# TIBIAL ULTRASONOMETRY IN CHILDREN

Maarten H. Lequin

ISBN 90-9014121-9

Lay-out: A.W. Zwamborn
Illustrations: A.W. Zwamborn
Photographs: T. Rijsdijk
Cover: Céline Lequin

Printed  by Ridderprint B.V. Ridderkerk

# TIBIAL ULTRASONOMETRY IN CHILDREN

# ECHOGRAFIE VAN DE TIBIA BIJ KINDEREN

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van Rector Magnificus

Prof.dr. Ir. J.H. van Bemmel

en volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
woensdag 4 oktober 2000 om 15.45 uur

door

Maarten H. Lequin
geboren te Dubbeldam

## PROMOTIECOMMISSIE

| | |
|---|---|
| Promotor: | Prof. dr. M. Meradji |
| Copromotor: | Dr. C. van Kuijk |
| Overige leden: | Prof. dr. S.L.S. Drop<br>Prof. dr. J.S. Laméris<br>Prof. dr. M.G.M. Hunink |

Aan mijn ouders

# CONTENTS

| | |
|---|---|
| AVU | apparent velocity of ultrasound |
| BMAD | bone mineral apparent density |
| BMC | bone mineral content |
| BMD | bone mineral density |
| BUA | broadband ultrasound attenuation |
| CA | calendar age |
| CUBA | contact ultrasound bone analyser |
| CV | coefficient of variation |
| DEQCT | dual-energy quantitative computed tomography |
| DPA | dual photon absorptiometry |
| DXA | dual-energy X-ray absorptiometry |
| MTX | methotrexate |
| $\mu$MR | magnetic resonance microscopy |
| PQCT | peripheral quantitative computed tomography |
| QCT | quantitative computed tomography |
| QMR | quantitative magnetic resonance |
| QUI | quantitative ultrasound index |
| QUS | quantitative ultrasound |
| RA | radiographic absorptiometry |
| SA | skeletal age |
| SEQCT | single-energy quantitative computed tomography |
| SOS | speed of sound |
| SPA | single photon absorptiometry |
| SXA | single-energy X-ray absorptiometry |
| UTV | ultrasound transmission velocity |

# BONE MASS ASSESSMENT TECHNIQUES IN CHILDREN

## 1.1 INTRODUCTION

It has been known for many years that early detection of osteoporosis is important for predicting its progression and response to therapy. Detection and monitoring of osteoporosis is essential because they have a major impact on morbidity, mortality and financial costs[1]. In 1994 the World Health Organization defined osteoporosis as "a disease characterized by low bone mass and a micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk"[2]. Considerable progress in the development of new, noninvasive techniques for assessing the bone quantity and quality makes early detection of osteoporosis possible[3]. These new methods make possible a better understanding of the development of osteoporosis and therefore can give an opportunity to prevent osteoporosis. Some studies suggest that the best prevention of osteoporosis is to maintain the highest possible bone mass (peak bone mass) because it will take longer to reach the osteoporotic state (at which level there is a higher fracture risk, especially at the hip and spine)[1,4,5]. The peak bone mass is the highest level of bone mass, attained as a result of normal growth. The age when peak bone mass is reached, especially in the proximal femur and the vertebral bodies, varies in different studies[6-9]. Most authors believe that the peak bone mass occurs in late adolescence[6,7,10]. Given the importance of this peak bone mass, bone assessment techniques in children have gained substantially in interest. Most noninvasive bone mass assessment techniques are now widely used in the adult population but many of these techniques still have to be validated in children.

Before discussing the techniques used to detect osteoporosis in an adult and pediatric population, we will discuss the modifiable and non-modifiable factors which influence the occurrence of osteoporosis.

The most important non-modifiable determinants are genetic-ethnic factors and gender[11]. However, only 46 to 62 percent of the biological variability in bone mass can be explained by those factors. The rest can be attributed to modifiable determinants, such as hormonal status, physical activity, weight and

nutrition[12]. All those determinants influence the level of peak bone mass. Therefore it is important to have a noninvasive technique for bone assessment in pediatric populations, as well as in adult populations. To be useful in pediatric populations a noninvasive bone assessment technique should be reproducible, accurate, easy to use, relatively cheap and if possible without radiation exposure.

## 1.2 REVIEW OF THE LITERATURE

A variety of techniques for noninvasive assessment of the skeleton are or have been used. All techniques are listed in Table 1 and their precision, accuracy and discrimination will be discussed, as will differences in their fundamental methodology, clinical and research utility, and general availability.

**Table 1** Bone mineral assessment techniques.

| |
|---|
| Radiogrammetry |
| Radiographic absorptiometry (RA) |
| Single photon and single-energy X-ray absorptiometry |
| Dual photon and dual-energy X-ray absorptiometry (DXA) |
| Quantitative computed tomography (QCT) |
| Quantitative magnetic resonance and magnetic resonance microscopy |
| Quantitative ultrasound bone assessment (QUS) |

*Radiogrammetry*

Radiogrammetry is a simple X-ray technique for the assessment of osteoporosis using bone dimensions. Since the introduction of this method in the 1960's, a large number of reports in this field have been published[13-15]. Several measurements are taken, such as total bone width, cortical thickness, the ratio of cortical width to total bone width, and the cortical area. The measurement sites are tubular bones, such as the phalanges and metacarpals. Due to the relatively low reproducibility (errors in the order of 3%) and the inability to measure intracortical porosity, well-known feature of bone loss, interest in

radiogrammetry has diminished. Recently there is a renewed interest in this technique from the introduction of a promising additional measurement site, the hip. The hip axis length is measured on conventional X-rays images of the hip or on images acquired by bone densitometers[16]. Until now radiogrammetry has been used mainly in the adult population rather than in the pediatric population[17].

*Radiographic absorptiometry*

Radiographic absorptiometry (RA), also known as photodensitometry, is relatively inexpensive and a widely accessible technique, used to assess bone mass (cortical and trabecular) quantitatively on conventional radiographs. In RA, radiographs of the hand are taken, using a special film and film cassette with an aluminum wedge. The films are analyzed with a digital or video densitometer. The bone mineral density (BMD) is calibrated relative to that of an aluminum wedge and is expressed in mm Al equivalent[18-23]. Most investigators use the middle phalanges, especially of the second digit, or the metacarpals. Initially this technique was characterized by a high reproducibility error of about 9-10%[24]. The introduction of computer-assisted methods reduced the reproducibility error and further constituted a remarkably fast way of measuring BMD. Several RA techniques have been developed. One technique uses centralized analyses of the posteroanterior made hand radiographs and averages the BMD of the second to fourth middle phalanges[18]. Another technique measures the diaphysis of the second metacarpal[19,20], while a third technique uses the diaphysis and proximal metaphysis of the second middle phalanx as measuring site in the posteroanterior and lateral view (LAT)[23,25]. The lateral view of the second middle phalanx is made on the same screen using a dedicated cassette with an aluminum reference wedge (Figure 1A and 1B). By combining the measurements of the perpendicular views at the same level of the second middle phalanx, a real bone density can be calculated and in addition a sophisticated soft-tissue correction is provided (Figure 1C) [26].
The precision errors of the computer-assisted RA techniques are lower in both *in vitro* and *in vivo* measurements. The range is between 0.6 and 1.7% *in vitro* and between 0.3 and 2.4% *in vivo*[19,20,22,23,27,28]. The precision of these RA techniques is similar to those obtained with other densitometric techniques[29,30].

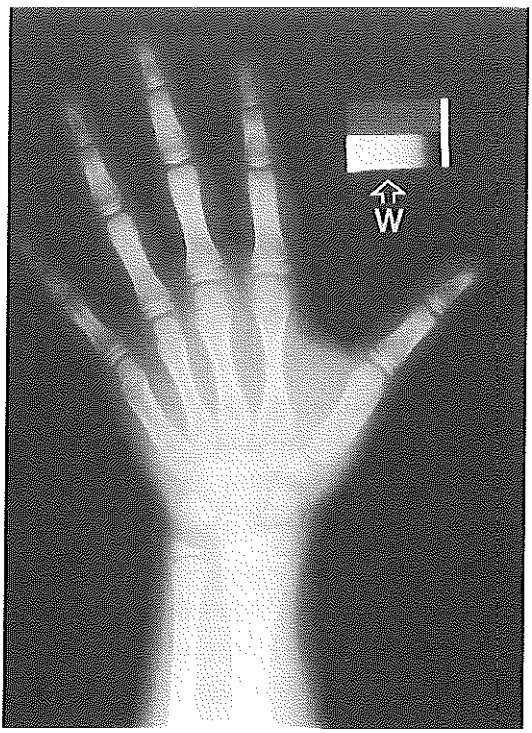



Fig. 1A

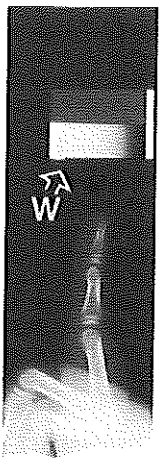



Fig. 1B

**Fig. 1** An example of the X-ray images used in this study.
**A.** Posteroanterior projection of the left hand (W = Aluminum reference wedge).
**B.** Lateral projection of the left index finger (W = Aluminum reference wedge).
**C.** Graphic representation of the principle behind phalangeal radiographic absorptiometry.

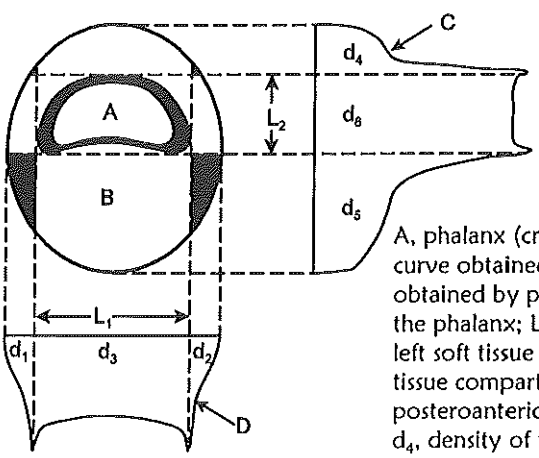



Fig. 1C

A, phalanx (cross-section); B, soft tissue; C, density curve obtained by lateral radiograph; D, density curve obtained by posteroanterior radiograph; $L_1$, width of the phalanx; $L_2$ height of the phalanx; $d_1$, density of the left soft tissue compartment; $d_2$, density of the right soft tissue compartment; $d_3$ density of medial posteroanterior compartiment (bone and soft tissue); $d_4$, density of the upper soft tissue compartment; $d_5$, density of lower soft tissue compartment; $d_6$ density of medial LAT compartiment (bone and soft tissue).

RA techniques, especially when computer-assisted, appear to be suitable for the assessment of BMD of phalanges and metacarpals not only in the adult but also in the pediatric population[26,31,32].

*Single photon and X-ray absorptiometry*

The first commercial single photon absorptiometry (SPA) unit was introduced in the 1960's [34]. Both this method and its recently developed successor, single-energy X-ray absorptiometry (SXA) make a quantitative assessment of the bone mineral content (BMC) at peripheral sites of the skeleton. A monoenergetic collimated photon beam, emitted from a radionuclide, low-energy, source (usually $^{125}I$), or a small X-ray tube is moved at a constant speed across bone and soft tissue measuring the radiation attenuation at the investigated site. The replacement of the radionuclide source by an X-ray tube has improved the spatial resolution and precision, and has reduced examination time[35-37]. A major disadvantage of these methods is its inability to separate trabecular and cortical bone because it is an area projectional technique. Moreover, SPA and SXA use a single-energy source and therefore cannot be used in areas in which neighboring tissues are inhomogeneous, such as the spine. The measuring sites of both SPA and SXA are mainly at the distal or ultradistal radius and calcaneus. The radial shaft includes more cortical bone and less trabecular bone, the latter being metabolically more active[38]. The distal part of the radius consists of 95% cortical bone and ensures a good range of precision; while the ultradistal radius, which includes more trabecular bone (up to 40%), gives poorer precision due to difficulty in targeting the region of interest precisely and the inhomogeneity of the trabecular bone content[39,40]. The more sophisticated rectilinear scanning devices now in use improve the precision at this site[41]. As bone mineral measuring site, the calcaneus was controversial in the beginning because of the uncertain relationship between BMC at this site and body weight or exercise[42]. However, recent studies show that SPA and SXA have good predictive value for osteoporotic fractures, using either the calcaneus or the radius as bone mineral measuring site[43-45].

Especially in the adult population SPA and SXA have proven to be an accurate method for the diagnosis of osteoporosis. Application of these techniques in children is low because of the lower precision of SPA and SXA in children, compared to the adult population, resulting from the smaller and less dense bone and from motion artifacts[46]. Another drawback of some units is the necessity to put the part of the skeleton under investigation in a water bath to

obtain a reliable bone mineral measurement. The radiation exposure also hampers its use in children.

*Dual photon and X-ray absorptiometry*

The measurement of two photon energies, in dual photon absorptiometry (DPA), instead of one (SPA/SXA) allows for the discrimination of bone mineral from soft tissue and air interfaces[47]. Thus DPA can control for variable path lengths in the body and can be used to measure BMD at the hip and lumbar spine, two sites with high trabecular bone content and of considerable clinical relevance to osteoporotic fractures. DPA uses a radionuclide source, typically $^{153}Gd$ at two radiation peaks. The successor of DPA, dual-energy X-ray absorptiometry (DXA), was introduced commercially in the late 1980's[48]. In DXA the radionuclide source is replaced by an X-ray system, giving DXA a shorter examination time and greater precision and accuracy due to a higher resolution[49]. The preferred measuring sites of DXA are the lumbar spine, the proximal femur, and/or the total body. Sometimes a peripheral site such as the radial shaft is also used for scanning. Because of the precision of the posteroanterior DXA examination of the lumbar spine *in vivo*, ranges between 0.5-1.5% with an accuracy error of 5-10%, the DXA systems are used all over the world[50-56]. Other reasons for this worldwide distribution of DXA systems are its low radiation dose (Table 2)[57] and ease of use, which makes it well applicable for clinical trials and epidemiological studies[58-60].

**Table 2** Effective dose for a pediatric scan mode using a lunar DPX-L.

| Scan | Mode | Patient size | Age | Scan time (min) | ESD (μGy) | ED (μSv) |
|---|---|---|---|---|---|---|
| PA spine | | 6-16 cm | 5 | 5 | 6.0 | 0.28 |
| PA spine | | | 10 | | 6.0 | 0.20 |
| Total body | medium | 15-25 kg | 5 | 9 | 0.12 | 0.03 |
| Total body | large | 25-35 kg | 10 | 12 | 0.1 | 0.02 |

However, DXA systems also have some important drawbacks. First, the over-projection of aortic calcifications, the presence of osteophytes, degenerative facet hypertrophy and intervertebral disc space narrowing, increase the measured BMD in an artificial way, especially in elderly patients. Second, when the lumbar spine is only scanned in posteroanterior direction the measured area not only contains trabecular bone but also cortical bone. This reduces its ability to discriminate between osteoporotic and non-osteoporotic subjects[61-64]. The discrimination increases when an additional lateral scan of the lumbar spine is used. The lateral examination measures trabecular bone almost exclusively and therefore has a stronger correlation with quantitative computed tomography (QCT) than posteroanterior DXA and QCT[65,66]. A disadvantage of this lateral method is the over-projection of ribs at the level of L2 and the overlap of the iliac crest at the level of L4. Another problem is the poor reproducibility of the lateral measurements of the lumbar spine due to the greater thickness and inhomogeneity of the soft tissues in the lateral decubitus position[67-70]. The development of a rotating tube-detector system allows a lateral spine scanning in a supine position. This reduces obliquity and thereby improves the reproducibility of the lumbar spine measurement[71,72]. Several studies showed a stronger association between the lateral method and prevalent vertebral fractures than between the posteroanterior DXA method and such fractures. Also the aged-related bone loss is more pronounced in the lateral method[73-75].

The third drawback of DXA is the problem of standardizing measurements. Variations result from differences in bone standards, edge detection algorithms used in different devices and regions of interest[76,77].

A recent study shows that total body bone mineral measurements done with DXA are error prone due to differences in fat distribution. This impedes the applicability of DXA, especially in children and adults with diseases or taking medications that influence fat distribution[77,78].

In addition to the general drawbacks of DXA, it has some special problems when used in a pediatric population. First, two types of software sets are needed in children: one below 30-kg weight and one above this level[79]. And second, scanning very small children has the additional problem of a greater influence of the head on measurement of the total body.

Third, standardization in children is a problem because many DXA machines have no good pediatric reference populations. A major drawback of DXA is its inability to measure the true volume density due to its projectional measurement technique. Especially in the growing child, where there is three-dimensional growth, this technique cannot reliable distinguish between a true increase in BMD or simple growth of the investigated part of the skeleton.
Moreover, DXA values are also influenced by the unknown composition of soft tissues in the beam path of the region of interest. Because corrections for the soft tissues are based on a uniform distribution of fat around the bone, longitudinal DXA values in children may reflect the changes in body size and composition that occur with growth more than true changes in bone mineral content. It has been determined that inhomogeneous fat distribution in soft tissues, resulting in a difference of two cm fat layer between soft tissue area and bone area, will influence DXA measurements by 10%[67,80]. Several studies of DXA in children have been published to date[81-86]. In summary, DXA is an accurate and precise quantitative bone assessment technique in the mature skeleton, but in the growing child DXA is unable to take into account the large changes in body and skeletal size and therefore is of limited use in pediatric studies[87].

*Quantitative computed tomography*
In contrast to all the bone mineral assessment techniques discussed above, computed tomography (QCT) measures true volumetric densities of trabecular bone and cortical bone at the investigated site separately. Cortical bone has a more protective and mechanical function, while trabecular bone has a metabolic function. Trabecular bone has a higher bone turnover[88,89]. Because of higher bone turnover in trabecular bone (50 to 70 %), its importance for vertebral strength, and clinically important osteoporotic fractures are in the vertebrae, QCT is principally used at the lumbar spine. This technique can assess vertebral fracture risk, and measure and follow-up age-related bone loss and bone loss due to metabolic bone diseases[66,90-93].
More than 4000 centers use this validated technique[3]. One of the reasons for the worldwide acceptance of QCT is that it can be performed on a standard clinical CT scanner with an external bone mineral reference phantom device.

This device calibrates the CT number measurements to bone-equivalent values. The region of interest is the vertebral body of L1-L3. The planning of the mid-vertebral slices and the axial tracing of the region of interest can be handmade, but automated tracing improves precision and decreases analyses time[94]. A typical automatic analyses time for a vertebral body is about five seconds, and the total patient examination time is several minutes.

Two methods of QCT are available: single-energy quantitative computed tomography (SEQCT) and dual-energy quantitative computed tomography (DEQCT). As well as their radiation differences, the accuracy and precision of bone mineral assessment differ between SEQCT and DEQCT[95].

The most important factor, which influences the accuracy of SEQCT, is the variable fat content in the vertebral body[96]. This is more of a problem for bone mineral assessment in the adult population, due to physiological increase of vertebral marrow fat content with age, than in the pediatric population[97]. DEQCT can solve the fat-error problem, which improves the accuracy but at the cost of precision *in vivo* and it requires a higher radiation dose[98-100]. Therefore DEQCT is recommended only for research studies[100].

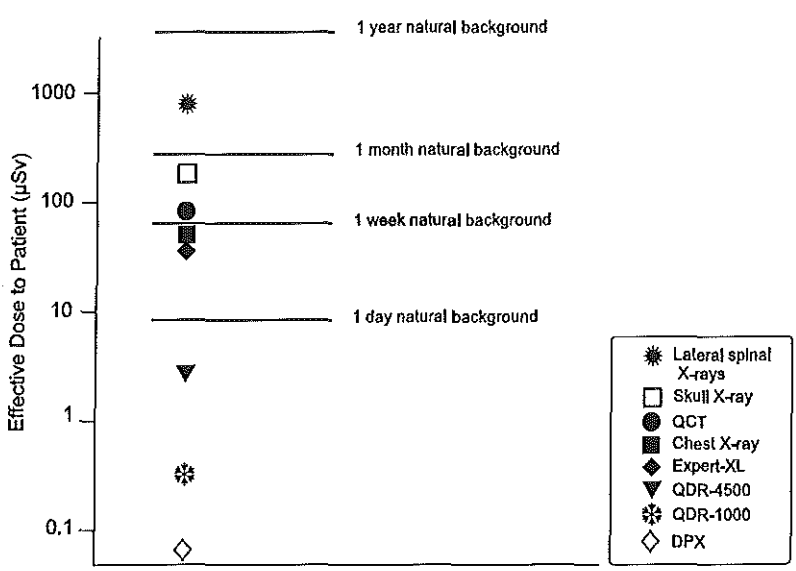



**Fig. 2** Effective dose to patient.

Because of the relative high radiation dose needed for QCT (compared to the dose used with DXA or other bone mineral assessment techniques, see Figure 2)[57] and the expensive equipment requiring costly maintenance, the use of QCT is low, especially in the pediatric population. Few studies with QCT have been performed in children[101-103].

In a growing child five QCT bone measurements can be obtained: the density of cancellous bone, the density of cortical bone, the size of the axial and peripheral skeleton and the volume of cortical bone in the appendicular bone. The coefficients of variation (CV) for these QCT measurements in a pediatric population range between 0.6 and 2%[101,102]. The accuracy of QCT in children is better than in adults due to a lower bone marrow fat content. The density of cancellous bone seems to be directly proportional to the bone volume fraction and inversely proportional to the porosity[104]. The relative large CV values of cancellous bone density are due to a considerable variation in the dimensions of the pores throughout the vertebral body. On the other hand, cortical bone mass depends on the concentration of osteoid and mineral. The nonmineral fraction may only contribute to minor fluctuations in cortical bone measurement. On average, CT values for cortical bone density are eight times higher than those for cancellous bone density, a finding consistent with histomorphometric studies indicating an equivalent difference in the porosity of these two forms of structural organization of bone tissue[105]. The material density of cortical bone and cancellous bone in the appendicular skeleton seems to increase during puberty and reaches its peak around the time of cessation of longitudinal growth and epiphyseal closure[101,106]. The difference in cancellous bone density in the axial skeleton between males and females is a result of an early gender difference in the size of the bones rather than of a real difference in the density of cancellous bone[107]. The size of a vertebral body is approximately 20% smaller in girls than in boys, even after accounting for differences in body size. In the appendicular skeleton no gender difference is seen in bone size. The major determinants of cortical volume of the appendicular skeleton are weight bearing and mechanical stresses and therefore correlate strongly with all anthropometric indices[107].

Peripheral QCT (pQCT) was developed for true volumetric density measurement of appendicular bone without overprojection of other tissues and

has the ability to localize exactly the desired measurement site. In contrast to QCT, pQCT is easier to use. Like QCT, pQCT has the ability to assess cortical and trabecular bone separately and to measure BMD and BMC. The measuring site is the forearm. A biomechanical study found that the cortical shell contributes substantially to the mechanical strength of the distal radius[108]. Because cortical rim thinning at the distal radius is a feature of osteoporosis, measuring BMC and thickness at this location is important[109]. Also other data suggest that pQCT measurements of cortical, rather than of trabecular, bone at the forearm has greater diagnostic sensitivity for fracture risk assessment[28]. There are about 1000 pQCT systems in use, mostly in Europe[3]. In the United States those systems are primarily limited to research. Like QCT, pQCT is mainly used in the adult population.

*Quantitative magnetic resonance imaging*

After the introduction of magnetic resonance (MR) in medical science in the early 1970's, the worldwide use of MR largely depends on its capability to image the anatomy of the human body very accurately. In the last years other abilities of MR have been explored, such as quantitative magnetic resonance (QMR) and magnetic resonance microscopy ($\mu$MR) used for bone mineral assessment and the study of trabecular bone architecture[110-113]. To date QMR and $\mu$MR have only been for research studies, due to their cost and time-consuming techniques.

*Quantitative ultrasound bone assessment*

Quantitative ultrasound (QUS) is a recently introduced bone assessment technique. This technique holds the prospect of a good reproducibility, ease of use, cost effectiveness and being radiation free. Several systems for QUS bone assessment are in production, almost exclusively used in and validated for adult populations[114,115]. Until now the measuring site of the QUS systems is the calcaneus, both in children and in adults[116]. QUS bone assessment measurements at the tibia, performed using a SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel), could also be implemented in adults as in children. This technique measures the speed of sound (SOS, ms-1) along the

cortex of the tibia,[114,117] see Figure 3. A more thorough overview of all QUS systems, used in children, will be outlined in Chapter 2.

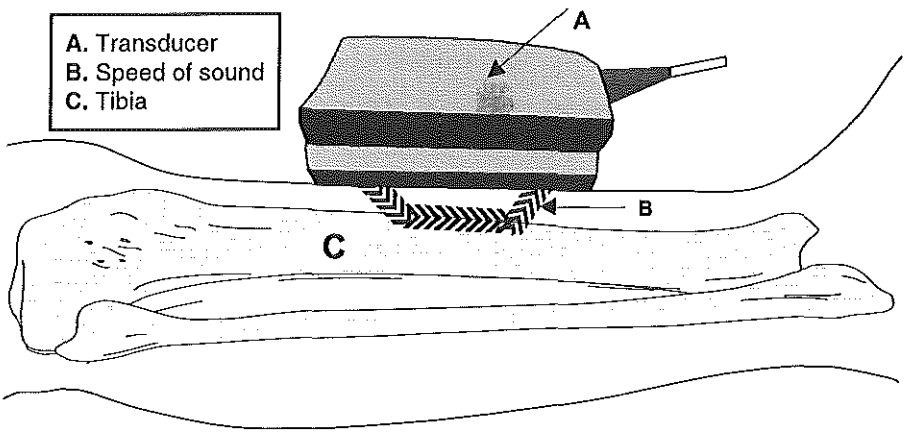



**Fig. 3** Principle of tibial quantitative ultrasonometry.

## 1.3 AIMS OF THE STUDY

The aims of the study presented in this thesis are to answer the following questions:

- Can quantitative bone assessment be validated in children, aged 6-19 years, using the tibia ultrasound device, the SoundScan®Compact?
- Can we generate normal, reference values in a healthy Caucasian pediatric population?
- What is the short- and long-term reproducibility of the system and which factors influence QUS bone assessment measurements?
- Do SOS values obtained with the tibia ultrasound device correlate well with bone density values obtained by phalangeal radiographic absorptiometry (method Trouerbach et al.) and DXA?
- What is the correlation between two different ultrasound devices, which measure at two different sites, the tibia and the calcaneus?
- Are there clinical applications for this tibial ultrasound bone assessment technique?

## 1.4 OUTLINE OF THIS DISSERTATION

In ***Chapter 2*** we review in depth all quantitative ultrasound (QUS) bone assessment techniques in children, especially the coupling gel technique using the SoundScan®Compact at the tibia. In ***Chapter 3*** the short- and long-term reproducibility of this system will be discussed, along with an overview of the factors which can influence tibial QUS bone assessment measurements. Normal values for quantitative tibial ultrasound bone assessment in a Caucasian pediatric population are shown in ***Chapter 4.*** Comparison of quantitative tibial ultrasound bone assessment with dual-energy X-ray absorptiometry (DXA) and radiographic absorptiometry (RA) in healthy Caucasian children are given in ***Chapter 5*** and ***Chapter 6*** respectively. In ***Chapter 7*** there is a comparison between the tibia ultrasound device and a calcaneus ultrasound device in normal healthy children. In ***Chapter 8*** longitudinal measurement results of a part of our normal, healthy children population will be presented. Clinical applications of tibial ultrasonometry in patients with acute lymphoblastic leukemia will be presented in the next two chapters: In ***Chapter 9*** cross-sectional results of our comparison between DXA and tibial ultrasonometry in long-term survivors of acute lymphoblastic leukemia in childhood will be presented. A longitudinal study using tibial ultrasonometry as a bone assessment technique in children with acute lymphoblastic leukemia will be presented in ***Chapter 10.***

Finally, a summary and conclusions of this dissertation, in both English and Dutch, will be presented and possibilities for future research are discussed in ***Chapter 11.***

## 1.5 REFERENCES


1. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. Lancet 1993;341:72-5.
2. WHO study group. Assessment of fracture risk and it's application to screening for postmenopausal osteoporosis. Geneva: WHO Technical reports series, 1994.
3. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: state of the art [review]. J Bone Miner Res 1996;11(6):707-30.
4. Kanis JA, Melton III LJ, Christiansen C, Johnston CC, Khaltaev N. Perspective: The diagnosis of osteoporosis. J Bone Miner Res 1994;9(8):1137-41.
5. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med 1991;325:1597-1600.
6. Bonjour J-P, Theintz G, Buchs B, Slosman D, Rizolli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991;73:555-63.
7. Glastre C, Braillon P, David L, Cochat P, Meunier PJ, Delmas PD. Measurement of bone mineral content of the lumbar spine by dual energy X-ray absorptiometry in normal children: correlations with growth parameters. J Clin Endocrinol Metab 1990;70:1330-3.
8. Lu PW, Briody JN, Ogle GD. Bone mineral density of total body, spine, and femoral neck in children and young adults: cross-sectional and longitudinal study. J Bone Miner Res 1994;9:1451-1458.
9. Teegarden D, Proulx WR, Martin BR. Peak bone mass in young women. J Bone Miner Res 1995;10:711-715.
10. Matkovic V, Jelic T, Wardlaw GM. Timing of peak bone mass in Caucasian females and its implication for prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994;93:799-808.
11. Luckey MM, Meier DE, Madeli JP, DaCosta MC, Hubbard ML, Goldsmith SJ. Radial and vertebral bone density in white and black women: evidence for racial differences in premenopausal bone homeostasis. J Clin Endocrinol Metab 1989;69(4):762-70.
12. Krall EA. Heritable and lifestyle determinants of bone mineral density. J Bone Miner Res 1993;8:1-9.
13. Barnett E, Nordin BEC. The radiological diagnosis of osteoporosis: a new approach. Clin Radiol 1960;11:166-174.
14. Horsman A, Simpson M. The measurement of sequential changes in cortical bone geometry. Br J Radiol 1973;48:471-476.
15. Bloom RA, Pogrund H, Libson E. Radiogrammetry of the metacarpal: a critical reappraisal. Skeletal Radiol 1983;10:5-9.
16. Faulkner KG, Cummings SR, Black D, Palermo L, Glüer CC, Genant HK. Simple measurement of femoral geometry predicts hip fracture: the study of osteoporotic fractures. J Bone Miner Res 1993;8(10):1211-7.

17. Yarbrough C, Martorell R, Klein RE. Stature and age as factors in the growth of second metacarpal cortical bone in moderatly malnourished children. Ann Human Biol 1977;4(1):43-8.
18. Cosman F, Herrington B, Himmelstein S, Lindsay R. Radiographic absorptiometry: a simple method for determination of bone mass. Osteoporosis Int 1991;2:34-8.
19. Hayashi T, Satoh H, Soga T, Tanaka D, Itabashi K, Okuyama K. Evaluation of bone density in newborn infants by computed X-ray densitometry. J Pediatr Gastroenterol Nutr 1996;23:130-4.
20. Matsumoto C, Kushida K, Yamazaki K, Imose K, Inoue T. Metacarpal bone mass in normal and osteoporotic Japanese women using computed X-ray densitometry. Calcif Tissue Int 1995;55:324-9.
21. Strid K, Kalebo P. Bone mass determination from microradiographs by computer-assisted videodensitometry I. Methodology. Acta Radiologica 1988;29:465-472.
22. Trouerbach WT, Birkenhäger JC, Schmitz PIM, et al. A cross-sectional study of age related loss of mineral content of phalangeal bone in men and women. Skeletal Radiol 1988;17:338-43.
23. Trouerbach WT, Grashuis JL, Zwamborn AW, Clermonts ECGM, Schouten JA. Microdensitometric analysis of bone structure in X-ray images. Skeletal Radiol 1987;16:190-5.
24. Morgan D, Spiers F, Pulvertaft C, Fourman P. The amount of bone in the metacrapal and phalanx according to age and sex. Clin Radiol 1967;18:101-108 .
25. Trouerbach WT, Birkenhäger JC, Collette BJA, Drogendijk AC, Schmitz PIM, Zwamborn AW. A study on the phalanx bone mineral content in 273 normal pre- and postmenopausal females (transverse study of age-dependend bone loss). Bone Miner 1987;3:53-62.
26. van Rijn RR, Lequin MH, van Leeuwen WJ, Hop WCJ, van Kuijk C. Radiographic absorptiometry of the Middle Phalanx (digitII) in a Caucasian pediatric population: normative data. Osteoporosis Int 2000;11(3):240-247.
27. Colbert C, Bachtell R. Non-invasive measurements of bone mass and their clinical applications. Boca Raton, Florida USA: CRC Press, 1981.
28. Steel SA, Thorpe JA, Walker R, Howey S, Langton CM. Development and evaluation of a phantom for morphometric X-ray absorptiometry. Osteoporosis Int 1999;9(1):38-44.
29. Grampp S, Lang P, Jergas M, et al. Assessment of the skeletal status by peripheral quantitative computed tomography of the forearm: short-term precision *in vivo* and comparison to dual X-ray absorptiometry. J Bone Miner Res 1995;10(10):1566-76.
30. Jergas M, Genant HK. Current methods and recent advances in the diagnosis of osteoporosis. Arthritis Rheum 1993;36:1649-1662.
31. Trouerbach WT, Man SAd, Gommers D, Zwamborn AW, Grobbee DE. Determinants of bone mineral content in childhood. Bone Miner 1991;13:55-67.

32. van Teunenbroek A, Mulder P, de Muinck Keizer-Schrama S, et al. Radiographic absorptiometry of the phalanges in healthy children and girls with Turner syndrome. Bone 1995;17(1):71-8.
33. Versluis RG, Petri H, Vismans FJ, van de Ven CM, Springer MP, Papapoulos SE. The relationship between phalangeal bone density and vertebral deformities. Calcif Tissue Int 2000;66(1):1-4.
34. Cameron JR, Sorensen J. Measurement of bone mineral *in vivo*: an improved method. Science 1963;143:230.
35. Bjarnason K, Nilas L, Hassager C, Christiansen C. Dual energy x-ray absorptiometry of the spine-decubitus lateral versus anteroposterior projection in osteoporotic women: Comparison to single energy x-ray absorptiometry of the forearm. Bone 1995;16:255-260.
36. Kelly TL, Crane G, Baran DT. Single X-ray Absorptiometry of the forearm: precision correlation and reference data. Calcif Tissue Int 1994;54:212-8.
37. Ross P, Huang C, Davis J, et al. Predicting vertebral deformity using bone densitometry at various skeletal sites and calcaneus ultrasound. Bone 1995;16(3):325-32.
38. Schlenker RA, Von Seggen WW. The distribution of cortical and trabecular bone mass along the lengths of the radius and ulna and the implications for *in vivo* bone mass measurements. Calcif Tissue Res 1976;20:41-52
39. Vogel JM. Application principles and technical considerations in SPA. In: Genant HK, ed. Osteoporosis Update 1987. San Francisco, California, USA: Radiology Research and Education Foundation, 1987: 219-231.
40. Wahner HW, Eastell R, Riggs BL. Bone mineral density of the radius: Where do we stand? J Nucl Med 1985;26:1339-1341.
41. Mazess RB. Computers used for single and dual absorptiometry. In: Gelfand MJ, Thomas SR, eds. Use of computers in nuclear medicine. New York: Hill, 1988: 503-19.
42. Suominen H, Heikkinen E, Vaino P, Lahtinen T. Mineral density of calcaneus in men at different ages: A population study with special reference to life-style factors. Age Ageing 1984;13:273-281.
43. Cheng S, Suominen H, Era P, Heikkinen E. Bone density of the calcaneus and fractures in 75- and 80-year-old men and women. Osteoporosis Int 1994;4:48-54.
44. Vogel JM, Wasnich RD, Ross PD. The clinical relevance of calcaneus bone mineral measurement: a review. Bone Miner 1988;5:35-58.
45. Wasnich R. Fracture prediction with bone mass measurements. In: Genant HK, ed. Osteoporosis Update 1987. San Francisco, California, USA: Radiology Research and Education Foundation, 1987: 95-101.
46. Henderson RC. Assessment of bone mineral content in children. J Pediatr Orthop 1991;11:314-7.
47. Wahner HW, Dunn WL, Mazess RB, et al. Dual-photon Gd-153 absorptiometry of bone. Radiology 1985;156:203-6.

