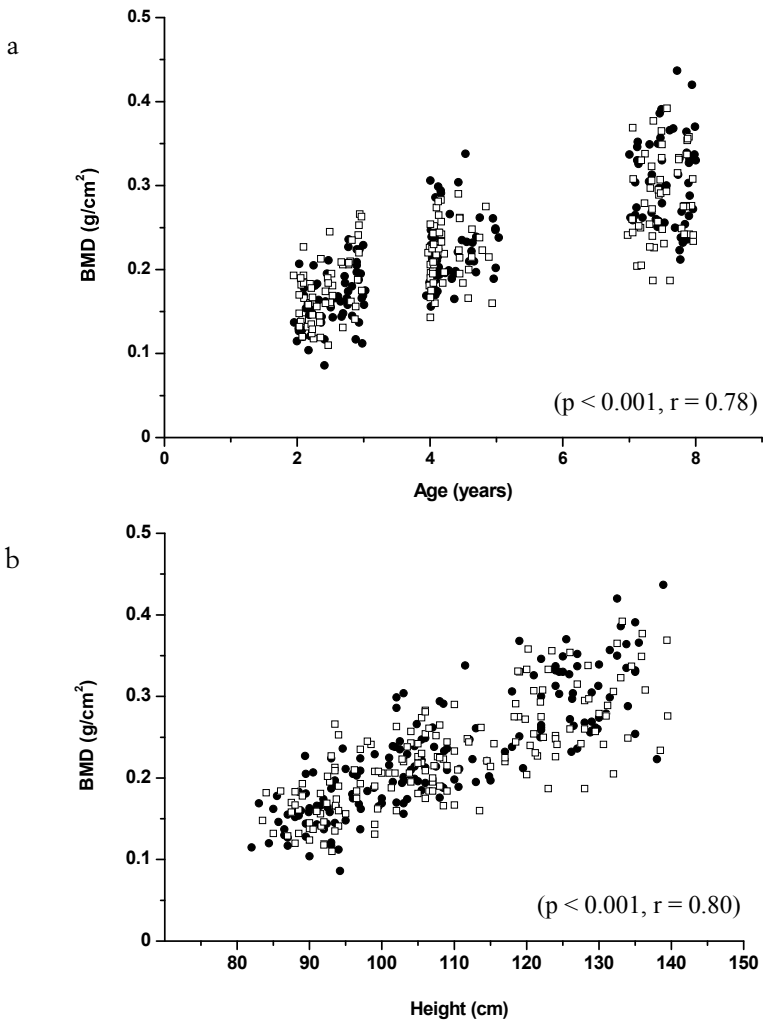
For comparisons between groups, Fisher's non-parametric permutation test was used for continuous variables. For comparisons of change in continuous variables over time within groups, Wilcoxon's signed rank test was used. Correlation analyses were performed with Spearman's rank correlation.

Results

Results

DXL evaluation and paediatric reference data (Study I)

All in all, 334 DXL heel bone measurements on young children aged two, four and seven were included in this cross-sectional study. Analyses revealed positive correlations between BMD values and age ($p < 0.001$, $r = 0.78$), height ($p < 0.001$, $r = 0.80$) and weight ($p < 0.001$, $r = 0.76$) (Figure 16 a-c); all factors that reflect an increase in size.



Results

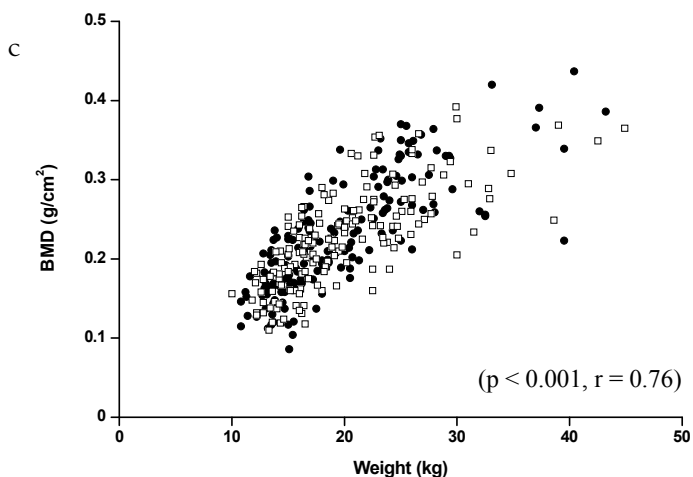


Figure 16 a-c – Scatter plots of BMD versus age (a), height (b) and weight (c) in children aged 2, 4 and 7 years (● girls, □ boys). Significant correlations as indicated in the figures.

Moreover BMAD values, calculated in this study in an attempt to adjust for different heel bone sizes, correlated significantly with age ($p < 0.001, r = 0.23$) (Figure 17), height ($p < 0.001, r = 0.22$) and weight ($p < 0.001, r = 0.22$).

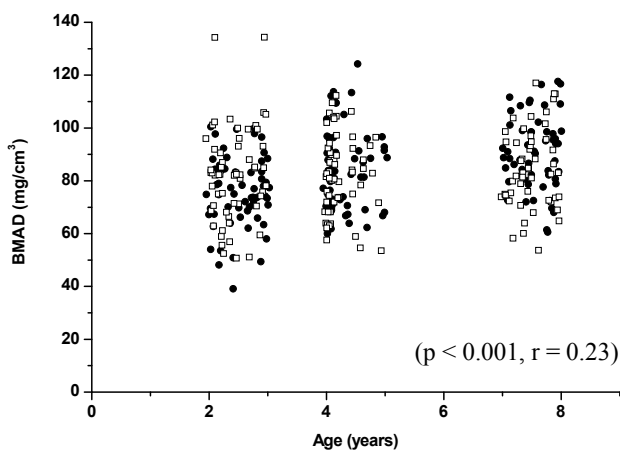


Figure 17 – Scatter plot of BMAD versus age in children aged 2, 4 and 7 years (● girls, □ boys). Significant correlation as indicated in the figure.

Results

The collected data are presented as percentile values for BMC, BMD and BMAD for each age group in Paper I, Table 2. Comparisons between boys and girls revealed no significant differences in BMD or BMC in the two- and four-year-old children; however, in the group of seven-year-old children, girls had significantly higher BMD and BMC than boys ($p=0.02$). To explore the utility of the DXL Calscan device, a few patients with disorders considered to involve the skeleton were scanned. The measurements were easily performed and all these patients had low values for BMD, BMC and BMAD compared with the reference values. Reference curves (mean \pm 2 SD), based on a total of 645 (328 girls/317 boys) DXL Calscan measurements, were produced for calcaneal BMD according to age and height in girls and boys aged two to 10 years (Figure 18).

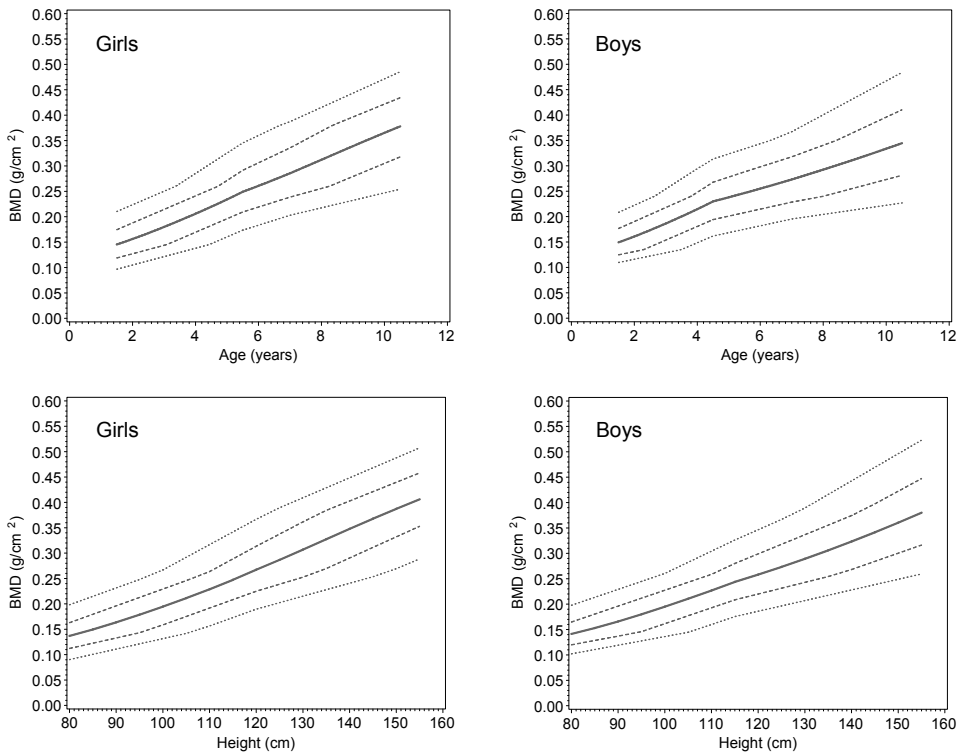


Figure 18 – Reference curves (mean \pm 2 SD) for calcaneal BMD according to age and height in two to 10 years old girls ($n = 328$) and boys ($n = 317$).

Results

Precision studies (Studies I and III)

DXL in-vitro precision

In Study I, the consistency of the DXL Calscan device was checked during the study by repeated measurements of an anthropometrical heel bone phantom. The CV (arithmetical mean) from these measurements was 1.1%.

DXL in-vivo precision

In-vivo precision values for the DXL Calscan measurements were assessed for each age group respectively. The intra-individual CV and the intra-individual SD (IISD) for BMD, BMC, calcaneal height and BMAD for children aged two, four and seven years are shown in Table 5.

Table 5 – In-vivo precision values for the DXL Calscan measurements. Coefficient of variation % (intra-individual SD).

	2 yr (n=23)	4 yr (n=26)	7 yr (n=27)
BMD (g/cm ²)	9.8% (0.016)	6.5% (0.015)	2.4% (0.007)
BMC (g)	10.1% (0.013)	8.1% (0.014)	2.6% (0.006)
Calcaneal height (mm)	4.0% (0.84)	1.8% (0.48)	3.7% (1.28)
BMAD (mg/cm ³)	10.7% (0.8)	6.2% (0.5)	3.7% (0.3)

The CV% decreased with increasing age, but, considering the BMD and BMC measurements, the IISD remained at the same level in the two- and four-year-old children and a smaller decrease was noticed in the seven-year-old children compared with the younger children. In ten children (aged 2-7 years) measured by two different operators, there was no significant mean difference (0.00) (SD=0.02, p=0.67) between the BMD values. The IISD was 0.013 with 95% limits of agreement ranging from -0.04 to 0.04 and the inter-operator CV for BMD was 5.3%. The intra-operator CV in heel height measurements that was utilised in volumetric evaluation of BMAD was 1.1%.

Results

DXA in-vivo precision

In Study III, the in-vivo precision for whole-body DXA was assessed through measurements of twenty healthy persons (aged 6-37) who were scanned twice on the same day. The CVs from these measurements were 0.6% for TB BMD (IISD 0.007), 0.9% for spine (L1-L4) BMD (IISD 0.01), 1.3% for right hip BMD (IISD 0.01) and 1.6% for left forearm BMD (IISD 0.01). The coefficients of variation for body fat mass and lean mass were 3.4% and 1.2% respectively (IISD 484 g and 520 g respectively).

The relationship between calcaneal DXL and whole-body DXA measurements (Study II)

Study II aimed to investigate the agreement between measurements performed using the axial DXA technique and the DXL Calscan technique. Conventional DXA measurements of TB, TB_{HE}, spine and hip BMD in the total study group were significantly correlated with measurements of calcaneal BMD using DXL at all sites measured. Correlation coefficients are shown in (Table 6).