48. Stein JA, Lazewatsky JL, Hochberg AM. Dual-energy x-ray bone densitometer incorporating an internal reference system. Radiology 1987;165:313.
49. Kelly TL, Slovik BM, Schoenfeld DA, Neer RM. Quantitative digital radiography versus dual photon absorptiometry of the lumbar spine. J Clin Endocrinol Metab 1988;67(4):839-44.
50. Wahner HW, Fogelmann I. The evaluation of osteoporosis: dual-energy X-ray absorptiometry in clinical practice. Cambridge: University Press, 1994.
51. Ho CP, Kim RW, Schaffler MB, Sartoris DJ. Accuracy of dual-energy radiographic absorptiometry of the lumbar spine: cadaver study. Radiology 1990;176:171-3.
52. Glüer CC, Steiger P, Selvidge R, Elliesen-Kliefoth K, Hayashi C, Genant HK. Comparative assessment of dual photon absorptiometry and dual-energy radiography. Radiology 1990;174(1):223-8.
53. Cullum ID, Ryder JP, Ell PJ. X-ray dual-photon absorptiometry: a new method for the measurement of bone density. Br J Radiol 1989;62:587-92.
54. Wahner HW, Dunn WL, Broen ML, Morin RL, Riggs BL. Comparison of dual-energy X-ray absorptiometry and dual photon absorptiometry for bone mineral measurement of the lumbar spine. Mayo Clin Proc 1988;63:1075-84.
55. Lilley J, Walters BG, Heath DA, Droic Z. *in vivo* and *in vitro* precision for bone density measured by dual-energy X-ray absorption. Osteoporosis Int 1991;1:141-6.
56. Orwoll ES, Oviatt SK. Longitudinal precision of dual-energy X-ray absorptiometry in a multicenter study. J Bone Miner Res 1991;6(2):191-7.
57. Njeh CF, Fuerst T, Hans D, Blake GM, Genant HK. Radiation exposure in bone mineral density assessment. Appl Radiat Isot 1999;50(1):215-36.
58. Black DM, Reiss TF, Nevitt MC, Cauley J, Karpf D, Cummings SR. Design of the fracture intervention trial. Osteoporosis Int 1993;3(suppl):S29-39.
59. Steiger P, Cummings SR, Black DM, Spencer NE, Genant HK. Age-related decrements in bone mineral density in women over 65. J Bone Miner Res 1992;7(6):625-32.
60. Sabatier JP, Guaydier-Souquieres G, Benmalek A, Marcelli C. Evolution of lumbar bone mineral content during adolescence and adulthood: a longitudinal study in 395 healthy females 10-24 years of age and 206 premenopausal women. Osteoporosis Int 1999;9(6):476-82.
61. Drinka PJ, DeSmet AA, Bauwens SF, Rogot A. The effect of overlying calcification on lumbar bone densitometry. Calcif Tissue Int 1992;50:507-10.
62. Frye MA, Melton III LJ, Bryant SC, et al. Osteoporosis and calcification of the aorta. Bone Miner 1992;19:185-94.
63. Whitehouse RW, Karantanas A, Adams JE. Discrepancies in spinal bone mass measured by QCT and DXA. In: Ring EFG, ed. Current research in osteoporosis and bone mineral measurement II. London, U.K.: British Institute of Radiology, 1992: 26.
64. Ryan PJ, Evans P, Blake GM, Fogeman I. The efffect of vertebral collaps on spinal bone mineral density measurement in osteoporosis. Bone Miner 1992;18:267-72.

65. Finkelstein J, Cleary RL, Butler J, et al. A comparison of lateral versus anterior-posterior spine dual x-ray absorptiometry for the diagnosis of osteopenia. J Clin Endocrinol Metab 1994;78:724-730.
66. Yu W, Glüer CC, Grammp S, et al. Spinal bone mineral assessment in postmenopausal women: a comparison between dual X-ray absorptiometry and quantitative computed tomography. Osteoporosis Int 1995;5:433-9.
67. Hangartner TN, Johnston CC. Influence of fat on bone measurement with dual-energy absorptiometry. Bone Miner 1990;9:71-81.
68. Tothill P, Pye DW. Errors due to non-uniform distribution of fat in dual X-ray absorptiometry of the lumbar spine. Br J Radiol 1992;65:807-13.
69. Larnach TA, Boyd SJ, Smart RC, Butler SP, Rohl PG, Diamond TH. Reproducibility of lateral spine scans using dual-energy X-ray absorptiometry. Calcif Tissue Int 1992;51:255-8.
70. Engelke K, Glüer CC, Genant HK. Factors influencing short-term precision of dual X-ray bone absorptiometry (DXA) of the spine. Calcif Tissue Int 1995;56:19-25.
71. Jergas M, Breitenseher M, Glüer CC, et al. Which vertebrae should be assessed using lateral dual-energy x-ray absorptiometry of the lumbar spine? Osteoporosis Int 1995;5:196-204.
72. Blake GM, Herd RJM, Fogelman I. A longitudinal study of supine lateral DXA of the lumbar spine: A comparison with posteroanterior spine, hip and total-body DXA. Osteoporosis Int 1996;6:462-70.
73. Duboeuf F, Pommet R, Meunier PJ, Delmas PD. Dual-energy x-ray absorptiometry of the spine in anteroposterior and lateral projections. Osteoporosis Int 1994;4:110-116.
74. Guglielmi G, Grimston SK, Fischer KC, Pacifici R. Osteoporosis: Diagnosis with lateral and posteroanterior dual X-ray absorptiometry compared with quantative CT. Radiology 1994;192:845-850.
75. Mazess RB, Barden HS, Eberle RW, Drue Denton M. Age changes of spine density in posterior-anterior and lateral projections in normal woman. Calcif Tissue Int 1995;56:201-205.
76. Genant HK, Grampp S, Glüer CC, et al. Universal standardization for dual x-ray absorptiometry: Patient and phantom cross-calibration results. J Bone Miner Res 1994;9:1503-1514.
77. Tothill P, Laskey MA, Orphanidou CI, van Wijk M. Anomalies in dual energy X-ray absorptiometry measurements of total-body bone mineral during weight change using Lunar, Hologic and Norland instruments. Br J Radiol 1999;72(859).
78. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997;12(11):1908-21.

79. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997;82:57-62.
80. Wang J, Thornton JC, Horlick M, Formica C, Wang W, Pierson RNJ. Dual-energy X-Ray Absorptiometry in Pediatric Studies: Changing Scan Modes Alters Bone and Body Composition Measurements. J Clin Densitom 1999;2(2):135-141.
81. del Rio L, Carrascosa A, Pons F, Gusinye M, Yeste D, Domenech FM. Bone mineral density of the lumbar spine in white Mediterranean Spanish children and adolescents: changes related to age, sex and puberty. Pediatr Res 1994;35:362-366.
82. Kröger H, Kotaniemi A, Vainio P, Alhava E. Bone densitometry of the spine and femur in children by dual-energy X-ray absorptiometry. Bone Miner 1992;17:75-85.
83. Boot AM, Bouquet J, de Ridder MAJ, Krenning EP, de Muink Keizer-Schrama SMPF. Determinants of body composition, measured by dual-energy x-ray absorptiometry, in Dutch children and adolescents. Am J Clin Nutr 1997;66:232-8.
84. Southard RN, Morris JD, Mahan JD, et al. Bone mass in healthy children: measurement with quantitative DXA. Radiology 1991;179:735-8.
85. Schonau E, Wentzik U, Michalk D, Scheidhauer K, Klein K. Is there an increase of bone density in children? Lancet 1993;324:689-690.
86. Zanchetta JR, Plotkin H, Alvarez Filgueira ML. Bone mass in children: normative values for the 2-20-years-old population. Bone 1995;14:35-3995.
87. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998;26:177-182.
88. Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy x-ray absorptiometry for total-body and regional bone mineral density and soft tissue composition. Am J Clin Nutr 1990;51:1106-12.
89. Genant HK, Cann CE, Ettinger B, Gordan GS. Quantitative computed tomography of vertebral spongiosa: a sensitive method for detecting early bone loss after oophorectomy. Ann Int Med 1982;97:699-705.
90. Genant HK. Quantitative computed tomography: update 1987. Calcif Tissue Int 1987;41:179-86.
91. Block JE, Piel CF, Selvidge R, Genant HK. Familial hypophosphatemic rickets: bone mass measurements in children following therapy with calcitrol and supplemental phosphate. Calcif Tissue Int 1989;44:86-92.
92. Kalender WA, Klotz E, Suess C. Vertebral bone mineral analysis: an integrated approach with CT. Radiology 1987;164:419-23.
93. Pacifici R, Rupich R, Griffin M, Chines A, Susman N, Avioli LV. Dual-energy radiography versus quantitative computer tomography for the diagnosis of osteoporosis. J Clin Endocrinol Metab 1990;70(3):705-10.
94. Kalender WA, Brestowsky H, Felsenberg D. Bone mineral measurement: automated determination of midvertebral CT section. Radiology 1988;168:219-21.

95. Genant HK, Boyd D. Quantitative bone mineral analysis using dual-energy computed tomography. Invest Radiol 1977;12(6):545-51.
96. Glüer CC, Reiser UJ, Davis CA, Rutt BK, Genant HK. Vertebral mineral determination by quantitative computed tomography (QCT): accuracy of single and dual-energy measurements. J Comput Assist Radiol 1988;12(2):242-58.
97. Glüer CC, Genant HK. Impact of marrow fat on accuracy of quantitative CT. J Comput Assist Radiol 1989;13(6):1023-35.
98. Goodsitt MM, Rosenthal DI, Reinus WR, Coumas J. Two postprocessing CT techniques for determining the composition of trabecular bone. Invest Radiol 1987;22(3):209-15.
99. van Kuijk C, Grashuis JL, Steenbeek JM, Schütte HE, Trouerbach WT. Evaluation of postprocessing dual-energy methods in quantitative computed tomography. Part II: practical aspects. Invest Radiol 1990;25(8):882-9.
100. van Kuijk C. Evaluation of postprocessing dual-energy quantitative computed tomography: Erasmus University, 1991.
101. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE. Peak trabecular vertebral density: a comparison of adolescent and adult females. Calcif Tissue Int 1988;43:260-2.
102. Kovanlikaya A, Loro ML, Hangartner TN, Reynolds RA, Roe TF, Gilsanz V. Osteopenia in children: CT assessment. Radiology 1996;198:781-784.
103. Yu W, Qin M, Xu L, et al. Normal changes in spinal bone mineral density in a Chinese population: assessment by quantitative computed tomography and dual-energy X-ray absorptiometry. Osteoporosis Int 1999;9(2):179-87.
104. Dyson ED, Jackson CK, Whitehouse WJ. Scanning electron microscope studies of human trabecular bone. Nature 1970;225:707-30.
105. Gong JK, Arnold JS, Cohn SH. Composition of trabecular and cortical bone. Anat Rec 1964;149:325-32.
106. Hangartner TN, Gilsanz V. Evaluation of cortical bone by computed tomography. J Bone Miner Res 1996;11(10):1518-25.
107. Gilsanz V, Boechat MI, Gilsanz R, Loro ML, Sayre JW, Goodman WG. Gender difference in vertebral body sizes in children and adolescents. Radiology 1994;190:673-7.
108. Sparado JA, Werner FW, Brenner RA, Fortino MD, Fay LA, Edwards WT. Cortical and trabecular bone contribute strength to the osteopenic distal radius. J Orthop Res 1994;12:211-218.
109. Ruegsegger P, Durand EP, Dambacher MA. Differantial effects of aging and disease on trabecular and compact bone density of the radius. Bone 1991;12:99-105.
110. Davis CA, Genant HK, Dunham JS. The effect of bone on proton NMR relaxation times of surrounding liquids. Invest Radiol 1992;21(6):472-7.
111. Majumdar S, Newitt D, Mathur A, et al. Magnetic resonance imaging of trabecular bone structure in the distal radius: relationship with X-ray tomographic microscopy and biomechanics. Osteoporosis Int 1996;6(5):376-85.

112. Chung HW, Wehrli FW, Williams JL, Wehrli SL. Three-dimensional nuclear magnetic resonance microimaging of trabecular bone. J Bone Miner Res 1995;10(10):1452-61.
113. Hong J, Hipp JA, Mulkern RV, Jaramillo D, Snyder BD. Magnetic resonance imaging measurements of bone density and cross-sectional geometry. Calcif Tissue Int 2000;66(1):74-8.
114. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995;17(4):363-7.
115. van Daele PLA, Burger H, de Laet CEDH, et al. Longitudinal changes in ultrasound parameters of the calcaneus. Osteoporosis Int 1997;7(3):207-12.
116. Herd RJM, Ramalingham T, Ryan PJ, Fogelman I, Blake GM. Measurement of broadband ultrasound attenuation in the calcaneus in premenopausal and postmenopausal women. Osteoporosis Int 1992;2:247-51.
117. Simkin A. Ultrasonic velocity along the tibial shaft as a diagnostic tool for osteoporosis and a predictor of hip fractures, 1993.

CHAPTER 2

# QUANTITATIVE ULTRASONOMETRY IN CHILDREN

## 2.1 INTRODUCTION

Almost all of the non-invasive techniques discussed in Chapter 1 provide information about the quantity of bone density, not about quality. Several studies suggest that quantitative ultrasound (QUS) has the ability to investigate not only bone density but also bone structure[1,2]. This is important because studies have shown that bone density accounts for only 70-80% of the variability in the strength of a bone. The remaining variance may be due to other factors such as ineffective bone architecture, fatigue damage, measurement artifacts and state of remodeling [3,4]. Therefore a non-invasive bone assessment technique, which can detect fragility and not just decreased bone mass, would be an important advance. QUS seems to be such a technique, as it can predict the fracture risk, using a combination of information on bone elasticity, structure and density [5,6]. Other advantages of QUS equipment are its low cost, ease of use, and patient friendly and radiation free nature. The combination of these benefits and promising preliminary clinical results concerning fracture prediction, encouraged further basic investigation and commercial development. Currently QUS devices have been used to a moderate degree in Europe and Asia but not in the United States. Recently the U.S. Food and Drug Administration (FDA), have approved some QUS equipment for clinical use which will encourage more basic and clinical investigations in this field.

Two ultrasound techniques for bone assessment have been developed: the reflection technique and the transmission technique [7,8]. Currently almost all commercial systems use the transmission technique. This method measures sound transmission in the tissue between two ultrasound transducers (a transmitter and receiver). QUS systems measure ultrasound parameters primarily in trabecular bone at the calcaneus and patella and in cortical bone at the tibia and integral at the phalanges.

QUS systems use a mechanical wave vibrating at a frequency range from 20,000 waves/s (20kHz) to 100,000,000 waves/s (100 MHz). These waves produce vibrations in the investigated bone on a micro-scale..

The physical and mechanical properties of this piece of bone change the shape, the intensity and the speed of the propagating mechanical wave. Therefore the measured QUS parameters are the ultrasound velocity and/or the frequency dependence of the attenuation of the ultrasound signal.

*Velocity of ultrasound*

Ultrasound velocity, or speed of sound (SOS), through bone is determined as the quotient of transmit time and body part width or length and is quoted in meters per second (m/s). SOS depends both on the material properties of the investigated medium through which the signal is propagated and on its mode of propagation. The greater the connectivity or complexity of the material, the greater will be the velocity of the ultrasound wave through this material. Thus, normal bone will have a higher velocity than osteoporotic bone. The relation between SOS and the mechanical properties of a material can be expressed by the equation:

$$SOS = \sqrt{\frac{E}{\rho}}$$

E is the modulus of elasticity (a measure of resistance to deformation) and is correlated with bone density (ρ) [7,9].

Using SOS as measuring tool introduces some problems. First, the value of SOS differs when using different instruments because disparate algorithms are employed. Second, there is confusion about the term "velocity of ultrasound", some manufacturers use SOS, while others use apparent velocity of ultrasound (AVU) or ultrasound transmission velocity (UTV). Moreover in calcaneus measurements three different methods of calculating velocity have been utilized. This results in three velocity measurements: the heel velocity (calcaneus plus soft tissue); bone velocity (calcaneus only); and time of flight velocity (between transducers positioned at a fixed distance and assuming a constant heel thickness)[10,11]. The three velocity calculations correlate strongly and have slightly different values within a range from 1400 to 1900 m/s [12,13].

*Attenuation and broadband ultrasound attenuation (BUA)*

Attenuation of ultrasound signal occurs as energy is removed from the wave by beam spreading (diffraction), scattering, mode conversion and absorption in the bone, marrow and soft tissue [14]. The predominant attenuation mechanism in trabecular bone is scattering while absorption predominates in cortical bone [8,15]. BUA is determined at the calcaneus and is a measure of the frequency dependence of the attenuation of ultrasound. This dependence is approximately linear over the range 0.1-1 MHz. The increase in attenuation as a function of frequency is measured by comparing the amplitude spectrum for a reference material with that for the measured sample. BUA is defined as the slope of attenuation given by linear regression of the spectral amplitude difference, expressed in the units of decibels per MHz. In contrast to velocity, which is influenced by bone density and elasticity, BUA is determined by bone density and bone microarchitecture. The more complex the bone structure, the more the ultrasound wave passing through it, will be attenuated. Therefore normal bone will have a higher attenuation than osteoporotic bone.

Recently some manufacturers of QUS equipment have introduced a newly calculated parameter, which is a combination of SOS and BUA. This parameter is named either "stiffness" (which should not be confused with the biomechanical term) or "quantitative ultrasound index" (QUI). This combination of SOS and BUA improved the precision of the QUS systems and simplified the interpretation of the measurements, especially for the clinicians working with these systems. The precision error of measurements *in vivo* using the transmission ultrasound velocity is about of 0.3-1.5% [12,16]. For BUA the precision error measurement is about 0.4-4.0% and for "stiffness" 0.1-2.1% [1,16-18].

The currently available QUS systems are:

- Calcaneus fixed, single point transmission system employing either a water-bath or ultrasonic coupling gel;
- Calcaneal transmission system using a pair of scanning focused transducers immersed in a water-bath at room temperature;
- Single point QUS devices measuring at the finger phalanges using coupling gel. The system measures the amplitude dependent speed of sound through the distal metaphysis of the phalanges of four fingers;

- Tibial ultrasound transmission system measuring the speed of sound longitudinally along the middle third of the anterior tibia;
- Devices measuring at the patella with the transmission technique; and
- Device measuring at the ulna using the reflection technique.

## 2.2 REVIEW OF THE LITERATURE

Clinical studies have employed QUS to assess bone mass in adult women, pregnant women, patients with primary hyperparathyroidism, and children [19-22]. The *in vivo* studies in the adult population confirm the relationship between the QUS parameters and bone density. Using a variety of techniques, numerous studies in the adult population found correlation coefficients, compared with DXA, ranging from 0.33 to 0.83 for the lumbar spine BMD and 0.30 to 0.87 for the femoral neck BMD [16,19,23,24]. The range of the correlation coefficient at site-matched comparison between BUA and BMD at the calcaneus is 0.56-0.75 [25,26]. These moderate, but significant, correlation coefficients between BUA and BMD confirm the findings, seen in the *in vitro* studies, that QUS has a stronger relationship with bone structure and strength than can be explained solely by BMD [24]. Because of this unexplained component, which may be related to bone strength or structure or to some other parameter unrelated to osteoporosis, the question arises whether QUS can accurately predict BMD *per se* [27].

Clinical studies showed that QUS is useful for discriminating between individuals with fractures and without [28]. Using the velocity parameter measurements at the patella, tibia, or phalanges one can identify individuals with prevalent vertebral fractures with the same effectiveness as conventional bone mass measurements at the spine, hip, or forearm [29-31]. Also some *in vitro* studies confirm the potential utility of QUS for bone assessment and fracture risk prediction [9,24,32,33]. The majority of these *in vitro* and *in vivo* studies have been done with QUS systems at the calcaneus. Prospective studies are still needed to evaluate the ability of the QUS systems at the tibia and phalanges to predict fracture risk. A large prospective study at the calcaneus showed that SOS and BUA have the same power to predict the risk of hip fractures as BMD measured with DXA at this site [34,35]. Even after adjusting BUA and SOS for neck BMD, both ultrasonic parameters were still significant independent predictors

of hip fractures. Also the combination of BUA and femoral neck BMD improves the detection of women at a high risk for hip fractures.
Whether QUS is useful for monitoring treatment of osteoporosis or drug effects is not yet certain [36]. Therefore, further longitudinal studies are required.
Currently the majority of the clinical studies have been done in adults, only a few in children [37-39].

## 2.3 SOUNDSCAN®COMPACT USED FOR TIBIAL ULTRASOUND SCANNING

Using the transmission technique with a 250 kHz pulse, the SoundScan® Compact (Figure 1) measures the transit time, or speed of sound (SOS), through the cortical layer at the anterior mid-tibial site over a defined linear distance of 50 mm, parallel to the tibial axis.

**Fig. 1** SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel).

Due to its special design, this system eliminates the soft tissue error when the distance between the transducer and the bone is less than 15 mm. The distance between the bone and the transducer is measured by two "depth finders" within the transducer, using 1 MHz echo pulses. The system is highly sensitive to changes in this distance, and takes a reading only when these two distances are equal, which is the definition of alignment. The threshold value of the signal level is 0.5 volts and the difference in signal level between the two depth finders may not exceed 1.0 volts. The transducer is placed at the anterior mid-tibial point. This point is midway between the distal apex of the patella

and medial malleolus, identified by palpation with the leg in extended position. At this level the transducer is moved back and forth perpendicular to the axis drawn between the apex of the patella and the medial malleolus. The aim of this finely-controlled manipulation is to find the peak of bone velocity. At least 150 readings are necessary to obtain a good result and the SOS (m/s) is the average of the five highest readings attained during the whole session. The procedure typically takes less than five minutes. To date this technique has only been validated in adults [40-43]. All of these studies report a good precision not only *in vitro* but also *in vivo*. Even using the standardized coefficient of variation, which corrects for measurement results with a large offset from zero (e.g. SOS at the tibia compared with the other ultrasound techniques at other sites of the body) the precision of the SoundScan®Compact is better compared to BMD measurement techniques [44]. Nonetheless this technique has also some drawbacks. Currently there is no consensus about the clinical use of this tibial ultrasound system or other ultrasound devices in diagnosis of osteoporosis. This is due to uncertainties in assessing accuracy of QUS and in the moderate correlation of densitometric and ultrasonic results [35]. In some studies the correlations of calcaneal SOS and BUA with lumbar spine and femoral neck BMD are better than the correlation between tibial SOS and BMD of the lumbar spine and femoral neck [27,44]. Second, the accuracy of the tibial system is still not clear due to lack of long-term prospective studies. Also monitoring of skeletal changes due to treatment or progression of the disease affecting the bone is difficult due to limited data, not only with the tibial system but also with other ultrasound techniques. In Chapter 10 we present a longitudinal study in children with acute lymphoblastic leukemia, who received high doses of steroids and methotrexate. Another important clinical use of QUS is fracture risk assessment. Several studies in adults show the similar ability of tibial ultrasound to distinguish between fracture patients and controls in comparison of calcaneus ultrasound SOS and BUA measurements and DXA. First a longitudinal study in normal healthy children should be done, to assess the influence of normal growth on the measurements. Such a study will be presented in Chapter 8 of this thesis.

## 2.4 REFERENCES


1. Glüer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 1994;55:46-52.
2. Duquette J, Lin J, Hoffman A, Houde J, Ahmadi S, Baran D. Correlations among bone mineral density, broadband ultrasound attenuation, mechanical indentation, and bone orientation in bovine femoral neck samples. Calcif Tissue Int 1997;60:181-6.
3. Kleerekoper M, Villanueva AR, Stanciu J, Sudhaker-Rao D, Parfitt AM. The role of three-dimensional trabecular microstructure in the pathogenesis of vertebral compression fractures. Calcif Tissue Int 1985;37:594-7.
4. Mosekilde L. Sex diffences in age-related loss of vertebral trabecular bone mass and structure - biomechanical consequences. Bone 1989;10:425-32.
5. Kaufman JJ, Einhorn TA. Perspective: ultrasound assessment of bone. Osteoporosis Int 1993;8:517-525.
6. Hans D, Schott AM, Meunier PJ. Ultrasonic assessment of bone: a review. Eur J Med 1993;2:157-163.
7. Rho JY, Ashman RB, Turner CH. Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements. J Biomechanics 1993;26:111-119.
8. Bamber JC, Tristam M. Diagnostic ultrasound. In: Webb S, ed. The physics of medical imaging. Bristol: Adam Hilger, 1988: 319-386.
9. Njeh CF, Hodgkinson R, Currey JD, Langton CM. Orthogonal relationships between ultrasonic velocity and material properties of bovine cancellous bone. Med Engin Phys 1996;18(5):373-81.
10. Hans D, Schott AM, Arlot ME, Sornay E, Delmas PD, Meunier PJ. Influence of anthropometric parameters on ultrasound measurements of calcaneus. Osteoporosis Int 1995;5:371-375.
11. Kotzki PO, Buyck D, Hans D, et al. Influence of fat on ultrasound measurements of calcaneus. Calcif Tissue Int 1994;54:91-95.
12. Miller CG, Herd RJM, Ramalingham T, Fogelman I, Blake GM. Ultrasonic velocity measurements through the calceneus: which velocity should be measured ? Osteoporosis Int 1993;3:31-5.
13. Waud CE, Lew R, Baran DT. The relationship between ultrasound and densitometric measurement of bone mass at the calcaneus of women. Calcif Tissue Int 1992;51:415-8.
14. Langton CM, Njeh CF, Hodgkinson R, Currey JD. Prediction of mechanical properties of the human calcaneus by broadband ultrasound attenuation. Bone 1996;18(6):495-503.
15. Kinsler LE, Frey AR, Coppens AB, Sanders JV. Fundamentals of acoustics. New York: Wiley, 1992.
16. Faulkner KG, McClung MR, Coleman LJ, Kingston-Sandahl E. Quantitative ultrasound of the heel: Correlation with densitometric measurements at different skeletal sites. Osteoporosis Int 1994;4:42-47.

17. Stewart A, Reid DM, Porter RW. Broadband ultrasound attenuation and dual-energy X-ray absorptiometry in patients with hip fractures: which technique discriminates fracture risk. Calcif Tissue Int 1994;54:466-9.
18. Krieg MA, Thiébaud D, Burckhardt P. Quantitative ultrasound of bone in institutionalized elderly women: a cross-sectional and longitudinal study. Osteoporosis Int 1996;6:189-95.
19. Baran DT. Quantitative ultrasound: a technique to target women with low bone mass for preventive therapy. Am J Med 1995;98(2a):48-51S.
20. Gambaccianni M, Spinetti A, Gallo R. Ultrasonographic bone characteristics during normal pregnancy: longitudinal and cross-sectional evaluation. Am J Obstet Gynecol 1995;173:890-893.
21. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995;56:368-71.
22. Minisola S, Rosso R, Scarda A. Quantitative ultrasound assessment of bone in patients with primary hyperparathyroidism. Calcif Tissue Int 1995;56:526-528.
23. Massie A, Reid DM, Porter RW. Screening for osteoporosis: Comparison between dual-energy x-ray absorptiometry and broadband ultrasound attenuation in 1000 perimenopausal women. Osteoporosis Int 1993;3:107-110.
24. Toyras J, Kröger H, Jurvelin JS. Bone properties as estimated by mineral density, ultrasound attenuation, and velocity. Bone 1999;25(6):725-31.
25. Glüer CC, Vahlensieck M, Faulkner KG, Engelke K, Black D, Genant HK. Site-matched calcaneal measurements of broadband ultrasound attenuation and single x-ray absorptiometry: Do they measure different skeletal properties? J Bone Miner Res 1992;7:1071-1079.
26. Salamone LM, Krall EA, Harris S, Dawson-Hughes B. Comparison of broadband ultrasound attenuation to single x-ray absorptiometry measurements at the calcaneus in post-menopausal women. Calcif Tissue Int 1994;54:87-90.
27. Young H, Howey S, Purdie DW. Broadband ultrasound attenuation compared with dual-energy x-ray absorptiometry in screening for post-menopausal low bone density. Osteoporosis Int 1993;3:160-164.
28. Peretz A, De Maertelaer V, Moris M, Wouters M, Bergmann P. Evaluation of Quantitative Ultrasound and Dual X-Ray Absorptiometry Measurements in Women With and Without Fractures. J Clin Densitom 1999;2(2):127-133.
29. Alenfeld F, Wüster C, Beck C, Ziegler R. Validity of ultrasound measurements of bone mineral density on the phalanges of the hand. Perth International Bone Meeting 1995, Fremanle, Australia: 54.
30. Orgee J, McCloskey EV, Foster H, Coombes G, Khan S, Kanis JA. Tibial ultrasound velocity; a useful clinical measure of skeletal status. J Bone Miner Res 1995;10(suppl 1).
31. Stegman MR, Heaney RP, Recker RR. Comparison of speed of sound ultrasound with single photon absorptiometry for determining fracture odds ratios. J bone Miner Res 1995;10:346-352.

32. Bouxsein ML, Courtney AC, Hayes WC. Ultrasound and densitometry of the calcaneus correlate with the failure loads of cadaveric femurs. Calcif Tissue Int 1995;56:99-103.
33. Bouxsein ML, Radloff SE. Quantitative Ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. J Bone Miner Res 1997;12(5):839-46.
34. Hans D, Dargent P, Schott AM. Ultrasonographic heel measurements to predict hip fracture in elderly women: The EPIDOS prospective study. Lancet 1996;348:511-514.
35. Tromp AM, Smit JH, Deeg DJ, Lips P. Quantitative ultrasound measurements of the tibia and calcaneus in comparison with DXA measurements at various skeletal sites. Osteoporosis Int 1999;9(3):230-5.
36. Rosenthall L, Caminis J, Tenehouse A. Calcaneal ultrasonometry: response to treatment in comparison with dual x-ray absorptiometry measurements of the lumbar spine and femur. Calcif Tissue Int 1999;64(3):200-4.
37. Bayer M, Kutílek S. Ultrasound transmission through the os calcis in children: which side should we measure. Calcif Tissue Int 1998;61(441-2).
38. Mora S, Pitukcheewanont P, Kaufman FR, Nelson JC, Gilsanz V. Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. J Bone Miner Res 1999;14(10):1664-71.
39. Taaffe DR, Duret C, Cooper CS, Marcus R. Comparison of calcaneal ultrasound and DXA in young women. Med Sci Sports Exerc 1999;31(10):1484-9.
40. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995;17(4):363-7.
41. van Daele PLA, Burger H, de Laet CEDH, et al. Longitudinal changes in ultrasound parameters of the calcaneus. Osteoporosis Int 1997;7(3):207-12.
42. Simkin A. Ultrasonic velocity along the tibial shaft as a diagnostic tool for osteoporosis and a predictor of hip fractures, 1993.
43. Funck C, Wüster C, Alenfeld FE, et al. Ultrasound velocity of the tibia in normal German women and hip fracture patients. Calcif Tissue Int 1996;58:390-4.
44. Rosenthal L, Caminis J, Tenehouse A. Correlation of ultrasound velocity in the tibial cortex, calcaneal ultrasonography, and bone mineral density of the spine and femur. Calcif Tissue Int 1996;58:415-8.

# EVALUATION OF SHORT-TERM PRECISION FOR TIBIAL ULTRASONOMETRY


## SUMMARY

Tibial quantitative ultrasonometry is a relatively novel technique in the field of bone sonometry, an emerging alternative to bone densitometry. The implementation of this technique in a pediatric population could prove valuable from a clinical, as well as from a research, point of view. It is necessary to know the precision of this technique and the possible influence on measurements before implementation in clinical practice. This study presents the precision in a Caucasian pediatric population and the influence of measurement site, dexterity, brand of coupling gel, and temperature of coupling gel.

To assess intra- and inter-observer variance duplicate measurements, with repositioning, were performed in ten children over a short period of time. The observers were blinded for the results of the other observer and after each measurement the skin markings were removed. Intra-observer variance for operator one (MHL) was CV 0.43%, and for observer two (SFGR) it was CV 0.43%. The inter-observer variance was CV 0.61%.

Left mid-tibial and right mid-tibial SOS measurements showed no significant differences. There were, however, significant differences in both boys and girls between right proximal vs. right mid-tibial, right mid-tibial vs. right distal and right proximal vs. right distal (for all $P<0.001$).

One-way analysis of variance showed that neither the use of different coupling gels nor an increase in gel temperature had a significant influence on measurements.

The results of our study show that tibial QUS is a highly reproducible technique in a Caucasian pediatric population.


---


M.H. Lequin, MD.[1], R.R. van Rijn, MD.[2], S.G.F. Robben, MD.[1], W.C.J. Hop, Msc. [3],S. Dijkhuis[4], M.M.E.G. Fijten[4], L.A.W. Meijer[4], C. van Kuijk, MD. PhD.[2&5]

Depts. of Pediatric Radiology, University Children's Hospital 'Sophia' Rotterdam[1], Experimental Radiology[2], Epidemiology and Biostatistics[3], Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, School of Higher Vocational Education, Medical Imaging and Radiotherapeutic Techniques[4], Haarlem, the Netherlands and Osteoporosis and Arthritis Research Group[5], University of California, San Francisco, USA.



Part of this study was presented at the Bone and Mineral Workshop, May 1997, Crieff, Scotland.
Published in: Calcif Tissue Int 1999; 64:24-27

## 3.1 INTRODUCTION

Bone densitometry in children has gained interest due to the theory that the higher the attained peak bone mass the longer it will take to reach an osteoporotic state later in life[1-3]. Several bone densitometry techniques are available for an adult population, but for the implementation in a pediatric population several problems have to be solved. First of all, the skeleton shows three-dimensional growth, making interpretation of two-dimensional bone densitometry techniques such as single-energy X-ray absorptiometry (SXA) or dual-energy X-ray absorptiometry (DXA) difficult [4-6]. Secondly, in a pediatric population, reproducibility can be a problem. This can mainly be attributed to motion artifacts, as younger children are usually unable to lay immobile during the investigation.

The implementation of quantitative ultrasonometry (QUS) in a pediatric population could provide a solution to the above mentioned problems. QUS is radiation free, which will enhance patients acceptance, easy to use and therefore patient friendly, and the interactive measurements allow for doctor patient interaction, thus limiting motion artifacts. Before QUS would be implemented in a pediatric population the technique must exhibit a good reproducibility.

There are several systems for bone ultrasonometry in production, almost all exclusively designed for, used in and validated for an adult population [7-13]. Several studies have shown a good correlation between tibial QUS, performed using a SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel), and age related bone loss in an adult population [9,14-18]. Two studies into the association of tibial QUS and appendicular fracture risk showed a significant association [9,15]. These results indicate that tibial QUS could be useful tool to detect osteoporosis at an early stage in the adult population.

In contrast to most QUS systems, tibial QUS by virtue of its design could easily be implemented in children, especially below the age of eight years in contrast to calcaneal ultrasonometry techniques. Two studies have previously reported data on tibial QUS in a pediatric population[19,20]. Both studies showed a positive correlation with age. In our study a linear regression of age versus mid-tibial speed of sound (SOS, $ms^{-1}$) yielded $r=0.69$ for boys and $r=0.79$ for girls[19]. The mean annual change in our Caucasian pediatric population (6-19 years) was

29.0 $ms^{-1}$ for boys and 27.9 $ms^{-1}$ for girls. Our study also showed an association of tibial QUS with Tanner stage, significant differences were for boys found between stages II-III and IV-V and for girls between stages I-II, III-IV and IV-V (all $P<0.01$). These two preliminary studies show that tibial QUS could be implemented in a pediatric population.

In this paper we present the precision of tibial QUS in a pediatric population and three important factors which, from our point of view, could influence measurements. The first factor influencing measurements is the site of measurement. According to standard operational procedures all tibial QUS measurements have to be performed on the right tibia at the mid-tibial point [21]. The second factor is dexterity, this influences the preference for leg use (eg. kicking in soccer or jumping) and may therefore influence bone density of the tibia. Third, the temperature and type of the coupling gel could influence the measurements. In our pediatric radiology department we commonly use coupling gel which is, for patient comfort, heated to body temperature. Throughout our department several different brands of coupling gel are used, if the brand of coupling gel influences the measurements caution should be taken when performing follow-up measurements.

## 3.2 MATERIAL AND METHODS

*Study subjects*

For all study subjects informed consent was obtained explicitly by parental/guardian consent and where appropriate the child's consent (in the Netherlands this is mandatory in children aged 12 years and over). This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines as stated by the Internal Review Board of the University Hospital Rotterdam and Erasmus University Rotterdam, Faculty of Medicine and Health Sciences, the Netherlands.

Precision was assessed according to the FDA guidelines in ten, healthy Caucasian, children (group A) using repeated measurements by two investigators (MHL and SGFR) [22]. The study group consisted of ten children, seven girls and three boys. The age range in this group was 6-19 years (mean: 13.1 years).

The study group (group B) for the assessment of dexterity and measurement site consisted of 53, healthy Caucasian, children. Twenty-three girls mean age 12.8 years (range 6-19 years) and 30 boys, mean age 10.7 years (range 6-17 years).
For the influence of the type and temperature of coupling gel on measurements, one investigator (MMEGF) performed measurements on the verification phantom (part Nº ASY10029 serial Nº 189) provided by Myriad Ultrasound Systems Ltd. For this the gel was heated in an incubator. Temperature was measured using a mercury thermometer placed at the core of the gel tube.

*Quantitative ultrasound*
Tibial QUS was performed using the SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel, software version 1.1e). All measurements were conducted by trained operators.
In group A, duplicate measurements by two investigators (ML and SR), i.e. in total four measurements, of the SOS at the mid-length of the right tibia were performed. These measurements, which took about 5 minutes, were made in a blinded fashion, i.e. the investigators removed the skin marks after each measurement and the results of tibial QUS were not available to the other investigator. Between measurements (time period between measurements for each operator at least one hour) the children were allowed to walk around. Statistical analysis was performed according to the equations recommended in the 'Draft Guidance for Review of Bone Densitometers' by the FDA [22].
Miller et al. proposed the use of standardized CV%'s (SCV%) [23]. This SCV% is defined as the CV% divided by the percentage of the range over the mean
Where the range is taken to be four times the population standard deviation [13].
In group B, the following four tibial QUS measurements were taken: (1). Right mid-tibial (RMT, i.e. the mid-point between the apex of the patella and the distal part of the medial malleolus; (2). Left mid-tibial (LMT); (3). Right proximal (RP) (2 cm above the mid-tibial point) and (4). Right distal (RD) (2 cm below the mid-tibial point). Dexterity was assessed by asking each child which hand was used for writing and medial tasks[24]. The ipsilateral leg was considered to be dominant [25].

For the influence of gel type and temperature, measurements were performed using four different brands of coupling gel:

1. Aquasonic 100 (Parker, Orange, NJ, USA),
2. Ezem Rooster (Ezem Rooster, Dordrecht, the Netherlands),
3. Aquarius 101 (Enraf Nonius, Dordrecht, the Netherlands), and
4. Sonogel (Enraf Nonius, Dordrecht, the Netherlands).

With each gel, triplicate measurements were performed at six different temperatures; 21°C (room temperature), 25°C, 29°C, 33°C, 37°C (body temperature), and 41°C.

*Statistical analyses*

Paired and unpaired data are compared using the paired and unpaired *t*-test respectively. For the evaluation of brands of coupling gel and gel temperature analysis of variance (ANOVA) was used. Correlation coefficients given are Pearson's. Partial correlation coefficients ($r_p$) are calculated to account for common correlations with age. Analysis of variance, taking account of age, was performed to compare boys and girls. $P$=0.05 (two-sided) was considered to be the limit of significance.

## 3.3 RESULTS

Intra-observer variance for operator one (MHL) was 16.3 $ms^{-1}$ (CV 0.43%, SCV 2.3%) and for observer two (SFGR) it was 16.4 $ms^{-1}$ (CV 0.43%, SCV 2.3%). The inter-observer variance was CV 0.61% (SCV 3.3%).

In group B, the measurements of all 53 children were available for analyses. Mean values for SOS, as shown in Figure 1, for none of the measurement sites did the mean SOS in girls differ significantly from boys. Also ANOVA, taking account of age, did not show significant differences between boys and girls. There was, however, a significant increase in SOS from right proximal to right distal ($P$<0.001). LMT and RMT SOS measurements show a significant correlation ($r_{boys}$=0.84, $r_{girls}$=0.92, both $P$<0.001, Figures 2 and 3).

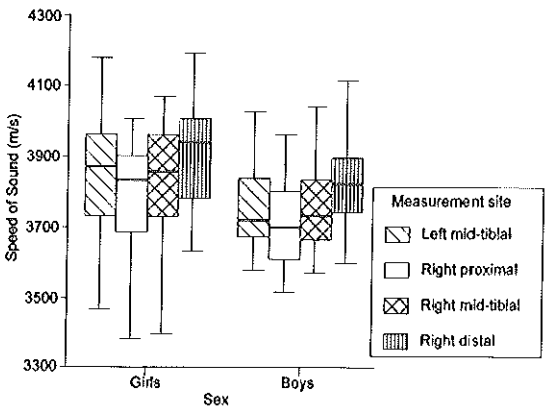



**Fig.1** Tibial QUS for four measurement sites in girls and boys.

These correlations remained highly significant when the correlations were determined while correcting for the mutual correlation with age ($r_{boys}$=0.65, $r_{girls}$=0.69, both $P$<0.001).

Out of 30 boys seven (23 %) and out of 23 girls three (13 %) were left handed. Comparing the mid-tibial SOS between the dominant and non-dominant leg no significant differences were found (paired $t$-test: $P$=0.72). This applied to boys as well as girls. There were also no significant differences between children grouped according to their dexterity regarding RP and RD SOS measurements.

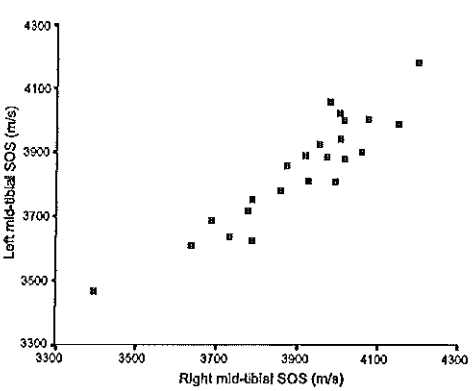



**Fig. 2** Left mid-tibial versus right mid-tibial SOS (m/s) in girls.

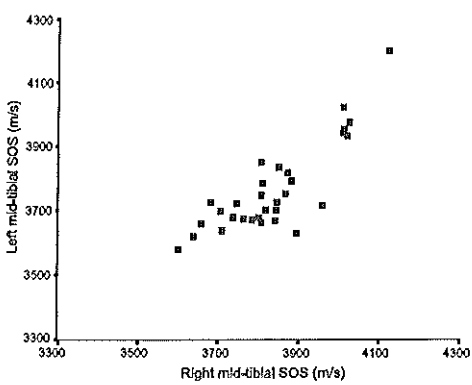



**Fig. 3** Left mid-tibial versus right mid-tibial SOS (m/s) in boys.

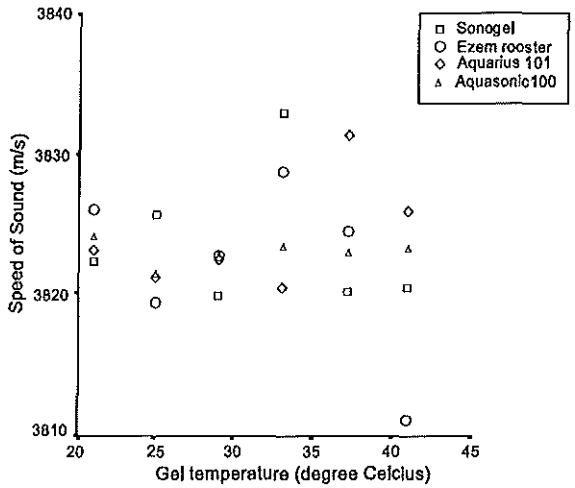



**Fig. 4** Speed of sound (ms-1) versus temperature (°C) for all four gel brands.

Figure 4 shows the influence of brands of coupling gel and temperature on SOS measurements. ANOVA showed no significant trends with increasing temperature. There were also no significant differences between brands of coupling gels.

## 3.4 DISCUSSION

In a study performed by Greenspan et al. the precision of six different calcaneal bone assessment techniques were compared [26]. Three of these techniques also measure SOS, the Achilles (Lunar Corp), the CUBA (McCue Ultrasonics Ltd.) and the UBA 575+ (Hologic Inc.). The intra-observer SCV% of these three techniques ranged from 1.94 to 3.58 % and the inter-observer SCV% from 2.68 to 4.14%. Our SCV% is in the same range as these techniques, showing that it is equally precise. Previous studies using tibial QUS in an adult population showed similar results with respect to the intra-observer precision (range 0.42 - 0.85%) [9,15].

It is well known that as well as all other measurements, tibial QUS has an inherent variability. However, it is unknown if these differences also exist between measurement sites within one person. Our data show that there is no significant difference in SOS between LMT and RMT in a pediatric population,

an influence of dexterity on these measurements was not found. Similar results have been shown in an adult population by Howard et al. (calcaneal ultrasound) and Yang et al. (DXA of the hip) and in a pediatric population by Faulkner et al. (DXA) and Leong et al. (tibial QUS) [27-30]. The higher, not significant, value for SOS in girls compared to boys could be explained by the higher mean age of the girls (12.8 versus 10.7 years).

The strength of our study is that we used physically active, healthy, young children in whom a discrepancy, if there is any, between the dominant and non-dominant leg should easily be found. As a result of this, LMT and RMT measurements can be used in a pooled fashion in future studies. This makes studies evaluating the effect of uni-lateral immobilisation possible.

A second important finding is that measurements 2 cm proximal and distal from the mid-tibial point differ significantly from measurements at the mid-tibial point. It is of the utmost importance to measure the tibial length accurately and measure exactly on the mid-tibial point. An explanation for this finding may be the influence of cortical thickness, which differs from proximal to distal, on ultrasound measurements. Our data show a significant increase in SOS from proximal towards distal. This finding is supported by data presented by Wu et al.[31] and Orgee et al.[13]. A drawback of this part of the study is that we did not investigate measurement sites between RP, RMT and RD. Therefore, we cannot predict the influence of small errors in the range of millimetres, on tibial QUS.

Another important factor in tibial QUS which could be of importance is the cortical thickness and geometry of the tibia [31]. Both factors were not measured in our population. However, if tibial QUS versus skeletal age would be used accordingly to normal growth curves then this factor might be of less importance.

To study the influence of temperature the phantom was chosen to minimize differences between measurements due to different measurement sites. Using ANOVA we found no significant trends of SOS with increasing temperature. There were also no significant differences between the different brands of coupling gel. These results show that for patient comfort the gel may be heated to body temperature, this might be especially important for young children.

In conclusion, this study shows that tibial QUS is a precise technique for bone assessment in a pediatric population. Further studies into the clinical relevance, including correlation with established methods, in this specific population are needed.

*Acknowledgements*
We would like to thank rector Rootstock of the 'Marnix' high school (Rotterdam, the Netherlands) and his pupils for their co-operation in our study. Furthermore we thank Myriad Ultrasound Systems Ltd., Rehovot, Israel, for the use of their equipment.

## 3.5 REFERENCES


1. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. Lancet 1993;341:72-5.
2. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med 1991;325:1597-1600.
3. Kanis JA. Osteoporosis. Oxford: Blackwell Science Ltd., 1994.
4. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE. Peak trabecular vertebral density: a comparison of adolescent and adult females. Calcif Tissue Int 1988;43:260-2.
5. Mazess RB, Cameron JR. Skeletal growth in school children: maturation and bone mass. Am J Phys Anthrop 1971;35:399-408.
6. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997;12(11):1908-21.
7. Cunningham JL, Fordham JN, Hewitt TA, Speed CA. Ultrasound velocity and attenuation at different skeletal sites compared with bone mineral density measured using dual-energy X-ray absorptiometry. Br J Radiol 1996;69:25-32.
8. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995;17(4):363-7.
9. Funck C, Wüster C, Alenfeld FE, et al. Ultrasound velocity of the tibia in normal German women and hip fracture patients. Calcif Tissue Int 1996;58:390-4.
10. Han IK, Yim CH, Chung HY, Kim SW, Han KO, Min HK. Ultrasound velocity measurement of cortical bone. J Bone Miner Res 1995;10(suppl 1).
11. Lorenc RS, Jaworski M. Sex related differences in growing children monitored by ultrasound measurements. Osteoporosis Int 1997;7(3):301.
12. Povoroznjuk VV, Tatarchuk TF, Mazur IP, Tkachenko LP, Orlik TV. Use of ultrasound bone densitometry for evaluation of bone tissue in children. Osteoporosis Int 1997;7(3):298.
13. Orgee JM, Foster H, McCloskey EV, Khan S, Coombes G, Kanis JA. A precise method for the assessment of tibial ultrasound velocity. Osteoporosis Int 1996;6:1-7.
14. Liberman UA, Rimon AB, Keinan D. Ultrasound measurements along the tibia: age-related changes in normal female and male populations and correlation to postmenopausal osteoporotic (PMO) patients. Bone 1995;16(suppl 1):68.
15. Stegman MR, Heaney RP, Travers-Gustafson D, Leist J. Cortical ultrasound velocity as an indicator of bone status. J Bone Miner Res 1995;10(suppl 1).
16. Lee SC, Coan BS, Bouxsein ML. Tibial ultrasound velocity measured in situ predicts the material properties of tibial cortical bone. Bone 1997;21(1):119-125.
17. Simkin A. Ultrasonic velocity along the tibial shaft as a diagnostic tool for osteoporosis and a predictor of hip fractures, 1993.

18. Liberman UA, Rimon A, Noff M, Otremski I, Edelstein S. Detection of osteoporotic bone fragility by measurement of speed of sound (SOS) in human cortical bone with a new ultrasound instrument, 1994.
19. Lequin MH, van Rijn RR, Robben SGF, Keinan DD, van Kuijk C. Quantitative Ultrasound of the tibia: precision in a pediatric population. Osteoporosis Int 1997;7(3):300.
20. Kaga M, Takahashi K, Suzuki H, et al. Utrasonic assessment of tibia in Japanese children and adolescents. Osteoporosis Int. 1997;7(suppl 2):67.
21. Myriad Ultrasound Systems Ltd. Soundscan 2000 user's guide (version v2.1). Rehovot: Myriad Ultrasound Systems Ltd., 1996.
22. Arnaudo JS. Draft guidance for review of bone densitometers 510(k). Guide to clinical trials. New York: Raven, 1992: 24-7.
23. Miller CG, Herd RJM, Ramalingham T, Fogelman I, Blake GM. Ultrasonic velocity measurements through the calceneus: which velocity should be measured ? Osteoporosis Int 1993;3:31-5.
24. Coren S. Measurement of handedness via self-report: it's relationship to limb dominance and age. Percept Mot Skills 1993;76:1035-42.
25. Didia BC, Nyenwe EA. Foot breath in children: its relationship to limb dominance and age. Foot Ankle 1988;8:192-202.
26. Greenspan SL, Bouxsein ML, Melton ME, et al. Precision and discriminatory ability of calcaneal bone assessment technologies. J. Bone Miner. Res. 1997;12(8):1303-1313.
27. Howard GM, Ngyuen TV, Pocock NA, Kelly PJ, Eisman JA. Influence of handedness on calcaneal ultrasound: implications for assessment of osteoporosis and study design. Osteoporosis Int 1997;7(3):190-4.
28. Yang R-S, Tsai K-S, Chieng P-K, Liu T-K. Symmetry of bone mineral density at the proximal femur with emphasis on the effect of side dominance. Calcif Tissue Int 1997;61:189-91.
29. Faulkner RA, Houston CS, Bailey DA, Drinkwater DT, McKay HA, Wilkinson AA. Comparison of bone mineral content and bone mineral density between dominant and non-domininant limbs in children 8-16 years of age. Am J Hum Biol 1993;5:491-9.
30. Leong KH. Ultrasound of the tibia--precision error, left versus right sides and correlation with bone mineral density. Ann Acad Med Singapore 1997;26(6):747-9.
31. Wu CY, Hans DB, He YQ, Fan B, Richards J, Genant HK. Ultrasound velocity reflects combination of cortical thickness, mineral density and surface feature in cortical bone. Osteoporosis Int 1997;7(Suppl 2):63.

## 3.6 ADDENDUM

*Evaluation of long-term precision for tibial ultrasonometry*

As mentioned in the short-term precision part of this chapter the tibial ultrasound device has an excellent short-term precision, also when using the standardized CV%'s (SCV%). Compared to other ultrasound bone mass assessment techniques used at the calcaneus, the tibial ultrasound device showed that our tibial SCV% is in the same range as these techniques, showing that it is equally precise.[1] Previous studies using tibial QUS in an adult population showed similar results with respect to the intra-observer precision (range 0.42 - 0.85%)[2,3]. For longitudinal studies it is essential to know the long-term precision of the technique been used for bone mass assessment. If the long-term measurement error of the technique is to big, it is essentially impossible to interpret results of longitudinal studies, especially when small changes are anticipated. This could be the case in longitudinal studies looking for changes in bone mass in subjects treated with drugs which can affect bone mineral status.

Until today no long-term precision of the tibial ultrasound device has been published. We have long-term phantom data over a period of almost 3 years. In this period 253 times the tibial ultrasound system was calibrated for daily practice. Every time the ultrasound probe was placed on a phantom and about 50 measurements were done. If the mean of these measurements was more than 25 above or below the standardized SOS of the phantom (3805 m/s) the measurement setup failed and the calibration procedure had to be done again till it passed. We did use two probes. With the first probe we did 171 phantom measurements with a mean SOS of 3814 m/s (SD 8 m/s, median 3815 m/s) and with the second probe we did 82 phantom measurements with a mean of 3823 m/s (SD 8 m/s, median 3825 m/s). There is no significant increase or decrease of the phantom measurements in time, looking at the probes separately. Looking at the total phantom measurement time we saw a little, but statistical significant increase of SOS of 9 m/s comparing the second probe against the first probe (Figure 1). Figure 2 shows that this 9 m/s increase in SOS is not clinical significant looking at the wide biological variance of the reference data of normal healthy children (Chapter 4) and the broad range of SOS in children with acute lymphoblastic leukemia (ALL) (Chapter 10).

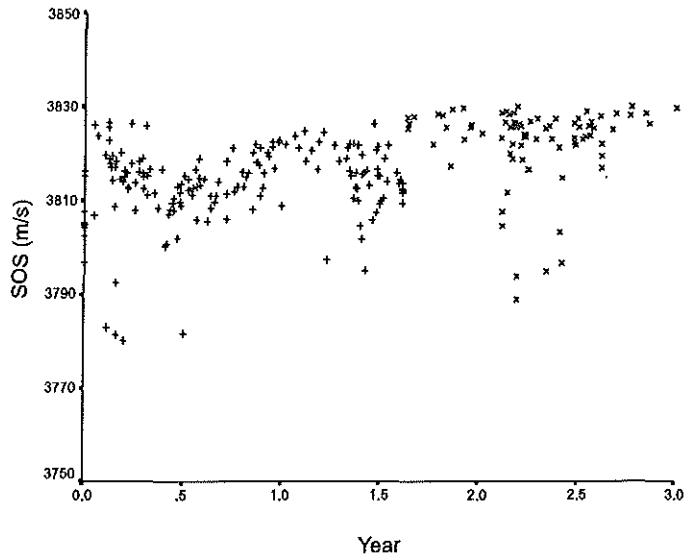



**Fig. 1** Long-term tibial ultrasound phantom data, expressed in mean SOS m/s. Measurements are done with probe 1 (+ sign) and with probe 2 (x sign).

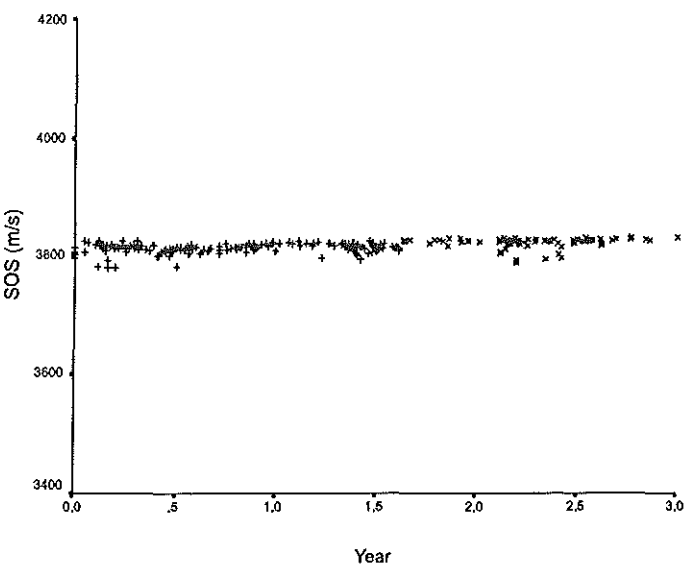



**Fig. 2** Long-term tibial ultrasound phantom data, expressed in mean SOS m/s, plotted in the clinical relevant scale of SOS, using probe 1(+ sign) and probe 2 (x sign).

Therefore we think that we can use the tibial ultrasound device not only in cross-sectional studies, but also in longitudinal studies. Looking at the very small change in mean phantom SOS in time, the tibial ultrasound device can be used not only in healthy children but also in children with diseases, which affect bone metabolism. The tibia ultrasound device can potentially also detect effects of treatment, known to affect bone metabolism.

## REFERENCES

1. Greenspan SL, Bouxsein ML, Melton ME, et al. Precision and discriminatory ability of calcaneal bone assessment technologies. J. Bone Miner. Res. 1997;12(8):1303-1313.
2. Funck C, Wüster C, Alenfeld FE, et al. Ultrasound velocity of the tibia in normal German women and hip fracture patients. Calcif Tissue Int 1996;58:390-4.
3. Stegman MR, Heaney RP, Travers-Gustafson D, Leist J. Cortical ultrasound velocity as an indicator of bone status. J Bone Miner Res 1995;10(suppl 1).

# NORMAL VALUES FOR TIBIAL QUANTITATIVE ULTRASONOMETRY IN CAUCASIAN CHILDREN AND ADOLESCENTS (aged 6 to 19 years)


## ABSTRACT

*Background:* Bone densitometry in children is a relatively new topic of interest within the field of osteoporosis. Bone densitometry techniques using an X-ray source have the disadvantage of radiation exposure. Also on some systems, motion artefacts are caused by long scan times. Tibial quantitative ultrasonometry (QUS) is ideally suited for children as it is radiation free and the interactive measurement provides real-time quality control.

In this prospective study, we present data acquired from 596 healthy children, 309 girls, mean age 12.9 years (range 6.1-19.9 years), and 287 boys, mean age 12.3 years (range 6.1-19.6 years) from Rotterdam, the Netherlands. For all subjects a short questionnaire regarding overall health was completed. To assess skeletal age, an X-ray of the left hand was taken and tibial QUS of the right tibia was performed using the SoundScan®Compact.

A statistical significant correlation was found between age and speed of sound (SOS) $r^2_{boys}=0.52$ and $r^2_{girls}=0.63$ (both $P<0.001$) and between skeletal age and SOS $r^2_{boys}=0.56$ and $r^2_{girls}=0.63$ (both $P<0.001$). In boys, significant increase of mean SOS is seen between Tanner stage II and III and between IV and V. In girls there is a significant increase of mean SOS between all Tanner stages, except between Tanner stage II and III.

This is the first study to present normative tibial QUS data for Caucasian children and adolescents. In this study, normative data relative to skeletal age are also provided, facilitating the implementation of this technique in children with growth disorders showing dissociation between calendar and skeletal age.


---


M.H. Lequin[1], R.R. van Rijn[1], S.G.F. Robben[1], W.C.J. Hop[2], C. van Kuijk[3]

Department of Pediatric Radiology[1], University Children's Hospital 'Sophia' Rotterdam, Department of Epidemiology and Biostatistics[2], Erasmus University Rotterdam, and Department of Radiology, Academic Medical Centre, Amsterdam[3], the Netherlands.



Part of this paper was presented at the Second International Congress of Osteoporosis, November 13-16, 1997, Osaka, Japan.

Published in: Calcif. Tissue Int 2000;67(2):101-105

## 4.1 INTRODUCTION

In recent years pediatric bone densitometry has become feasible, and with it, the importance of knowing the mechanism of bone. The concept of peak bone mass in normal growth and development has especially received increased attention[1-3]. Peak bone mass is dependent on genetic and environmental factors, such as nutrition and activity. These factors provide opportunities for interventional strategies to ensure the highest peak bone mass. Pediatric bone densitometry is also used increasingly for follow-up in children with diseases or treatment modalities (e.g. corticosteroids) influencing bone metabolism and growth [4-7].

Bone mass assessment in children has specific technical problems. First, the skeleton grows in three-dimensions, making interpretation of the results obtained by two-dimensional (areal) bone densitometry techniques, such as dual-energy X-ray absorptiometry (DXA), difficult [8,9]. With these techniques it may be hard to discern between the effect of actual bone density gain and changes in body or skeletal size[10]. To alleviate this problem the use of so-called bone mineral apparent density (BMAD) has been propagated [11]. However, further studies into the use of this parameter in children and adolescents are needed.

In recent publications Tothill et al.[12] and Bollotin[13] have highlighted a second drawback to DXA. Detrimental effects of changes in body composition on DXA measurements were shown, making the applicability of DXA in a pediatric population uncertain[12,13]. It has been determined that inhomogeneous fat distribution in soft tissues, resulting in a difference of 2 cm fat layer between soft tissue area and bone area, will influence DXA measurements by 10%[14]. Therefore, longitudinal DXA values in children may reflect the changes in body size and composition that occur with growth more than true changes in bone mineral density[12,13]. Moreover, reproducibility of those measurements is a problem in children, attributed mainly to motion artefacts due to long scanning times.

Theoretically, three-dimensional bone densitometry techniques such as quantitative computed tomography (QCT) are preferred[15]. The use of QCT means a higher level of radiation exposure compared with DXA, although the radiation exposure is still lower than with conventional X-rays [16].

A relatively new technique for bone mass assessment is quantitative ultrasonometry (QUS). Currently, several systems for QUS are in production, but are used almost exclusively in and validated for adult populations[17-20]. Because of their design, some systems are not suitable for use in children or they need extensive modification. Tibial QUS was performed using a SoundScan®Compact which is a system designed to facilitate measurements in children aged 6 years and older. It measures the speed of sound (SOS, $ms^{-1}$) along the cortex of the tibia (Figure 1)[18]. Research has shown that the SOS measurements are affected by the amount of bone density as well as by the structural properties of bone, i.e. anisotropy, elasticity and lamellar orientation [18,21].

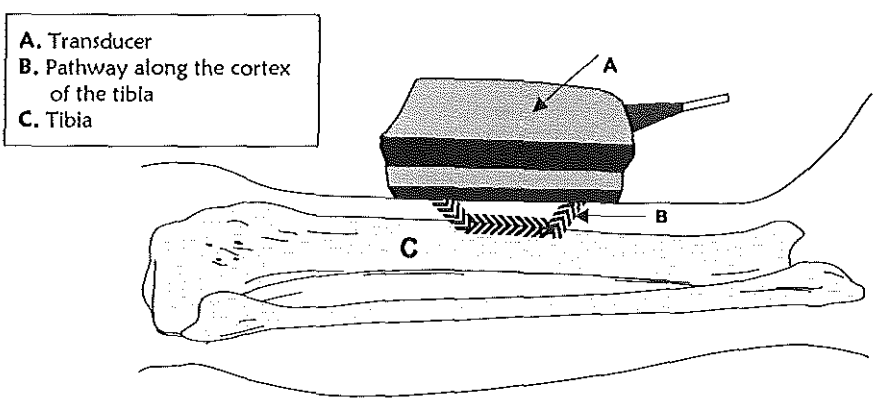



**Fig.1** The principle of quantitative ultrasound tibial bone assessment.

We present a study in healthy, Dutch, Caucasian children and adolescents (aged 6-19 years) establishing normal values for tibial QUS. The SOS values are normalized for skeletal age.

## 4.2 MATERIAL AND METHODS

*Study subjects*

Six hundred and twenty children and adolescents were recruited from two different populations by advertisement. The first group was recruited from employees of the University Hospital Rotterdam, the Netherlands.

And the second group from pupils of two high schools in Rotterdam. All volunteers were asked to complete a questionnaire relating to hand dominance, date of birth, sex and race. It also included questions pertaining to chronic illness, medication or a diet affecting bone growth and/or metabolism. A history of previous fractures and their cause was also recorded. Girls were asked their age at menarche and if they have had 6 or more months without menstruation. All children who suffered from diseases or used medications, known to affect bone growth and/or metabolism, were excluded from the study. A total of 24 children were excluded from the study because of chronic diseases: exostosis (1), Henoch-Schönlein (1), epilepsy (2), renal failure (1), prolonged immobilization (1), muscular dystrophy (1), and chronic respiratory disease (17). In total 309 girls, mean age 12.9 years (range 6.1-19.9 years), and 287 boys, mean age 12.3 years (range 6.1-19.6 years), participated in this study. Informed consent was obtained from parents or guardians and where appropriate from the child (in the Netherlands, this is mandatory in children aged 12 years and over). This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines of the Internal Review Board of the University Hospital Rotterdam and Erasmus University Rotterdam, Faculty of Medicine and Health Sciences, the Netherlands.

Tanner stages were evaluated through self-assessment, according to Duke et al. [22]. Subjects were shown pictures and written information illustrating breast and pubic hair development for girls, and genital and pubic hair development for boys. They were asked to select the one that closely resembled their own status. If there were discrepancies between variables, emphasis was placed on the breast development in girls and genital development in boys [23].

Height was measured, without shoes, using a wall-mounted ruler [24] and weight was measured, without shoes, on an electronic weight scale. Body mass index (BMI) as an indicator of nutritional status was calculated as the ratio of weight to height $^2$ ($kg \cdot m^{-2}$).

*Radiography*

To assess skeletal age (SA) radiographs of the left hand were taken for all participants (Philips diagnost H, Imation GT film, α-II screen, film-focus distance

1.5 m, 45 kV, 16 mAs). All radiographs were evaluated and scored according to the Greulich and Pyle hand atlas [25].

*Quantitative ultrasonometry*

Tibial QUS was performed using the SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel, software version 1.1e). Following standard operational procedures, all QUS bone assessments were done on the right tibia at the mid-tibial point, defined as the mid-point of the line between the apex of the medial malleolus and the distal patellar apex (see Lequin et al. [26] for a full-length discussion of this technique).

Measurements were made by trained operators (MHL, RRVR, SGFR). The intra- and inter-observer variation had been tested previously, according to the Food and Drugs Administration's guidelines, and the coefficient of variation was less than 0.5 % [26,27]. These results correspond well with previously published results obtained in adults [18,28,29].

*Statistical analyses*

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 6.1.3, Chicago, IL. USA). Regression analysis was used to assess the relation between SOS and age. Ninety-five percent reference intervals were calculated as mean ±2 SD of residuals. Student's *t*-test was used to test for differences between boys and girls. One-way analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons, was used to compare the various Tanner stages separately for boys and girls. Multiple regression, taking age into account, was used to explore differences between children with and without a fracture history.

## 4.3 RESULTS

Cubic regression analysis showed the best fit (see results Table 1 and Figures 2 through 5). The anthropomorphic and QUS data stratified by Tanner stage for boys and girls are shown in Table 2. In boys, a significant increase of mean SOS is seen between Tanner stages II and III and between stages IV and V. In girls, there is a significant increase of mean SOS among all Tanner stages, except

between stages II and III. A significant gender difference of mean SOS was found for Tanner stages IV ($P<0.05$) and V ($P<0.05$).

**Table 1.** Results of cubic regressions of SOS versus calendar and skeletal age.

| | Calendar age | | Skeletal age | |
|---|---|---|---|---|
| | Boys | Girls | Boys | Girls |
| Constant | 3113 ± 236 | 4042 ± 227 | 3243 ± 161 | 3820 ± 269 |
| A | 129.97 ± 60.93 | -138.13 ± 57.33 | 105.47 ± 45.06 | -73.61 ± 71.06 |
| B | -10.86 ± 4.97 | 13.22 ± 4.59 | -9.42 ± 3.89 | 7.47 ± 5.97 |
| C | 0.34 ± 0.13 | -0.32 ± 0.12 | 0.32 ± 0.11 | -0.16 ± 0.16 |
| $r^2$ | 0.52 | 0.63 | 0.56 | 0.63 |
| SD | 98.6 | 98.2 | 94.8 | 98.3 |

Data shown are coefficients ± standard error, with $r^2$ and SD of residuals:

(SOS = Constant + A x age + B x age$^2$ + C x age$^3$)

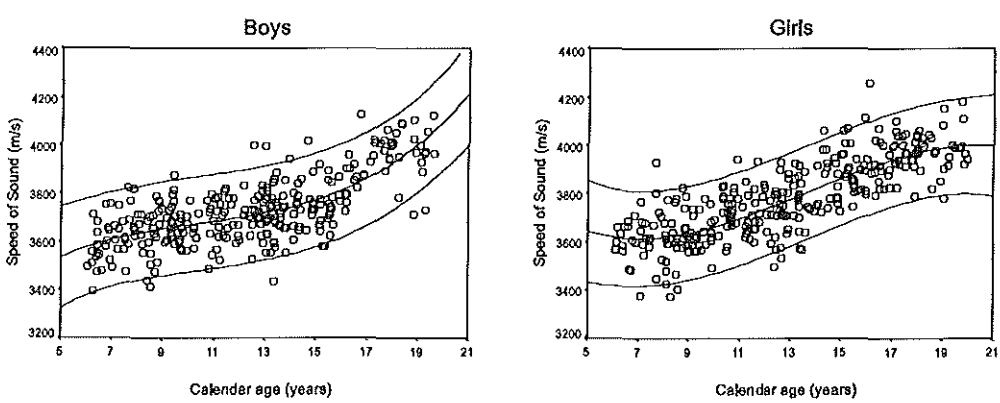



**Fig. 2 and 3** Cubic regression and 95% reference intervals of speed of sound for boys and girls according to calendar age.

**Table 2.** Anthropomorphic data stratified by Tanner stage for boys and girls.

| Measurement | Sex | Tanner stage I | II | III | IV | V |
|---|---|---|---|---|---|---|
| Age (years) | ♂ | 8.3 (1.6) | 10.6 (2.3)[3] | 12.8 (1.2)[3] | 14.4 (1.6)[3] | 16.6 (1.9)[3] |
| | ♀ | 8.2 (1.5) | 10.8 (1.9)[3] | 12.0 (1.0)[3] | 14.0 (1.0)[3] | 16.6 (1.8)[3] |
| Skeletal age (years) | ♂ | 8.3 (2.0) | 10.6 (2.4)[3] | 13.0 (0.8)[3] | 14.8 (1.4)[3] | 17.1 (1.7)[3] |
| | ♀ | 8.4 (1.5) | 10.7 (1.2)[3] | 12.4 (1.0)[3] | 14.6 (1.5)[3] | 16.8 (1.3)[3] |
| Length (m) | ♂ | 1.37 (0.12) | 1.49 (0.12)[3] | 1.60 (0.08)[3] | 1.75 (0.08)[3] | 1.83(0.05)[3] |
| | ♀ | 1.38 (0.090) | 1.50 (0.08)[3] | 1.60 (0.09)[3] | 1.67 (0.08)[3] | 1.70(0.06)[1] |
| Weight (kg) | ♂ | 31.8 (8.5) | 39.2 (10.2)[3] | 48.1 (8.9)[3] | 58.7 (8.0)[3] | 72.3(8.6)[3] |
| | ♀ | 31.2 (6.5) | 40.1 (7.4)[3] | 44.7 (7.2)[1] | 56.4 (7.9)[3] | 61.4(8.3)[3] |
| BMI ($kgm^{-2}$) | ♂ | 16.6 (1.9) | 17.4 (2.5)[1] | 18.6 (2.5)[1] | 19.1 (1.8)[NS] | 21.5(2.3)[3] |
| | ♀ | 16.3 (1.9) | 17.9 (2.6)[1] | 17.3 (1.9)[NS] | 20.2 (2.1)[3] | 21.3(2.6)[3] |
| Mean SOS ($ms^{-1}$) | ♂ | 3656 (93) | 3654 (108)[NS] | 3749 (99)[3] | 3755 (126)[NS] | 3902(127)[3] |
| | ♀ | 3637 (97) | 3692 (105)[2] | 3713 (102)[NS] | 3845 (103)[3] | 3945(103)[3] |
| Number | ♂ | 100 | 47 | 37 | 53 | 49 |
| | ♀ | 96 | 41 | 22 | 63 | 86 |

Difference between this and previous Tanner stage,' SD between brackets:
NS = not significant, [1]=$P<0.05$, [2]=$P<0.01$, [3]=$P<0.001$

These significant differences were also present when age was taken into account using multiple regression. For those girls (n= 129), who had their first period, menarche had occurred at 12.7 years (range 10-16 years).