Table 6 – Pearson's pair-wise correlation coefficients for BMD measured at different sites. All correlations are significant ($p < 0.001$).

	Heel BMAD	TB BMD	TB _(HE) BMD	Spine BMD	Hip BMD
Heel BMD	0.91 n=128	0.73 n=124	0.83 n=93	0.72 n=124	0.90 n=81
Heel BMAD		0.45 n=124	0.62 n=93	0.47 n=124	0.76 n=81
TB BMD			0.96 n=93	0.95 n=124	0.85 n=81
TB _(HE) BMD				0.92 n=93	0.93 n=81
Spine BMD					0.85 n=81

Results

Likewise, significant correlations ($p < 0.001$) were also found, when the study group was divided into children younger than 10 years of age and children 10 years of age and older (Figure 19).

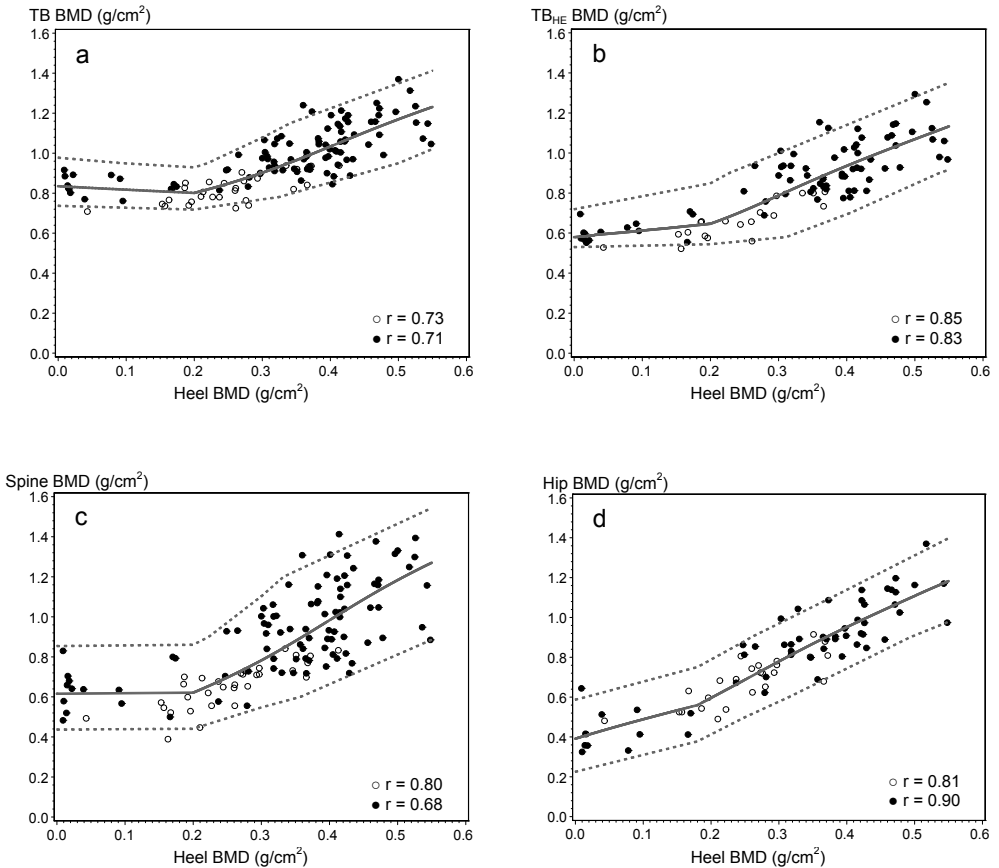


Figure 19 – Non-linear relationship between BMD values assessed from calcaneal DXL measurements and DXA measurements of: (a) the total body (TB) ($n=124$), (b) the TB head excluded (TB_{HE}) ($n=93$), (c) the spine ($n=124$) and (d) the left hip ($n = 81$) and 95% tolerance intervals (\circ children <10 yrs, and \bullet children >10 yrs), significant ($p < 0.001$) r -values for each group are presented in the figures.

Results

Receiver operating characteristic curves (ROC) analyses revealed that both the sensitivity levels (0.9-1.0) and the specificity levels (0.86-0.95) were high (Table 7 and Figure 20), which implies that the lowest 25th percentile of the DXA measurements could be well classified by the heel DXL measurements.

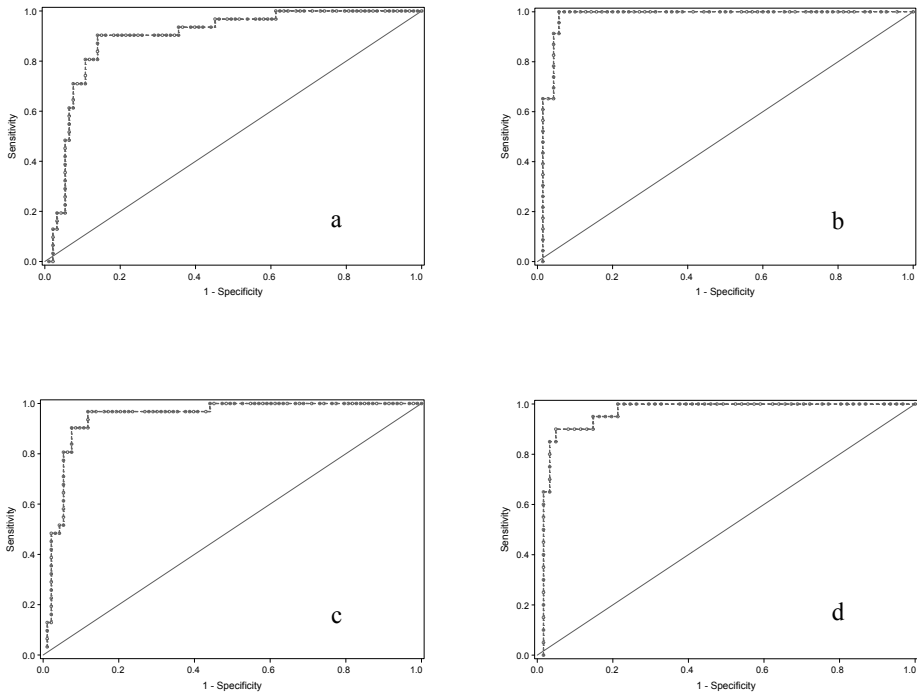


Figure 20 – ROC curves for predicting the 25th lowest percentile of BMD values measured using DXA in (a) the total body (TB), (b) the TB head excluded (TB_{HE}), (c) the spine and (d) the left hip with calcaneal BMD measured using DXL.

Results

Tabell 7 – ROC Analysis Study II. AUC = Area Under the ROC Curve

Left heel for prediction of BMD values below the 25 th percentile at:	Cut off Heel BMD	AUC	Sensitivity	Specificity	BMD value for 25 th percentile
TB	0.28	0.90	0.90	0.86	0.85
TB _(HE)	0.26	0.98	1.00	0.94	0.69
Spine	0.26	0.95	0.90	0.92	0.70
Hip	0.22	0.96	0.90	0.95	0.63

Duchenne and Becker muscular dystrophy studies (Studies III and IV)

In the cross-sectional study (Study III), 24 patients with Duchenne muscular dystrophy (DMD) (2.3-19.7 years) were compared with 24 age- and gender-matched healthy controls from a wide perspective with reference to bone health, and with particular emphasis on BMD measured at different sites, bone turnover, and calciotropic hormones.

In the four-year longitudinal study (Study IV), 18 patients with DMD from Study III (2.3-19.7 years at baseline) and six patients with Becker muscular dystrophy (MD) (10.8-18.9 years at baseline) were followed with respect to bone mass, body composition, motor function and muscle strength. Since there were obvious age differences between the groups, the DMD group was divided into two groups and comparisons between DMD and Becker MD patients above 10 years of age could be performed.

Characteristics (Studies III and IV)

To summarise growth data from these studies (Studies III and IV), there were no significant differences between DMD patients (aged 11.9 ± 5.2 (2.3 - 19.7)) and healthy age-matched controls (aged 11.8 ± 5.1 (2.7-19.6)) in terms of weight and height at birth or at one year of age. With increasing age, however, the DMD patients were found to be significantly shorter ($p < 0.001$) and lighter ($p < 0.001$) compared with healthy controls. The DMD_{>10yr} patients were also shorter ($p < 0.01$) and lighter ($p < 0.05$) compared with the Becker MD patients at follow-up.

Results

DXL and DXA measurements (Studies III and IV)

Despite growth during childhood and adolescence, BMD values obtained from the hip and the heel decreased with age in the DMD group. The values assessed from the spine, TB, TB_{HE} and forearm appeared to remain at a similar level with increasing age (Study III). These findings were confirmed for the hip and the heel BMD in the four-year longitudinal study (Study IV). The decrease was, however, only significant for hip BMD values in DMD patients above 10 years of age (DMD_{>10yr}). In the healthy control group, increasing BMD values with age were demonstrated at all the sites that were measured (Figure 21).

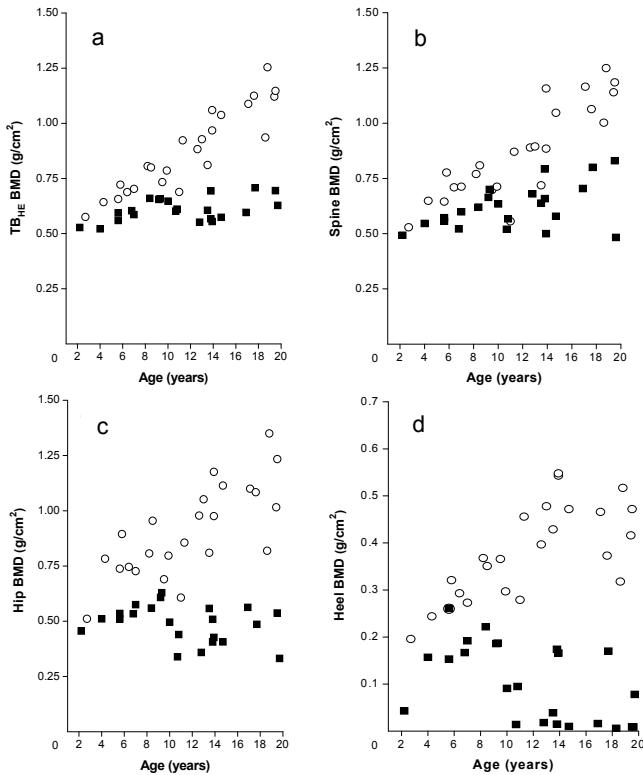


Figure 21 – BMD for different sites; (a) the TB head excluded (TB_{HE}), (b) the spine (c), the right hip and (d) the left heel, in relation to age. DMD patients (■) and healthy controls (○). The differences in BMD values between the patients and the controls increased significantly with increasing age (TB_{HE} $p < 0.001$, $r = 0.92$, spine $p < 0.001$, $r = 0.78$, hip $p < 0.001$, $r = 0.89$, heel $p < 0.001$, $r = 0.82$).

Results

In the Becker MD group, the BMD values increased with age in most individuals at all the sites that were measured; however, there was only a significant increase in TB BMD and BMC and spine BMD (Table 8) (Figure 22).

Table 8 – Changes (Δ) in DXA and DXL measurement parameters from baseline to follow-up in Study IV. Total body (TB), TB head excluded (TB_{HE}), right (R) and left (L), ns=non-significant

	Becker MD n=6		DMD n=18		
	mean (SD) median (min;max) n=	p-value within group	mean (SD) median (min;max) n=	p-value within group	p-value between groups
Δ TB BMD	0.116 (0.057) 0.125 (0.028; 0.190) n=6	<0.05	0.036 (0.041) 0.033 (-0.011; 0.114) n=15	<0.01	0.002
Δ TB_{HE} BMD	0.150 (0.034) 0.136 (0.118; 0.199) n=5	ns	0.027 (0.035) 0.025 (-0.042; 0.092) n=14	0.01	<0.001
Δ TB BMC	781 (385) 758 (236; 1330) n=6	<0.05	191.3 (141.1) 218.2 (-144.3; 380.6) n=15	<0.001	<0.001
Δ TB_{HE} BMC	823 (300) 850 (511; 1257) n=5	ns	142.7 (139.7) 174.2 (-174.0; 321.2) n=14	<0.01	<0.001
Δ Z-score TB	-0.4 (0.6) -0.2 (-1.1; 0.2) n=6	ns	-0.9 (0.8) -1.1 (-1.7; 0.8) n=13	<0.01	ns
Δ spine BMD	0.255 (0.086) 0.260 (0.133; 0.356) n=6	<0.05	0.064 (0.074) 0.089 (-0.091; 0.174) n=14	<0.01	<0.001
Δ Z-score spine	0.4 (1.0) 0.6 (-0.8; 1.4) n=6	ns	-0.5 (0.9) -0.6 (-1.9; 1.6) n=12	ns	ns
Δ R FN BMD	0.120 (0.116) 0.097 (-0.040; 0.310) n=6	ns	-0.071 (0.114) -0.064 (-0.355; 0.094) n=15	<0.05	<0.01
Δ L heel BMD	0.050 (0.055) 0.030 (-0.006; 0.130) n=6	ns	-0.019 (0.070) -0.002 (-0.159; 0.130) n=18	ns	<0.05
Δ L heel BMC	0.037 (0.042) 0.020 (-0.006; 0.095) n=6	ns	-0.015 (0.055) 0.000 (-0.122; 0.108) n=18	ns	<0.05

Results

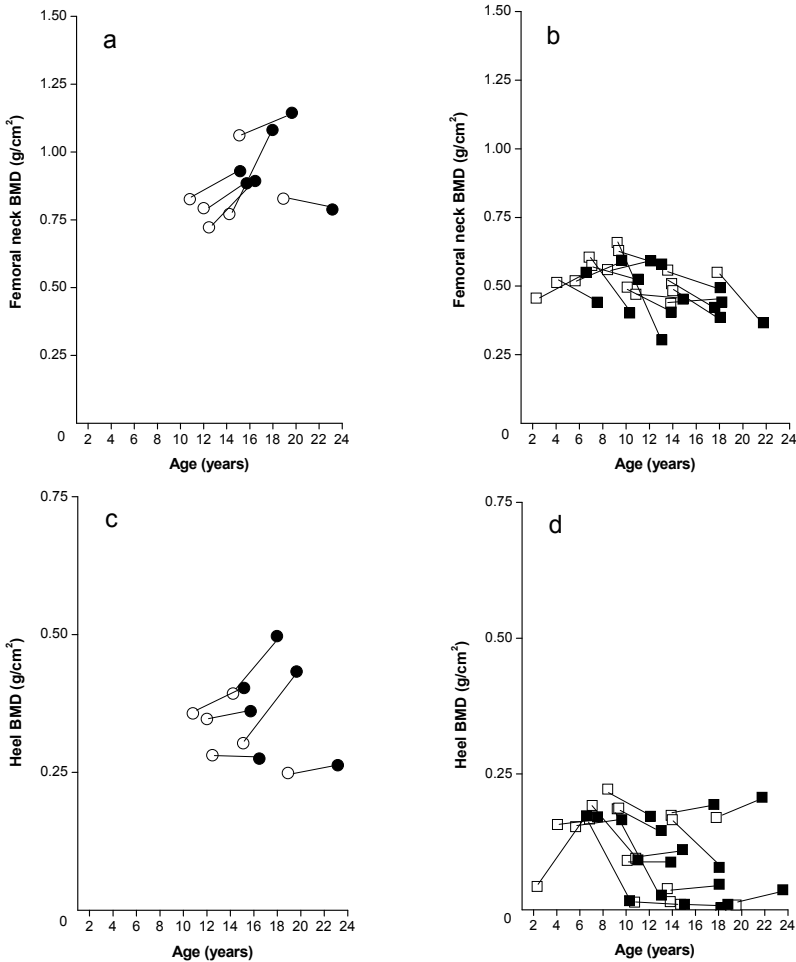


Figure 22 – BMD for different sites for Becker MD patients and DMD patients; (a), (b) the right femoral neck and (c), (d) the left heel in relation to age at baseline (empty symbols) and at follow-up (filled symbols). Becker MD patients (\circ and \bullet) and DMD patients (\square and \blacksquare). The BMD values were significantly lower in the DMD group compared with the Becker MD group at all sites at both baseline and follow-up ($p < 0.001$). For the total DMD group, a significant decrease in femoral neck BMD was noted during Study IV ($p < 0.05$).

Results

BMD and BMC values for TB, TB_{HE}, spine, heel and hip were all significantly lower in the DMD patient group in comparison with the healthy control group ($p < 0.001$) (Study III), and as shown in Figure 21, this cross-sectional study demonstrated that the differences in BMD values between DMD patients and healthy controls increased significantly with age (TB_{HE} $p < 0.001$, $r = 0.92$, spine $p < 0.001$, $r = 0.78$, hip $p < 0.001$, $r = 0.89$, heel $p < 0.001$, $r = 0.82$). Study IV demonstrated that the BMC and BMD values were significantly lower also in the DMD_{>10yr} group compared with the age-related Becker MD patients at all the sites that were measured both at baseline and at follow-up (see Table 2 in Paper IV). The DMD_{tot} group showed a significant decrease in the femoral neck BMD during this four-year period ($p < 0.05$) (Figure 22). In comparison with healthy age- and gender-matched controls, additional results collected from the follow-up study revealed that patients with Becker MD also had significantly lower BMD at most sites, even if this was less pronounced than in the DMD patients (Figure 23).

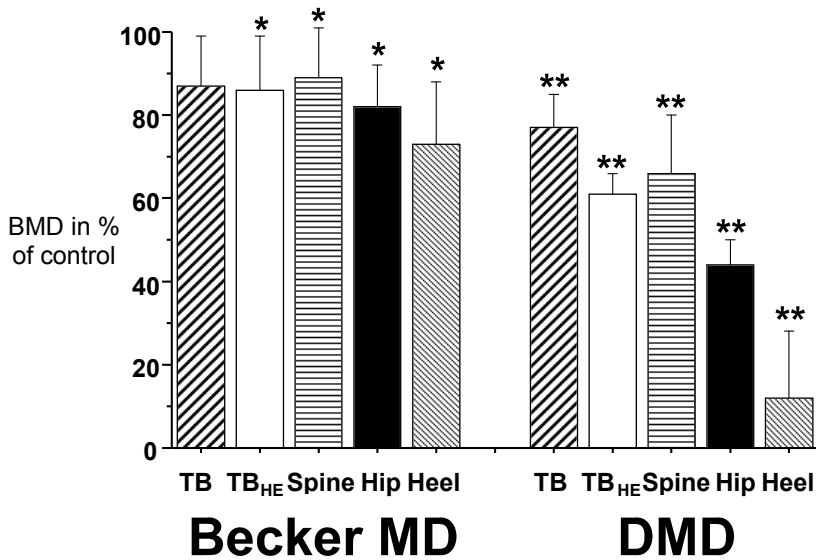


Figure 23 – The Becker MD patients and the DMD patients were compared with healthy age- and gender-matched controls. The figure shows BMD values as a percentage of control for each patient group and for different skeletal sites. Significant differences within the groups * $p < 0.05$, ** $p < 0.01$.

Results

Bone measurements and motor function (Studies III and IV)

Both the cross-sectional Study (III) and the longitudinal Study (IV) demonstrated a significant influence by motor function and muscle strength on bone mass measurement results. In Study III, motor function according to Vignos grade correlated significantly with heel BMD ($p < 0.001$, $r = -0.80$). In the same way, there was a significant correlation between heel BMD and isometric muscle strength ($p < 0.001$, $r = 0.76$). For the patients, the percentage of isometric muscle strength in the knee extensors in relation to reported reference values for healthy individuals of the same ages were calculated^{11,12}. In Study III, these quotients correlated significantly with the BMD values in the hip ($p = 0.016$, $r = 0.54$) and even more strongly with the heel BMD values ($p < 0.001$, $r = 0.82$). However, no significant correlations were found between the results of the motor function or muscle strength tests and the BMD values in TB, TB_{HE} or spine in Study III. In Study IV, it was demonstrated that motor function (Figure 24) and muscle strength decreased with increasing age during a period of four years.

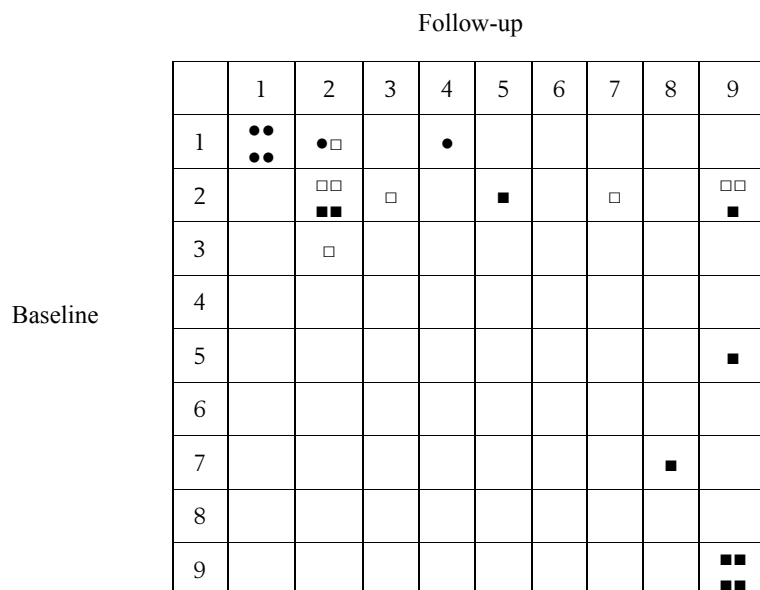


Figure 24 – Vignos grades for Becker MD patients (●) and DMD patients (□ < 10 yrs and ■ > 10 yrs) at baseline and at follow-up in Study IV.

Results

The quotients for knee extensor muscle strength in relation to reference values^{11,12} were significantly lower at baseline in the DMD_{>10yr} group ($15 \pm 15\%$) compared with the age-related Becker MD group ($76 \pm 31\%$) ($p < 0.001$). During the study period these quotients decreased in both groups; for the DMD_{>10yr} group $-10 \pm 12\%$ (median -8% (-36 to 0%)) ($p < 0.01$) and for the Becker MD group $-19 \pm 25\%$ (median -16% (-63 to 7%)) ($p = 0.12$). However, between the DMD_{>10yr} and the Becker MD groups, these changes did not differ significantly ($p = 0.39$). Correlation analyses between muscle function parameters (Vignos grade, knee extensor strength and knee extensor quotient) and BMD values (TB_{HE}, FN and heel) in the total patient group (all DMD and Becker MD patients taken together) performed in Study IV revealed a strong association between BMD values particularly in the lower extremities and the muscle function parameters assessed in the study; for Vignos grade at baseline: FN ($p < 0.001$, $r = -0.85$), heel ($p < 0.001$, $r = -0.86$), at follow-up: FN ($p < 0.001$, $r = -0.72$), heel ($p < 0.001$, $r = -0.77$); for the knee extensor parameters versus heel BMD both at baseline and at follow-up (all $p < 0.001$, $r = 0.82-0.89$). In this study there was also a significant correlation between TB_{HE} BMD and Vignos grade at baseline ($p < 0.001$, $r = -0.56$) and at follow-up ($p < 0.001$, $r = -0.78$) and between TB_{HE} BMD and knee extensor parameters both at baseline and at follow-up (p -values varying between < 0.05 to < 0.001 and r -values between $0.50-0.74$) (see Paper IV, Table 3).