One hundred and fourteen children (63 girls and 51 boys) had a history of fractures (fractures which are typical for children, e.g. sports traumas and falls from bikes).

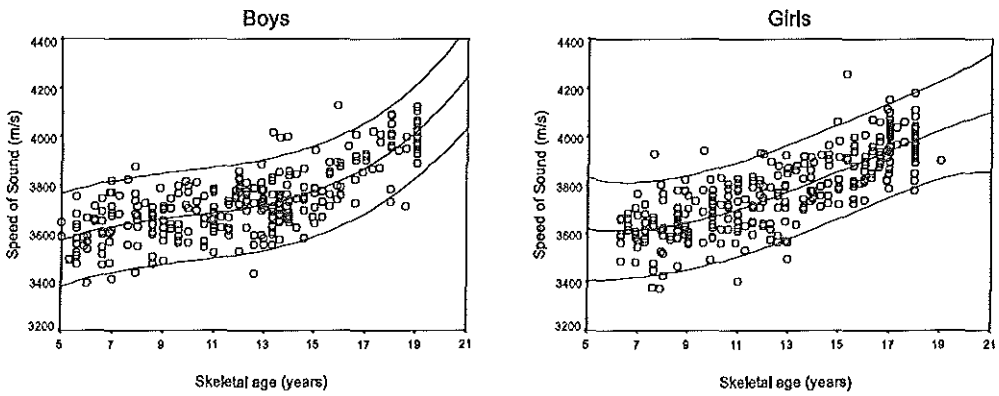



**Fig. 4 and 5** Cubic regression and 95% reference intervals of speed of sound for boys and girls, according to skeletal age.

All of these fractures occurred at least one year prior to our investigation. Neither for boys nor for girls there was a significant difference in mean SOS (measured by multiple regression, adjusting for age) between those with and those without fracture. There was also no significant influence of the interval of fracture on the SOS in children with a history of fracture.

## 4.4 DISCUSSION

This is the first paper to present normative data for tibial QUS in Caucasian children in the age range of 6-19 years with this specific device. The attractiveness of QUS, especially in children, lies in its low cost, portability, ease of use and lack of ionizing radiation. Several studies using calcaneal ultrasound have been done in children and adolescents, two of which report pooled data obtained from groups of children of different gender and ethnicity[30,31]. As bone development is influenced by both gender and ethnicity, these study results are hard to interpret. Both studies used a specially adapted version of commercially available systems. The advantage over the more widely used calcaneal QUS systems is the applicability of the tibial QUS in smaller and/or younger children. The minimum age at which children can be investigated at the mid-tibial site is about 6 years or, because of the relatively large transducer head, a minimal tibial length of approximately 20 cm. For calcaneal QUS systems the average age at which the device is applicable is 8 years. Furthermore, hypothetically the

change of the mid-tibial site location will be less than the change in the region of interest at the calcaneus [32].

Our data showed significant increase of the SOS value between all Tanner stages in girls except between Tanner stage II and III which is in contrast with the data reported by Mughal et al.[30]. An explanation could be that there is a difference in mean age at Tanner stages II and III between our investigated group of girls and the group investigated by Mughal. Furthermore Mughal's study included fewer girls. In our study, the highest increase of SOS in girls is found between Tanner stages III and IV, within mean age range of 12 to 14 years (Table 2).

Our results show no constant increase of SOS in boys, but significant increases between Tanner stages II and III and stages IV and V. The highest increase of SOS is between Tanner stage IV and V, with a mean age range of 14.4 and 16.6 years and therefore approximately two years after the highest increase of SOS in girls. The significant gender differences of SOS remain when age is taken into account using multiple regression. Our results show that the SOS of boys is significantly lower than girls in Tanner stage IV ($P<0.001$) and V $P<0.05$). However, our data shown in Figures 2 and 3, suggest that after the age of 20 years males may have the highest SOS, in agreement with other studies done with tibial QUS in adults[18]. The same trends have been shown by Boot et al. [23] who generated normal values for their DXA machine. They found no difference in BMD of the lumbar spine, or total body between boys and girls, but a lower volumetric bone mineral density (BMAD) in boys than in girls in Tanner stage V. They do not give a good explanation for this difference, but suggest that it may be due to a difference in female hormone levels in boys and girls in this age group. Gilsanz et al [10] using QCT of the femur, did not find a lower BMD in boys than in girls in the age range of Tanner stage V, therefore it is more likely that there is a difference in bone architecture in this age group between the two sexes rather than a real bone density difference. Furthermore, looking at the Figures 2-5, boys seem to catch up with this difference later in life, in their early twenties. Our findings are in agreement with the result of the study performed by Kaga et al.[33].

The standard deviation, and consequently the 95% reference intervals, presented in Figures 2-5 show quite an extensive biological variability also

reported for other bone densitometry techniques such as DXA [34]. It has been suggested that this broad variability is due partly to genetic and environmental factors [35]. As expected in this healthy population, no significant difference in the biological variability of the QUS measurements was seen, comparing skeletal age and biological age. To our knowledge, this is the first study to assess bone density and skeletal age simultaneously, thus facilitating the clinical use of this technique in pediatric patients afflicted with diseases affecting bone growth and/or metabolism, causing dissociation between calendar and skeletal age. In this group it is customary to make radiographs of the hand in order to assess skeletal age. Depending on the dissociation of skeletal and calendar age therapeutic regimen may be initiated. We therefore feel that it is imperative to use data normalised for skeletal age.

Measured tibial QUS values can probably be used in the same way as actual growth curves in children. If it can be shown that children in general follow their specific percentile during their progress to adulthood, then very early in life children with QUS values below 5% percentile can be identified as having a low bone mass, and the appropriate interventions could be explored to ensure the highest peak bone mass possible. This however, necessitates longitudinal studies investigating the normal variation in bone gain in children, as well as exploring the usefulness of such interventions.

In our opinion, the best bone mass assessment technique by design is QCT, not only in adults but also in children. But the major disadvantage, which limits its use, is its availability. To a lesser extent, patients discomfort due to claustrophobia and radiation burden plays a role. Also, the use of QCT for follow-up studies is hindered by the relatively high radiation dosages, which makes patient compliance low.

Longitudinal studies performed using DXA could be cumbersome in childhood as several authors have shown that DXA is sensitive to changes in soft tissue composition and bone size occurring during growth. Therefore, QUS could be an attractive technique. The tibial site especially shows great promises in children, as the mid-point of long bones remains fairly constant throughout childhood and adolescence. At the moment we are conducting a longitudinal study assessing the normal variation in bone gain in children and its clinical applicability.

**Acknowledgements**

We are indebted to Myriad Ultrasound Systems Ltd., Rehovot, Israel, and in particular Dr. D.D. Keinan, for providing us with the SoundScan®Compact. Our thanks to parents of the children as well as the pupils of the 'Erasmiaans' and 'Marnix' high schools for their co-operation in our study. We also would like to thank Ms. W. van Leeuwen, Ms. J. Hogen-Esch and the radiological technicians of the departments of Pediatric Radiology, University Hospital 'Sophia' Rotterdam, and Experimental Radiology, Erasmus University Rotterdam, the Netherlands.

## 4.5 REFERENCES


1. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. Lancet 1993; 341:72-5.
2. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med 1991; 325:1597-1600.
3. Kanis JA, Melton III LJ, Christiansen C, Johnston CC, Khaltaev N. Perspective: The diagnosis of osteoporosis. J Bone Miner Res 1994; 9:1137-41.
4. Behnke B, Altrogge H, Delling G, Kruse HP,Muller-Wiefel, D.E. Bone mineral density in pediatric patients after renal transplantation. Clin Nephrol 1996; 46:24-29.
5. Block JE, Piel CF, Selvidge R, Genant HK. Familial hypophosphatemic rickets: bone mass measurements in children following therapy with calcitrol and supplemental phosphate. Calcif Tissue Int 1989; 44:86-92.
6. Saggese G, Baroncelli GI, Bertelloni S, Barsanti S. The effect of long-term growth hormone (GH) treatment on bone mineral density in children with GH deficiency. The role of GH in attainment of peak bone mass. J Clin Endocrinol Metab 1996; 81:3077-83.
7. Zamboni G, Soffiati M, Giavarina D, Tato L. Mineral metabolism in obese children. Acta Pediatr Scand 1988; 77:741-6.
8. Barden HS, Mazess RB. Bone densitometry in infants [review]. J Pediatr 1988; 113:172-7.
9. Mazess RB, Cameron JR. Skeletal growth in school children: maturation and bone mass. Am J Phys Anthrop 1971; 35:399-408.
10. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998; 26:177-182.
11. Kröger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 1995; 17:157-9.
12. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997; 12:1908-21.
13. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in vivo bone mineral density. J Bone Miner Res 1998; 13:1739-1746.
14. Hangartner TN, Johnston CC. Influence of fat on bone measurement with dual-energy absorptiometry. Bone Miner 1990; 9:71-81.
15. Gilsanz V, Gibbens DT, Roe TF, et al. Vertebral bone density in children: effect of puberty. Radiology 1988; 166:847-50.
16. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: state of the art [review]. J Bone Miner Res 1996; 11:707-30.
17. Cunningham JL, Fordham JN, Hewitt TA, Speed CA. Ultrasound velocity and attenuation at different skeletal sites compared with bone mineral density measured using dual-energy X-ray absorptiometry. Br J Radiol 1996; 69:25-32.

18. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995; 17:363-7.
19. Funck C, Wüster C, Alenfeld FE, et al. Ultrasound velocity of the tibia in normal German women and hip fracture patients. Calcif Tissue Int 1996; 58:390-4.
20. Orgee J, McCloskey EV, Foster H, Coombes G, Khan S, Kanis JA. Tibial ultrasound velocity; a useful clinical measure of skeletal status. J Bone Miner Res 1995; 10.
21. Bouxsein ML, Radloff SE. Quantitative Ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. J Bone Miner Res 1997; 12:839-46.
22. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980; 66:918-20.
23. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997; 82:57-62.
24. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997; 12:531.
25. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Stanford, California: Stanford University Press, 1959.
26. Lequin MH, van Rijn RR, Robben SGF, et al. Evaluation of short-term precision of tibial ultrasonometry. Calcif Tissue Int 1999; 64:24-27.
27. Arnaudo JS. Draft guidance for review of bone densitometers 510(k). Guide to clinical trials. New York: Raven, 1992:24-7.
28. Foldes AJ, Danziger A, Constantini N, Popovtzer MM. Reduced ultrasound velocity in tibial bone of young dancers. Int J Sports Med 1997; 18:296-9.
29. Fan B, Zucconi F, Fuerst T, Gluër CC, Genant HK. Precision assessment: ultrasonic velocity measurement of the mid-tibia versus other techniques. J Bone Miner Res 1995; 10.
30. Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL. Comparison between broad-band ultrasound attenuation of the calcaneus and total body bone mineral density in children. Acta Pediatr 1996; 85:663-5.
31. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995; 56:368-71.
32. Laugier P, Fournier B, Berger G. Ultrasound parametric imaging of the calcaneus: in vivo results with a new device. Calcif Tissue Int 1996; 58:326-31.
33. Kaga M, Takahashi K, Suzuki H, et al. Utrasonic assessment of tibia in Japanese children and adolescents. Osteoporosis Int. 1997; 7:67.
34. Boot AM. Bone mineral density and body composition of children and adolescents in health and disease. Pediatrics. Rotterdam: Erasmus University Rotterdam, 1997.
35. Fässler AC, Bonjour J. Osteoporosis as a pediatric problem. Pediatr Clin North Am 1995; 42:663-5.

# TIBIAL QUANTITATIVE ULTRASOUND VERSUS TOTAL BODY AND LUMBAR SPINE DXA IN A DUTCH PEDIATRIC AND ADOLESCENT POPULATION


## ABSTRACT

*Rationale and objectives:* To understand normal bone development, studies in healthy children and adolescents are important. To assess the applicability of tibial quantitative ultrasound measurements (QUS) in children, we performed a study that compared dual-energy X-ray absorptiometry (DXA) of the lumbar spine and total body with tibial QUS.

*Methods:* For this study we recruited 146 Dutch children and adolescents, 58 boys (median age, 14.1 years; range 7.6 - 23.4 years) and 88 girls (median age, 18.0 years; range 7.6 - 23.5 years). Tanner stage, weight and height were assessed for all participants. Bone mineral mensity (BMD; $g \cdot cm^{-2}$) of the total body and lumbar spine ($L_2$-$L_4$) and bone mineral apparent density (BMAD) of the lumbar spine ($g \cdot cm^{-3}$) were assessed by using the Lunar DPXL. For tibial QUS, the SoundScan®Compact system was used.

*Results:* Both lumbar BMD as well as total body BMD showed a strong, significant correlation with tibial QUS in boys and girls: $r_{\text{total body boys}}=0.81$, $r_{\text{total body girls}}=0.77$, $r_{\text{lumbar spine boys}}=0.79$, and $r_{\text{lumbar spine girls}}=0.72$. Lumbar spine BMAD also showed significant correlations with tibial QUS: $r_{\text{boys}}=0.63$ and $r_{\text{girls}}=0.63$ (for all correlations, $P<0.001$).

*Conclusions:* Our study showing strong significant correlations between DXA and tibial QUS measurements suggests that tibial QUS is a technique that may be applicable in children and adolescents.



R.R. van Rijn, MD. PhD.[1], I.M. van der Sluis, MD.[2], M.H. Lequin, MD.[1], S.G.F. Robben, MD. PhD.[1], S.M.P.F. de Muinck Keizer-Schrama, MD. PhD.[2], W.C.J. Hop, MSc.[3], and C. van Kuijk, MD. PhD.[4]

Departments of Radiology[1] and Pediatric Endocrinology[2], University Hospital Rotterdam, department of Epidemiology and Biostatistics, Erasmus University Rotterdam and department of Radiology[3], Academic Medical Center, Amsterdam, the Netherlands


Accepted for publication in: Investigative Radiology 35:(9) 2000

## 5.1 INTRODUCTION

Because osteoporosis is an increasing problem worldwide, it has become the focus of attention of many studies. Variation in the attainment of maximal peak bone mass has recently been recognized as a risk factor for the development of osteoporosis later in life[1]. Because a high peak bone mass is important, studies in healthy children are necessary to understand normal bone development [2].

Currently most studies of bone mass development in children use dual-energy X-ray absorptiometry (DXA). Although DXA shows excellent results in the adult population, its implementation in children might be hindered by the way it assesses bone mineral density (BMD; $g \cdot cm^{-2}$) in a two-dimensional fashion. Because bone grows in three dimensions the two-dimensional approach measures not only true BMD gain but also growth, making the results of DXA difficult to interpret [3]. To overcome this problem, the use of bone mineral apparent density (BMAD; $g \cdot cm^{-3}$) has been advocated [3-5]. To calculate BMAD, the vertebral body is assumed to be cylindrical. By using the projected image of this vertebral body, the volume can be approximated, thereby yielding a volumetric density. For longitudinal studies in children, DXA has an additional drawback, in the fact that it uses different software versions in children and adolescents. There is a considerable difference between the BMD values obtained by these two software versions [6].

Besides longitudinal growth there are also significant changes in body composition during childhood. Three recent publications have drawn attention to the negative effect of changes in fat and lean body mass on DXA measurements [7-9]. Another minor drawback is the fact that DXA uses ionizing radiation; although the dose is extremely low, it still is something one would prefer not to use in children [10, 11].

Recently, quantitative ultrasound (QUS) has emerged as a promising technique in the field of bone densitometry. This technique might be suitable in a pediatric population [12-17]. QUS has been validated in the adult population and it has been shown to be as reliable as other techniques in predicting osteoporotic fractures in the elderly [4,18-20]. However, QUS implementation in a pediatric population has specific problems to overcome. Currently, there are several QUS systems on the market that assess at different skeletal sites.

By design, the tibial ultrasound system (SoundScan®Compact, Myriad Ultrasound Systems Ltd., Rehovot, Israel) is suited for use in children and adolescents. The approach used in this specific system differs from the calcaneal and phalangeal systems in that it measures the speed of sound (SOS) along the cortex of the tibia instead of the SOS and broadband attenuation through the calcaneus or the phalanx[21]. The predefined distance along the tibia is short enough to accommodate the tibia of children aged 4 years and older. Moreover, because the measurement is performed at the mid-point of the tibia, the region of interest does not change significantly during growth, unlike in other systems in which the calcaneus is measured.

To investigate the applicability of this system we previously tested its precision in a pediatric population and collected normative data [22, 23]. To further assess the applicability in this specific population we performed a study comparing the results of DXA of the spine and total body with those of tibial QUS in a healthy, young, Dutch population. Because most clinicians consider DXA to be the "gold standard" in bone assessment, a strong positive correlation between DXA and tibial QUS might convince them to adapt tibial QUS as a diagnostic modality.

## 5.2 MATERIAL AND METHODS

*Participants*

For this study we recruited 146 Dutch children and adolescents from the Rotterdam region of the Netherlands: 58 boys, with a median age of 14.1 years (range 7.6 - 23.4 years), and 88 girls, with a median age of 18.0 years (range 7.6 - 23.5 years). The parents or guardians, and all children aged 12 years and older, signed an informed consent form according to the Helsinki agreement and to the guidelines stated by the Internal Review Board of the University Hospital Rotterdam, the Netherlands [24].

All participants filled out a questionnaire, regarding overall health. None of the children suffered from any disease known to affect bone metabolism and/or growth. Tanner stage was assessed by using photographs depicting the five stages; children were asked to point out those pictures that showed the best resemblance with there own pubertal status. In cases of discrepancies between variables, emphasis was placed on breast development in girls and genital

development in boys [25]. This technique has previously been validated by Duke et al. and is widely used because it relieves the child from the psychological burden of undressing in front of an adult [26]. Weight was assessed with a standard clinical balance and height was assessed using a fixed stadiometer. As an indicator of nutritional status, body mass index (BMI) was calculated as the ratio mass to the square of height ($kg \cdot m^{-2}$). To test for normality, standard deviations, defined as the (measured value minus mean value for the normal population) divided by the standard deviation of the normal population and matched for age and sex from the normal Dutch population, for both height and BMI were calculated.

*Dual-energy X-ray absorptiometry*

BMD ($g \cdot cm^{-2}$) of the lumbar spine ($L_2$-$L_4$) and total body was measured by DXA (Lunar DPXL, Lunar corp., Madison, WI). BMAD ($g \cdot cm^{-3}$) was calculated by using the lumbar spine BMD and the projected dimensions of the vertebral body. For this measure, the equation postulated by Kröger et al.[5] was used:

$$BMAD = BMD \times \left( \frac{4}{\pi \times width} \right)$$

For measurement of the spine, the natural lumbar lordosis was flattened by elevation of the knees. All measurements were performed and analyzed by one operator (I.M. v/d S). For this study, the precision of DXA was not assessed, separately, because it was considered unethical to repeat those measurements in children with a technique that uses ionizing radiation. However, the published precision for DXA in children has been reported to be 1.8% [27].

*Quantitative ultrasound*

For tibial QUS, the SoundScan®Compact system (Myriad Ultrasound Systems Ltd., Rehovot, Israel) was used. By following standard operational procedures, all QUS bone assessments were done on the right tibia at the mid-tibial point. All participants underwent one single measurement for the study. The mid-tibial point was defined as the mid-point of the line between the apex of

the medial malleolus and the distal patellar apex. Along a specified length, the SOS (in $m \cdot s^{-1}$) through the cortex of the tibia was measured[21]. A single measurement takes less than 5 minutes to perform. Two trained operators (RRvR and MHL) performed all measurements. For tibial QUS the intra- and inter-observer variances were tested beforehand in a healthy, white, Dutch pediatric and adolescent population. The inter-observer variance for this population is 0.61%, and the intra-observer variance is 0.43%[16].

*Statistical analyses*

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS version 7.5.2, SPSS Inc., Chicago, IL, USA). Multiple regression analysis was used for evaluation of the relation between tibial QUS, total body DXA, and lumbar spine BMD. Body weight, body height, BMI and Tanner stage were entered into the regression model to test for modification of relationships.

## 5.3 RESULTS

The data of all children were available for analysis. The mean and SD of height for boys was 0.42 (SD=1.31) and for girls 0.27 (SD=1.29). The mean and SD of BMI for boys was 0.88 (SD=1.01) and for girls 0.34 (SD=1.02). In the male group 52 children (89.7%) were white, 3 (5.2%) were black, 2 (3.4%) were Hispanic, and 1 (1.7%) was of mixed ethnicity. In the female group, 71 children (80.7%) were white, 4 (4.5%) were black, 3 (3.4%) were Asian, and 10 (11.4%) were Hispanic. Both the lumbar spine BMD as well as the total body BMD showed strong, significant correlations with tibial QUS in boys and girls: $r_{\text{total body boys}}=0.81$, $r_{\text{total body girls}}=0.77$, $r_{\text{lumbar spine boys}}=0.79$, and $r_{\text{lumbar spine girls}}=0.72$ (all $P<0.001$; Table 1 and Figures 1 and 2). Introduction of additional parameters such as height, body weight, BMI or Tanner stage into the multiple regression analysis failed to reach significance. Lumbar spine BMAD also showed moderate but significant correlations with QUS: $r_{\text{boys}}=0.63$ and $r_{\text{girls}}=0.63$ ($P<0.001$), although the correlations were weaker than those for BMD of the spine or total body (Table 1 and Figure 3).

**Table 1.** Linear regression parameters for DXA BMD ($g \cdot cm^{-2}$) and DXA BMAD ($g \cdot cm^{-3}$) versus tibial SOS ($m \cdot s^{-1}$).

| Sex | Site | r | *P*-value | a | b |
|---|---|---|---|---|---|
| Boys | Lumbar BMD | 0.79 | <0.001 | 3318.3 | 468.2 |
| | Total body BMD | 0.81 | <0.001 | 2948.6 | 775.5 |
| | Lumbar BMAD | 0.63 | <0.001 | 3191.9 | 1853.9 |
| Girls | Lumbar BMD | 0.72 | <0.001 | 3378.8 | 449.9 |
| | Total body BMD | 0.77 | <0.001 | 2963.4 | 834.2 |
| | Lumbar BMAD | 0.63 | <0.001 | 3294.6 | 1578.3 |

Regression equation: SOS = a + b x BMD or BMAD. r=regression coefficient. DXA: dual-energy X-ray absorptiometry; BMD: bone mineral density; BMAD: bone mineral apparent density; and SOS: speed of sound.

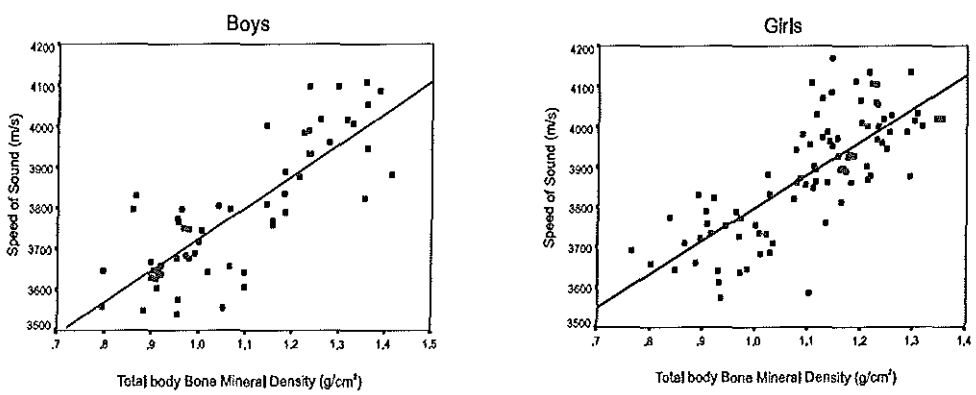



**Fig. 1** Total body bone mineral density versus tibial quantitative ultrasound measurements in boys and girls.

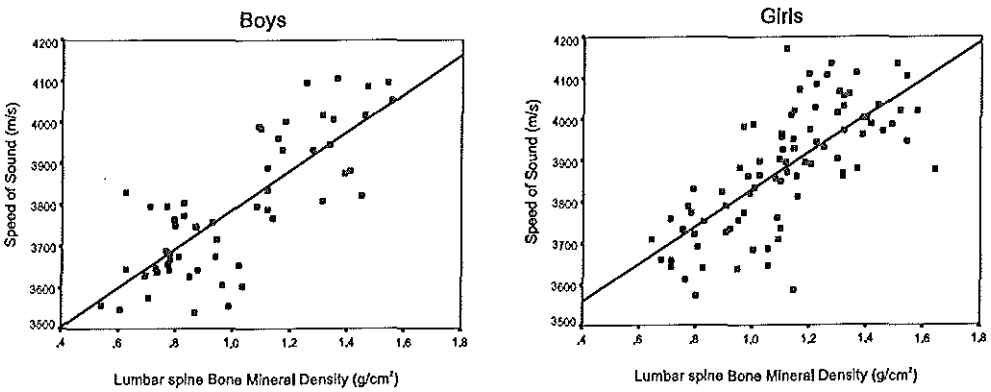



**Fig. 2** Lumbar spine bone mineral density versus tibial quantitative ultrasound measurements in boys and girls.

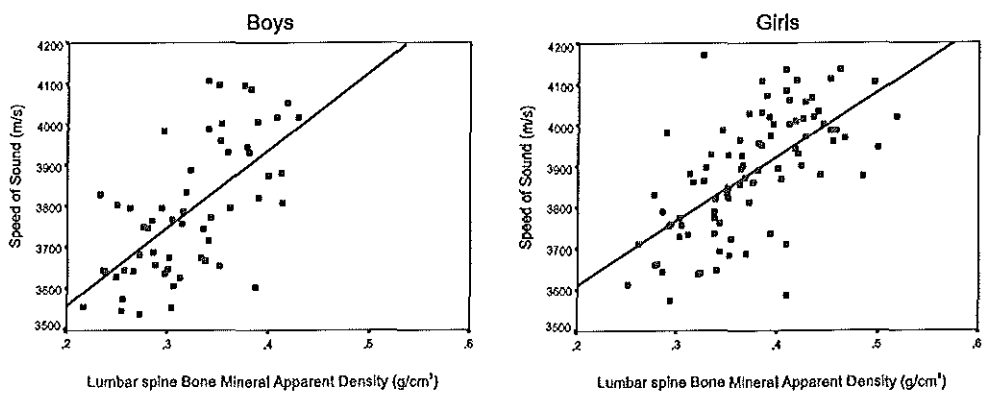



**Fig. 3** Lumbar spine bone mineral apparent density versus tibial quantitative ultrasound measurements in boys and girls.

## 5.4 DISCUSSION

To date, there are only a few published studies regarding the correlation between DXA and QUS in this specific age group. All of these studies have concentrated on the correlation between calcaneal QUS and DXA [12, 28]. In the study of Jaworski et al.[12] containing pooled data, for both healthy and osteopenic children, the authors presented correlations between calcaneal QUS and total body BMD (r =0.67), lumbar BMD (r =0.67) and calcaneal BMD (r =0.67) (*P*-values were not reported). For their study, Jaworski et al.[12] measured the distance between the tuberosity of the fifth metatarsal and the back of the heel. At one third of this distance from the back of the foot, a point 1 cm above and perpendicular to this first point was marked. The foot was positioned in the Achilles ultrasound densitometer (Lunar Corp., Madison, WI, USA), by using one to four footpads underneath or behind the foot. Mughal et al.[28] used a prototype pediatric contact ultrasound bone analyzer and compared its results with those from a total body DXA (Hologic QDR-1000W; Hologic, Waltham, MA). In that study a significant, positive correlation was found between DXA-derived values and calcaneal SOS (r =0.74 $P<0.001$); furthermore, both total body BMD and calcaneal BUA were significantly correlated with age. Both studies used systems that were especially adapted for a pediatric population, either by using footpads or by design. Tromp et al. [29] recently presented a study of an adult population that compared tibial QUS and DXA. Their study yielded the following correlations: tibial QUS versus lumbar spine BMD r =0.54 ($P<0.001$) and tibial QUS versus total body BMD r =0.58 ($P<0.001$). Our study, which used a standard, commercially available system in children and adolescents, shows significant correlations ranging between r =0.60 and r =0.83 ($P<0.001$). We expected to find the most significant correlations between QUS and total body BMD, because the latter consists of approximately 80% cortical bone. In both boys and girls there was no significant difference in correlation between lumbar or total body BMD and tibial QUS.

The significant correlations between DXA and QUS suggest that both techniques measure a component of growth as well as some change in bone mass and composition. Although we found a small difference in correlation between QUS and DXA of the spine or total body in boys and girls (in favour of boys), this was not significant.

As mentioned in the introduction, the applicability of DXA in a pediatric and adolescent population is still a matter of debate [3,7]. However, until now, DXA has been considered the "gold" standard by most clinicians [4]. With QUS a new way of assessing bone has been introduced. Although QUS has proved to be as reliable as DXA in predicting fractures of the hip in the elderly, what exactly is measured is still a matter of debate [4,19,30-32]. From basic physics, it is known that SOS is dependent on the complexity of the material through which the ultrasound waves travel.

Although bone is an anisotropic material, we may, by approximation, apply the following equation:

$$SOS = \frac{1}{2} \times \frac{E}{\rho}$$

where E is the modulus of elasticity and ρ is bone density. Therefore, the SOS reflects not only bone density but also the structural properties of bone [33,34].

With respect to the effect of bone maturation and osteoporosis on ultrasound parameters, several authors have already stated that further fundamental research is necessary [33,35,36].

Normative data gathered in more than 500 healthy, white children and adolescents showed a significant, positive correlation between skeletal age and tibial QUS in both boys and girls [22]. Our results showing a significant correlation between DXA and QUS, indicate that QUS may be an addition to the diagnostic tools of the physician.

**Acknowledgement**

The authors would like to thank Myriad Ultrasound Systems Ltd., Rehovot, Israel for lending us the equipment to perform this study. The authors received no financial support from any company for this study.

## 5.5 REFERENCES


1. Fässler AC, Bonjour J-P. Osteoporosis as a pediatric problem. Pediatr Clin N Am 1995;42(4):811-824.
2. Nelson DA, Koo WWK. Interpretation of absorptiometric bone mass measurements in the growing skeleton: issues and limitations. Calcif Tissue Int 1999;65:1-3.
3. Gilsanz V. Bone density in children: a review of the available techniques and indications. Eur J Radiol 1998;26:177-182.
4. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: State of the art [review]. J Bone Miner Res 1996;11(6):707-730.
5. Kröger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 1995;17(2):157-159.
6. Laskey MA, Prentice A. Comparison of adult and paediatric spine and total body software for the Lunar dual energy X-ray absorptiometer. Br J Radiol 1999;72(862):967-976.
7. Bolotin HH. An analytic and quantitative exposition of patient-specific systematic inaccuracies inherent in planar DXA-derived in-vivo BMD measurements. Med Phys 1998;25(2):139-151.
8. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in-vivo bone mineral density. J Bone Miner Res 1998;13(11):1739-1746.
9. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997;12(11):1908-1921.
10. Freeman WL. Research with radiation and healthy children: greater than minimal risk. National Indian Health Service IRB, Albuquerque, N.M. 1994;16(5):1-5.
11. Njeh CF, Fuerst T, Hans D, Blake GM, Genant HK. Radiation exposure in bone mineral density assessment. Appl Radiat Isot 1999;50(1):215-236.
12. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995;56:368-371.
13. Barden HS, Mazess RB. Bone densitometry in infants [review]. J Pediatr 1988;113(1):172-177.
14. Eggerts P. Eine neue Methode zur Mineralgehaltsbestimmung von Knochen mittels Ultraschall. Monatsschr Kinderheilkd 1992;140(Supp 1):S28-31.
15. Bayer M, Kutílek S. Ultrasound transmission through the os calcis in children: Which side should we measure? Calcif Tissue Int 1998;61(441-442).
16. Lequin MH, van Rijn RR, Robben SGF, et al. Evaluation of short-term precision of tibial ultrasonometry. Calcif Tissue Int 1999;64:24-27.
17. Lorenc RS, Jaworski M. Sex related differences in growing children monitored by ultrasound measurements. Osteoporosis Int 1997;7(3):301.
18. Bouxsein ML, Courtney AC, Hayes WC. Ultrasound and densitometry of the calcaneus correlate with the failure loads of cadaveric femurs. Calcif Tissue Int 1995;56:99-103.

19. Turner CH, Peacock M, Timmerman L, Neal JM, Johnston jr CC. Calcaneal ultrasonic measurements discriminate hip fracture independently of bone mass. Osteoporosis Int 1995;5:130-135.
20. Njeh CF, Boivin CM, Langton CM. The role of ultrasound in the assessment of osteoporosis: A review. Osteoporosis Int 1997;7(1):7-22.
21. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: A novel approach for assessment of bone status. Bone 1995;17(4):363-367.
22. Lequin MH, van Rijn RR, Robben SGF, van Kuijk C. Normal values for quantitative tibial ultrasound bone assessment in a Caucasian pediatric population (aged 6 to 19 years). Calcif Tiss Int 2000;67(2):101-105.
23. Lequin MH, van Rijn RR, Robben SGF, Keinan DD, van Kuijk C. Quantitative ultrasound bone assessment of the tibia normative data for a pediatric, 6 to 19 years Caucasian population. J Clin Densito 1998;1(1):102.
24. 41 st World Medical Assembly. Declaration of Helsinki: Recommendations guiding physicians in biomedical research involving human subjects. Bull Pan Am Health Organ 1990;24:606-609.
25. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: Relation to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997;82:57-62.
26. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66(6):918-920.
27. Pintauro SJ, Nagy TR, Duthie CM, Goran MI. Cross-calibration of fat and lean measurements by dual energy x-ray absorptiometry to pig carcass in the pediatric body weight range. Am J Clin Nutr 1996;63:293-298.
28. Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL. Comparison between broad-band ultrasound attenuation of the calcaneus and total body bone mineral density in children. Acta Pediatr 1996;85:663-665.
29. Tromp AM, Smit JH, Deeg DJH, Lips P. Quantitative ultrasound measurements of the tibia and calcaneus in comparison with DXA measurements at various skeletal sites. Osteoporosis Int 1999;9:230-235.
30. Bouxsein ML, Boardman KC, Pinilla TP, Myers EM. Ability of bone properties at the femur, forearm, and calcaneus to predict the structural capacity of the proximal femur during a sideways fall. J Bone Miner Res 1995;10(suppl 1):178.
31. Hans D, Njeh CF, Genant HK, Meunier J-P. Quantitative ultrasound in bone status assessment. Rev. Rhum [Engl. Ed.] 1998;65(7-9):489-498.
32. Schott AM, Weil-Engerer S, Hans D, Duboeuf F, Delmas PD, Meunier PJ. Ultrasound discriminates patients with hip fracture equally well as dual X-ray absorptiometry and independently of bone mineral density. J Bone Miner Res 1995;10(2):243-249.
33. Glüer CC, Barkmann R, Heller M. Quantitativer Ultraschall; status 1999. Radiologe 1999;39:213-221.