Body composition (Studies III and IV)

In Study III, the DMD group had a significantly larger amount of body fat than the healthy control group ($p = 0.007$). The amount of lean mass was, however, significantly lower in the DMD group compared with the healthy controls ($p < 0.001$). In Study IV, there were no significant differences in fat mass between the DMD and the Becker MD groups at baseline or at follow-up. The Becker MD group did, however, have significantly ($p < 0.001$) more lean mass both at baseline and at follow-up compared with DMD_{>10yr} and the lean mass in the Becker MD group also increased significantly more than in the age-related DMD_{>10yr} group during follow-up ($p = 0.035$) (Table 9).

Results

Table 9 – Changes (Δ) for lean mass (kg), muscle mass (kg) and weight (kg) in Becker MD and DMD patients during follow-up (Study IV). Significant differences within the groups * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	All DMD n=18	Becker n=6	DMD>10 years n=10	DMD<10 years n=8
	mean (SD) median (min;max) n=	mean (SD) median (min;max) n=	mean (SD) median (min;max) n=	mean (SD) median (min;max) n=
Δ Lean mass (kg)	3.8 (3.3)*** 2.9 (-2.1; 10.1) n=15	9.2 (7.1) 12.5 (-3.5; 14.7) n=6	2.1 (1.5)* 1.8 (5.6; 5.1) n=7	5.2 (3.8)* 5.9 (-2.1; 10.1) n=8
Δ Fat mass (kg)	12.3 (9.5)*** 11.0 (5.8; 37.3) n=15	4.6 (13.3) 6.1 (-19.3; 16.7) n=6	8.6 (4.2)* 76 (3.7; 13.8) n=7	15.6 (11.8)* 14.6 (5.83; 37.3) n=8
Δ Weight (kg)	14.9 (11.2)*** 13.4 (-2.5; 42.3) n=18	13.9 (14.9) 17.3 (-13.9; 30.0) n=6	10.2 (6.4)** 9.2 (-2.5; 20.0) n=10	20.7 (13.4)* 19.6 (0.7; 42.3) n=8

When the DMD and the Becker MD patients were pooled, correlation analyses revealed that TB BMC was positively correlated to lean mass at baseline ($p < 0.001$, $r = 0.93$, $n = 24$) and at follow-up ($p < 0.001$, $r = 0.77$, $n = 21$) (Figure 25). Further correlation analyses revealed that the changes in lean mass in all patients taken together during the follow-up period correlated significantly with the changes in TB BMC ($p < 0.05$, $r = 0.52$, $n = 21$), which is shown in Figure 26. In these figures, however, a difference can be seen between the DMD patients and the Becker MD patients, who appear in part to form two separated groups during the study period.

Results

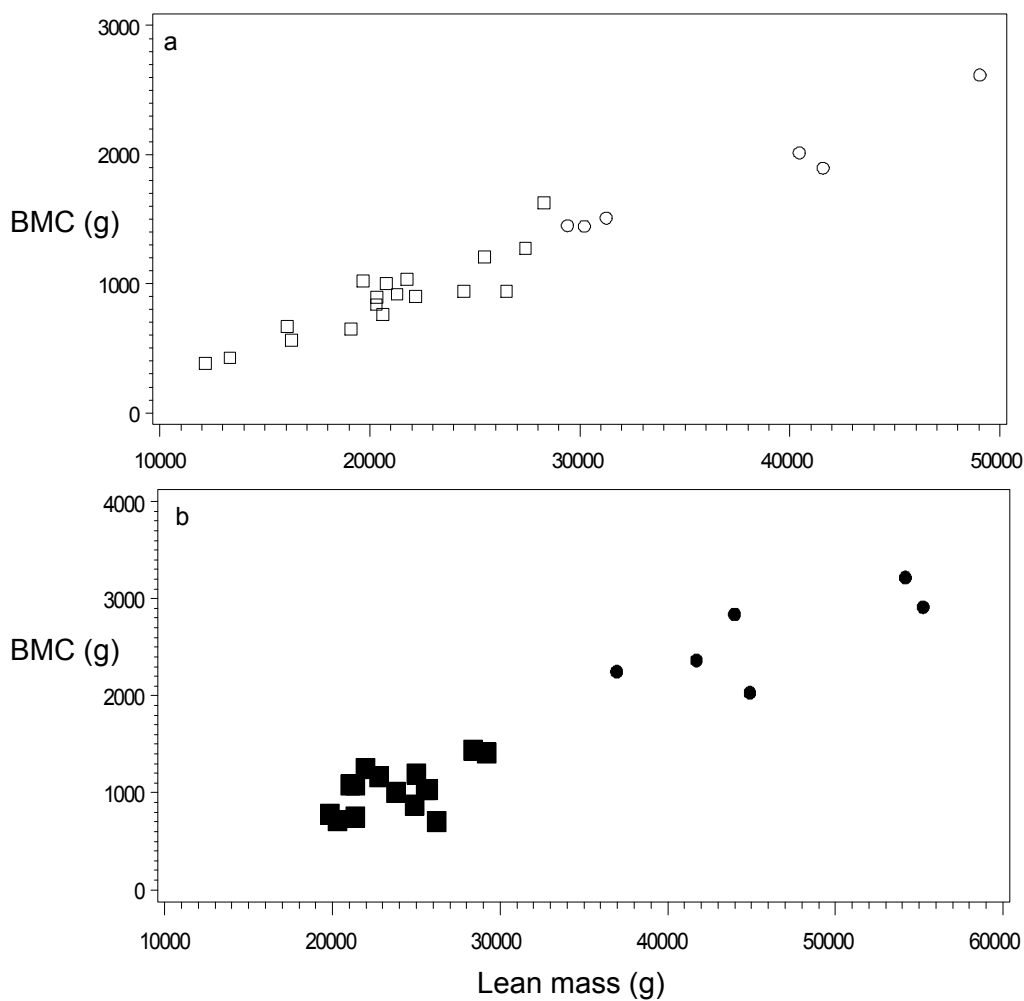


Figure 25 – Correlation analyses for BMC (g) according to lean mass (g) including both Becker MD (○ and ●) and DMD (□ and ■) patients in Study IV; (a) at baseline ($p < 0.001$, $r = 0.93$, $n = 24$) (empty symbols) and (b) at follow-up ($p < 0.001$, $r = 0.77$, $n = 21$) (filled symbols).

Results

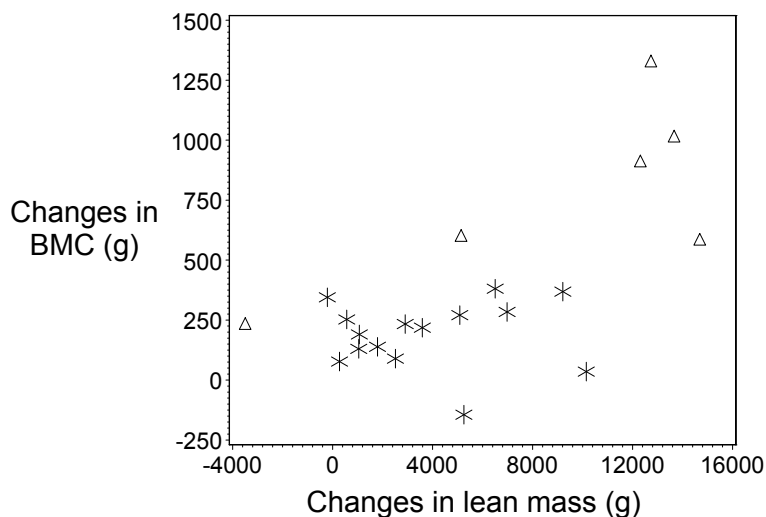


Figure 26 – A significant correlation was noted ($p < 0.05$, $r = 0.52$, $n = 21$) between the BMC changes (g) and the lean mass changes (g) during the four-year longitudinal Study IV; all patients together (Becker MD Δ and DMD $*$).

Biochemical markers of bone metabolism (Studies III and IV)

In the cross-sectional study (Study III), comparisons between DMD patients and healthy controls revealed that all bone formation markers were significantly lower in the patient group (BALP ($p = 0.002$), PINP ($p < 0.001$) and OC ($p < 0.001$)). Significantly lower values for the bone resorption markers CTX ($p < 0.001$) and TRACP-5b ($p = 0.002$) were also found in the patient group, but not for ICTP ($p = 0.09$). The CTX/TRACP-5b quotient (a suggested index for osteoclast activity in relation to the number of osteoclasts)¹²⁵, indicated that the majority of patients (11/16) had a lower quotient than their corresponding controls ($p = 0.025$). The levels of bio-intact PTH were within the normal range for all subjects and did not differ between the patients and the healthy controls. Significantly lower levels of 25(OH) D were found in the patient group (21 ± 6 $\mu\text{g/L}$) compared with the controls (30 ± 8 $\mu\text{g/L}$) ($p < 0.001$). In the longitudinal study (Study IV), the bone formation marker BALP levels and the bone resorption marker CTX levels were only significantly lower in the DMD_{>10yr} group compared with the BMD group at baseline ($p < 0.05$).

Results

Food questionnaire (Study III)

In Study III, dietary records were kept for four days, but only a very small number of participants completed these records (15 patients and 8 controls) and no further statistical analysis was performed on these data. The data that were obtained revealed, that the patient group obtained a mean of 72% of the daily intake of energy (kJ) recommended (RDI) in Sweden (controls 94%)¹³², 83% of the protein RDI (controls 107%), 71% of the fat RDI (controls 98%) and 69% of the carbohydrate RDI (controls 87%). The dietary calcium intake was more than 100% in both groups (patients 114% and controls 119%) and the vitamin D intake was 94% in the patients and 103% in the controls.

Fractures (Studies III and IV)

Both Studies III and IV indicate that patients with muscular dystrophy more frequently experience a fracture in the lower limbs compared with the upper limbs and compared with healthy individuals.

In Study III, the fracture rate was 25% in the DMD group (n=6) (7/11 fractures in the lower limbs) and 37.5% in the healthy control group (n=9) (4/13 fractures in the lower limbs); however, the numbers of fractures were small and the numerical differences were not statistically significant.

During the four-year follow-up study period (Study IV), six of 18 DMD patients sustained one or more fractures. Three of the DMD patients who had previously sustained fractures had another four fractures; two patients with one foot fracture each and one patient with both an ankle and a femur fracture. Three DMD patients sustained one fracture each for the first time; one in the foot, one in the ankle and one vertebral fracture. One of the BMD patients, who had previously had a foot fracture, sustained a tibial fracture during follow-up. At follow-up, there was a relatively higher rate of fractures in the Becker MD group (67%) (n=4) compared with the DMD group (39%) (n=7). Fractures were mostly observed in the lower extremities; 9/13 fractures in the DMD patients and 3/5 fractures in the Becker MD patients. However, this study was also small and no further statistical analysis of the fractures was performed.

Discussion

General discussion

In this thesis, it has been shown that the DXL Calscan is convenient and easy to use in a paediatric population, containing children as young as two years of age. In addition, it has been demonstrated that it is possible effortlessly to perform measurements of the calcaneus using this device in children and adolescents with severe disabilities.