34. Van den Bergh JP, van Lenthe GH, Hermus AR, Corstens FH, Smals AG, Huiskes R. Speed of sound reflects Young's modulus as assessed by microstructural finite element analysis. Bone 2000;26(5):519-524.
35. Hans D, Schott AM, Meunier PJ. Ultrasonic assessment of bone: a review. Eur J Med 1993;2:157-163.
36. Hans D, Fuerst T, Duboeuf F. Quantitative ultrasound bone measurement. Eur Radiol 1997;7(Suppl 2):43-50.

# QUANTITATIVE TIBIAL ULTRASONOMETRY VERSUS RADIOGRAPHIC PHALANGEAL ABSORPTIOMETRY IN A CAUCASIAN PEDIATRIC POPULATION


## ABSTRACT

*Introduction:* There is a need for a reliable bone assessment technique in children. In this study, we compare an existing technique used in children, radiographic absorptiometry (RA), with a relatively novel technique, quantitative tibial ultrasonometry (QUS).

*Material and Methods:* In a prospective cohort study, we enrolled 290 girls (mean age 12.7 years) and 273 boys (mean age 12.4 years). Radiographs of the left hand and the left index finger were taken with an aluminium reference wedge within the field of exposure. Radiographic absorptiometry on the second middle phalanx at the mid-level ($BMD_{50\%}$) and proximal quarter ($BMD_{25\%}$) was performed with interactive software. Tibial QUS was performed using the SoundScan®Compact.

*Results:* Multiple regression analyses showed that SOS correlated significantly with $BMD_{25\%}$ for both boys ($r = 0.65$, $P<0.001$) and girls ($r = 0.59$, $P<0.001$), taking into account age and gender. The same applied for the correlation between speed of sound (SOS) and $BMD_{50\%}$ in boys ($r = 0.62$, $P<0.001$) and girls ($r = 0.67$, $P<0.001$). Cubic regression between calendar age and $BMD_{25\%}$ showed the best fit for both boys ($r^2 = 0.60$) and girls ($r^2 = 0.60$). For $BMD_{50\%}$ a difference in regression was found between boys and girls. Quadratic regression gave a satisfactory fit for boys ($r^2 = 0.61$) while for girls a cubic relation was best ($r^2 = 0.59$). Overall, there was a significant correlation between $BMD_{25\%}$ and $BMD_{50\%}$ for boys $r = 0.89$ and for girls $r = 0.91$ (both $P<0.001$).

*Conclusion:* Our data show a significant correlation between two different bone assessment techniques. In addition, these data suggest that both tibial ultrasonometry and RA are useful bone assessment techniques in children.


---


Maarten H. Lequin, MD.[1], Rick R. van Rijn MD. PhD.[1], Simon G.F. Robben MD. PhD[1], Wibeke J. van Leeuwen, RT.[1], Wim C. Hop, PhD.[2], Cornelis van Kuijk MD. PhD.[3]

Departments of Pediatric Radiology[1], Sophia Children's Hospital, Erasmus University, Rotterdam, Biostatistics and Epidemiology[2], Erasmus University, Rotterdam and Radiology[3], Academic Medical Centre, Amsterdam, the Netherlands



Part of this study has been presented at the 84th meeting RSNA, 1998, Chicago, USA. Submitted for publication: Calcif Tissue Int 2000.

## 6.1 INTRODUCTION

Several cross-sectional and longitudinal studies show that osteoporosis is no longer a disease of elderly. Not only bone loss in adult life is an important factor for future fracture risk due to osteoporosis, but also the height of peak bone mass (PBM), attained in early adulthood [1]. The emphasis is now more on prevention through maximising PBM and reducing subsequent bone loss, because none of the present treatments can significantly restore the amount of bone lost in severe osteoporosis. Therefore, it is important to increase our knowledge on the attainment of peak bone mass by using quantitative bone mineral density (BMD) assessment techniques. Other indications to measure BMD in a child are quantification of bone mass changes due to various diseases and drug therapies inducing osteopenia [2,3].

Several non-invasive techniques for BMD assessment have been used in the adult population. However, only a few techniques are available for a pediatric population due to several reasons.

The most widely used BMD assessment technique is dual-energy X-ray absorptiometry (DXA). The reasons for its frequent use include its low radiation dose, good availability and ease of use. The major drawback of this technique is it being a projectional method, which measures the radiation attenuation at the measured site. Therefore, DXA measures an area density, calculated as the quotient of the bone mineral content and the area, rather than a true volumetric density. For instance, DXA will measure a higher areal bone density in a larger vertebra of a child than in a smaller vertebra, when they have both the same constant volumetric bone density. Therefore this two-dimensional technique cannot discern between a change in true bone density and normal growth of the skeleton, because the skeleton of a child grows in three-dimensions.

Second, weight changes, which normally occur during growth, have a tremendous impact on the DXA measurements [4,5].

Quantitative computed tomography is a technique, which measures true volumetric BMD independent of surrounding soft tissue, and therefore could be a very accurate method for measuring BMD changes in a growing child [6]. Its relative high level of radiation exposure hampers the implementation in

childhood. Furthermore, the need for a relative long immobility period of the child during scanning is an additional drawback.

Radiographic absorptiometry (RA) is a technique, which combines assessment of skeletal age (SA), an important indicator of bone maturation, and BMD assessment. This method has existed for many years and it is the oldest quantitative bone mass assessment technique [7-9]. RA quantitatively assesses bone mass (cortical and trabecular) on conventional radiographs, mostly of the hand. Several studies have shown that RA is as good in predicting fracture risk in postmenopausal women as other densitometry techniques, like DXA and quantitative ultrasonometry [10,11]. RA appears also to be appropriate for the assessment of BMD of phalanges and metacarpals in the pediatric population [12-14]. Moreover, it is relatively inexpensive and widely accessible. It is, however, not frequently used. One of the reasons being the necessity of dedicated postprocessing equipment and lack of normal reference values.

Recently another bone assessment technique has been introduced: quantitative ultrasonometry (QUS). Several studies suggest that quantitative ultrasonometry (QUS) has the capability to investigate not only bone density but also bone structure [15,16]. This is important because several studies have shown that bone density can account for only 70-80% of the variability in bone strength. The remaining variance in bone strength could be due to other factors such as ineffective bone architecture, fatigue damage, measurement artifacts and state of remodeling [17,18]. Therefore a non-invasive bone assessment technique, which might detect fragility and not only decreased bone mass, would be preferred. QUS seems to be such a technique, using the combination of the information on bone elasticity, structure and density [19,20]. Other advantages of QUS are its low cost, ease of use, patient comfort and absence of radiation. In this study, we use the tibial QUS, which can be performed in young children without problems. We choose not to use the calcaneal QUS method because difficult positioning and immobilisation of small feet introduce inaccurate measurements. In addition, the change of the measuring site at the calcaneus due to growth, will introduce another uncontrolled factor. In this study, we address the correlation between BMD measurements with QUS and RA, acquired in a healthy Caucasian pediatric population, aged 6-19 years.

## 6.2 MATERIAL AND METHODS

*Study subjects*

In a prospective cohort study, we enrolled 290 girls, (mean age 12.7 years; range 6.0-19.9 years) and 273 boys, (mean age 12.4 years; range 6.0-19.5 years). Exclusion criteria were diseases and/or medication therapies, which affect bone architecture and bone metabolism.

Informed consent was obtained explicitly from parents or guardians and where appropriate from the child (in the Netherlands this is mandatory in children aged 12 years and over). This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines of the Internal Review Board of the Erasmus Medical Centre (Rotterdam, the Netherlands).

Overall health, sex, Tanner stage, calendar (CA) and skeletal (SA) were assessed for each participant. Tanner stages were evaluated through self-assessment; this technique was validated by Duke et al. [21]. Subjects were shown pictures, written information illustrating breast and pubic hair development for girls, and genital and pubic hair development for boys. They were asked to select the one that had the closest resemblance to their own status. When there were discrepancies between variables, emphasis was placed on the breast development in girls and genital development in boys [22].

Height was measured, without shoes, using a wall-mounted ruler [23]. Weight was measured, without shoes, on an electronic weight scale. Body mass index (BMI) as an indicator of nutritional status was calculated as the ratio of weight to height$^2$ (kg·m$^{-2}$).

*Radiography*

A standardizedised posteroanterior (PA) radiograph of the left hand, and an additional lateral radiograph of the left index finger (LAT) were taken (Philips diagnost H, Imation GT film, α-II screen, film-focus distance 1.5 m, 45 kV, 16 mAs). To assess skeletal age, all radiographs of the left hand were evaluated and scored according to the Greulich and Pyle atlas [24].

*Radiographic absorptiometry*

The RA technique measures the diaphysis and proximal metaphysis of the second middle phalanx as measuring site in the posteroanterior (PA) and lateral view (LAT). The lateral view of the second middle phalanx is made on the same screen using a dedicated cassette with an identical aluminium reference wedge (Figure 1).

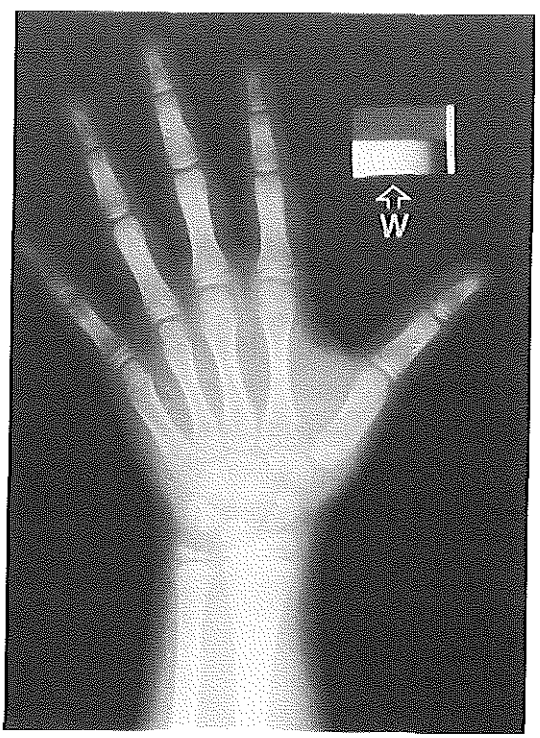



Fig. 1A

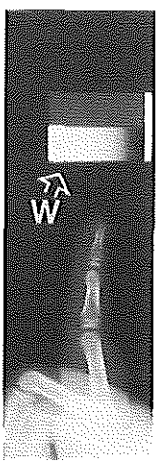



Fig. 1B

**Fig. 1** An example of the X-ray images used in this study.
**A.** Posteroanterior projection of the left hand (W = Aluminum reference wedge).
**B.** Lateral projection of the left index finger (W = Aluminum reference wedge).

Interactive software developed in-house (Departement of Experimental Radiology, Erasmus University, Rotterdam) was used to analyse all radiographs, using a 286 PC equipped with a modular frame grabber (Imaging Technology) in combination with a CCD camera (SWK-31, DIFA).

Analyses, performed by one operator (WvL), consisted of scanning the two identical aluminium wedges (thickness 0-12 mm). A reference curve was obtained with this scanning. The regions of interest (ROI's) are automatically determined from the digitized X-ray, after defining the distal and proximal edges of the second phalanx. The software also automatically determines the length and the outer contours of the phalanx. The two ROI's were a 3-mm wide area across the mid-point of the total length of the phalanx ($BMD_{50\%}$) and a 3-mm wide area located at the proximal quarter-point of this phalanx ($BMD_{25\%}$). The mid-phalangeal ROI consists mainly of cortical bone (80%), the proximal quarter-point ROI mainly of trabecular bone (60%)[12,25,26]. The software combines the measurements at the two ROI's in the PA and LAT projection, to obtain a volumetric BMD relative to the aluminium wedge ($mgAL/mm^3$).

*Quantitative ultrasonometry (QUS)*

Tibial QUS was performed using the SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel, Software Version 1.1e) (Figure 2). Following standard operational procedures, all QUS bone assessments were done on the right tibia at the mid-tibial point. The mid-tibial point was defined as the mid-point of the line between the apex of the medial malleolus and the distal patellar apex.

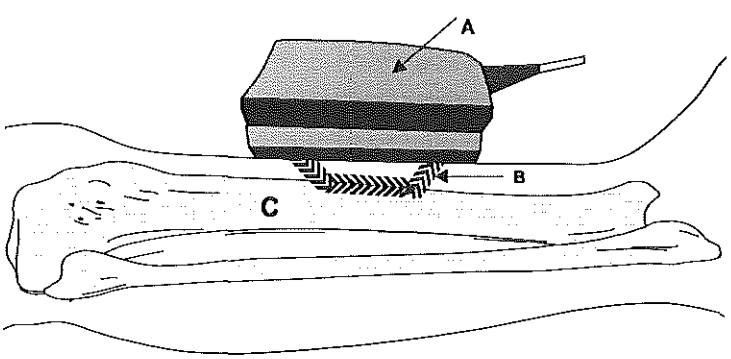



**Fig 2.** Principle of tibial quantitative ultrasonometry.
A. Transducer
B. Speed of sound
C. Tibia

*Statistical analyses*

Polynomial regression was used to assess the relation between SOS and age. The same method was used to evaluate age-effects on $BMD_{25\%}$ and $BMD_{50\%}$. Multiple regression analyses were performed to test the relations between SOS on one hand and $BMD_{25\%}$ and $BMD_{50\%}$ on the other hand. Correlation coefficients given are Pearson's, $P$=0.05 (two-sided) was considered the limit of significance.

## 6.3 RESULTS

Quadratic regression between CA and SOS showed the best fit for both boys ($r^2$=0.52) and girls ($r^2$=0.63). Cubic regression between $BMD_{25\%}$ and CA showed the best fit for both boys ($r^2$=0.60) and girls ($r^2$=0.60). For $BMD_{50\%}$, a difference in regression was found between boys and girls: quadratic regression gave a satisfactory fit for boys ($r^2$=0.61) while for girls a cubic relation was best ($r^2$=0.59).

Overall, there was a strong linear correlation between $BMD_{25\%}$ and $BMD_{50\%}$, (for boys r =0.89 and for girls r =0.91; both $P$<0.001). SOS correlated moderately with $BMD_{50\%}$ for both boys (r =0.62, $P$<0.001, Figure 3) and for girls (r =0.67, $P$<0.001, Figure 4).

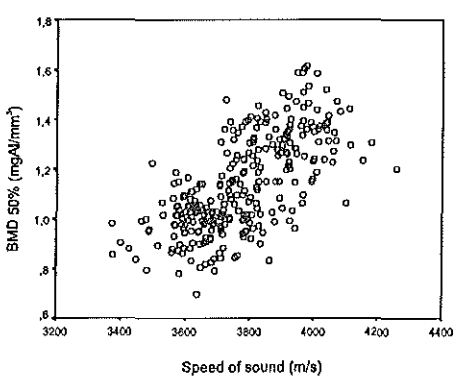



**Fig 3.** $BMD_{50\%}$ versus SOS in Boys.

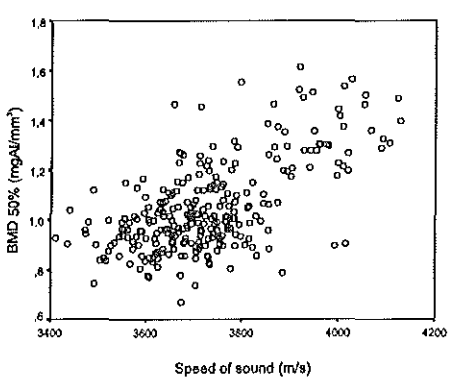



**Fig 4.** $BMD_{50\%}$ versus SOS in Girls.

Table 1. Results of multiple regression analyses of SOS (m/s).

| Factor | Regression coefficient | | *P* value |
|---|---|---|---|
| Constant | 3286 | (± 28.6) | |
| $BMD_{50\%}$ (mgAl/mm$^3$) | 212.8 | (± 53.7) | <0.001 |
| $BMD_{25\%}$ (mgAl/mm$^3$) | -75.5 | (± 81.9) | 357 |
| Sex (male-female) | -19.7 | (± 8.8) | 26 |
| Skeletal age (years) | 24.2 | (± 1.8) | <0.001 |
| $R^2$ | 0.58 | | |

Data given are regression coefficients (± standard error)

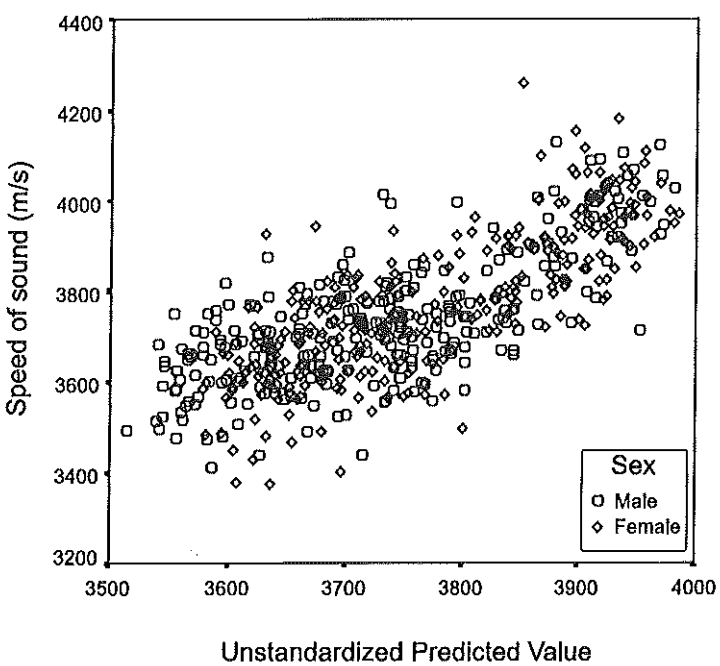



**Fig 5.** SOS versus unstandardized predicted value.

Analyses of $BMD_{50\%}$ and $BMD_{25\%}$ in regard their predictive value of SOS, taking into account age and sex, showed that $BMD_{50\%}$ was of significant predictive value, while $BMD_{25\%}$ was of no additional value (see Table 1 and Figure 5). This result was also found when CA was used instead of SA. Anthropomorphic data, SOS and BMD stratified by Tanner stage are presented in Tables 2A and 2B.

**Table 2A.** Anthropomorphic data (means) according to Tanner stage for boys.

| | Tanner stage | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Age (years) | 8.9(1.7) | 11.2(2.2) | 13.2(1.2) | 14.8(1.6) | 16.9 (1.8) |
| Skeletal age (years) | 8.3(2.0) | 10.7(2.3) | 13.0(0.8) | 14.8(1.4) | 17.0(1.7) |
| $BMD_{25\%}$ ($mgAl/mm^3$) | 0.63(0.06) | 0.62(0.06) | 0.66(0.06) | 0.79(0.09) | 0.87(0.09) |
| $BMD_{50\%}$ ($mgAl/mm^3$) | 0.94(0.09) | 0.94(0.09) | 0.96(0.07) | 1.15(0.14) | 1.31(0.14) |
| SOS (m/sec) | 3657.5(87.0) | 3648.7(108.6) | 3747.0(101.8) | 3755.4(125.7) | 3899.8(128.7) |
| BMI ($kg/m^2$) | 16.5(1.8) | 17.4(2.5) | 18.6(2.5) | 19.1(1.8) | 21.5(2.3) |
| Number of subjects | 91 | 47 | 35 | 53 | 47 |

SD between brackets

**Table 2B.** Anthropomorphic data (means) according to Tanner stage for girls.

| | Tanner stage | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Age (years) | 8.6(1.6) | 10.9(1.4) | 12.5(1.1) | 14.4(1.5) | 17.1(1.8) |
| Skeletal age (years) | 8.5(1.4) | 10.7(1.2) | 12.3(1.0) | 14.6(1.5) | 16.8(1.3) |
| $BMD_{25\%}$ ($mgAl/mm^3$) | 0.64(0.06) | 0.66(0.06) | 0.74(0.08) | 0.83(0.09) | 0.87(0.09) |
| $BMD_{50\%}$ ($mgAl/mm^3$) | 0.98(0.09) | 1.01(0.10) | 1.08(0.12) | 1.24(0.16) | 1.32(0.13) |
| SOS (m/sec) | 3638.2(98.3) | 3688.8(102.7) | 3703.9(97.9) | 3841.1(100.4) | 3945.5(106.9) |
| BMI ($kg/m^2$) | 16.2(2.0) | 18.0(2.6) | 17.3(2.0) | 20.1(2.1) | 21.2(2.5) |
| Number of subjects | 90 | 41 | 20 | 61 | 78 |

SD between brackets

## 6.4 DISCUSSION

Many studies in adults have shown significant but weak correlation between several different BMD techniques at a variety of measuring sites [2,27-29]. Relatively few studies have been performed in children to compare various BMD techniques [30-33].

Ideally, tibial QUS should be compared to QCT that is considered the gold standard of true volumetric bone densitometry. However, several objections can be raised to the use of QCT as a bone mass assessment method in a large healthy pediatric population: the high costs and the relatively high radiation dose compared to other radiological BMD techniques. We did not choose the more popular DXA technique for comparison, because this method seems to be unreliable in the growing child[4,5]. Therefore RA was used for comparison,

because this technique gives a low radiation dose, is easy to perform and in many pediatric patients X-rays of the hand have been made to determine skeletal maturity and to look for disturbances in bone mineralization.

Our data show a statistically significant correlation between SOS and $BMD_{25\%}$ and SOS and $BMD_{50\%}$. This correlation increases significantly when calendar or skeletal age and gender are taking into account. Using either SA or CA, and gender, $BMD_{50\%}$ gives a good predictive value of SOS. In contrast, $BMD_{25\%}$ has no additional significant influence on SOS. Therefore, $BMD_{50\%}$ seems the most important parameter for predicting the SOS. In addition, this parameter was measured at the location where the percentage of cortical bone is the highest. This could explain why $BMD_{50\%}$, and not $BMD_{25\%}$, is the best predictor of SOS. With the assumption that RA is a true measurement of bone density, increase in the predicted SOS may be explained not only by the increase in bone density but possibly also by growth, more specifically, increase in cortical thickness or changes in bone architecture (see Table 1). This suggests that tibial QUS measures more than bone density. Several other studies have shown that QUS gives information not only on bone density but also on bone elasticity and structure [19,20]. This is important because bone elasticity and structure accounts for 20-30% of the variability in bone strength. Lee et al. showed that tibial QUS measured in situ correlated with the material properties of tibial cortical bone nearly as strongly as did bone density [34].

In conclusion, our data show a significant correlation between two different bone assessment techniques, RA and tibial QUS. This is in agreement with other correlation studies with different techniques. The correlation coefficient (r) ranges between 0.6 and 0.8 [27,28,30,31]. Our data suggests that tibial QUS and RA are useful and complementary bone assessment techniques in children. Due to its limited availability and the necessity for dedicated software, RA is not a widely used bone assessment technique in pediatric populations. Tibial QUS may be a more appropriate bone assessment technique in children due to its lack of radiation and its mobility. The clinical application of tibial QUS should be investigated further in prospective studies.

*Acknowledgements*

We would like to thank all volunteers and their parents and guardians, for their co-operation in this study. Special gratitude is due to the 'Erasmiaans' and 'Marnix' high schools in Rotterdam, for their willingness to co-operate in our study. We also thank the radiological technicians of the Department of Radiology, Erasmus Medical Centre, Rotterdam.

## 6.5 REFERENCES


1. Heaney RP. Bone mass and osteoporotic fractures. Calcif Tissue Int 1990;47:63-5.
2. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: state of the art [review]. J Bone Miner Res 1996;11(6):707-30.
3. Nilsson BE, Johnell O, Petersson C. In vivo bone-mineral measurement. How and why -- a review. Acta Orthop Scand 1990;61(3):275-81.
4. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997;12(11):1908-21.
5. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in vivo bone mineral density. J Bone Miner Res 1998;13(11):1739-1746.
6. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med 1991;325:1597-1600.
7. Mack PB, O'Brien AT, Smith JM, Bauman AW. A method for estimating the degree of mineralization of bone from tracings of roentegongrams. Science 1939;89:467.
8. Stein I. The evaluation of bone density in the roentgenogram by the use of ivory wedges. An J Roentegenol Radiat Ther 1937;37:678-82.
9. Warren SL, Bishop FW, Hodge HC, van Huysen G. A quantitative method for studying the roentgen-ray absorption of tooth slabs. Am J Roentgenol Radiat Ther 1934;31:663-72.
10. Grampp S, Lang P, Jergas M, et al. Assessment of the skeletal status by peripheral quantitative computed tomography of the forearm: short-term precision in vivo and comparison to dual X-ray absorptiometry. J Bone Miner Res 1995;10(10):1566-76.
11. Jergas M, Genant HK. Current methods and recent advances in the diagnosis of osteoporosis. Arthritis Rheum 1993;36:1649-1662.
12. Trouerbach WT, Man SAd, Gommers D, Zwamborn AW, Grobbee DE. Determinants of bone mineral content in childhood. Bone Miner 1991;13:55-67.
13. van Teunenbroek A, Mulder P, de Muinck Keizer-Schrama S, et al. Radiographic absorptiometry of the phalanges in healthy children and girls with Turner syndrome. Bone 1995;17(1):71-8.
14. Rijn RRv, Lequin MH, Leeuwen WJv, Kuijk Cv. Radiographic absorptiometry of the phalanx in a Caucasian pediatric population; normative data for ages 6 to 19. Osteoporosis Int 2000;11:240-247.
15. Glüer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 1994;55:46-52.
16. Duquette J, Lin J, Hoffman A, Houde J, Ahmadi S, Baran D. Correlations among bone mineral density, broadband ultrasound attenuation, mechanical indentation, and bone orientation in bovine femoral neck samples. Calcif Tissue Int 1997;60:181-6.

17. Kleerekoper M, Villanueva AR, Stanciu J, Sudhaker-Rao D, Parfitt AM. The role of three-dimensional trabecular microstructure in the pathogenesis of vertebral compression fractures. Calcif Tissue Int 1985;37:594-7.
18. Mosekilde L. Sex diffences in age-related loss of vertebral trabecular bone mass and structure - biomechanical consequences. Bone 1989;10:425-32.
19. Kaufman JJ, Einhorn TA. Perspective: ultrasound assessment of bone. Osteoporos Int 1993;8:517-525.
20. Hans D, Schott AM, Meunier PJ. Ultrasonic assessment of bone: a review. Eur J Med 1993;2:157-163.
21. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66(6):918-20.
22. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997;82:57-62.
23. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997;12(Suppl 1):S31.
24. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2nd ed. Stanford, California: Stanford University Press, 1959.
25. Trouerbach WT, Grashuis JL, Zwamborn AW, Clermonts ECGM, Schouten JA. Microdensitometric analysis of bone structure in X-ray images. Skeletal Radiol 1987;16:190-5.
26. Trouerbach WT, Birkenhäger JC, Collette BJA, Drogendijk AC, Schmitz PIM, Zwamborn AW. A study on the phalanx bone mineral content in 273 normal pre- and postmenopausal females (transverse study of age-dependend bone loss. Bone Miner 1987;3:53-62.
27. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995;17(4):363-7.
28. Rosenthal L, Caminis J, Tenehouse A. Correlation of ultrasound velocity in the tibial cortex, calcaneal ultrasonography, and bone mineral density of the spine and femur. Calcif Tissue Int 1996;58:415-8.
29. Fan B, Zucconi F, Fuerst T, Glüer CC, Genant HK. Precision assessment: ultrasonic velocity measurement of the mid-tibia versus other techniques. J Bone Miner Res 1995;10(suppl 1).
30. Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL. Comparison between broad-band ultrasound attenuation of the calcaneus and total body bone mineral density in children. Acta Pediatr 1996;85:663-5.
31. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995;56:368-71.
32. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998;26:177-182.

33. Slosman DO, Rizzoli R, Bonjour J-P. Bone absorptiometry: a critical appraisal of various methods. Acta Paediatr Suppl 1995;411:9-11.
34. Lee SC, Coan BS, Bouxsein ML. Tibial ultrasound velocity measured in situ predicts the material properties of tibial cortical bone. Bone 1997;21(1):119-125.

# COMPARISON BETWEEN QUANTITATIVE CALCANEAL AND TIBIAL ULTRASOUND IN A DUTCH CAUCASIAN PEDIATRIC AND ADOLESCENT POPULATION


## ABSTRACT

*Introduction:* In the field of bone densitometry, attention has recently focused on the pediatric population. Quantitative ultrasonometry (QUS) is a bone assessment technique that has many advantages for children in comparison with bone assessment techniques that use ionizing radiation. In this pilot study, we compared the use of a calcaneal QUS system and tibial QUS system in a healthy Caucasian pediatric population.

*Material and Methods:* We studied 120 healthy Caucasian Dutch children between 7 and 19 years, 53 boys (mean age 12.5 years; range 4.5 - 18 years) and 67 girls (mean age 13.5 years; range 7.1 - 19 years). We recruited children from a large population, who had previously participated in a bone assessment study performed at our hospital. Two operators performed calcaneal QUS of the right calcaneus using the Sahara®Osteometer and tibial QUS of the right tibia using the SoundScan®Compact.

*Results:* The correlation between calcaneal ultrasonometry and tibia ultrasonometry was modest, but significant ($r = 0.29$, $P<0.01$). Using the calcaneal device, in girls we found weak positive correlations between skeletal age and speed of sound (SOS) ($r = 0.38$), broadband ultrasound attenuation (BUA) ($r = 0.57$) and quantitative ultrasound index (QUI) ($r = 0.46$), all with a $P<0.01$. For boys all parameters failed to reach significance (ns). Using the tibia device, we found a good correlation between skeletal age and SOS in girls ($r = 0.76$) and modest correlation in boys ($r = 0.50$) (both with a $P<0.01$).

*Discussion:* This is one of the first studies to present a comparison between two quantitative ultrasonometry techniques in children. In light of the poor correlation with skeletal age, calcaneal ultrasound is ineffective in children, while tibia ultrasonometry seems to be a good bone assessment technique in children.


---


Maarten H. Lequin, MD.[1], Rick R. van Rijn, MD., PhD.[1],Marieke C.H.W. Bukkems, RT.[2], Leonieke L.J. Verhaak, RT.[2], Simon G.F. Robben, MD., PhD.[1], Wim C.J. Hop, PhD.[3] Cees Van Kuijk, MD., PhD.[4]

Depts of Radiology[1], and Epidemiology and Biostatistics[3], University Children's Hospital 'Sophia' Rotterdam, School of Higher Vocational Education[2], Medical Imaging and Radiotherapeutic Techniques, Eindhoven and Department of Radiology[4], Academic Medical Centre Amsterdam, the Netherlands.


Accepted for publication in J Clin Densito :2001.

## 7.1 INTRODUCTION

In the field of bone densitometry, attention has recently focused on the pediatric population, as attainment of a high peak bone mass seems to decrease the risk of osteoporosis later in life [1]. This is especially true in children who have specific disorders affecting structural bone growth with or without metabolic disorders of bone and who are therefore considered to be at risk for osteoporosis [2]. Screening by means of bone assessment at a young age could provide a basis for early intervention. For children a bone assessment technique preferably possesses the following characteristics: it should be patient friendly; it should be accurate, and have a good reproducibility; it should provide clinically useful parameters. Recent publications by Tothill, Svendsen and Bollotin showed that changes in body composition have detrimental effect on DXA measurements [3-7]. In healthy children with normal weight for height, this effect will be minimal, but in sick children, receiving high dose corticosteroids for therapy changes in body composition could influence the DXA measurements. Also measuring bone density of the skeleton of a child, which grows in three-dimensions, a two-dimensional technique could give erroneous results and therefore the applicability of DXA in a pediatric population is uncertain.

Quantitative computed tomography (QCT) is a technique that assesses bone mass in three-dimensions [8,9]. As the skeleton of children grows in three-dimensions, QCT is to be preferred over a two-dimensional technique. Furthermore, QCT is capable of distinguishing between trabecular and cortical bone. However, with QCT, children are exposed to a high level of radiation and this technique also has problems with reproducibility, because of motion artifacts due to long scanning time [10].

Quantitative ultrasound (QUS) might be a good alternative to the above-mentioned techniques. Several studies showed that QUS systems measure not only the amount of bone density, but also structural properties of bone, i.e. anisotropy, elasticity and lamellar orientation [11,12]. This could be of clinical relevance, because fracture risk later in life depends not only on bone density but also on bone architecture [13,14]. There are several systems available for QUS, measuring either the tibia, calcaneus or the phalanx [9,10]. Calcaneal ultrasound systems are most widely used in adult subjects, internationally [9].

However, now there are only sparse data available on the efficacy of these systems in pediatric populations [15,16]. We chose to use a calcaneal ultrasound device with a dry system. A dry system makes the application in children easier then those requiring a water-bath. Furthermore the use of coupling-gel instead of a water-bath removes one of the most important factors influencing the variability of measurements, the variable temperature of the water-bath [17].
The aim of this study was to compare a calcaneal QUS system with the tibial QUS system, we have used for several years. In addition, the correlations between skeletal age (SA) and speed of sound (SOS), broadband ultrasound attenuation (BUA) and the quantitative ultrasound index (QUI) were investigated. The latter is a parameter derived from both BUA and SOS, which is claimed superior to the standardized coefficient of variation of either SOS or BUA alone.

## 7.2 MATERIAL AND METHODS

*Study subjects*

A group of 53 Caucasian boys (mean age 12.5 years; range 4.5-18 years), and 67 Caucasian girls (mean age 13.5 years; range 7.1-18 years) was studied. Study subjects were children recruited from a larger group, who previously participated in bone ultrasonometry studies at our hospital [18]. Informed consent was obtained explicitly from parents or guardians and where appropriate from the child (in the Netherlands this is mandatory in children aged 12 years and over). This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines of the Internal Review Board of the University Hospital, Rotterdam and of the Erasmus University Rotterdam, Faculty of Medicine and Health Sciences, the Netherlands.
All children met the following criteria: They had no recent injury of tibia or calcaneus and they did not suffer from any disease known to affect bone growth and/or metabolism.
Data concerning sex, age, height, weight and Tanner stage were collected. Height was measured, without shoes, using a wall-mounted ruler [19]. Weight was measured, without shoes, on an analog weight scale. Tanner stages were evaluated through self-assessment, a technique validated by Duke et al. [20].

*Radiography*

To asses skeletal age (SA), X-rays of the left hand were taken from all participants (Philips Diagnost H, Imation GT film, (α II screen, film-focus distance 1.5 m, 45 kV, 16 mAs). All X-rays were evaluated and scored according to the Greulich and Pyle atlas, by one investigator (RvR) [21].

*Quantitative ultrasonometry*

Calcaneal QUS was performed using the Sahara® Osteometer (Hologic Inc., Bedford, MA, USA). This device from Hologic is a fixed calcaneal method, which can be used (according to the company) in a pediatric population aged eight years and over. This system takes less than ten seconds to perform the measurement without need for a water-bath. The Sahara® system measures not only the speed of sound (SOS in m/s) and broadband ultrasound attenuation (BUA, in dB/MHz), it also calculates a so-called quantitative ultrasound index (QUI). Two operators performed all measurements (MCHWB and LLJV). To assess intra- and inter-observer variability, each investigator measured ten volunteers twice. Intra- and inter-observer variances were calculated according to the guidelines stated by the FDA [22].

We have already validated tibial ultrasonometry in a normal pediatric population, using the SoundScan®Compact [23]. This system uses a transmission technique with coupling gel, placed at the mid-point of the right tibia, to measure the speed of sound (SOS in m/s) through a fixed distance of the tibia.

*Statistical analyses*

Linear regression analyses were used to assess correlations between measurements and age. Correlation coefficients given are Pearson's. Multiple regression analyses were done to investigate the predictive value of various characteristics regarding outcome parameters. $P$=0.05 (two-sided) was considered the limit of significance.

## 7.3 RESULTS

Tables 1A and 1B show the results of the tibial and calcaneal ultrasound measurements stratified by Tanner stage for both boys and girls.