ROC analyses revealed that heel DXL BMD measurements can predict low whole-body DXA BMD values with high reliability.

The study has provided percentile values for calcaneal BMC, BMD and BMAD in children aged two, four and seven years, and the continuous work of collecting normative data in young children has recently made it possible to present reference curves (mean \pm 2 SD) for BMD in the calcaneus for children aged two to 10 years, for girls and boys separately.

The DXL Calscan method, together with analyses of bone turnover, muscle strength and motor function, was applied to DMD and Becker MD patients, whose muscles become increasingly weak with age. The bone mass as measured by DXA and DXL, was significantly reduced in the DMD patients compared with healthy individuals and the differences between the groups increased with age. In addition, reduced bone turnover was found in the DMD patients compared with healthy controls. The fracture rate was similar in the DMD group and the healthy control group, but the DMD patients experienced fractures in the lower extremities more frequently than the healthy individuals. Differences in bone mass accrual were observed between DMD patients and Becker MD patients, the latter group experiencing a milder course of the disease. Longitudinal data revealed a decrease in hip BMD in the DMD patients with time. Significant correlations between bone mass parameters and muscle strength and motor function were observed.

Methodological considerations - DXL and DXA

There are several factors of importance for bone strength. Bone mass is one of them, but high BMD or BMC is not synonymous with stronger bone. This is one reason why the DXA technique has been called into question. However, DXA has been proven to be able to predict fracture risk in adults⁷⁷ and there is also an

Discussion

association between low BMD and fracture risk during childhood ^{29,55}. The disadvantages of using the DXA technique, such as its being an imaging technique without the opportunity to measure bone in the geometrical way the bone is constructed, may well be overcome if this tool is good enough to identify risk individuals and safely predict fracture risk. Experience of using the DXA technique has now been widespread for many years and most users are well aware of the difficulties associated with interpreting measurement data, especially in growing individuals ^{64,139}. Although QCT or pQCT measurements are able to provide information on bone geometry and cross-sectional muscle area, these techniques have not been shown to be superior to DXA in terms of fracture prediction ¹⁸. The development of the DXA technology, especially the finding of methods that are more suitable for children, which were one of the aims of this study, might therefore be well advised.

Finding the most appropriate method for measuring children is challenging. Certain difficulties are involved in performing measurements in children, irrespective of the technique that is used, particularly in very young children and in children with different disorders or disabilities. Movements cause artefacts on a scan and internal metallic implants, as well as feeding tubes or ventilator connectors, also interfere with the scan. Study I revealed that the DXL Calscan method was easy to use and that it was well tolerated by all subjects, even by the very young two-year-old children. Since the device is portable, it was also convenient to carry out measurements at schools and day-care centres. No more than 16 of 350 scans, had to be excluded because of movement artefacts, which illustrates that the measurements were easy to perform. In Studies II-IV, several patients could not be measured at all sites in the whole-body DXA due to metallic implants, contractures or difficulty moving the patient from the wheelchair to the DXA bunk, which is a limitation in these studies and also to this DXA method. All the patients in these studies could be measured in the DXL Calscan device.

Since DXA and DXL are two-dimensional techniques, they do not allow the volumetric measurement of the bone, which makes it even more complicated to measure and follow growing individuals. Different calculation models for bone mineral apparent density (BMAD) have therefore previously been proposed^{24,162}. In Study I, it is suggested that the information on calcaneal height should be added for the adjustment of differences in size at different ages and in different individuals. In Study I, this BMAD (mg/cm^3), was shown to be less influenced by age, and in Study II, it was independent of the age, weight and height of the

Discussion

children. This indicates that this BMAD may be used as an independent measurement when assessing the bone density of the growing skeleton. The fact that the BMAD increase is less evident with age compared with the BMD increase, may also indicate that bone mass accrual is more dependent on longitudinal growth than on the skeleton becoming more dense or mineralised at tissue level, at least in prepubertal stages.

A recent report ⁵⁴ recommended that densitometry values should be assessed from the lumbar spine and TB_{HE} (total body head excluded), when investigating bone mass in children. The present Studies III and IV, however, demonstrate the importance of also assessing values from other skeletal sites, depending on how the specific disease affects the body. The studies of DMD and Becker MD patients clearly demonstrate the differences in bone mass development at different sites, probably generated by the gradual deterioration in muscle in these patients, primarily affecting the lower extremities.

Normative data

In recent decades, an increasing amount of reference or normative data for DXA measurements in children have been reported ^{21,26,81,105,164} and every kind of DXA device or company with DXA equipment probably now also has its own reference material within the software. Ideally, reference data should be representative of the overall population and derived from a large enough sample size of individuals, adequately to characterise the variability of the bone measurements. At the present time, normative data reported in the literature comprise very few individuals in each age group, especially in the youngest ages (2-5 years). In the update on growth reference values ¹⁶¹, data from measurements of 4,488 boys and girls from birth up to 18 years of age were collected. In that study, the smallest number of individuals used in any age group was 55 girls and the largest number was 1,849 boys. In the light of that study, even the sample size of more than 100 children (50% girls and 50% boys) in each age group (2, 4, and 7 years) in Study I, appears to be too small. However, compared with other bone densitometry reference data, where 5-27 children of each gender and age between 2 and 7 years of age are measured in the lumbar spine and femoral neck ¹⁶⁴ or, in a study of the calcaneus, where a total 70 children between 5 and 8 years of age were measured ²⁶, the present study appears to include a more relevant population size. Kalkwarf and co-workers recently published normative data from a large ongoing longitudinal multicentre study ⁸¹. In that study 70-178 non-black girls or boys and 21-61

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black girls or boys up to the age of 17 were included; however, that study comprised no children younger than 7 years of age.

It is recommended that SD scores for paediatric bone densitometry reference data should be calculated¹⁶⁵. This is definitely important for the evaluation of measurement results when there are very low or very high values, which could be outside the reference population distribution (i.e. > 100th percentile or < 0th percentile) and it is also of importance for the longitudinal follow-up of children. From a three-year longitudinal study following Study I, we have recently been able to add calcaneal bone densitometry data for the calculation of SD scores in children 2-10 years of age. This may be of value for future research studies and for clinical use.

Precision data

Precision is often expressed as the coefficient of variation, $CV\% = SD/\text{mean value}$. If the mean value is very small, it follows that the CV% will be relatively larger, when SD remains constant. This was well exemplified in the DXL study (Study I), where we observed a fairly high CV% in the two-year-old children and a decrease in the CV% up to age of seven. This highlights the probable importance of also documenting the intra-individual SD (IISD), because these values tell us more about the true variance between measurements in individuals of different sizes or ages. We found that there was a small IISD in each age group, but this variance was similar in two- and four-year olds. It did, however, decrease in the seven-year-olds compared with the two younger groups. In some way these findings reduced the suspicion that there were considerable variances between measurements in these young children. At the same time, it confirmed the suspicion that the precision was somewhat weaker in the youngest children. DXA and DXL techniques may have difficulty recognising the more cartilaginous tissue in very young children compared with bone tissue in older individuals. The results could also be explained by the fact that measurements of a smaller foot are likely to be more sensitive to intra-individual differences in the positioning of the foot.

The relatively high CVs that were found in Study I, especially for the youngest children, are a limitation to the method. CVs at similar levels are seen also for pQCT and this is probably due to the small amount of bone (small mean values) measured at a peripheral site. Since a change of 2.8 x CV% is usually required to be statistically significant (the least significant change), a high CV% reduces the

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possibility to monitor changes in BMD in an individual over time. On the other hand, the trabecular bone, which is the major type of bone in the calcaneus, is more sensitive to metabolic changes than cortical bone. Therefore changes are probably seen first in this location, making the calcaneus an appropriate site for measuring and monitoring BMD after all.

In reporting DXA results, authors often refer to the CV% from the manufacturer ¹³¹, from other studies ¹⁵³, or the results from a group of individuals of different ages ^{89,106,164}. The way and in what individuals (ages, sex etc.) these values have been calculated is not always reported ⁴¹, which unfortunately largely precludes proper comparisons between different studies. For example, in another study of the calcaneus, Martini and co-workers ⁹⁸ reported a difference in the CV% between osteoporotic and non-osteoporotic women; the CV% was 2.4% and 1.7%, respectively. The IISD was, however, not reported and it is only possible to speculate about whether there are differences in the SD between the groups or if the difference in the CV% is only caused by the lower mean BMD value in the osteoporotic women.

Correlation between different bone densitometry techniques

For many years, the whole-body DXA technique has been regarded as the “gold standard” for assessing bone mass. However, as there are situations in which it is not possible to use this technique, other techniques have to be evaluated and validated. Chinn and co-workers ²⁶ previously presented data on heel BMD measured using peripheral DXA and found a linear, strong correlation to TB BMD in healthy children aged 5-18 ($r=0.82$ and 0.88 for girls and boys respectively). In Study II, similar correlations are presented; pair-wise correlation coefficients for BMD values measured in the calcaneus using the DXL Calscan and BMD values measured at other sites using the whole-body DXA varied between 0.72–0.90. However, the correlations appeared to be weaker, the lower the BMD (Figure 19). Study II also demonstrated correlations between whole-body DXA and heel DXL BMD measurements that were similar to those presented in previous studies performed in adults ^{98,131}. The studies in adults have used the T-score for calculations, which is not used in children. For this reason, our study results are not easy to compare with those in adults. Previous comparative studies of quantitative ultrasound (QUS) measurements and BMD measurements have generally yielded weaker correlations, especially when peripheral QUS measurements have been correlated with axial DXA. The correlation coefficients have ranged between 0.3 and 0.7 ^{40,130}. Sundberg and co-

Discussion

workers¹⁴⁵ reported correlations (r-values) ranging between 0.44 and 0.70 for QUS and BMD at different sites in a study of 280 children, who were 11-16 years of age. In comparison with DXA and DXL technologies, QUS technology appears to be more sensitive to environmental influences¹⁵⁹ and the poorer precision of QUS in comparison with DXA limits its applicability for longitudinal use in progressive disorders or in therapeutic interventions⁸⁶.

Physical activity and physical inactivity

During childhood and adolescence, changes in long bone size are determined by both longitudinal growth and the expansion of the cross-sectional bone area⁴⁶. Periosteal modelling leads to wider and larger bone. Endocortical changes lead to a wider marrow space; however, during female puberty, the site-specific apposition of bone occurs¹⁰⁸. A number of cross-sectional and longitudinal studies have shown that increased physical activity in growing children is associated with a higher areal BMD measured using DXA^{60,61,89,90,96,101}. Studies that have been performed using pQCT measurements^{95,160} indicate that increased mechanical loading improves bone strength through cross-sectional geometry changes at the diaphyseal sites and through an increase in BMD at the epiphyseal sites. It has also been reported that sports activity during childhood and adolescence shows remaining bone geometry effects in young adulthood even though the sports activity has ceased¹¹³. Even if there are adults who have been included in bed-rest studies^{127,128} and space flight projects⁷⁹ for investigations of the skeleton after a period of unloading or no gravitation, physical inactivity as an intervention in healthy children would probably be regarded as unethical. In a group of individuals, like DMD and Becker MD patients, where muscle activity decreases with time even though the patients are receiving the treatment of choice, it is, however, possible to study how bone develops with no, less or progressively decreasing muscular influence also in children. In contrast to studies of a sporting population, studies on a severe disabled population are more difficult to extrapolate to the general population as there may be several confounding factors (such as lack of nutrition, specific medication and/or selection bias due to small study groups). Despite these limitations, such studies, like Studies III and IV, can provide an insight into skeletal consequences resulting from loading or unloading. During a period of four years, the DMD patients lost more muscle strength and motor function compared with the Becker MD patients. This may have contributed to the lower increase, and even decrease, in BMD at certain sites, which was noted in the DMD patients.