**Table 1A.** Ultrasound parameters obtained by tibia ultrasonometry and calcaneal ultrasonometry for boys.

| Tanner Stage | N (%) | | Tibia SOS (m/s) | | Calcaneus SOS (m/s) | BUA (dB/MHz) | QUI |
|---|---|---|---|---|---|---|---|
| I | 22 | 42 | 3719 | (103) | 1558 (18) | 59 (11) | 92 (11) |
| II | 5 | 9 | 3768 | (64) | 1534 (16) | 53 (11) | 80 (10) |
| III | 5 | 9 | 3748 | (82) | 1560 (24) | 58 (10) | 92 (13) |
| IV | 11 | 21 | 3741 | (114) | 1554 (25) | 56 (12) | 89 (10) |
| V | 10 | 19 | 3877 | (123) | 1555 (20) | 67 (17) | 96 (12) |

SD between brackets

**Table 1B.** Ultrasound parameters obtained by tibia ultrasonometry and calcaneal ultrasonometry for girls.

| Tanner Stage | N (%) | | Tibia SOS (m/s) | | Calcaneus SOS (m/s) | BUA (dB/MHz) | QUI |
|---|---|---|---|---|---|---|---|
| I | 12 | 18 | 3711 | (100) | 1550 (18) | 54 (10) | 86 (11) |
| II | 8 | 12 | 3750 | (130) | 1546 (15) | 58 (7) | 86 (8) |
| III | 11 | 16 | 3832 | (122) | 1556 (32) | 60 (13) | 91 (17) |
| IV | 15 | 22 | 3913 | (107) | 1561 (23) | 68 (12) | 97 (14) |
| V | 21 | 31 | 3982 | (88) | 1567 (22) | 72 (13) | 101 (14) |

SD between brackets

In Figure 1, the correlations between the calcaneus device and tibia device are plotted (girls: r =0.29, $P<0.05$; boys: r =0.26, $P>0.05$).

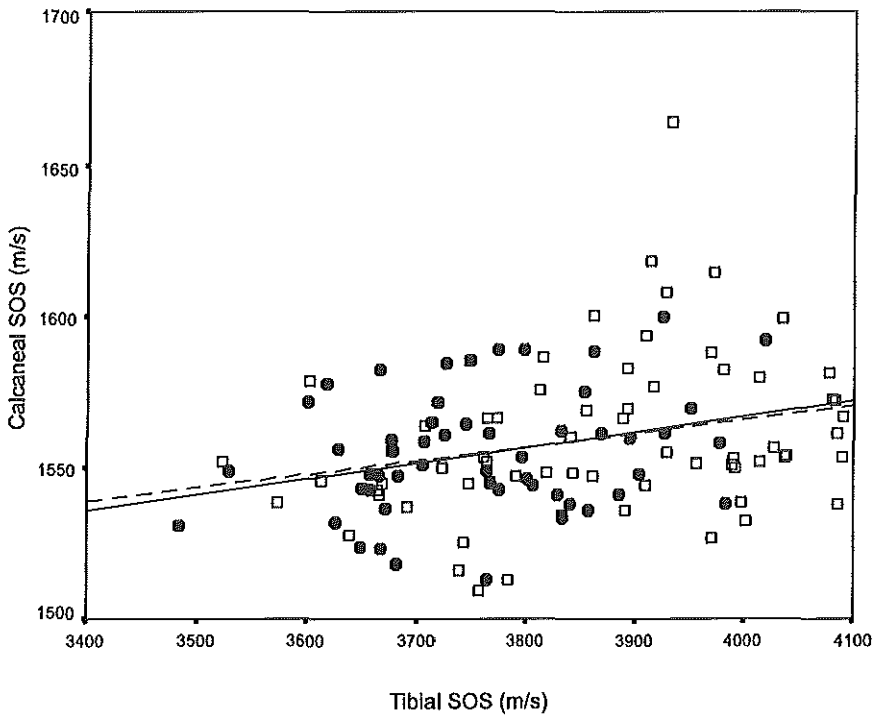



**Fig 1.** Comparison between calcaneal speed of sound (m/s) and tibial speed of sound (m/s)(r =0.29, $P<0.01$), dots represent boys and squares girls.

Results comparing SA versus SOS, BUA and QUI for boys and girls are shown in Figures 2 (A and B) through 4 (A and B). For boys all correlations with SA failed to reach significance. For girls these correlations were all significant (SOS r =0.38, BUA r =0.57, and QUI r =0.47; all $P<0.01$). The correlation coefficient for girls between SA and BUA (0.57) is significantly higher than for SOS (0.38) or for QUI (0.47). In girls, neither length, weight or body mass index (BMI) added additional predictive value to SA with regard to any of these measurements. In girls, only Tanner stage is a significant determinant for SOS, BUA and QUI. Using the tibia device, we found a good correlation between skeletal age and SOS in girls (r =0.76; $P<0.01$) and modest correlation in boys (r =0.50; $P<0.01$) (Figures 5 A and B respectively).

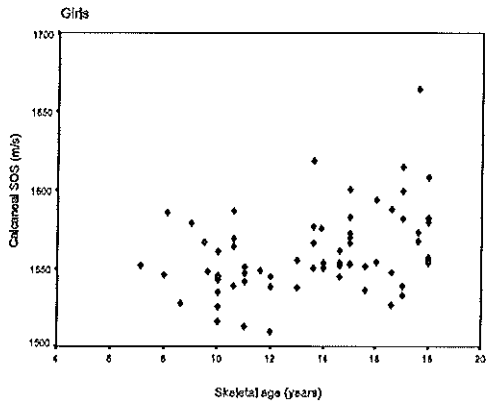



**Fig 2A.** Calcaneal speed of sound versus skeletal age (r =0.38; $P<0.01$).

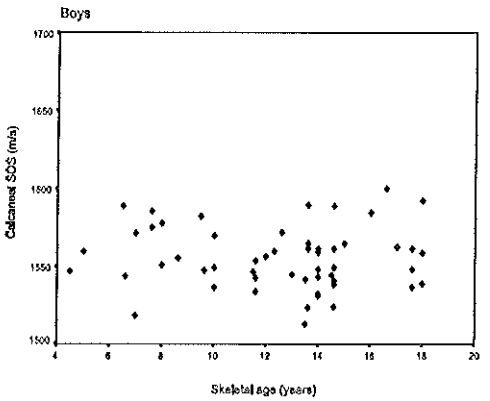



**Fig 2B.** Calcaneal speed of sound versus skeletal age (r =0.26, $P>0.05$).

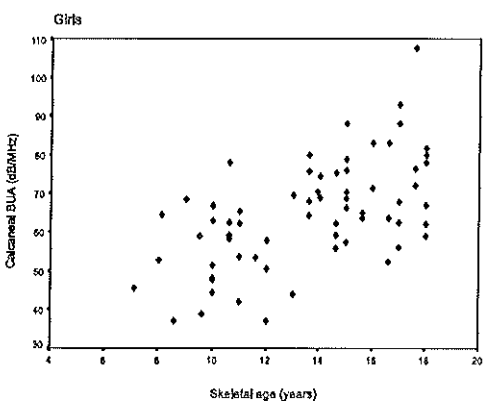



**Fig 3A.** Calcaneal broadband ultrasound attenuation versus skeletal age (r =0.57; $P<0.01$).

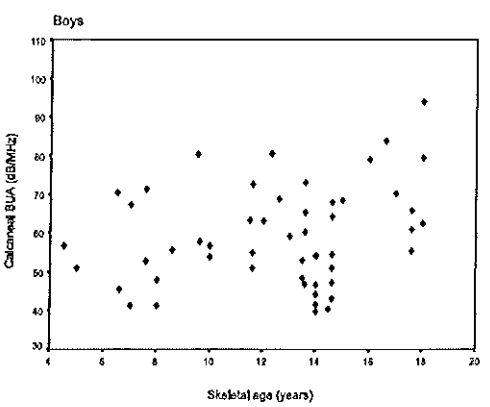



**Fig 3B.** Calcaneal broadband ultrasound attenuation versus skeletal age (r =0.26, $P>0.05$).

The precision of inter-observer variability for SOS was 0.2%, for BUA 3.5% and for QUI 1.9%. The intra-observer variability of the first investigator (MCHWB) was 0.2% for SOS, 2.6% for BUA and 1.7% for QUI. For the second investigator (LLJV) the precision was 0.3% for SOS, 3.7% for BUA and 1.9% for QUI. The intra-observer precisions in our population are comparable to those seen in an adult population [24].

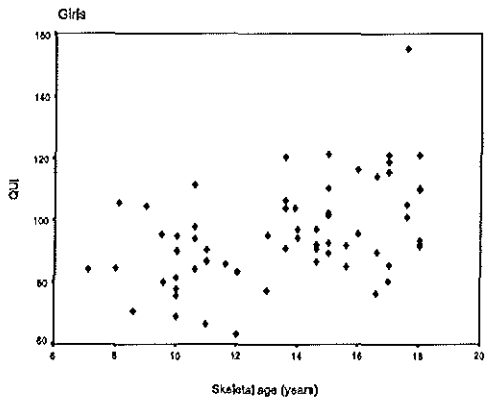



**Fig 4A.** Quantitative ultrasound index versus skeletal age (r =0.47; $P<0.01$).

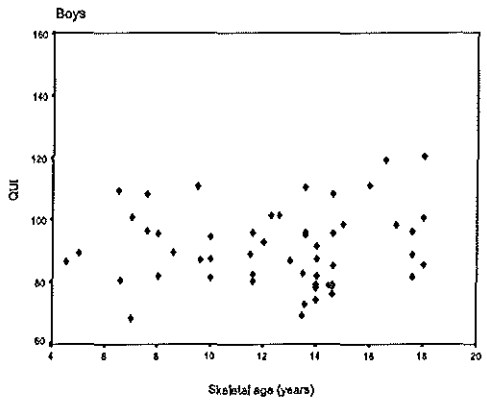



**Fig 4B.** Quantitative ultrasound index versus skeletal age (r =0.08, $P>0.05$).

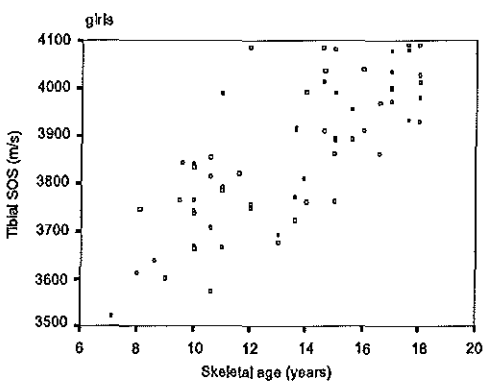



**Fig 5A.** Tibial speed of sound (SOS m/s) versus skeletal age (r =0.76; $P<0.01$) in girls.

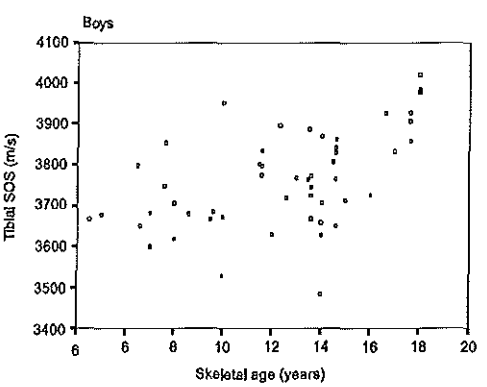



**Fig 5B.** Tibial speed of sound (SOS m/s) versus skeletal age (r =0.50; $P<0.01$) in boys.

The precisions of inter-observer and intra-observer variabilities of the tibia device have already been published and are in the same range as those of the calcaneal device[25].

## 7.7 DISCUSSION

Our study shows a weak, but statistically significant, correlation between the SOS measured with the tibia device and the SOS, BUA and QUI, measured with the calcaneal device. The main reason for this modest correlation is that this calcaneal device does not work as well as the tibia device in our pediatric population. The calcaneal ultrasound in boys, showed no significant correlation with SA or CA. In girls, all parameters showed significant correlations. This difference might be explained by the more uniform distribution of Tanner stage in girls compared to boys. The group of boys has a disproportional high percentage (51%) of Tanner stage I and II (Table 1A).

We studied a reasonable number of subjects and the correlations are low; it is unlikely that a larger number of subjects would show strong correlations.

In a review article, Hans et al. made clear that it is very difficult to define the fundamental accuracy of QUS [26]. The complex bone structure of the calcaneus and its inhomogeneity may result in variable transmission times. In light of the growth in children, this might have a stronger influence in this specific population. Unpublished data of our longitudinal part of the study in these 120 healthy children measured with our tibial ultrasound technique, suggest that tibial growth has no significant influence in the QUS measured parameters. It seems that the tibial ultrasound technique measures not growth but a real increase in bone strength expressed in an increase in SOS (m/s).

In both boys and girls, the strongest correlations were found between SA and BUA. BUA, as opposed to SOS, has no theoretical relation to the properties of bone [24]. However, using fractal dimensions, Rho et al. demonstrated a significant positive correlation between the elastic modulus and BUA [27].

In a recent review article, foot positioning was presented as an important factor influencing precision [26]. This was considered to be the most important source of error in BUA assessment, resulting from the inhomogeneity in spatial distribution of calcaneal trabecular bone. In a pediatric population reproducible foot positioning, especially in longitudinal studies, is difficult. In our study the intra- and inter-observer variance for BUA was significantly higher than for SOS. In light of this factor, we expect BUA to show weaker correlations with SA than with SOS; we have no explanation for our contrasting findings.

Our results are in conflict with those presented by Jaworski et al., who used an adapted version of the Achilles densitometer (Lunar Corp., Madison, WI, USA) [15]. They measured the distance between the tuberosity of the fifth metatarsal and the back of the heel: at one-third on this line (measured from the heel) a point was marked. The measurement point was located one cm perpendicular to the above-mentioned line. They narrowed the originally 2.5 cm broad beam to one cm by using a rubber ring. In this study of 71 children, they reported an increase of SOS and BUA over time. However, they do not present any statistical data on the correlation or significance of this increase. Furthermore, they pooled the data for both boys and girls. Had we done so with our data set, the following correlations would be found: SOS vs. SA r =0.22 ($P$=0.016); BUA vs. SA r =0.45 ($P$<0.001); and QUI vs. SA r =0.32 ($P$<0.001). However, we feel that pooling of data in growing children has an adverse effect on reliability because of differences in pubertal development in boys and girls. Calculation of Z-scores from these pooled data will have no clinical relevance. Mughal et al. also used an adapted version of a commercially available calcaneal system: the McCue ultrasound bone analyser [16]. They however did not present data correlating age and BUA (SOS data were not presented in their publication) but only data correlating total body DXA and BUA (r =0.74, $P$<0.0001). In this study not only were the data of boys and girls pooled, but their group also consisted from children of different racial backgrounds making interpretation of their results problematic.

We feel that, in contrast to the SoundScan®Compact tibia device, the Sahara® Osteometer cannot be used in a pediatric population without specific pediatric adaptations. Further investigation into placement of the foot (i.e. the use of footpads for different foot sizes) and into the spatial distribution of trabecular bone in the growing calcaneus are needed. The use of imaging ultrasound systems, which provide a graphic display of the region of interest, might provide a possible solution to this problem [28].

## 7.5 REFERENCES


1. Fässler AC, Bonjour J. Osteoporosis as a pediatric problem. Pediatr Clin North Am 1995; 42:663-5.
2. Carracosa A, Gussinye M, Yeste D, del Rio L, Audi L. Bone mass acquisition during infancy, childhood and adolescence. Acta Paediatr 1995; 411:18-23.
3. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in vivo bone mineral density. J Bone Miner Res 1998; 13:1739-1746.
4. Bolotin HH. An analytic and quantitative exposition of patient-specific systematic inaccuracies inherent in planar DXA-derived in-vivo BMD measurements. Med Phys 1998; 25:139-51.
5. Svendsen OL, Hassager C, Skodt V, Christiansen C. Impact of soft tissue on in vivo accuracy of bone mineral measurements in the spine, hip, and forearm: a human cadaver study. J Bone Miner Res 1995; 10:868-73.
6. Tothill P, Pye DW. Errors due to non-uniform distribution of fat in dual X-ray absorptiometry of the lumbar spine. Br J Radiol 1992; 65:807-13.
7. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997; 12:1908-21.
8. Genant HK, Ettinger B, Cann CE, Reiser U, Gordan GS, Kolb FO. Osteoporosis: assessment by quantitative computed tomography. Othop Clin N Amer 1985; 16:557-68.
9. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: state of the art [review]. J Bone Miner Res 1996; 11:707-30.
10. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998; 26:177-182.
11. Foldes AJ, Rimon A, Keinan DD, Popovtzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. Bone 1995; 17:363-7.
12. Bouxsein ML, Radloff SE. Quantitative Ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. J Bone Miner Res 1997; 12:839-46.
13. Kaufman JJ, Einhorn TA. Perspective: ultrasound assessment of bone. Osteoporosis Int 1993; 8:517-525.
14. Hans D, Schott AM, Meunier PJ. Ultrasonic assessment of bone: a review. Eur J Med 1993; 2:157-163.
15. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995; 56:368-71.
16. Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL. Comparison between broad-band ultrasound attenuation of the calcaneus and total body bone mineral density in children. Acta Pediatr 1996; 85:663-5.
17. Glüer CC, Barkmann R, Heller M. Quantitativer Ultraschall; status 1999. Radiologe 1999; 39:213-221.

18. Lequin MH, van Rijn RR, Robben SGF, Keinan DD, van Kuijk C. Quantitative ultrasound bone assessment of the tibia normative data for a pediatric, 6 to 19 years Caucasian population. J Clin Densito 1998; 1:102.
19. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997; 12:S31.
20. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980; 66:918-20.
21. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Stanford, California: Stanford University Press, 1959.
22. Arnaudo JS. Draft guidance for review of bone densitometers 510(k). Guide to clinical trials. New York: Raven, 1992:24-7.
23. Lequin MH, van Rijn RR, Robben SGF, Keinan DD, van Kuijk C. Quantitative Ultrasound of the tibia: precision in a pediatric population. Osteoporosis Int 1997; 7:300.
24. Njeh CF, Boivin CM, Langton CM. The role of ultrasound in the assessment of osteoporosis: a review. Osteoporosis Int 1997; 7:7-22.
25. Lequin MH, van Rijn RR, Robben SGF, et al. Evaluation of short-term precision of tibial ultrasonometry. Calcif Tissue Int 1999; 64:24-7.
26. Hans D, Njeh CF, Genant HK, Meunier J-P. Quantitative ultrasound in bone status assessment. Rev. Rhum [Engl. Ed.] 1998; 65:489-98.
27. Rho JY, Flaitz D, Swarnakar V, Acharya RS. The characterization of broadband ultrasound attenuation and fractal analysis by biomechanical properties. Bone 1997; 20:497-503.
28. Roux C, Fournier B, Laugier P, et al. Broadband ultrasound attenuation imaging: a new imaging method in osteoporosis. J Bone Miner Res 1996; 11:1112-8.

# A LONGITUDINAL STUDY USING TIBIAL ULTRASONOMETRY IN HEALTHY CAUCASIAN CHILDREN AND ADOLESCENTS

## 8.1 INTRODUCTION

In Chapter 4, reference data of children and adolescents were presented, using tibial ultrasonometry. These data are cross-sectional and therefore may have given a biased estimate of the true rate of increase of speed of sound (SOS), measured with this tibia device. To control for this possible bias, we performed a longitudinal study. In this chapter, we determined the associations between calendar age, skeletal age and SOS as well as differences in association between cross-sectional and longitudinal data for both sexes. If there are no significant differences between the data acquired in the cross-sectional and longitudinal study, we can use the cross-sectional normative data as a standard for serial tibial bone assessment in healthy and sick children. This is essential for assessing the clinical application of this tibia device and therefore we performed a longitudinal study in healthy children, using tibial ultrasonometry as bone assessment technique.

## 8.2 MATERIAL AND METHODS

*Participants*

We recruited 120 healthy Caucasian children and young adults, 53 boys (mean age 12.5 years; range 4.5-18 years) and 67 girls (means age 13.5 years; range 7.1-18 years). They all were part of a previous larger cross-sectional normative study. The follow-up time was about two years (mean 1.8 years, range 1.3-2.1 years) for all participants. All participants were chosen at random by the computer, from our normative study database. Each participant filled out a questionnaire regarding overall health. None of the selected children suffered from any disease known to affect bone metabolism and/or growth. Each participant was assessed for sex, Tanner stage, calendar age (CA) and skeletal age (SA). Tanner stages were evaluated through self-assessment [1]. The same procedures (described in Chapter 4) were used to measure height and weight [2].

Body mass index (BMI) as an indicator of nutritional status was also calculated as the ratio of weight to height$^2$ (kg·m$^{-2}$).

*Tibial ultrasonometry*

The same tibial ultrasound device as in the previous cross-sectional study (Chapter 4) was used, SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel). Following standard operating procedures, all ultrasound bone measurements were done on the same right tibia at newly measured mid-tibial points. The same operators (MHL and RRvR) performed all measurements.

*Statistical analyses*

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 9.0, SPSS Inc, Chicago, IL, USA). Comparison of changes of Z-scores, or increases of SOS, between groups was done using the *t*-test. Correlation coefficients given are Spearman's.

## 8.3 RESULTS

We compared the calculated regression curves of the cross-sectional data study (Chapter 4) with all the follow-up measurements of the girls and all fit between the 5% and 95% interval curves, except two cases (Figure 1). In the boys, all measurements fit between the 5% and 95% interval curves (Figure 2). Considering the Z-scores of mean SOS, there is a slight, but significant, difference between the first measurement and the second measurement. The first Z-score of SOS is, as expected, about zero, the second Z-score of SOS is 0.4 higher. The delta (second minus first measurement) Z-score of SOS is therefore 0.4 ($P$<0.001). Tanner stage, height, weight and BMI had no additional influence on the delta Z-score. The correlation coefficients between the first and second Z-score of SOS were good, for girls 0.69 ($P$<0.001) and for boys 0.70 ($P$<0.001) (Figure 1).

Longitudinal data show an average annual gain in SOS of 50 m/s for boys and 58 m/s for girls. Between the two investigation times the mean increases in height and tibial length in girls were 6.5 cm and 1.8 cm, in boys 10.2 cm and 3.0 cm.

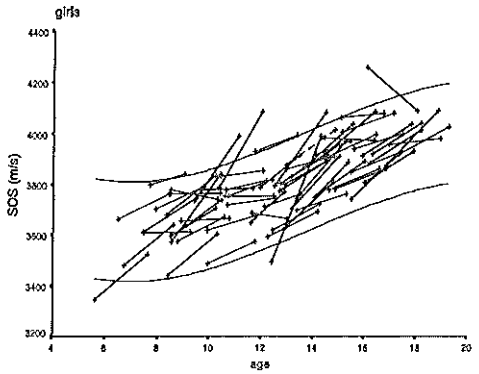



**Fig. 1** Long-term tibial ultrasound data, expressed in mean SOS m/s between the 5% and 95% interval curves for girls.

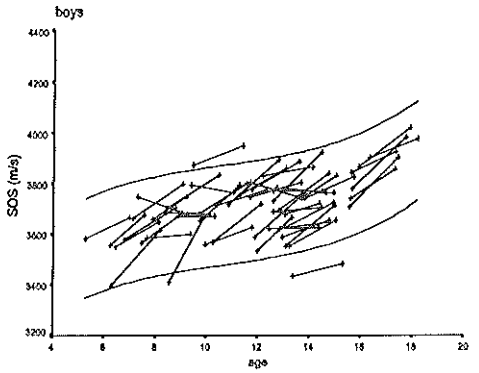



**Fig. 2** Long-term tibial ultrasound data, expressed in mean SOS m/s between the 5% and 95% interval curves for boys.

## 8.4 DISCUSSION

Most longitudinal bone assessment studies in the literature have focused on prevention and treatment of osteoporosis in adults, especially in postmenopausal women[3-8]. Longitudinal studies in children and young adults are few[9-12]. Most of these published studies use dual-energy X-ray absorptiometry (DXA) for bone mass assessment. To our knowledge, this is the first longitudinal bone assessment study done in healthy children and adolescents with this tibial device. We choose this tibia ultrasound device, rather than the more popular calcaneal ultrasound device, for two reasons. First, we think that the change in bone density and/or architecture in time will be less in the tibia than in the calcaneus because the tibia consists mainly of cortical bone and the calcaneus consists mainly of trabecular bone[13]. Second, the precision of the measured mid-tibial point seems better than the precision of localizing the same spot for the calcaneus measurement (see Chapter 7).

Besides the choice of bone assessment technique, there is another important issue to consider, when performing follow-up studies: the longitudinal sensitivity of the bone mass assessment technique[13]. There is on-going disagreement about the standardized method to be used for assessing longitudinal sensitivity of a bone assessment technique[14]. Using the simple approach of dividing the precision error (using the long-term phantom data presented in the addendum of Chapter 3) by the age-related change in our

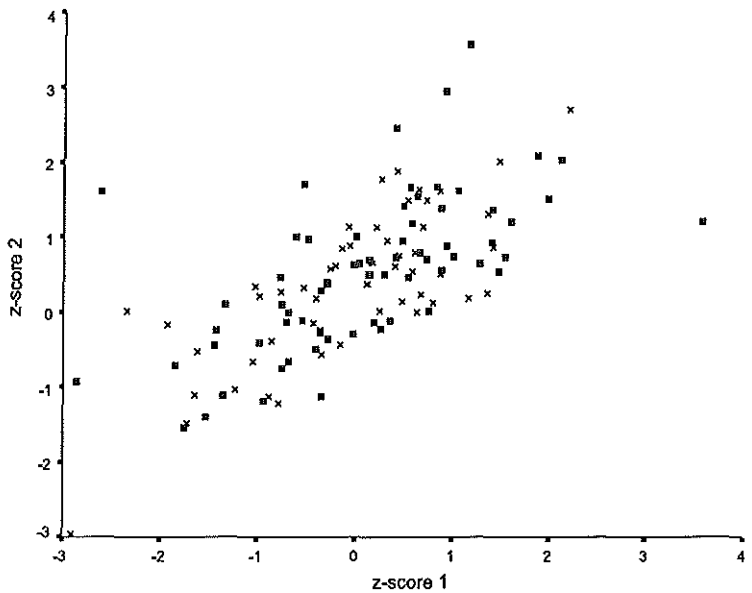



**Fig. 3** Correlation between Z-scores at measurement time 1 and 2 for boys (crosses) and girls (squares).

subject group, the tibia device seems to have a good longitudinal sensitivity[13]. Therefore this tibia device can be used in a clinical setting.

Our results show no significant differences between the cross-sectional data and the longitudinal data, looking only at the mean SOS on measuring time one and two (Figure 1 and 2). Lu et al. had similar results using a DXA bone assessment technique in 266 normal children and young adults (136 boys and 130 girls, aged 4-27) and of those 53 (25 boys and 28 girls, aged 4-16.9) had a follow-up study[11]. They also found no significant differences in the associations between age and bone mineral density (BMD), or between cross-sectional and longitudinal data of either sex. In their study they note that the peak BMDs of total body, lumbar spine and femoral neck are earlier in girls than in boys, probably due to earlier puberty.

But using Z-scores, and therefore ruling out age and gender, we saw a slight increase of delta Z-score of 0.4. This means that average all measurements of the second Z-score are higher than the first Z-score. An explanation for this increase is not easily given. The change of probes can only be a part of the explanation, because we already stated that the increase of the phantom measurements with the second probe is very small (9 m/s; Chapter 3). Other

differences in time, like increase in Tanner stage or length are statistically no explanation for this increase in Z-score.

Assuming that Tanner stage and length are representatives of growth, we find that this tibial ultrasound device is not measuring growth, but a real difference in tibial bone mass. Knowing that this tibial ultrasound device is not measuring growth and looking at the correlation between the first and second Z-score measurement (Figure 3), we can assume that if a child has a low Z-score at the first measured time point, the Z-score is also in the low range at the second measured time point. Other characteristics, such as Tanner stage and length, have no influence on this increase in Z-score. It seems that every child has a unique curve of increase of Z-score with time. Each child will have a curve in the higher or lower range of Z-scores not dependent of his or her Tanner stage or length, but probably of his or her genes.

We did choose a follow-up period of about two years, to minimize the possibility of measurement errors from variation in the rates of change. A longer follow-up time gives a decrease in variability due to a lesser role of imprecision relative to true variability, but in our subject population two years follow-up time seemed to be sufficient[14].

In conclusion, our longitudinal results are only slightly different from our cross-sectional normative data (Chapter 4). Because this difference is small, we think we can use our cross-sectional data for calculating Z-scores, which can be used for assessment of the bone mass status in sick children. The calculated Z-scores can also be used for monitoring treatment and evaluating disease course. Our longitudinal results also showed that the tibial ultrasound device measures not growth, but a real change in tibial bone mass. Every child follows his or her own curve of bone status, which is independent of growth influences but is more likely determined by his or her genes.

## 8.5 REFERENCES


1. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997;82:57-62.
2. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997;12(Suppl 1):S31.
3. van Daele PLA, Burger H, de Laet CEDH, et al. Longitudinal changes in ultrasound parameters of the calcaneus. Osteoporosis Int 1997;7(3):207-12.
4. Krieg MA, Thiébaud D, Burckhardt P. Quantitative ultrasound of bone in institutionalized elderly women: a cross-sectional and longitudinal study. Osteoporosis Int 1996;6:189-95.
5. Storm T, Thamsborg G, Steiniche T, Genant HK, Sorensen OH. Effect of intermittent cyclical etinodrate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. New Eng J Med 1990;322:1265-71.
6. Watts NB, Harris ST, Genant HK, et al. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. New Eng J Med 1990;323:73-9.
7. Liberman UA, Weiss SR, Bröll J, et al. Effect of oral Alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. New Eng J Med 1995;333:1437-43.
8. Lufkin EG, Wahner HW, O'Fallon WM, et al. Treatment of postmenopausal osteoporosis with transdermal estrogen. Ann Int Med 1992;117(1):1-9.
9. Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992;75(4):1060-5.
10. Faulkner RA, Bailey DA, Drinkwater DT, McKay HA, Arnold C, Wilkinson AA. Bone densitometry in Canadian children 8-17 years of age. Calsif Tissue Int 1996;59:344-51.
11. Lu PW, Briody JN, Ogle GD. Bone mineral density of total body, spine, and femoral neck in children and young adults: cross-sectional and longitudinal study. J Bone Miner Res 1994;9:1451-1458.
12. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998;26:177-182.
13. Genant HK, Engelke K, Fuerst T, et al. Noninvasive assessment of bone mineral and structure: state of the art [review]. J Bone Miner Res 1996;11(6):707-30.
14. Glüer CC. Monitoring Skeletal Changes by Radiological Techniques. Bone Miner Res 1999;14(11):1952-1962.
15. Seeman E, Wahner HW, Offord KP, Kumar R, Johnson WJ, Riggs BL. Differential effects of endocrine dysfunction on the axial and the appendicular skeleton. J Clin Invest 1982;69:1302-9.

16. Cadossi R, Canè V. Pathways of transmission of ultrasound energy through the distal metaphysis of the second phalanx of pigs: an in vitro study. Osteoporosis Int 1996;6:196-206.

# BONE MINERAL ASSESSMENT WITH TIBIAL ULTRASONOMETRY AND DUAL-ENERGY X-RAY ABSORPTIOMETRY IN LONG-TERM SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDHOOD


## ABSTRACT

*Introduction:* Acute lymphoblastic leukemia (ALL) in childhood is a serious disease which can affect growth and the attainment of maximal peak bone mass, a risk factor for the development of osteoporosis later in life. To determine long-term effects of the disease itself and its treatment, we assessed the bone status of a group of long-term survivors of childhood ALL, all treated with high doses steroids and methotrexate (MTX) but without cranial irradiation. To study the efficacy of a tibial ultrasound device in this patient group results were compared with the bone mineral density measured with a dual-energy X-ray absorptiometry (DXA) device.

*Material and Methods:* All 21 subjects enrolled in this cross-sectional study had diagnosis of non-high-risk precursors acute lymphoblastic leukemia (12 boys and 9 girls; mean age 16.5 years; range 12.2- 25.4 years). Standard deviation (SD) scores were calculated using a tibial ultrasound device and DXA device as bone assessment technique. These calculated SD scores of those two different bone assessment techniques were compared.

*Results:* The mean SOS (speed of sound) SD score of the tibia (mean 0.11, SD 1.02) were not significant different from our reference value of zero. There was no significant difference between the SOS SD scores in boys and girls. With DXA, no significant difference was seen between the mean BMD SD scores and the reference data and no significant difference in BMD between boys and girls was found. Spearman's correlation between mean SOS SD scores and mean BMD of lumbar spine was 0.49, and mean SOS SD score and mean BMD of total body 0.51. These correlations were significant at the 0.05 level (2-tailed). Spearman's correlation between SOS SD score and mean BMAD SD score was not significant at 0.39.

*Conclusion:* Despite high doses steroids and MTX, used for treatment of children with ALL, no long-term side effects on the bone mineral status of the subjects, measured with both DXA or tibial ultrasonometry, could be determined. Tibial ultrasonometry can be considered as a reliable technique for bone assessment in children.

M.H. Lequin[1], I.M. van der Sluis[1,4], R.R. van Rijn[1], W.C.J. Hop[2], M.M. van den Heuvel-Eibrink[5], S.M.P.F. MuinckKeizer-Schrama[4], C. van Kuijk[3]

Departments of Pediatric Radiology[1], Endocrinology[4], Pediatric Oncology and Hematology[5], Sophia Children's Hospital Rotterdam, Department Epidemiology and Biostatistics[2], Erasmus University Rotterdam and Department of Radiology, Academic Medical Centre, Amsterdam[3]
Submitted for publication: Bone 2000

## 9.1 INTRODUCTION

The increasing number of children surviving leukemia has focussed attention on long-term side effects of this disease and its treatment [1-4]. Acute lymphoblastic leukemia (ALL) in childhood is a serious disease which can affect growth and the attainment of maximal peak bone mass, a recognized risk factor for the development of osteoporosis later in life[5]. Several studies have shown that not only the leukemic process itself, but also ectopic production of parathyroid hormone[6], paracrine secretion of lymphokines[7,8] and decreased physical activity contribute to decreased bone mineral density (BMD)[1]. Another major cause of decreased bone mineral density in ALL is its treatment, especially chemotherapy with dexamethasone[2] and methotrexate[3,9]. Also, cranial irradiation (sometimes part of the treatment) induces growth retardation most likely by growth hormone deficiency[10].

Until now, few studies investigated BMD in long-term survivors of ALL. These longitudinal studies showed BMD in the normal, reference range as well as in low range[11-14]. In these studies the BMD assessment was performed with dual-energy X-ray absorptiometry (DXA). The results of those studies are difficult to interpret because, especially during growth, changes in body composition and size have confounding influence on the BMD measurements [15]. This could be a significant problem in follow-up studies of children with ALL, who commonly have been treated with high dose corticosteroids. Apart from avoiding cranial irradiation, the use of high dose dexamethasone for a long period of time makes this treatment protocol unique and is thought to be responsible for the remarkably good outcome [16].

A bone assessment technique, in which the measurements are not significantly influenced by body composition or size, would be desirable in longitudinal studies. Quantitative computed tomography (QCT) might be such a method, but because of competition with normal patient care in the CT unit, the need for extra software and, to a lesser extend, the radiation burden, its use is restricted largely to academic settings. Ultrasonography seems to be a good alternative method. In addition to its ease to use, and its absence of radiation burden, another advantage would be that ultrasonometry gives information on bone architecture as well as bone mineral density. Another advantage is the shorter investigation time of ultrasound techniques compared to other bone

assessment techniques. Most studies, not only in adults but also in children, have used a calcaneal device[17-20]. But because of the unpredictable changes in bone mineral density and growth in the calcaneus during childhood, longitudinal studies with this kind of device are problematic.

The purpose of this study was to assess the bone status of a group of long-term survivors of childhood ALL, all treated with a unique treatment protocol involving high dose steroids and methotrexate and avoiding cranial irradiation (ALL-6 protocol of the Dutch Childhood Leukemia Study Group, (DCLSG)), with the use of a tibia ultrasound device and to compare the results with dual-energy X-ray absorptiometry (DXA).

## 9.2 MATERIAL AND METHODS

All 21 patients enrolled in this cross-sectional study were diagnosed with non-high-risk acute lymphoblastic leukemia (12 boys and 9 girls; mean age 16.5 years; range 12.2- 25.4 years). The mean follow-up period after cessation of therapy was 9.6 years (range 7.9-11.4 years).