Discussion

Several authors have reported reduced BMD or BMC in children with different disabilities such as cerebral palsy ^{3,68}, myelomeningocele ^{6,7,10,120} and muscular dystrophies ^{5,15,88}. Both Aparicio and co-workers ⁵ and Larson and Henderson ⁸⁸ demonstrated that DMD patients lose bone mass in the proximal femur while they are still ambulant, all of which agrees with the findings in Studies III and IV.

In their study of patients with spinal cord injuries, Giangregorio and McCartney ⁵¹ confirmed what is also seen in the clinical setting, that neuromuscular impairment from birth plays a vital role in the development of the size and shape of the bone in a different way compared with the situation when the impairment occurs later in life. Without normal neuromuscular stimuli the bone becomes thinner, more slender and shorter. Animal studies of hind limb unloading have underscored the importance of normal muscle function for the maintenance of bone mass ⁵⁹ and there are indications of the age-dependent effect of immobilisation and remobilisation on bone tissue ¹⁵¹. Eser and co-workers ³⁹ investigated spinal cord injured men, two months–50 years after injury. They observed an exponential decrease in different bone parameters, which reached steady states after 3-8 years. In the epiphyses, the loss of bone mass was caused by reduced BMD. In the diaphyses, however, bone mass was lost due to the thinning of the cortical wall as a result of endosteal resorption.

The width of the bone is important for bone strength and the capacity of bone to withstand forces of different kinds ¹²⁶. In studies of bed-rest-induced bone losses, a recovery of bone mass has been observed when mobilisation is resumed ¹²⁷, which indicates that the adult skeleton also has the capability to adapt to mechanical stimuli. However, the degree to which bone mass can be recovered in adulthood is limited by the PBM reached during growth. Thus, achieving the optimal predisposed PBM and bone size during growth appears of utmost importance. In Studies III and IV, it was not feasible to evaluate whether or not or how the cross-sectional area of the bones in the muscular dystrophy patients change with age. This may be a limitation when studying this patient group and information relating to that dimension at least is missing. Nevertheless, the studies of DMD and Becker MD patients indicate that these patients achieve far from the expected optimal predisposed PBM as observed in healthy controls. This was particularly evident in the lower extremities, which are also the sites most affected by fractures.

The muscle and bone unit

In 2002, Schönau and co-workers¹³⁸ proposed a two-step diagnostic algorithm to evaluate the clinical application of the muscle and bone relationship. In step 1, the question is whether *muscle mass is adequate for body height* and in step 2, the question is whether *BMC is adequate for muscle mass*. These questions form four diagnostic groups:

- 1) **Normal system** with normal muscle mass for body height and adequate BMC for that muscle mass.
- 2) **Primary bone defect** with normal muscle mass for body height, but the BMC is lower than expected for muscle mass.
- 3) **Secondary bone defect** with low muscle mass for body height, but the BMC is normally adapted to a smaller muscle mass and to a lower mechanical demand.
- 4) **Mixed bone defect** (primary and secondary) with low muscle mass for body height and lower BMC than expected for that reduced muscle mass.

According to this diagnostic algorithm it is important to have information on muscle function and muscle mass in addition to the information on bone mass. The studies of DMD and Becker MD patients in this thesis indicate a secondary bone defect, but there could also be some mixed bone defect, which the design of the studies is unable fully to demonstrate, due to confounding factors, for example, GC treatment.

It has been suggested that the insufficient increase in bone mass and longitudinal growth with age in the DMD patients could originate from the genetic deletion on the Xp21 gene¹⁰⁷. However, to the author's knowledge, this theory has not yet been confirmed. Short stature is found in DMD patients, also without GC treatment, as shown by Nagel and co-workers¹⁰⁷, who also found reduced bone-specific ALP (BALP), which could be a result of the reduced muscle mass and lower biomechanical load, which would lead to reduced bone turnover. However, they did not find any correlation between height and motor function and between height and BALP. They therefore concluded that short stature in DMD is unlikely to be a result of muscular weakness. In Study III, we found reduced bone turnover in the DMD patients compared with healthy age- and gender-matched controls. Furthermore, a significant correlation was demonstrated between both calcaneal BMD and Vignos grade, and calcaneal BMD and isometric muscle strength in the knee extensors in the DMD patients. These findings were confirmed in the four-year longitudinal study, where strong

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correlations were also found between hip BMD values and muscle function parameters.

In DMD patients, muscle strength is reduced very early, often before the disease is diagnosed. The DMD patients generally start to walk later than healthy children; this was also confirmed in the present study. During the first years of life, their muscle strength is sufficient to acquire new motor function skills, but with time their motor function is gradually impaired. The Vignos scale (Table 4) is a method for grading motor function and, even if it appears to be a crude measure, this grading effectively classifies the progress of the disease with time and has been used for several decades¹⁵⁷. For the evaluation of muscle strength, the hand-held myometry is a well-accepted method in DMD patients. It has been shown to be a reliable tool in mentally retarded patients¹⁴⁶, but the child still has to co-operate and the investigator needs to be experienced. In the present study, one experienced physiotherapist performed all these measurements.

Taken together, our results indicate that bone development during childhood and adolescence is likely to be affected by the muscular status in DMD patients, although firm evidence of causality could not be provided.

Other factors influencing bone

During the four-year longitudinal study period (Study IV), DMD patients appeared to form their own group in terms of the development of BMC in relation to lean mass (muscle mass) development (Figures 25-26). In this group, the relationship between muscle mass and BMC appears to be weaker with time. This may be explained by the fact that the contractility and strength per mass unit is lower in DMD patients, hence reducing the muscle mass influence on BMC development. At the same time, the insufficient increase in bone mass with age in the DMD patients could also be caused by other factors such as inadequate nutrition, vitamin D insufficiency and the GC treatment. Study III indicated that the DMD patients received too little nutritional energy, protein, fat and carbohydrate compared with recommended daily intake (RDI) in Sweden. The vitamin D intake was more satisfactory, with 94% of the RDI in the DMD patients. Nevertheless, Studies III and IV indicated that DMD patients, as well as Becker MD patients, had low vitamin D (25(OH) D) levels, which could have a direct impact on the mineralisation of newly synthesised osteoid within bone, as well as affecting the muscle strength in these

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patients^{44,45,66,71}. The definition of 25(OH) D-insufficiency is, however, still unclear. It has been suggested that the diagnosis of vitamin D insufficiency (25(OH) D levels of < 30 µg/L) should be based on the physiological response/elevation of PTH⁶⁶. Others⁷¹ concur, that serum 25(OH) D levels of < 20 µg/L should be regarded as vitamin D deficiency. In the studies III and IV, the 25(OH) D levels were not sufficiently reduced to cause elevated PTH levels for most patients, and this might be due to the adequate daily intake of calcium.

Biochemical markers of bone metabolism

The diagnostic role of bone turnover markers in bone diseases or during growth is still not fully established, but bone turnover is reflected through different levels of bone formation markers and bone resorption markers, which were analysed in Studies III and IV. The results indicated a reduction in bone turnover in the DMD group. In contrast to our findings, Bianchi and co-workers¹⁵ measured OC and urinary NTX (a marker of bone resorption) and interpreted their results as increased bone turnover in DMD patients and particularly in those treated with GC. However, values for urinary NTX are dependent on urinary creatinine and muscle mass. Since DMD patients have reduced muscle mass, urinary creatinine will be markedly decreased⁵⁸, and this may thereby entail falsely elevated NTX values. Serum CTX, which is not dependent on urinary creatinine or muscle mass, therefore reflects the activity of bone resorption more accurately than urinary markers of bone resorption in DMD patients. In Study III, the CTX levels indicated that there was reduced bone resorption in the DMD patients compared with healthy individuals. The treatment with GC may have contributed to the reduced bone turnover, as shown previously by Ton and co-workers¹⁵⁰. The disease itself, with the weaker muscles triggering lower strain and less mechanical loading on the skeleton, may also have contributed to the reduction in bone remodelling and modelling in the DMD patients.

Fractures in children

Before the 1980s, little attention was paid to fractures in children. In 1983, Landin performed a very thorough study of fracture patterns in children in southern Sweden⁸⁷. This study is still most frequently cited in new studies of fractures in children and, in the last few decades, an increasing number of studies discussing fracture risk in childhood⁵⁷, epidemiology³¹, cause, treatment

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and prevention²⁵ and the risk of future fracture after a childhood fracture have been published¹¹⁹.

Goulding reported that 19.8% of children accounted for 66% of all fractures during childhood and adolescence⁵⁷. The reasons for this can only be speculated on, but an inherent skeletal weakness or lower bone mineralisation, low physical activity, high risk-taking behaviour or abnormal body weight could underlie the results. That study does not, however, report the kind of trauma causing fracture, the localisation of fractures, whether new fractures in the same individual are located in the same or another extremity or how much time has passed between two fractures in these individuals. All this can certainly influence the risk of repeated fractures.

An association has been suggested between fracture and low BMD in childhood^{28,29} as well as in adulthood⁷⁷ and it has also been suggested that there is an increased risk of fracture associated with neuromuscular diseases¹⁵⁶. Vestergaard and co-workers reported a similar risk of fracture in individuals with muscular dystrophy or muscular atrophy (RR=1.9) as Goulding and co-workers reported in healthy children when it came to experiencing a second fracture⁵⁷. In our studies of DMD and Becker MD patients, 25% of the DMD patients had experienced one or more fractures in the cross-sectional baseline study. At follow-up, the fracture rate was 39% in the DMD group, which is similar to the results of a previous Swedish study of healthy children⁸⁷. In the Becker MD group the incidence of fractures was 67%. This patient group was, however, small (n=6), but if this figure also holds true in a larger sample, the high fracture incidence could possibly be due to weaker bone in a still relatively physically active group. The question of whether or not these patients have osteoporosis still remains unanswered. According to the ISCD 2007 Pediatric Official Positions of the International Society for Clinical Densitometry¹²³, Study IV would leave only two DMD patients with a required type of fracture and Z-scores less than or equal to -2.0, who would consequently be regarded as having osteoporosis. Nevertheless, a retrospective chart review (n=143) revealed that patients with DMD on long-term corticosteroid treatment ran an increased risk of vertebral and lower limb fractures compared with steroid-naïve patients⁸⁵. As other authors have previously reported^{88,156}, we also observed that the majority of fractures in the DMD patients were located in the lower extremities. This differed clearly from the healthy control group, where the majority of fractures occurred in the upper extremities. This difference may be caused by the weakness in the lower extremities that appears at an earlier stage compared with

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the rest of the body in DMD and Becker MD patients. This goes hand in hand with the observations that BMD was particularly reduced in the lower extremities and even showed a tendency to decrease with increasing age in the DMD patients.

Osteoporosis in children is still not clearly defined. The definition of “true osteoporosis” suggested by Frost ⁴⁶, certainly makes sense; however, that definition does not facilitate the prediction of fractures. Who knows when a bone is weaker than it should be for its daily usage, prior to a fracture?

Limitations

Clinical non-intervention studies are generally based on associations, making it complicated firmly to establish causality in several situations. Further, studying human beings, as compared to experimental animals, is invariably associated with large biological variation which in turn requires large samples. In this respect, studying rare disorders poses limitations. In spite of the fact that DMD is the most common muscular dystrophy, it is still a rare disorder. Becker MD is even more uncommon. Moreover, with age, the DMD patients in particular are often severely hampered by the disease, making it difficult for them to take part in a clinical study or trial. As a result, few patients were available for the studies of DMD and Becker MD (Studies III and IV). Small study populations complicate the statistics and set the limits for the interpretation and generalisation of the results.