Non-high-risk ALL was defined as peripheral white blood cell count< 50*109/l, and absence of both mediastinal mass and cerebromeningeal leukemia at diagnosis. All subjects of this homogeneous group of immunophenotypic precursor-B ALL are treated in our hospital, according the ALL-6 protocol of the the Dutch Childhood Leukemia Study Group (DCLSG); systemic chemotherapy involved dexamethasone, MTX, 6-mercaptopurine, asparaginase, and vincristine (Figure 1).

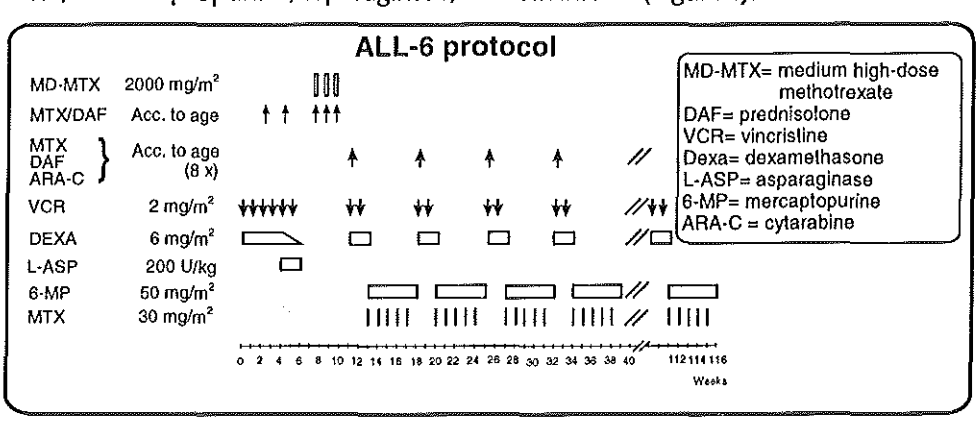



**Fig. 1** Treatment scheme in children with ALL, according to ALL-6 protocol of the Dutch Childhood Leukemia Study Group.

As stated above, no cranial irradiation, but only high dose i.v. MTX and prolonged intrathecal triple therapy (MTX, prednisolone, and cytarabine) was used as central nervous system prophylaxis. A more extensive description of the treatment used, has been given by Veerman et al[16]. The duration of the total treatment was approximately two years.

The study protocol was approved by the ethics committee of the University Hospital Rotterdam. Informed consent was obtained explicitly from parents or guardians and where appropriate from the child (in the Netherlands this is mandatory for children aged 12 years and over). This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines of the Internal Review Board of the University Hospital Rotterdam and Erasmus University Rotterdam, Faculty of Medicine and Health Sciences, the Netherlands.

*Anthropometry*

Height was measured, without shoes, using a stadiometer [21]. Weight was measured, without shoes, on an electronic weight scale. Body mass index (BMI) as an indicator of nutritional status was calculated as the ratio of weight to height$^2$ (kg/m$^2$). A questionnaire concerning overall health and history of fractures was also taken.

Using an X-ray of the left hand, skeletal age was assessed in all children in which there was still growth, by one investigator (ML), according to the Greulich and Pyle method [22].

Tanner stages were evaluated through self-assessment, according to Duke et al [23]. Patients were shown pictures and written information illustrating breast and pubic hair development for girls, and genital and pubic hair development for boys. They were asked to select the one that had the closest resemblance to their own status. If there were discrepancies between variables, emphasis was placed on the breast development in girls and genital development in boys [24].

*Bone assessment*

Of all 21 subjects examined with DXA, SD scores were calculated using reference data, generated by Boot et al [25]. Of those 21, 17 ( 9 boys and 8 girls) were in the age range of our normative data group [26], examined with our tibial

ultrasound device, and therefore SD scores were only calculated for these subjects.

Tibial ultrasonometry was performed using the SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel, Software Version 1.1e). Following standard operating procedures, all bone assessments were done on the right tibia at the mid-tibial point. The mid-tibial point was defined as the mid-point of the line between the apex of the medial malleolus and the distal patellar apex. This tibial ultrasound device measures the speed of sound (SOS) through a fixed length of five centimeters around the mid-tibial point. The results are compared to healthy age- and sex-matched Dutch controls, expressed as SOS SD scores (Z-scores).

Bone mineral densities (BMD) of lumbar spine (LS) and total body (TB) were assessed by dual-energy X-ray absorptiometry (DXA) (Lunar DPXL, Madison, WI, USA). Also apparent BMD (BMAD) of the lumbar spine was calculated as a "volumetric bone mass measurement" to correct for growth, a method validated by Kröger et al [27].

*Statistical analyses*

All measurements are expressed as SD scores. Mean SD scores were compared with the reference value of zero, using the one-sample *t*-test. Mean values were compared between groups, using the *t*-test. $P=0.05$ (two-sided) was considered the limit of significance and the correlations given are Spearman's.

## 9.3 RESULTS

*Tibia ultrasound*

The individual SOS SD scores are presented in Figure 2. The mean SOS SD scores of the tibia (mean 0.11, SD 1.02) were not significant different from reference value of zero. There is no significant difference between the SOS SD score in boys and girls.

*DXA*

The individual SD score for BMD of lumbar spine (mean 0.22, SD 1.03), total body (mean 0.15, SD 1.03) and BMADls (mean 0.03, SD 1.04) are presented in Figure 3. No significant difference was seen between the mean BMD and the reference data and no significant difference in BMD between boys and girls was found.

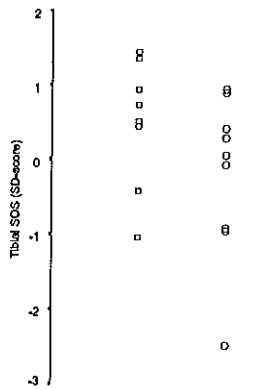



**Fig. 2** Individual tibial ultrasound SOS SD scoress, squares represent girls, circles represent boys.

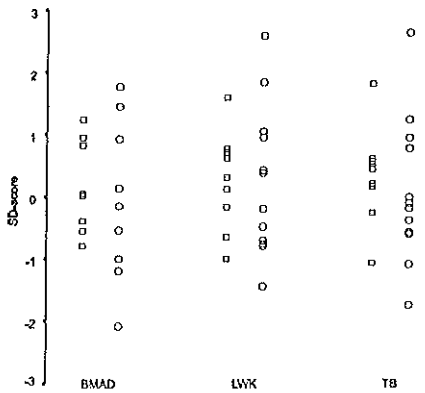



**Fig. 3** Individual SD scores for BMD of lumbar spine (LWK), total body (TB) and BMAD, squares represent girls, circles represent boys.

The correlation between mean SOS SD score and mean BMD of lumbar spine is 0.49, and mean SOS SD scores and mean BMD of total body 0.51, both significant (Figure 4 and 5). The correlation between SOS SD scores and mean BMAD SD scores is not significant and 0.39 (Figure 6).

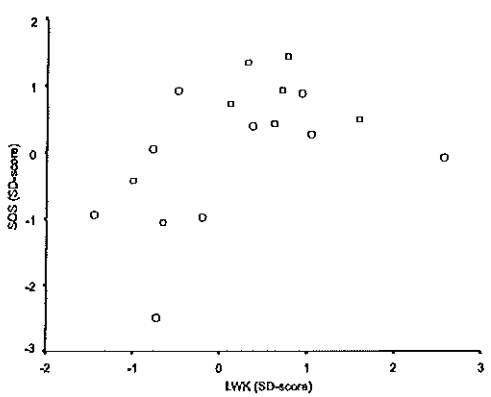



**Fig. 4** Correlation between SD scores of tibial ultrasound SOS and BMD SD scores of lumbar spine, squares represent girls, circles represent boys.

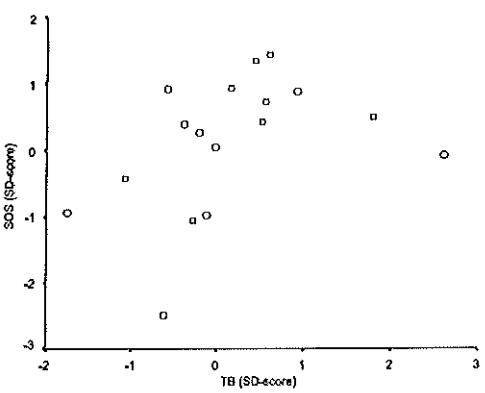



**Fig. 5** Correlation between SD scores of tibial ultrasound SOS and BMD SD scores of total body, squares represent girls, circles represent boys.

Of the 21 subjects seven had one fracture, two subjects had a history of two fractures. No fractures at the axial skeleton were reported, all were at the appendicular skeleton. Most of the fractures occurred during or shortly after treatment, all after trauma.

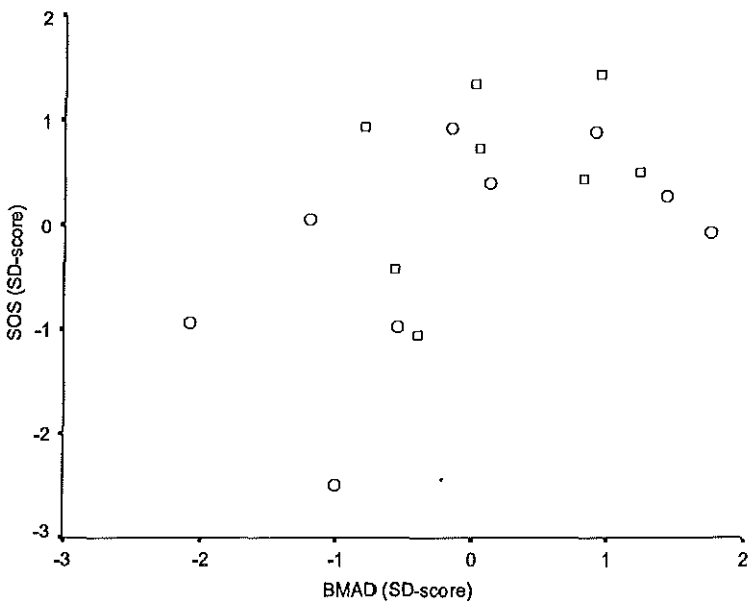



**Fig. 6** Correlation between SD scores of tibial ultrasound SOS and BMAD SD scores of DXA, squares represent girls, circles represent boys.

## 9.4 DISCUSSION

In children with ALL, the disease itself and its treatment might affect the bone status of the child on a short- or long-term basis. Using the tibial ultrasound device, we show no significant changes in mean SOS SD scores in a group of long-term survivors of childhood ALL compared to our reference population. No significant difference in SOS SD scores has been found between girls and boys. The results from our tibial ultrasound device were consistent with the results from the DXA device in this group of long-term survivors of childhood ALL. This could mean that not only the amount of bone mineral density (measured by DXA) but also the bone strength (measured by the tibial

ultrasound device) of long-term survivors of childhood ALL, return into normal ranges. This is the first study using an ultrasound device in long-term survivors of acute lymphoblastic leukemia in childhood.

The correlation between DXA and tibial ultrasonometry is significant but moderate, in healthy children as well as in children with a cured ALL (Figure 4, 5 and 6). The correlation between SOS SD scores and BMAD SD scores is not significant. As expected the correlation between SOS SD score and total body SD score is the greatest, because these measure both a large amount of cortical bone, like tibial ultrasonometry. Van der Sluis et al. already reported that the BMD measurements with the DXA performed in this patient group, showed no significant differences with the reference values [28]. Many studies in the literature show a decrease in BMD in ALL patients, but most of all in subjects treated with cranial irradiation [11,14,29]. DXA is considered the "gold-standard" bone mass assessment technique, but several studies show that the implementation of this technique in children is hampered by several factors [15,30,31]. The most important factor is that total body bone mineral measurements done with DXA is confounded by differences in fat distribution. This impedes the applicability of DXA, especially in children with diseases or on medications, which influence fat distribution. Therefore BMD measurements with DXA in children with ALL, treated with high dose dexamethasone, known to have great influences on the body fat distribution, should be interpreted cautiously. Although Van der Sluis et al. mentioned that all anthropomorphic characteristics (including the fat distribution) of the investigated children in this study showed no significant difference from the normal reference population [28].

By design, the tibial ultrasound device is unaffected by body fat distribution, because the changes in subcutaneous fat at the anterior site of the tibia are minor. The tibial ultrasound device may be more practical than the more popular calcaneal ultrasound devices. In a review article, Hans et al. made clear that it is very difficult to define the fundamental accuracy of QUS [32]. The complex bone structure of the calcaneus and its inhomogeneity may result in variable transmission times. In light of the growth in children, this might have a stronger impact in this specific population.

Differences in long-term effects on the bone status of children, as published in the literature, depend on sample size, follow-up time, and homogeneity of

patient groups with regard to diagnosis and treatment [1,2,11,12,29]. Pediatric patients with ALL, treated with cranial irradiation, showed a significant decrease in BMD [14,33]. The mechanism causing this reduced BMD after cranial irradiation is not totally understood, but growth hormone deficiency as a consequence of an injured hypothalamic-pituitary axis, may be a major factor 10. Growth hormone deficiency can also induce short stature and thus can partly explain the reduced BMD, measured with a two-dimensional technique such as DXA[34]. We did not find a significant difference between the mean height SD scores of our subjects and our reference group after therapy [28]. An explanation could be that cranial irradiation was replaced by intrathecal chemotherapy in the for that time period unique treatment protocol. Holm et al. noted "catch-up" growth within two years of cessation of therapy without cranial irradiation; this could be the reason for not finding significant difference in mean height SD scores with the reference population after an average follow-up time of 10 years [35].

In conclusion, despite high dose corticosteroids and MTX used for treatment of children with ALL, no long-term side effects on their bone status were noted, measured with either tibial ultrasonometry or DXA. All anthropometric characteristics were within the reference values. Given the correlation between tibial ultrasonometry and DXA, and specially the difference in the SD scores in the individual cases, it is not clear which bone assessment technique is the preferable technique, but looking at its lack of radiation burden and its accuracy in a growing child, tibial ultrasonometry might be the premier bone assessment technique.

## 9.5 REFERENCES


1. Halton J, Atkinson SA, Fraher L, et al. Mineral homeostasis and bone mass at diagnosis in children with acute lymphoblastic leukemia. J Pediatr 1995; 126:557-564.
2. Halton JM, Atkinson SA, Fraher L, et al. Altered mineral metabolism and bone mass in children during treatment for acute lymphoblastic leukemia. J Bone Miner Res 1996; 11:1774-1783.
3. Ragab AH, Frech RS, Vietti TJ. Osteoporotic fractures secundary to methotrexate therapy of acute leukemia in remission. Cancer 1970; 25:580-585.
4. May KM, West SG, McDermott MT, et al. The effect of low-dose methotrexate on bone metabolism and histomorphometry in rats. Arthritis Rheum 1994; 37:201-206.
5. Clayton PE, Shalet SM, Morris-Jones PH, et al. Growth in children treated for acute lymphoblastic leukaemia. Lancet 1988; i:460-462.
6. Cohn SL, Morgan ER, Malette LE. The spectrum of metabolic bone disease in lymphoblastic leukemia. Cancer 1987; 59:346-350.
7. Mundy GR, Luben RA, Raisz LG, et al. Bone resorbing activity in supernatants from lymphoid cell lines. N Engl J Med 1974; 290:867-871.
8. Vassilopoulou-Sellin R, Brosnan P, Delpassand A, et al. Osteopenia in young adult survivors of childhood cancer. Med Pediatr Oncol 1999; 32:272-8.
9. Zonneveld IM, Bakker WK, Dijkstra PF, et al. Methotrexate osteopathy in long-term low-dose methotrexate treatment for psoriasis and rheumatoid arthritis. Arch Dermatol 1996; 132:184-187.
10. Nussey SS, Hyer SL, Brada M, et al. Bone mineralization after treatment of growth hormone deficiency in survivors of childhood malignancy. Acta Paediatr Suppl 1994; 399:9-14.
11. Henderson RC, Madsen CD, Davis C, et al. Longitudinal evaluation of bone mineral density in children receiving chemotherapy. J Pediatr Hematol Oncol 1998; 20:322-326.
12. Gilsanz V, Carlson ME, Roe TF, et al. Osteoporosis after cranial irradiation for acute lymphoblastic leukemia. J Pediatr 1990; 117:238-244.
13. Arikoski P, Komulainen J, Voutilainen R, et al. Reduced bone mineral density in long-term survivors in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol 1998; 20:234-240.
14. Brennan BM, Rahim A, Adams JA, et al. Reduced bone mineral density in young adults following cure of acute lymphoblastic leukaemia in childhood. Br J Cancer 1999; 79:1859-63.
15. Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. J Bone Miner Res 1997; 12:1908-21.

16. Veerman AJP, Hählen K, Kamps WA, et al. High cure rate with moderate intensive treatment regimen in non- high risk childhood lymphoblastic leukemia: Results of protocol ALL VI from the Dutch Childhood leukemia study group. J Clin Oncol 1996; 14:911-918.
17. Gilsanz V. Bone density in children: a review of the available techniques and indications. EJR 1998; 26:177-182.
18. Jaworski M, Lebiedowski M, Lorenc RC, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int 1995; 56:368-71.
19. Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL. Comparison between broad-band ultrasound attenuation of the calcaneus and total body bone mineral density in children. Acta Pediatr 1996; 85:663-5.
20. Mazess RB, Wiener SA, Hanson JA, Trempe J, Bonnick SL. Utrasound measurement of the os calcis: Lunar corp. / Cooper clinic, Dallas, 1991.
21. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997; 12:S31.
22. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Stanford, California: Stanford University Press, 1959.
23. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980; 66:918-20.
24. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997; 82:57-62.
25. Boot AM. Bone mineral density and body composition of children and adolescents in health and disease. Pediatrics. Rotterdam: Erasmus University Rotterdam, 1997.
26. Lequin MH, van Rijn RR, Robben SGF, Hop WCJ, van Kuijk C. Normal values for tibial quantitative ultrasonometry in Caucasian children and adolescents (aged 6 to 19 years). Calcif Tissue Int 2000; 67(2):101-105.
27. Kröger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 1995; 17:157-9.
28. Van der Sluis IM, van den Heuvel-Eibrink MM, Hählen K, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density, body composition, and height in long-term survivors of acute lymphoblastic leukemia in childhood. Medical and Pediatric Oncology 2000:In press.
29. Nysom K, Holm K, Fleischer Michaelsen K, et al. Bone mass after treatment for acute lymphoblastic leukemia in childhood. J Clin Oncol 1998; 16:3752-3760.
30. Tothill P, Laskey MA, Orphanidou CI, van Wijk M. Anomalies in dual energy X-ray absorptiometry measurements of total-body bone mineral during weight change using Lunar, Hologic and Norland instruments. Br J Radiol 1999; 72.

31. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in vivo bone mineral density. J Bone Miner Res 1998; 13:1739-1746.
32. Hans D, Njeh CF, Genant HK, Meunier JP. Quantitative ultrasound in bone status assessment. Rev. Rhum [Engl. Ed.] 1998; 65:489-98.
33. Brennan BMD, Rahim A, Mackie EM, et al. Growth hormone status in adults treated for acute lymphoblastic leukaemia in childhood. Clinical Endocrinolgy 1998; 48:777-783.
34. Mazess RB, Barden H, Mautalen C, et al. Normalization of spine densitometry. J Bone Miner Res 1994; 9:541-548.
35. Holm K, Nysom K, Hertz H, et al. Normal final height after treatment for acute lymphoblastic leukemia without irradiation. Acta Paediatr 1994; 83:1287-1290.

# A LONGITUDINAL STUDY USING TIBIAL ULTRASONOMETRY AS A BONE ASSESSMENT TECHNIQUE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

## 10.1 INTRODUCTION

In Chapter 9, we discussed the possible changes in bone mass quantity and quality in long-term survivors of childhood acute lymphoblastic leukemia. In this chapter we will look at the difference in bone assessment between t=0 (time of diagnosis) and follow-up dates t=1 (6 months), t=2 (12 months) and t=3 (24 months) in children with the diagnosis and treatment of acute lymphoblastic leukemia (ALL). The bone assessment technique used is the tibial ultrasound device SoundScan®Compact.

As reviewed in Chapter 9, there are multiple causes of decreased bone mineral density in ALL. The most important causes are the leukemic process itself, and the use of chemotherapy, such as high doses steroids and methotrexate (MTX) [1-5]. Also cranial irradiation may induce low bone mineral density due to growth hormone deficiency, but in our investigated group of children with ALL, this did not play a role, because cranial irradiation is not part of the treatment protocol [6-8]. Due to the high cure rate and to its lack of major negative effects on the bone status in the long-term survivors of ALL, the same moderately intensive treatment protocol without cranial irradiation has been used in this new group of children with ALL [9].

Despite the fact that the long-term side effects of this disease and its treatment may have no significant long-term influence on bone mass quality or quantity, it is still of interest to know more about the short-term effects. This may give us the opportunity to intervene with dietary changes or medical substitution, like calcium suppletion. The final goal is to establish the highest peak bone mass possible, which is the best prevention for the development of osteoporosis later in life.

## 10.2 MATERIAL AND METHODS

*Participants*

In the period between January 1997- December 1999, 33 subjects (24 boys, 9 girls, mean age 8.5 years, range 3.0 - 16.2 years) were included in a longitudinal study. Of these 33 children, 30 were assessed at the start of therapy (t=0), 21 at a follow-up time of 6 months (t=1), 17 at a follow-up time of 12 months (t=2) and four at a follow-up time of 24 months (t=3). Of the ten subjects, who had only one measurement, two of them had relapses of ALL at t=0 and one at t=1, and therefore no further follow-up measurements were done, because they were treated according to another treatment protocol. One subject ceased after investigation time t=3, one entered the study at t=3, the rest (n=6) has not had time for follow-up. All subjects enrolled in this part of the study were diagnosed with either non-high-risk (n=24) or high-risk (n=9) acute lymphoblastic leukemia. Non-high-risk ALL was defined as peripheral white blood cell count < 50*109/l, absence of mediastinal mass and/or cerebromeningeal leukemia, at diagnosis. Systemic chemotherapy involved dexamethasone, MTX, 6-mercaptopurine, asparaginase, and vincristine. As stated before, no cranial irradiation has been done, but only moderate dose i.v. MTX and prolonged intrathecal triple therapy (MTX, prednisolone, and cytarabine) as central nervous system prophylaxis. Cumulative doses of dexamethasone and of MTX were 1370 mg/ $m^2$ and 8100 mg/ $m^2$ (orally and intravenously) respectively.

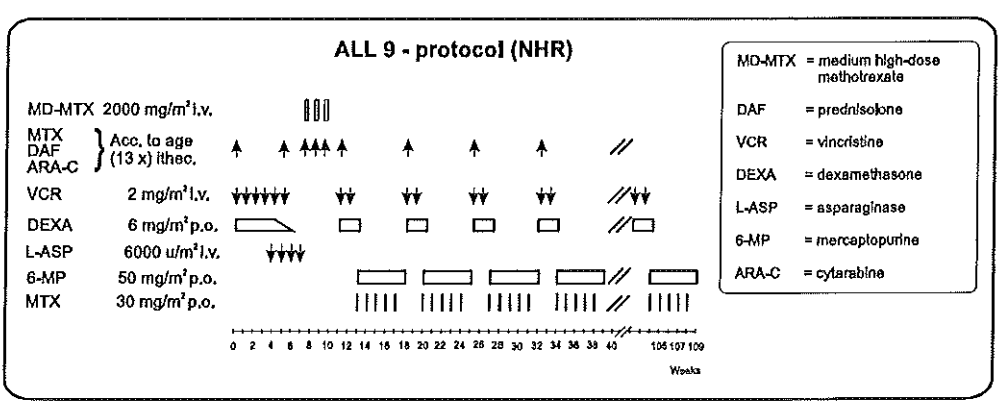



**Fig. 1** The scheme of doses of the major chemotherapy medications as part of the used treatment protocol in non-high-risk (NHR) ALL at investigation time t=0 until t=3.

Dexamethasone therapy during remission induction consisted of 6 mg/m$^2$ daily divided into three doses, for 4 weeks, then tapered off to 0 mg in 10 days. After complete remission was achieved, three weekly courses of i.v. MTX (2000 mg/m$^2$ ) were administered. Maintenance treatment consisted of MTX 30 mg/m$^2$/week orally or i.v. for 5 weeks, alternated with dexamethasone in a dose as for induction treatment for two weeks (Figure 1). High-risk ALL patients received an extra intensive treatment. The choice of therapy depended on the initial location of the disease. Also the cumulative systemic dose of MTX is higher (13600 mg/m$^2$ orally and intravenously) while the cumulative dose of dexamethasone was the same (1244 mg/m$^2$). Only when there was an initial CNS spread of ALL an extra triple therapy was given intrathecal with medication which could effect bone metabolism, (i.e. MTX, and prednisolone) (Figure 2). The treatment time is approximately two years and therefore only a few subjects received the complete treatment.

The study protocol was approved by the ethics committee of the University Hospital Rotterdam. Informed consent was obtained explicitly from parents or guardians and where appropriate from the child (in the Netherlands this is mandatory in children aged 12 years and over).

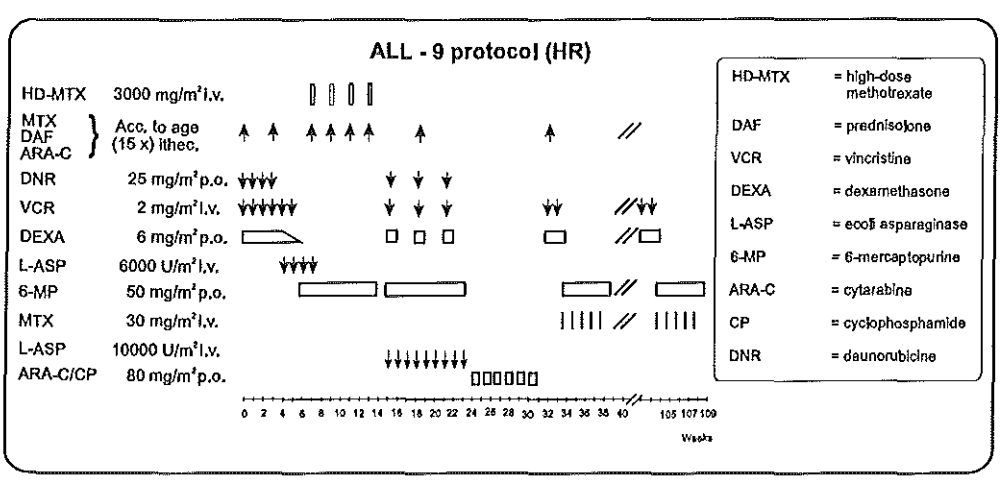



**Fig. 2** The scheme of doses of the major chemotherapy medications as part of the used treatment protocol in high-risk (HR) ALL at investigation time t=0 until t=3.

This was done according to the guidelines recommended by the Declaration of Helsinki (Hong Kong, 1989) and the guidelines of the Internal Review Board of the University Hospital Rotterdam and Erasmus University Rotterdam, Faculty of Medicine and Health Sciences, the Netherlands.

*Anthropometry*

Tanner stages were evaluated through self-assessment, according to Duke et al. [10]. Subjects were shown pictures and written information illustrating breast and pubic hair development for girls, and genitalia and pubic hair development for boys. They were asked to select the one that had the closest resemblance to their own status. If there were discrepancies between variables, emphasis was placed on the breast development in girls and genital development in boys[11].
Height was measured, without shoes, using a wall-mounted ruler[12]. Weight was measured, without shoes, on an electronic weight scale. Body mass index (BMI) as an indicator of nutritional status was calculated as the ratio of weight to height$^2$ ($kg \cdot m^{-2}$). Also a questionnaire concerning overall health and history of fractures was taken.
Skeletal age was assessed by one investigator (MHL), using an X-ray of the left hand, according to the Greulich and Pyle method [13].

*Bone assessment*

Tibial ultrasonometry was performed using the SoundScan®Compact (Myriad Ultrasound Systems Ltd., Rehovot, Israel, Software Version 1.1e). Following standard operating procedures, all bone assessments were done on the right tibia at the mid-tibial point, except in one subject, which had broken his right tibia: the left tibia at the mid-tibia point was measured. The mid-tibial point was defined as the mid-point of the line between the apex of the medial malleolus and the distal patellar apex.
The results are compared to healthy age- and sex-matched Dutch controls described in Chapter 4 and are expressed as SD scores (Z-scores) [14].

*Statistical analyses*

Comparison of outcomes at different investigation times (t=0, t=1, t=2) was done using Wilcoxon's signed rank test. The same test was used to compare mean values of SD scores with the mean reference values of zero.

## 10.3 RESULTS

The individual SOS SD scores at investigation times, t=0, t=1, t=2 and t=3 are given in Figure 3. The mean SOS SD scores at t=0, t=1 or t=2 were not significantly different from the reference value of zero. No reliable statistical analyses can be done at investigation time t=3 because of the small number of subjects. The mean SOS SD scores as a group are significant lower at t=1 and t=2 than at t=0. The biggest change was between t=0 and t=1. There was no significant change of mean SOS SD scores between t=1 and t=2.

Two subjects had fractures in their extremities: one at the right tibia after minor trauma and one at a metacarpal, between investigation time t=2 and t=3.

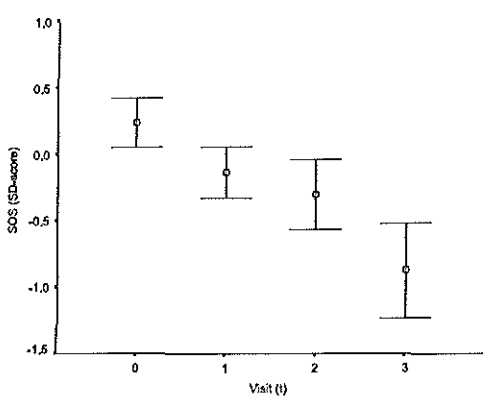



**Fig. 3** The SOS SD scores on investigation time t=0, t=1, t=2 and t=3, mean ± standard error of mean.

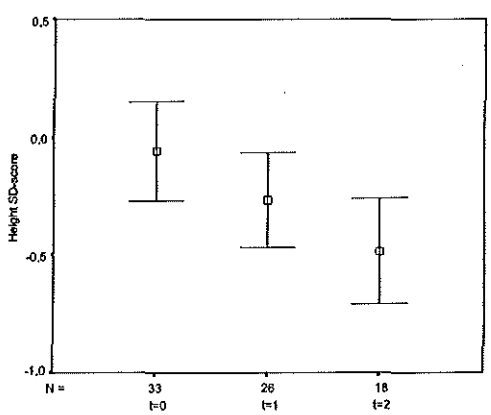



**Fig. 4** Changes of differences between the baseline and mean height SD scores during t=0, t=1 and t=2, mean ± standard error of mean.

The mean height SD scores at t=0, t=1 or t=2 were not significantly different from our references value of zero, showing that the leukemic disease did retard growth before the time of diagnosis (t=0) (Figure 4). There is a significant difference of mean height SD scores between t=0 and t=1,

and t=0 and t=2 ($P<0.05$), but not between t=1 and t=2. No significant correlation has been found between the changes of mean height SD scores and mean SOS SD scores at t=1 or t=2 (Figure 5).

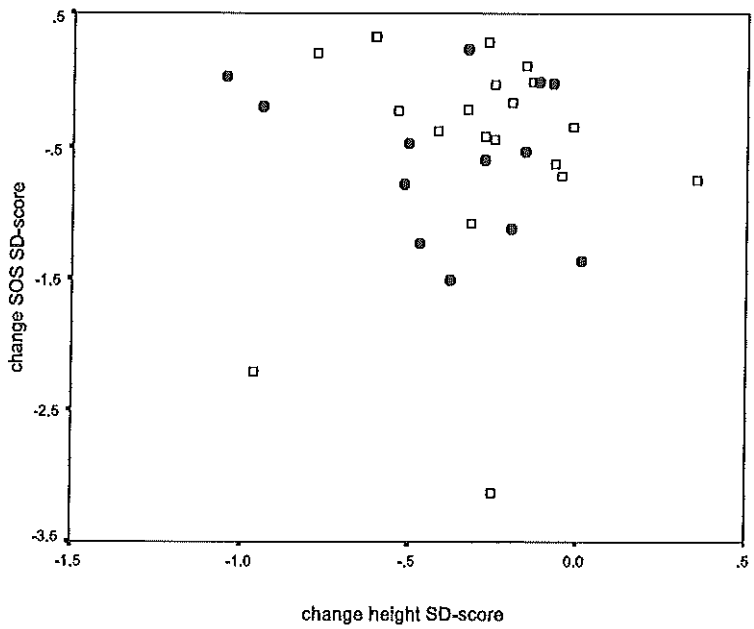



**Fig. 5** Changes of differences between the baseline and mean height SD scores and mean SOS SD scores, during t=1 (squares) and t=2 (dots).

The young subjects with ALL were all in Tanner stage I at t=0. In the follow-up period none of them matured to Tanner stage III, in which there is a marked increase in SOS in girls (see Chapter 4). The older subjects were already in Tanner stage V at t=0.

## 10.4 DISCUSSION

As discussed in Chapter 9 the long-term effects on the bone status of ALL itself and its treatment are negligible. Part of the differences in outcome of those long-term effects published in the literature depends on the sample size, follow-up time, homogeneity of subject groups with regard to diagnosis and treatment [1,2,8,15,16]. Specifically, subjects treated with cranial irradiation showed a reduced bone mineral density (BMD) [7,17]. As stated in Chapter 9, the mechanism causing this reduced BMD after cranial irradiation is uncertain, but growth

hormone deficiency as a consequence of an injured hypothalamic-pituitary axis, appears to be a major factor [18]. This growth hormone deficiency can result in short stature which can also partly explain the reduced BMD as measured with a two-dimensional technique like dual-energy X-ray absorptiometry [19]. To our knowledge this is the first longitudinal study with a tibia ultrasound device, which looks at the short-term effects of the disease itself and its therapy.

In our treatment protocol, cranial irradiation was not given but we still found a significant difference between the mean height SD scores at t=0 and t=1 and at t=0 and t=2. As shown in Figure 4, this shows a growth retardation of our subjects during therapy. This is no surprise, as Hokken-Koelega et al. already reported a temporary growth retardation during therapy of moderate dose MTX intravenously [20]. Holm et al. noted a catch-up growth within two years after cessation of ALL therapy without cranial irradiation, and this could be a reason that we did not see a significant difference in mean height SD scores with the reference population after a ten years follow-up time (see Chapter 9)[21]. Significantly the disease itself is not a cause of growth retardation, as at t=0 all subjects are not significantly different from the references value of zero.

There was a significant reduction in mean SOS SD scores in the first 6 months (t=1) and this significant reduction was still visible at t=2 (12 months). The biggest reduction is in the first 6 months of therapy. In this short period it is unlikely that growth retardation alone can cause this decrease of SOS. A better explanation could be the high dose of steroids administered during this time period. Halton et al. already showed that corticosteroids are the main cause of reduction in BMD during ALL treatment due to decreased bone formation and increased bone resorption [2]. MTX can also contribute to this BMD reduction as MTX is able to cause osteopenia by suppression of osteoblast activity and stimulation of osteoclast recruitment, resulting in increased bone resorption [4]. There was no significant change in mean SOS SD scores between t=1 and t=2. Whether there was a equilibrium between bone formation and bone resorption during this time period is hard to say, but a further decline of bone mass, expressed in a lower mean SOS SD scores, was not observed. Because of the small numbers of subjects at t=3 (24 months) it was impossible to look for further significant changes in mean SOS SD scores, but the trend seems to indicate that no catch-up in mean SOS SD scores occurs in this timeframe.

Henderson et al. even found that a follow-up time of 1 year after completion of chemotherapy was not enough to see a catch-up in BMD [8]. Figure 5 shows that the change of mean SOS SD scores between t=0, t=1 and t=2, was not caused by the observed growth retardation, but by a real decrease in SOS through the cortex of the tibia. Whether this decrease was due to a lower bone mass quantity, or bone mass quality of the tibia or a combination is not known, but it is clear that the tibia device is not measuring growth, but a real change in cortical bone. Interestingly in this two year time period we found an increase in skeletal age, not a decrease. This is important because this, also the decrease in mean SOS SD scores between t=0 and t=1, and t=2 is a real decrease in bone quantity in the tibia and not only simply growth retardation, expressed as a cessation of height. Whether this decrease in bone quantity of the tibia can be used to predict the whole bone status of the investigated person is a subject of a on-going study.