Dual-energy X-ray absorptiometry (DXA) is an indirect, two-dimensional imaging technique for measuring bone mass in the three-dimensional bone. Using this technique, it is therefore not possible exactly to study the bone geometry or the true BMD, both of which are important for bone strength and are influenced by growth and physical activity, for example. This limits every study including DXA measurements and all the studies in this thesis.

In growing individuals, every part of the body changes in form and size with time, which makes interpretation of DXA measurements more difficult. As a result, age, height, weight, puberty and bone age all influence the results of a DXA measurement. In this thesis, the DXA and DXL results were not adjusted for bone age, height, or weight, which limits the opportunity to compare individuals of different size or age. For DXL measurements, it was suggested to calculate the BMAD (mg/cm^3) (the areal BMD (g/cm^2) value was divided by the

Discussion

height (cm) of the calcaneal bone) in an attempt to adjust for different heel bone sizes in Study I.

Performing whole-body DXA measurements in individuals with different disorders or disabilities is challenging. Not every individual was able to accomplish a full whole-body DXA measurement for several different reasons, such as joint contractures and metallic implants. This was especially relevant in Studies II-IV. However, it was possible to perform heel bone measurements of all individuals in these studies, using the DXL Calscan.

DXA and DXL are methods with high accuracy; however, the intra-individual variation between measurements may differ and this then produces a considerable coefficient of variation (CV%). This is seen in particular in the peripheral measurements of the body, where bone mass values are small. Measuring the heel bone in young children with small bones and small amounts of bone mass therefore implies the risk of a higher CV%, which was also observed in Study I. A high CV% will set the limit for discovering changes during follow-up studies. This may be one reason why significant changes in heel bone BMD were not discovered during the DMD and Becker MD follow-up study (Study IV).

There are many possible ways of matching DMD patients with healthy controls; they include weight, height, BMI, testis size, bone age, muscle strength, motor function, Tanner stage, gender and age. Matching for all these parameters would have been better (Study III), but we choose gender and age. Matching for age makes it possible to follow the development of the group over time. As the disease progresses, it is of interest to compare the individuals in this way. We would have liked to match for developmental parameters (for example height and weight) as well, but that would have required screening of a large number of individuals to find the right matching controls.

The fact that we did not perform a spinal radiograph on the study individuals in order to evaluate vertebral fractures could be a limitation in Studies III and IV. However, among our DMD patients in Study III, 12 patients and all the non-ambulant boys had been investigated with spinal radiographs in connection with clinical follow-up within one year before inclusion and no vertebral fractures were reported. During the follow-up (Study IV), one vertebral fracture was reported.

Discussion

Furthermore, we did not have a well-defined patient group without GC treatment, which made it difficult to determine whether some results in Studies III and IV were due to GC or to the muscular dystrophy itself.

Another limitation in Studies III and IV could have been that the patients differed in age and disability; however, this was part of the study outcome.

In Study IV, the Becker MD patients were generally older than the DMD patients, which made it impossible correctly to compare Becker MD with all DMD patients. The DMD group was therefore broken down into two subgroups; one below and one above 10 years of age. For these comparisons, Study IV was limited to the smaller population of Becker MD patients.

Future perspective

During the work on this thesis, I have had the opportunity to become well acquainted with the patients with Duchenne and Becker muscular dystrophies and their parents. I have encountered an impressive joy of life and a positive attitude to life in these families, even though they are confronted by a severe and life-threatening disorder. In the future, both patients and physicians will be looking for new treatment alternatives to better preserve or improve muscle function in muscular dystrophy patients. This future approach includes genetic studies and the possibility of novel gene therapies.

When it comes to bone densitometry, future studies should include precision studies in children at different ages, adolescents and adults with different kinds of disability, to investigate which method is the most suitable for these individuals.

The development of normative data based on larger samples of healthy children and adolescents representing all ages is of particular importance for every bone densitometry technique. Representative reference data will provide a better opportunity to interpret measurements in paediatric patients.

The calculation of SD scores for paediatric bone densitometry reference data should be performed. This is particularly important for the evaluation of measurement results when very low or very high values are outside the reference population distribution (i.e. > 100th percentile or < 0th percentile) and for the longitudinal follow-up of children. Future studies should therefore extend the

Discussion

SD scores for bone densitometry data produced by the DXL Calscan device from 10 years of age up to PBM.

For DMD and Becker MD patients and other similar patient groups, longitudinal studies using pQCT and QCT techniques will reveal further information on skeletal development in the event of progressive or manifest muscular impairment during growth.

Randomised, controlled intervention studies applying increased physical activity or skeletal loading should investigate whether or not, and how, it is possible to improve bone strength in children with disabilities. The muscular and skeletal effects of low-frequency vibration training in DMD patients could be investigated, for example.

The present study indicates that patients with DMD have reduced bone turnover compared with healthy individuals. This could be due to the disease itself with weaker muscles triggering lower strain and less mechanical loading on the skeleton; however, the GC treatment could also have this effect on the skeleton. Future studies should focus on new information relating to the total effect of GC treatment in DMD patients, including the muscular effect, the skeletal effect and the bone- and muscle-unit effect. The reduced bone turnover may indicate that medical treatment with drugs, such as bisphosphonates, should not be used in these patients. However, very soon after beginning the GC treatment, the bone turnover in these patients may be increased and, within this initial therapeutic phase, bisphosphonates may be indicated for a shorter period to prevent vertebral fractures in particular.

It appears to be highly relevant to analyse the way in which the information from DXA and DXL measurements overlap with the information from QCT and pQCT measurements. It is often not possible to perform several different measurements in the clinical setting. It could therefore be valuable to study the way in which these techniques are complementary and when one of the techniques is to be preferred.

It is also important to create ROC curves to determine the sensitivity and specificity of standard DXA and DXL measurements of aBMD and BMAD; QCT and pQCT measures of vBMD and bone dimensions in the discrimination of fracture from non-fracture cases.

Discussion

In the continuous work in this field, it would be desirable to establish clinical guidelines considering children who warrant bone densitometry and the way the measurements that are obtained should be handled. The important future questions are; at what levels is treatment needed and which treatment should be given, how and when? Improved guidelines can probably be best accomplished by larger studies, with long follow-up and including information on specific disorders, fractures and medical treatment. This would most probably improve the potential for predicting fracture risk in children.

Conclusions

Conclusions

- The DXL Calscan method for bone densitometry is quick, convenient, and easy to use in children, even in children as young as two years of age and in children with disabling conditions.
- Reference data for calcaneal BMC, BMD and BMAD in children aged two, four and seven were produced and presented as percentile values. Additional data promoted the presentation of reference curves as the mean \pm 2 SD in children aged two to 10 years, for girls and boys respectively. These curves can be used for further research and in the clinical setting.
- High correlations were found between whole-body DXA BMD measurements at different sites and heel DXL BMD measurements. Receiver operating characteristics revealed both high sensitivity levels and high specificity levels at different sites, indicating that the lowest 25th percentile of the DXA measurements can be classified by the heel DXL measurements with a high reliability.
- Patients with DMD demonstrated lower BMD and BMC values compared with healthy age- and gender-matched controls. DMD patients showed less increase in BMD with increasing age as well as a decrease in the hip BMD values with time.
- The DMD patients also demonstrated generally lower BMD values and poorer bone mass accrual compared with patients with the milder Becker MD. The Becker MD patients, in turn, demonstrated lower BMD values compared with healthy controls, however not to the same degree as the DMD patients.

Conclusions

- DMD patients displayed reduced bone turnover as compared with healthy age- and gender-matched controls.
- The fracture rate in the DMD patients was no higher than in the healthy control group; however, in the DMD patients, the fractures were more frequently located in the lower extremities.
- The reduced BMD in both DMD and Becker MD patients was strongly correlated with the degree of muscular dystrophy, motor function and muscle strength. The lower extremities in particular are affected at an early stage, indicating the importance of site-specific information on bone mass development and that interventions aimed at strengthening this site could prove valuable.
- This thesis indicates the importance of a normally and adequately functioning muscle tissue for an optimal development of the skeleton.

Summary in Swedish (sammanfattning på svenska)

Bakgrund

Under tillväxten i barndomen grundläggs en stor del av den skelettvävnad, som vi skall leva på och av under vårt resterande liv. Många faktorer påverkar skelettet under livet, där ärftlighet är en av de mest betydelsefulla, men där även nutrition, hormoner, fysisk aktivitet, viss medicinering och vissa sjukdomar kan ha avgörande betydelse. Duchennes muskeldystrofi (DMD), som är en X-kromosom-bunden sjukdom och som bara drabbar pojkar, innebär tilltagande muskelsvaghet med tilltagande svårighet att gå och i tidiga tonår blir dessa barn beroende av rullstol. Muskelsvagheten leder till minskad belastning på skelettet och risk för utveckling av skört skelett, som i sin tur innebär ökad risk för frakturer. För att kunna följa skelettets utveckling hos barn krävs enkla och smidiga metoder, som barn tolererar, men samtidigt mätinstrument med god precision och noggrannhet. Att mäta bentäthet hos barn är en utmaning, eftersom skelettet ändrar form och storlek kontinuerligt, vilket försvårar tolkningen av mätresultat. Vilken metod som är den mest lämpliga för bentäthetsmätning hos barn är omdebatterat.

Syfte

Avhandlingsarbetets syfte var att utvärdera en ny röntgenmetod, DXL Calscan (dual-energy X-ray absorptiometry och laser), för bentäthetsmätning i hälbenet hos barn och ungdomar, att undersöka metodens användbarhet vid undersökning av mycket unga barn och barn med sjukdomar och funktionshinder, och att upprätta ett pediatrikt referensmaterial för hälsens bentäthet. Vidare syftade arbetet till att genomföra en kartläggning av benhälsan hos pojkar med sjukdomen DMD och att studera hur olika faktor inverkar på skelettets utveckling i denna population.

Metodik

Inledningsvis utfördes en noggrann genomgång av en sedan tidigare befintlig metod, DXL Calscan, avsedd för bentäthetsmätning i hälbenet hos vuxna. Genom metodutveckling kunde röntgentekniken anpassas för barn, med sänkt strömstyrka och minskad stråldos, och vidare utveckling av ett fotstöd för att kunna placera även de minsta fötterna korrekt. DXL Calscan för barn användes därefter genomgående i samtliga studier.

Summary in Swedish

Studie I

För att upprätta ett pediatrikt referensmaterial för bentäthet i hälbenet undersöktes 334 friska svenska barn i respektive åldrar; 2, 4 och 7 år. Upprepade mätningar utfördes dels på en konstgjord "referens-fot" (så kallad fantom-mätning), dels på 23-27 barn i varje åldersgrupp för att kunna beräkna metodens noggrannhet och mätningarnas variabilitet. För att kunna uppskatta tillväxtens inflytande på det uppmätta bentäthetsvärdet (BMD – bone mineral density), utarbetades en möjlighet att mäta hälbenets höjd på röntgenbilden. Då detta höjdmått dividerades med BMD, kunde ett storleksjusterat bentäthetsvärde (BMAD – bone mineral apparent density) beräknas.

Studie II

För ytterligare utvärdering av DXL metoden jämfördes hälbensmätningar med mätningar från andra delar av kroppen samt helkroppsmätningar utförda med DXA (dual-energy X-ray absorptiometry), vilket sedan längre tid ansetts vara "gold standard" för bentäthetsmätning. För denna studie samlades data in från tre separata studier, där samtliga individer under en och samma dag genomgått undersökning med både häl-DXL och helkropp-DXA. I studien undersöktes 112 personer (2-21 år); vissa individer undersöktes ytterligare en gång efter ett år, vilket medförde att totalt 128 mätningar med de bägge teknikerna kunde jämföras.

Studie III

I denna tvärsnittsstudie jämfördes 24 pojkar med DMD (2-20 år) med en kontrollgrupp bestående av 24 åldersmatchade friska pojkar. Grupperna undersöktes avseende benmassa, kropps-konstitution och hormoner. Dessutom analyserades specifika markörer i blodet för bedömning av skelettets omsättning (nedbrytning respektive uppbyggnad).

Studie IV

En uppföljningsstudie, där 18 pojkar med DMD från Studie III och sex pojkar med Beckers muskeldystrofi (MD) undersöktes på nytt efter fyra år. Beckers MD innebär en skada på samma gen som vid DMD, men gendefekten är av ett mildare slag, varför sjukdomsförloppet också är mildare och dessa pojkar behåller alltid gångförmågan över 16 års ålder. Dessa två patientgrupper kontrollerades longitudinellt och jämfördes avseende utveckling av benmassa, kropps-konstitution, motorisk funktion och muskelstyrka.

Summary in Swedish

Resultat

Mätningar av hälbenets bentäthet med DXL Calscan var lätta att utföra på barn och ungdomar, även på barn i mycket låg ålder (2 år) och på barn med funktionshinder. Studie I presenterar normaldata i percentiler för BMD, BMC (bone mineral content) och BMAD för barn i åldrarna 2, 4 och 7. BMD korrelerade signifikant med ålder, längd och vikt. BMAD var i lägre grad associerat till ålder, längd och vikt, vilket indikerar att detta mått kan vara användbart då man följer individer under tillväxt och vid mätning av olika stora individer.

Tilllägg av data från uppföljande årliga mätningar av drygt 30 % av barnen från varje åldersgrupp i Studie I, under ytterligare två år, medgav konstruktion av referenskurvor (medelvärde \pm 2 standarddeviationer) för flickor respektive pojkar i åldrarna 2-10 år. Dessa referenskurvor baseras på totalt 645 hälbensmätningar (328 flickor/317 pojkar) med DXL Calscan.

Resultat av Studie II indikerade en hög överensstämmelse mellan bentäthetsvärden uppmätta med DXL-teknik i hälbenet och värden uppmätta med DXA-teknik vid både helkroppsmätning och mätning av ländryggen, men framför allt beträffande bentäthetsvärden uppmätta i höften. Studie II visade vidare, att det med hög tillförlitlighet går att förutspå låga värden vid en DXA-mätning av hela kroppen, ländryggen eller höften, genom att utföra en DXL-mätning av hälbenet.

Patienter med DMD uppvisade generellt lägre bentäthetsvärden jämfört med friska ålders- och könsmatchade kontroller. Skillnaden i bentäthet mellan dessa grupper ökade med ökande ålder och detta var framför allt tydligt i höft och häl. Patienterna uppvisade en sänkt benomsättning, det vill säga sänkt såväl formation som nedbrytning av ben, jämfört med de friska kontrollerna. Man kunde inte finna någon skillnad i antalet frakturer som drabbat de bägge grupperna, men patienterna hade oftare brutit sig i de nedre extremiteterna, jämfört med de friska kontrollerna, som oftare brutit sig i de övre extremiteterna.

Patienter med DMD uppvisade även generellt lägre bentäthetsvärden jämfört med patienter med Beckers MD, vilka dock i sin tur uppvisade lägre bentäthetsvärden jämfört med friska ålders- och könsmatchade kontroller, dock inte lika uttalat som patienterna med DMD. Långtidsuppföljningen visade på en signifikant minskning av bentätheten i höften hos patienterna med DMD med

Summary in Swedish

ökande ålder. Studien visade på en stark association mellan bentäthetsvärden uppmätta i de nedre extremiteterna och muskelstyrka, tillika motorisk funktion.

Slutsatser

DXL Calscan är en användbar teknik för bentäthetsmätning på barn även i låga åldrar och på barn med funktionshinder. Mätning av bentäthet i hälbenet kan indikera en allmän risk för benskörhet, eftersom det är möjligt att med DXL-teknik identifiera individer, som uppvisar låga bentäthetsvärden vid helkropps-DXA-mätning. Barn med motoriskt funktionshinder, av typen Duchennes muskeldystrofi, utvecklar under uppväxten ett tunnare skelett i förhållande till motoriskt friska barn till följd av minskad kraftutveckling och belastning på skelettet. Graden av funktionshinder har stor betydelse för skelettets utveckling och beroende på funktionshindrets art blir vissa delar i skelettet mer benägna att utveckla benskörhet än andra.

Acknowledgements

Acknowledgements

I would like to express my sincere gratitude to the many persons without whom this thesis never would have been possible, for their work and encouragement. In particular I wish to thank:

Diana Swolin-Eide, MD, PhD, my tutor and friend, for introducing me to the bone field and to science, for your encouragement and enthusiasm, for all funny moments putting up the DXL method, for your support and for your true belief in doing good for patients through research.

Jon Karlsson, MD, professor, my co-tutor, orthopaedic colleague and friend, for professional guidance in the clinics and scientific matters, for your never-ending enthusiasm for research, for your impressive capacity and your round-the-clock absolutely-on-top support.

Kerstin Albertsson Wikland, MD, professor, my co-tutor and friend, for introducing me to science, for support, for valuable advice and kind, constructive criticism.

Per Magnusson, associate professor, my co-author and friend, for professional guidance in the bone marker field, for fruitful scientific discussions, encouragement, endless patience, support and invaluable help in preparing this work.

Ragnar Kullenberg, PhD, my co-author and friend, for your creativity in inventing the DXL Calscan device and for support and valuable scientific discussions.

Mar Tulinius, MD, professor, **Anne-Christine Åhlander**, MD, PhD, and **Anna-Karin Kroksmark** PT, PhD, my co-authors, for friendship, support and valuable discussions throughout the work on Duchenne muscular dystrophy.

Anne Dohse, for assuming so much responsibility in the “bone-measurement room” and invaluable help with DXL and DXA measurements throughout the years, for your friendship and encouragement.

Acknowledgements

Nils-Gunnar Persson, Gunnar Ekeröth, Mattias Molin and Aldina Pivodic, for invaluable help with statistical questions.

Roland Thomeé PT, associate professor, and **Pia Thomeé** PT, PhD, for friendship, enjoyable scientific discussions and valuable help with a lot of everything. I especially want to thank you, Roland, for your time and patience, when preparing the layout of this book.

Bengt I Eriksson, MD, professor, colleague and friend, for invaluable scientific advice, deep discussions and some salsa dancing.

Ulrika Sterky, for your obliging manner, friendship and valuable help in preparing all the blood samples.

Tommy Hansson, MD, professor, for valuable advice and constructive criticism and for sharing some of your knowledge on earlier bone densitometry technologies.

Björn Rydevik, MD, professor, for your constant kind and friendly attitude, for your professional scientific guidance and for providing the opportunity to do this work.

Jovanna Dahlgren, MD, associate professor, for support and friendship, for valuable advice and constructive criticism.

Katarina Nilsson-Helander, MD, PhD, for a close and wonderful friendship, days and nights full of laughter, all the talking (sometimes about science) and everything else. I am so grateful to you.

Fredrik Einarsson, MD, PhD, colleague and friend, for valuable discussions concerning not only research through the years.

The “**Gran Canaria research group**” for making January a very nice month for several years.

Ninni Sernert, PT, associate professor, for support and friendship and for letting me stay in your house during the autumn of 2007.

Acknowledgements

Colleagues at the **Orthopaedic Clinic in Borås**, for first introducing me to orthopaedic surgery.

All the colleagues who over the years have been a part of the **Children's Orthopaedic Team** at Östra Hospital; I especially want to thank **Bertil Romanus**, MD, associate professor, **Ragnar Jerre**, MD, associate professor, **Aina Danielsson**, MD, associate professor, **Andreas Runge**, MD, **Paula Berg**, MD, and **Ola Bing**, MD. I am really grateful for your support and encouragement. Only with your help and effort was it possible for me to take time off from the clinical work to complete this thesis.

Colleagues in the **Children's Orthopaedic Surgery Trauma Team**, **Queen Silvia's Hospital** for friendship and great support.

All the staff at the **Orthopaedic Department**, especially the **Orthopaedic Outdoor Clinic** and our secretaries, **Åsa Nordmark** and **Maile Gröndahl** at **Östra Hospital**, and the **Queen Silvia's Hospital** for making a day at work a joy, and all the staff at **GP-GRC** for help with something of everything through the years.

All the staff at the **Children's Habilitation Centre**; I especially want to thank **Barbro Westerberg**, MD, PhD, for being a wonderful person and an expert physician, for guidance in the neurological field through the years.

Linda Johansson, for kind support and invaluable help in all kinds of practical matters.

Marlene Mussmann, for kind help with administrative matters at the Pediatric Department.

Ann-Sofie Pettersson, for always having time to lend a helping hand with poster preparations and other practical things.

Jeanette Kliger, for excellent language revision.

Annette Dahlström, for professional illustrations.

Eve-Lotte, **Maude** and **Krystyna** at the Medical Library, SU/Östra Hospital, for always being so kind and helpful.

Acknowledgements

Anders Oldfors, MD, professor, for performing all the immunohistochemical analyses on the muscular dystrophy patients.

Anette Ekberg, for your friendly attitude and valuable help in preparing the transport of blood samples to Linköping.

All the, nowadays, ladies in “**Spinnarna**”; **Maria Berggren, Helene Brandrup-Wognsen, Susanne Ellbjär, Ingalill Friis-Liby, Marie Sverkersdotter, Ulrika Wall and Christina Ysander**, for “womanising” the world, for wonderful friendship full of joy and laughter, for your cheerful support and for all your extraordinarily nice partners.

My colleague **Olof Lundin**, MD, PhD, and **Mona Lundin**, dear friends, for being an incessant source of inspiration, for support and encouragement and for all the “hole-in-nines”.

Bollebygds Idrottsförening, Bollebygds Budo-Kai and the City Gym in Göteborg, for providing me with the opportunity to keep my BMD at an optimal level.

Gaddenskolan, for being an important part of my life and for keeping our children safe at school.

Eva Bengtsson, for friendship and wonderful co-operation within the school health care at Änglagårdsskolan.

All friends in Bollebygd, for wonderful friendship, a lot of laughter and nice times together and always being there when needed for our children during the years; especially **Gunilla and Anders Eliasson, Anki and Mikael Standar, Ulrika Andreasson and Lars Wiss** and **Eva and Robert Sellgren**.

The carpenters **Tommy and Olle** for helping us build a new home up the hill in Bollebygd; you added to my biomechanics.

My mother, **Ros-Marie and Björn**, my father, **Lars-Göran and Margaretha**, my mother-in-law **Inga-Maj and Ulf**, my recently deceased farther-in-law, for everything you have done for me. Words are not enough.

Acknowledgements

My sister, **Marie-Louise** and her family, and my sister-in-law, **Karin and Peter** and their family, for many happy, nice moments, for encouragement and support.

Josefin, Carl and Erik, the meaning of life, for your love and wonderful friendship, for your happiness, enthusiasm and encouragement, which eventually helped me complete this work.

Bosse, my everything, my beloved and best friend, for being not only a great scientist and physician but also a wonderful father and husband, for sharing the life with me, for your endless love, encouragement and never-ending belief in me.

All the participants for spending so much time in the “bone-measurement room” and for making the studies possible.

This study was supported by grants from the Swedish Society of Medicine, the Göteborg Medical Society, the Majblomman Foundation, the Folke Bernadotte Foundation, the Swedish Association of Persons with Neurological Disabilities and the Government under the LUA agreement.

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