The largest decrease in SOS SD scores was in the first year of treatment, especially the first six months, and therefore this is the best period to intervene with medication such as calcium suppletion (Figure 3). This could give a higher peak bone mass, expressed in a higher SOS later in life.

As mentioned in Chapter 9 there seems to be a higher fracture risk during and shortly after discontinuation of chemotherapy due to reduced BMD[16]. The fractures were all in the appendicular skeleton, no vertebral compression fractures were reported. This is unexpected, because a vertebral compression fracture is a classical presentation of a child with ALL. In this study one subject had two fractures (tibia and metatarsal) in the appendicular skeleton, and one subject had a fracture at his metatarsal. One cause can be the still short follow-up time of no more than two years and therefore missing the amount of fractures which do occur not only during treatment but also shortly thereafter.

In conclusion, tibia ultrasonometry detects short-term changes in mean SOS SD scores in children with ALL. The best intervention time to avoid a transient decrease bone mass, expressed in a higher SOS, seems to be the first 6 months after starting treatment. The real gain in peak SOS will be minor, as we already know that the long-term effects of children with the same type of ALL treated according the same protocol are insignificant.

## 10.5 REFERENCES


1. Halton J, Atkinson SA, Fraher L, et al. Mineral homeostasis and bone mass at diagnosis in children with acute lymphoblastic leukemia. J Pediatr 1995; 126:557-564.
2. Halton JM, Atkinson SA, Fraher L, et al. Altered mineral metabolism and bone mass in children during treatment for acute lymphoblastic leukemia. J Bone Miner Res 1996; 11:1774-1783.
3. Ragab AH, Frech RS, Vietti TJ. Osteoporotic fractures secundary to methotrexate therapy of acute leukemia in remission. Cancer 1970; 25:580-585.
4. May KM, West SG, McDermott MT, et al. The effect of low-dose methotrexate on bone metabolism and histomorphometry in rats. Arthritis Rheum 1994; 37:201-206.
5. Zonneveld IM, Bakker WK, Dijkstra PF, et al. Methotrexate osteopathy in long-term low-dose methotrexate treatment for psoriasis and rheumatoid arthritis. Arch Dermatol 1996; 132:184-187.
6. Kirk JA, Stevens MM, Menser MA, et al. Growth failure and growth-hormone deficiency after treatment for acute lymphoblastic leukaemia. Lancet 1987; i:190-193.
7. Brennan BMD, Rahim A, Mackie EM, et al. Growth hormone status in adults treated for acute lymphoblastic leukaemia in childhood. Clinical Endocrinolgy 1998; 48:777-783.
8. Henderson RC, Madsen CD, Davis C, et al. Longitudinal evaluation of bone mineral density in children receiving chemotherapy. J Pediatr Hematol Oncol 1998; 20:322-326.
9. Veerman AJP, Hählen K, Kamps WA, et al. High cure rate with moderate intensive treatment regimen in non- high risk childhood lymphoblastic leukemia: Results of protocol ALL VI from the Dutch Childhood leukemia study group. J Clin Oncol 1996; 14:911-918.
10. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980; 66:918-20.
11. Boot AM, Ridder de MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone mineral density in children and adolescents: relatition to puberty, calcium intake and physical activity. J Clin Endocrinol Metab 1997; 82:57-62.
12. McClung B, Parkins N, McClung MR. Comparison of height measurements made with a Harpenden stadiometer and a wall-mounted ruler on patients with vertebral compression fractures. J Bone Miner Res 1997; 12:S31.
13. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Stanford, California: Stanford University Press, 1959.
14. Lequin MH, van Rijn RR, Robben SGF, Hop WCJ, van Kuijk C. Normal values for tibial quantitative ultrasonometry in Caucasian children and adolescents (aged 6 to 19 years). Calcif Tissue Int 2000; 67(2):101-105.
15. Gilsanz V, Carlson ME, Roe TF, et al. Osteoporosis after cranial irradiation for acute lymphoblastic leukemia. J Pediatr 1990; 117:238-244.
16. Nysom K, Holm K, Fleischer Michaelsen K, et al. Bone mass after treatment for acute lymphoblastic leukemia in childhood. J Clin Oncol 1998; 16:3752-3760.

17. Brennan BM, Rahim A, Adams JA, et al. Reduced bone mineral density in young adults following cure of acute lymphoblastic leukaemia in childhood. Br J Cancer 1999; 79:1859-63.
18. Nussey SS, Hyer SL, Brada M, et al. Bone mineralization after treatment of growth hormone deficiency in survivors of childhood malignancy. Acta Paediatr Suppl 1994; 399:9-14.
19. Mazess RB, Barden H, Mautalen C, et al. Normalization of spine densitometry. J Bone Miner Res 1994; 9:541-548.
20. Hokken-Koelega ACS, Van Doorn WD, Hählen K, et al. Long-term effects of treatment for acute lymphoblastic leukemia with and without cranial irradiation on growth and puberty: a comparative study. Pediatr Res 1993; 33:577-582.
21. Holm K, Nysom K, Hertz H, et al. Normal final height after treatment for acute lymphoblastic leukemia without irradiation. Acta Paediatr 1994; 83:1287-1290.

# GENERAL DISCUSSION, CONCLUSIONS, AND POSSIBILITIES FOR FUTURE RESEARCH

## 11.1 GENERAL DISCUSSION

In recent years, several ultrasound systems have been developed for bone mass assessment because compared to existing bone assessment techniques they are relatively cheap, easy to use, radiation free and patient friendly. Ultrasonometry techniques were validated first in adults. In adults those techniques seem to be accurate in identifying osteoporosis, its progression and response to therapy, as well as in identifying subjects with a high fracture risk. It will be a powerful tool and a competitor to the established techniques, like DXA.

Chapter 1 is an overview of all bone mineral assessment techniques, developed in the last decades. The advantages and disadvantages of those techniques are discussed.

Chapter 2 gives an overview of the different ultrasound devices and techniques, which are used in daily practice. As with most of the bone mass assessment techniques mentioned in Chapter 1, most of the ultrasound techniques are first validated in adults. The tibia ultrasound device used in this thesis had not been validated in children and therefore this validation is one of the aims of this thesis. The data for this validation are presented in Chapter 3 and later.

To be a real competitor to other bone mass assessment techniques, tibial ultrasonometry should have good short- and long-term reproducibility. The reproducibility of this system is in the same range as other ultrasound equipment and can compete with bone mass measurement techniques such as radiographic absorptiometry and dual-energy X-ray absorptiometry (DXA). The short-term precision of this tibia device, influence of measurement site, dexterity, brand of coupling gel, and temperature of coupling gel are presented in Chapter 3. Intra-observer variance was CV 0.43%, the inter-observer variance was CV 0.61%. Left mid-tibial and right mid-tibial speed of sound (SOS) measurements showed no significant differences. There were, however, significant differences in both boys and girls between SOS measured in right

proximal versus right mid-tibial, right mid-tibial versus right distal and right proximal versus right distal (for all $P<0.001$). One-way analysis of variance showed that neither the use of different coupling gels nor an increase in gel temperature had a significant influence on measurements. The results of our study show that tibial ultrasonometry is a highly reproducible technique in a Caucasian pediatric population. Long-term precision of the tibia ultrasound device is good and is described in the addendum of Chapter 3.

In Chapter 4, we present a prospective study acquired from 596 healthy children, 309 girls, mean age 12.9 years (range 6.1-19.9 years), and 287 boys, mean age 12.3 years (range 6.1-19.6 years). For all subjects a short questionnaire regarding overall health was completed. To assess skeletal age, an X-ray of the left hand was taken. Tanner stage was done by self assessment. Trained operators performed ultrasonometry of the right tibia with the SoundScan®Compact. A statistical significant correlation was found between age and SOS $r^2_{boys}=0.52$ and $r^2_{girls}=0.63$ (both $P<0.001$) and between skeletal age and SOS $r^2_{boys}=0.56$ and $r^2_{girls}=0.63$ (both $P<0.001$). In boys significant increase of mean SOS was seen between Tanner stages II and III, and between IV and V. In girls there is a significant increase of mean SOS between all Tanner stages, except between Tanner stage II and III.

In the next three chapters, tibial ultrasonometry was compared to three different bone mass assessment technique, all in healthy Caucasian children and adolescents.

In Chapter 5 we compare ultrasonometry with DXA. For this study we recruited 146 Caucasian children and adolescents, 58 boys (median age 14.1 years, range 7.6 - 23.4 years) and 88 girls (median age 18.0 years, range 7.6 - 23.5 years). Tanner stage, weight and height were assessed for all participants. Using the Lunar DPXL, a DXA machine, bone mineral density (BMD) ($g \cdot cm^{-2}$) of the total body and lumbar spine ($L_2$-$L_4$) and bone mineral apparent density (BMAD) of the lumbar spine ($g \cdot cm^{-3}$) were assessed. Again the tibial ultrasound technique, the SoundScan®Compact, was used to assess SOS of the tibial cortex. Both lumbar BMD as well as total body BMD showed strong significant correlations in boys and girls with tibial SOS: $r_{total\ body\ boys}=0.81$, $r_{total\ body\ girls}=0.77$, $r_{lumbar\ spine\ boys}=0.79$, and $r_{lumbar\ spine\ girls}=0.72$. Lumbar spine BMAD also showed significant correlations with tibial SOS: $r_{boys}=0.63$ and $r_{girls}=0.63$ (for all

correlations $P<0.001$). Our study shows strong significant correlations between DXA and tibial SOS, suggesting that tibial SOS is a technique, which may be applicable in children and adolescents.

In Chapter 6 we presented a prospective cohort study, in which we enrolled 290 girls (mean age 12.7 years) and 273 boys (mean age 12.4 years). Radiographs of the left hand and the left index finger were taken and an aluminum reference wedge was placed within the field of exposure. We performed radiographic absorptiometry (RA) on the second middle phalanx at the mid-level ($BMD_{50\%}$) and proximal quarter ($BMD_{25\%}$). Tibial ultrasonometry was performed using the SoundScan®Compact. Multiple regression analyses showed that SOS correlated significantly with $BMD_{25\%}$ for both boys ($r=0.65$, $P<0.001$) and girls ($r=0.59$, $P<0.001$), taking into account age and gender. The same applied for the correlation between SOS and $BMD_{50\%}$ in boys ($r=0.62$, $P<0.001$) and girls ($r=0.67$, $P<0.001$). Cubic regression between calendar age and $BMD_{25\%}$ showed the best fit for both boys ($r^2=0.60$) and girls ($r^2=0.60$). For $BMD_{50\%}$ a difference in regression was found between boys and girls. Quadratic regression gave a satisfactory fit for boys ($r^2=0.61$) whilst for girls a cubic relation was best ($r^2=0.59$). Overall, there was a significant correlation between $BMD_{25\%}$ and $BMD_{50\%}$ for boys $r=0.89$ and for girls $r=0.91$ (both $P<0.001$).

In conclusion, our data demonstrate a significant correlation between two different bone assessment techniques. Our data also show that both tibial ultrasonometry and RA are useful bone assessment techniques in children.

In Chapter 7 we compare another ultrasound device, the Sahara®(Hologic Corp. Bedford, MA, USA), which measures at the calcaneus with the tibia ultrasound device. We studied 120 healthy Caucasian Dutch children between 7 and 19 years, 53 boys (mean age 12.5 years ; range 4.5 - 18 years) and 67 girls (mean age 13.5 years; range 7.1 - 19 years). The correlation between calcaneal ultrasonometry and tibia ultrasonometry is modest ($r=0.33$). Using the calcaneal device, we found in girls a weak positive correlation between skeletal age and SOS ($r=0.61$), broadband ultrasound attenuation (BUA) ($r=0.57$) and quantitative ultrasound index (QUI) ($r=0.46$). For boys all parameters failed to reach significance. Using the tibia device, we found a good correlation between skeletal age and SOS in girls ($r=0.76$) and modest correlation in boys ($r=0.50$).

At present we feel that, in light of the poor correlation with skeletal age, calcaneal ultrasound has yet to prove its efficacy in children. Thus tibia ultrasonometry seems to be a better choice for bone assessment in children.

Chapter 8 shows the longitudinal data from part of the study population presented in Chapter 4, 120 healthy children and young adults, 53 boys and 67 girls enrolled in this study. The follow-up time is about two years of all participants. Using the calculated regression curves of the cross-sectional data study, all the follow-up measurements of the girls fit between the 5% and 95% interval curves, except in two cases. In the boys all measurements are between the calculated regression curves. There is a slight significant difference between the Z-scores of the first measurement and the last measurement, the delta Z-score is 0.4. Length, weight and body mass index (BMI) have no additional influence on the bone assessment measurements. Therefore this tibial device measures not growth, but real increase in bone mass, expressed as an increase in SOS SD score. The good correlation between the first and second Z-score means that a child follows its bone mass curve and seems independent of the investigated characteristics but mainly genetically determined. In conclusion, our longitudinal results are only slightly different from our cross-sectional normative data, presented in Chapter 4. The tibial ultrasound device measures not growth but real increase in bone mass. The increase of bone mass in time fits a "bone mass curve" unique for the child and mainly determined by his or her genes.

In the next two chapters, the clinical applications, using the tibia ultrasound device, are presented. In Chapter 9 a cross-sectional study has been done in 21 children which were cured of their acute lymphoblastic leukemia (ALL). The follow-up time is approximately ten years. No significant differences were found in mean height and BMI between the children with ALL and our reference group (Chapter 4). No significant change in mean SOS SD score was found in boys and in girls. Spearman's correlation between mean SOS SD score and mean BMD of lumbar spine is 0.49, and mean SOS SD score and mean BMD of total body is 0.51. These correlations are significant at the 0.05 level (2-tailed). Spearman's correlation between SOS SD score and mean BMAD SD score is 0.39 and not significant. No significant difference in Tanner stage was seen compared to the reference group, but we found a tendency towards

delayed puberty; but our group of pubertal patients was too small for proper analysis. We did find a higher fracture risk in the patient group compared to our reference group.

In Chapter 10 we show data of an on-going longitudinal study in other children with the diagnose of ALL. We started with thirty-nine patients, but only in 29 tibia ultrasonometry was performed. There were multiple investigation times, t=0 (time of diagnosis), t=1 (6 months), t=2 (12 months) and t=3 (24 months). We saw a significant decrease in mean SOS SD score in the patient group between t=0 and t=1, and t=0 and t=2. The biggest decrease was between t=0 and t=1. This lower mean SOS can not be explained by growth retardation, but as a real decrease in bone mass. Therefore tibial ultrasonometry is a good method for detecting changes in bone mass, expressed in mean SOS SD scores, in children receiving chemotherapy for treatment.

## 11.2 CONCLUSIONS

After validation of the tibial ultrasound device SoundScan®Compact, we found tibial ultrasonometry to be a technique with good precision and longitudinal sensitivity. Comparison with DXA and radiographic absorptiometry suggests that tibial ultrasonometry is also a good bone assessment technique. Two great advantages are its ease to use and its lack of radiation burden. Tibial ultrasonometry measures not growth, but a real increase in tibial bone mass. After comparison with a calcaneus ultrasound device, we found that the tibia ultrasound device by design is a better choice as bone assessment technique in children. Our longitudinal data suggest that tibial ultrasonometry can also be implemented in a clinical setting. Monitoring changes in bone metabolism caused by a disease or treatment are now possible without radiation.

## 11.3 FUTURE RESEARCH

*In vivo*

As stated in Chapter 8, tibial ultrasonometry is a bone assessment technique with a good longitudinal sensitivity. In Chapter 10 we saw that tibial ultrasonometry can also detect changes in time in the bone mass of children with ALL. All those children received high dose steroids and methotrexate (MTX). It would be of interest if we find the same results as in Chapter 10, in children with other diagnoses such as rheumatoid arthritis, inflammatory bowel disease, or in children with kidney transplantation, who also receive high dose steroids and sometimes MTX. Children with abnormal bone formation like osteogenesis imperfecta could also be an interesting study group. By design the tibia device is the only possible bone assessment technique to quantify the bone status of paraplegic children. Due to their spasticity, the use of other bone mass assessment techniques, such as DXA and QCT, are impossible. Also development of smaller probes for use in younger children and even in neonates would expand the use of this device.

Defining a correlation with a bone mass assessment device which looks at bone sites, with a high fracture risk, like the femoral neck, would be interesting.

*In vitro*

An ultrasound technique also gives information on the quality of the bone; this could be of use in children with diseases which affect bone metabolism. Theoretically, it could be possible in a specific bone disorder, that a technique such as DXA measures a normal bone density and tibia ultrasound finds lower SOS caused by less bone quality but normal bone quantity. Paget disease is such a disorder, in which the bone quantity is even higher than normal, but the bone quality is decreased, which is a good explanation for the increased fracture risk in patients with Paget disease. It would be of interest to know which part of the measured SOS with the tibial ultrasound depends on the tibial BMD and which part of the SOS is influenced by the elasticity and by the architecture of the tibia. A part could be elucidated by comparing QCT and ultrasonometry of tibial bone specimens.

# SAMENVATTING, CONCLUSIES, EN TOEKOMSTIGE ONTWIKKELINGEN

## 11.4 SAMENVATTING

De laatste jaren zijn er verschillende echoapparaten ontwikkeld om botmassa te meten, die vergeleken met de bestaande botmassa meettechnieken relatief goedkoop, gemakkelijk in het gebruik, stralingsvrij en patiëntvriendelijk zijn. Deze nieuwe echotechnieken moesten eerst gevalideerd worden in volwassenen. In volwassenen blijken deze technieken accuraat te zijn in de detectie van osteoporose, de progressie en respons op therapie, als ook in de identificatie van personen met een verhoogd fractuurrisico. Het zijn krachtige methodes en een concurrent van de meer gevestigde technieken, zoals dual-energy X-ray absorptiometry (DXA).

Hoofdstuk 1 geeft een overzicht van alle botmassa metende technieken, die de afgelopen jaren zijn ontwikkeld. De voor- en nadelen van deze technieken worden genoemd.

Hoofdstuk 2 geeft een overzicht van de verschillende echoapparaten en technieken, die in de dagelijkse praktijk worden gebruikt. Net zoals de meeste van de in Hoofdstuk 1 genoemde botmassa meettechnieken, zijn ook de meeste echotechnieken eerst gevalideerd in volwassenen. De tibia (scheenbeen) echo-methode gebruikt in dit proefschrift was nog niet gevalideerd in kinderen. Daarom is deze validering een van de doelstellingen van dit proefschrift. De data van deze validering worden gepresenteerd in Hoofdstuk 3 en volgende hoofdstukken.

Om een volwaardige concurrent te zijn van de andere botmassa meettechnieken, moet de tibia echografie een goede korte en lange termijn reproduceerbaarheid hebben. De reproduceerbaarheid van dit systeem valt in dezelfde orde als van andere echoapparaten en kan concurreren met botmassa meettechnieken zoals radiografische absorptiometrie en DXA. De korte termijn precisie van dit tibia echoapparaat, de invloed van de plaats van meting, rechtshandigheid, merk echogel, en de temperatuur van de echogel worden gepresenteerd in Hoofdstuk 3. De intra-observer variabiliteit was CV 0.43%, de inter-observer variabiliteit was CV 0.61%.

De linker mid-tibia en rechter mid-tibia speed of sound (SOS) metingen lieten geen significant verschil zien. Er was wel een significant verschil bij zowel jongens als meisjes tussen de rechter proximale versus de rechter mid-tibia, de rechter mid-tibia versus de rechter distale en de rechter proximale versus rechter distale metingen. One-way analysis of variance laat zien dat noch het gebruik van verschillende echogels noch een toename in echogel temperatuur een significante invloed hebben op de metingen. De resultaten van onze studie laten zien dat de tibia echografie een goed reproduceerbare techniek is in Kaukasische kinderen. Lange termijn precisie van het tibia echoapparaat is goed, en is beschreven in het addendum van Hoofdstuk 3. In een prospectieve studie presenteren we data die we hebben verkregen bij 596 gezonde kinderen, 309 meisjes, gemiddelde leeftijd 12.9 jaar (6.1-19.9 jaar), en 287 jongens, gemiddelde leeftijd 12.3 jaar (6.1-19.6 jaar). Bij alle onderzochte kinderen werd een enquête betreffende de gezondheid afgenomen. Om de skeletleeftijd te bepalen werd er een röntgenfoto van de linkerhand gemaakt. Het puberteitsstadium (Tanner) wordt bepaald door het individueel vergelijken van de eigen beharing en uitwendige genitaliën met die van de gepresenteerde referentiefoto's. Getrainde onderzoekers verrichtten de echografie van de rechter tibia met de SoundScan®Compact. Een statistisch significante correlatie wordt gevonden tussen leeftijd en SOS jongens ($r^2$ =0.52) en meisjes ($r^2$ =0.63) ($P$<0.001) en tussen skeletleeftijd en SOS jongens ($r^2$ =0.56) en meisjes ($r^2$ =0.63) ($P$<0.001). In jongens is de toename van de gemiddelde SOS significant tussen Tanner stadium II en III en tussen IV en V. Bij meisjes is de toename van de gemiddelde SOS significant tussen alle Tanner stadia, behalve tussen Tanner stadium II en III.

In de volgende drie hoofdstukken wordt de tibia echografie vergeleken met drie verschillende botmassa meettechnieken, allemaal in gezonde Kaukasische kinderen en jong volwassenen.

In Hoofdstuk 5 is er een vergelijking met de DXA. Voor deze studie hebben we 146 Kaukasische kinderen en jong volwassenen onderzocht, 58 jongens met een mediaan leeftijd van 14.1 jaar (7.6 - 23.4 jaar) en 88 meisjes met een mediaan leeftijd van 18 jaar (7.6 - 23.5 jaar). Tanner stadium, gewicht en lengte werden bij allen bepaald. Gebruikmakend van de Lunar DPXL, een DXA machine, werd de botdichtheid (BMD) ($g \cdot cm^{-2}$) van het totale skelet en de

lumbale wervelkolom ($L_2$-$L_4$) en de bone mineral apparent density (BMAD) van de lumbale wervelkolom ($g \cdot cm^{-3}$) bepaald. Opnieuw werd het tibia echoapparaat de SoundScan®Compact gebruikt om de SOS van de tibia cortex te bepalen. Zowel de lumbale wervelkolom (lwk) BMD als ook het totale skelet (ts) BMD laten een sterk significante correlatie zien in jongens en in meisjes met de tibia SOS: $r_{ts\ jongens}$ =0.81, $r_{ts\ meisjes}$ =0.77, $r_{lwk\ jongens}$ =0.79, en $r_{lwk\ meisjes}$ =0.72. De lumbale wervelkolom BMAD laat ook een significante correlatie zien met de tibia SOS: $r_{jongens}$ =0.63 en $r_{meisjes}$ =0.63 (alle correlaties $P$<0.001). Onze studie laat sterk significante correlaties zien tussen DXA en tibia SOS, en bewijst dat tibia SOS een techniek is die kan worden toegepast in kinderen en jong volwassenen.

In Hoofdstuk 6 presenteren we een prospectieve cohort studie, waarin we 290 meisjes, gemiddelde leeftijd 12.7 jaar en 273 jongens, gemiddelde leeftijd 12.4 jaar hebben onderzocht. Röntgenfoto's van de linkerhand en linkerwijsvinger werden gemaakt en een aluminium referentie wig werd in het belichtingsveld geplaatst. Radiografische absorptiometrie (RA) van het middelste kootje van de wijsvinger op het mid-deel ($BMD_{50\%}$) en het proximale kwart ($BMD_{25\%}$) werden uitgevoerd. Tibia echografie werd gedaan met de SoundScan®Compact. Multiple regressie analyse laat zien dat SOS significant correleert met $BMD_{25\%}$ bij jongens (r =0.65, $P$<0.001) en meisjes (r =0.59, $P$<0.001), leeftijd en geslacht hierin betrekkend. Hetzelfde geldt voor de correlatie tussen SOS en $BMD_{50\%}$ in jongens (r =0.62, $P$<0.001) en meisjes (r =0.67, $P$<0.001). Kubische regressie tussen kalenderleeftijd en $BMD_{25\%}$ laat de beste correlatie zien bij jongens ($r^2$ =0.60) en meisjes ($r^2$ =0.60). Bij $BMD_{50\%}$ is er een verschil in regressie gevonden tussen jongens en meisjes. Kwadratische regressie was een bevredigende vorm bij de jongens ($r^2$ =0.61) terwijl voor de meisjes de kubische relatie het beste was ($r^2$ =0.59). Over het geheel was er een significante correlatie tussen $BMD_{25\%}$ en $BMD_{50\%}$ bij de jongens r =0.89 en bij de meisjes r =0.91 (beide $P$<0.001).

Concluderend laten onze data zien dat er een significante correlatie is tussen twee verschillende botmassa meettechnieken. Onze data bewijzen dat zowel tibia echografie als RA bruikbare botmassa meettechnieken zijn in kinderen.

In Hoofdstuk 7 is er een vergelijking met een ander echoapparaat, de Sahara® (Hologic Inc, Bedford, MA., USA), dat meet ter hoogte van de hiel (calcaneus).

We onderzochten 120 gezonde Kaukasische kinderen tussen 7 en 19 jaar, 53 jongens (gemiddelde leeftijd 12.5 jaar; 4.5 - 18 jaar) en 67 meisjes (gemiddelde leeftijd 13.5 jaar; 7.1 - 19 jaar). De correlatie tussen de calcaneus echografie en de tibia echografie is matig (r =0.33). We vonden, gebruikmakend van het calcaneus apparaat, in meisjes een zwak positieve correlatie tussen skeletleeftijd en SOS (r =0.61), broadband ultrasound attenuation (BUA) (r =0.57) en quantitative ultrasound index (QUI) (r =0.46). Bij de jongens was geen van de parameters significant. We vonden wel een goede correlatie tussen skeletleeftijd en SOS bij meisjes (r =0.76) wanneer we gebruik maakten van het tibia apparaat, en een matige correlatie bij jongens (r =0.50). Wij zijn van mening dat, in het licht van de slechte correlatie met de skeletleeftijd, de calcaneus echografie zijn bruikbaarheid in kinderen nog moet bewijzen. Tibia echografie blijkt wel een goede keuze te zijn als bot meettechniek in kinderen.

Hoofdstuk 8 laat de longitudinale data zien van een deel van de studie populatie die gepresenteerd is in Hoofdstuk 4. 120 Gezonde kinderen en jong volwassenen, 53 jongens en 67 meisjes namen deel aan dit onderdeel van de studie. De follow-up tijd van de deelnemers is gemiddeld bijna 2 jaar. Alle follow-up metingen van de meisjes vallen tussen de 5% en 95% interval curve, behalve in twee gevallen, wanneer gebruik gemaakt wordt van de berekende regressie curven van de cross-sectionele data studie. Bij de jongens vallen alle metingen binnen de berekende regressie curven. Er is een gering, maar significant verschil tussen de Z-scores van de eerste meting en de laatste meting, de delta Z-score is 0.4. Lengte, gewicht en body mass index (BMI) hebben geen additionele invloed op de botmassa metingen. Daarom meet dit tibia echoapparaat geen groei, maar een werkelijke toename in botmassa, uitgedrukt in een toename van SOS SD score (Z-score). De goede correlatie tussen de eerste en de tweede Z-score betekent dat een kind zijn of haar eigen "botmassa curve" volgt en dat deze niet afhankelijk is van de onderzochte variabelen, maar hoofdzakelijk van zijn of haar genen. Concluderend, onze longitudinale resultaten zijn gering maar significant verschillend van onze cross-sectionele normaalwaarden, zoals gepresenteerd in Hoofdstuk 4. Het tibia echoapparaat meet geen groei maar een werkelijke toename van botmassa.

De toename van de botmassa in tijd verloopt volgens een "botmassa curve", uniek voor het kind en hoofdzakelijk bepaald door zijn of haar genen. In de volgende twee hoofdstukken worden de klinische toepassingen van het tibia echoapparaat gepresenteerd. In Hoofdstuk 9 is er een cross-sectionele studie gedaan in 21 kinderen, die zijn genezen van acute lymfoblastisch leukemie (ALL). De follow-up tijd is ongeveer 10 jaar. Geen significante verschillen werden gevonden in gemiddelde lengte en BMI tussen de kinderen met ALL en onze referentiegroep (Hoofdstuk 4). Er werd geen significant verschil in gemiddelde SOS SD score gevonden bij de jongens dan wel bij de meisjes. Spearman's correlatie tussen de gemiddelde SOS SD score en de gemiddelde BMD van de lumbale wervelkolom is 0.49 en de gemiddelde SOS SD score en gemiddelde BMD van het totale skelet is 0.51. Deze correlaties zijn significant op het 0.05 niveau (2-tailed). Spearman's correlatie tussen SOS SD score en de gemiddelde BMAD SD score is 0.39 en niet significant. Er was geen significant verschil in Tanner stadium gezien, vergeleken met de referentiegroep, maar we vonden wel een neiging tot vertraagde puberteit, al was onze groep van patiënten in de puberteit te klein voor een gedegen analyse. We vonden een verhoogd fractuurrisico in de patiëntengroep, vergeleken met de referentiegroep.
Hoofdstuk 10 laat data zien van een nog lopende, longitudinale studie in kinderen met de diagnose ALL. We startten met 39 patiënten, waarvan slechts 29 een tibia echografie ondergingen. Er zijn meerdere onderzoekstijden, t=0 (tijdstip van diagnosis), t=1 (6 maanden), t=2 (12 maanden) en t=3 (24 maanden). We zagen een significante daling in gemiddelde SOS SD score in de groep patiënten tussen t=0 en t=1, en t=0 en t=2. De grootste daling is tussen t=0 en t=1. Deze gemiddeld lagere SOS kan niet verklaard worden door groei retardatie, maar berust op een werkelijke afname in botmassa. Daarom blijkt tibia echografie een goede methode te zijn om veranderingen in botmassa te detecteren, uitgedrukt in gemiddelde SOS SD score, in kinderen die chemotherapie ondergaan.

## 11.5 CONCLUSIES

Na validering van het tibia echoapparaat de SoundScan®Compact, blijkt tibia echografie een techniek met een goede precisie en longitudinale sensitiviteit. De vergelijking met DXA en radiografische absorptiometrie suggereert dat de tibia echografie ook een goede botmassa meettechniek is. Twee grote voordelen zijn gebruiksgemak en afwezigheid van röntgenstraling. Tibia echografie meet geen groei maar een werkelijke toename van de botmassa van de tibia. Na vergelijking met een calcaneus apparaat, denken we dat het tibia echo-apparaat, gezien het ontwerp, een betere keuze is als bot meettechniek in kinderen. Onze longitudinale data suggereren dat de tibia echografie ook in een klinische setting kan worden toegepast. Vastleggen van veranderingen in bot metabolisme, veroorzaakt door ziekte of behandeling, blijkt ook mogelijk.

## 11.6 TOEKOMSTIGE ONTWIKKELINGEN

*In vivo*

Zoals genoemd in Hoofdstuk 8 blijkt de tibia echografie als bot meettechniek een goede longitudinale sensitiviteit te hebben. In Hoofdstuk 10 zagen we dat de tibia echografie ook veranderingen in tijd ten aanzien van de botmassa van kinderen met ALL kan detecteren. Al deze kinderen kregen hoge doses steroïden en methotrexaat (MTX). Het zou interessant kunnen zijn of we dezelfde resultaten zouden vinden zoals beschreven in Hoofdstuk 10, in kinderen die hoge doses steroïden en soms MTX krijgen met een andere diagnose zoals reumatoïde artritis, ontstekingen van de darmen, dan wel na niertransplantaties. Ook kinderen met een abnormale botformatie zoals osteogenesis imperfecta kunnen een interessante studiegroep zijn. Door het ontwerp zou het tibia echoapparaat de enige bot meettechniek kunnen zijn die de botstatus van paraplegische kinderen kan kwantificeren. Als gevolg van hun spasticiteit is het gebruik van andere botmassa meettechnieken als DXA en quantitative computed tomography (QCT) niet altijd mogelijk. Ook de ontwikkeling van kleinere transducers, te gebruiken in kleine kinderen en zelfs in neonaten, zou het onderzoeksveld van dit apparaat sterk kunnen vergroten. Een correlatie met een ander botmassa meetapparaat dat plaatsen bekijkt waar een hoog fractuurrisico is, zoals de femurnek, zou interessant kunnen zijn.

*In vitro*

Een echotechniek geeft tevens informatie over de kwaliteit van het bot; dit kan van belang zijn bij kinderen met een ziekte die het botmetabolisme beïnvloedt. Theoretisch is het mogelijk dat bij een specifieke botstoornis een techniek zoals de DXA, een normale bot densiteit meet en de tibia echografie een lagere SOS vindt, veroorzaakt door een verminderde bot *kwaliteit*, maar met een normale bot *kwantiteit*. De ziekte van Paget is zo'n botziekte waarbij de bot *kwantiteit* zelfs hoger is dan normaal, maar de bot *kwaliteit* lager. Dit is een goede verklaring voor het verhoogde fractuurrisico bij patiënten met de ziekte van Paget. Het zou interessant zijn te bepalen welk deel van de gemeten tibia SOS bepaald wordt door de BMD en welk deel door de elasticiteit en de architectuur van de tibia. Een deel zou verduidelijkt kunnen worden wanneer QCT en ultrasonometrie van tibia kadavers vergeleken worden.

Lieve Gaby, ik weet dat menig mannelijke collega jaloers was op mijn vrijheden die ik thuis kreeg om mijn medische carrière op te bouwen. Meerdere congressen met aantrekkelijke locaties heb je moeten missen. Ook ten aanzien van dit proefschrift ben ik op mooie plekjes geweest. Ik sta bij je in het krijt. Ik hoop dat onze San Francisco tijd al iets heeft goed gemaakt.
Lieve Céline, Yorick en Florian, ik hoop dat de zin "je moet zeker weer naar het ziekenhuis" of "je kan ons zeker niet naar school toe brengen" in de toekomst minder vaak te horen is. Beloven kan ik het niet.

Morteza Meradji: beste Morteza, ik weet nog goed dat je tijdens mijn sollicitatie gesprek zei dat je hoopte dat ik een wetenschappelijke carrière zou maken. Het liefst in de kinderneuroradiologie want daar was grote behoefte aan, niet alleen op onze afdeling, maar ook landelijk. Helaas Morteza, hierin heb ik je met dit proefschrift teleurgesteld, het onderwerp heeft niets met dit superspecialisme van doen, maar met echografie en kinderen zoals je weet. Ik hoop dat je toch tevreden bent over mijn keuze voor dit onderwerp.

Kees van Kuijk: beste Kees, een betere co-promotor kon ik me niet wensen. Ondanks dat je voor een groot gedeelte van de tijd in San Francisco zat, had je nog altijd de regie in handen. Deels door goed voorbereidend werk voor je vertrek naar de States, deels door continue aanwezigheid via moderne media als e-mail, fax of gewoon de telefoon. Je deed je naam als co-promotor eer aan. Als ik het even niet zag zitten omdat er weer geen medewerking was uit Israël, stuwde je me, na een echte "peptalk" met vernieuwde energie weer vooruit. Sinds het laatste jaar, na je terugkeer naar ons polderlandje, waardeer ik ook je "lijfelijke gesprekken". Je hebt mij altijd doen geloven dat uit dit onderwerp zeker een proefschrift moest voortkomen en inderdaad het is ons gelukt.

Simon Robben: beste Simon, zoals je al suggereerde in jouw proefschrift, mijn tijd zou nog komen. Ik ziet het overigens niet als zoete wraak, ik vind het eerder leuk dat we waarschijnlijk de enige subspecialisatie in de radiologie in Rotterdam zijn waar alle stafleden gepromoveerd zijn.

Wat een wetenschappelijke output met zo weinig tijd voor wetenschap. Als we ons maar niet in de eigen vingers snijden met dit gegeven. Dat zou gevaarlijk zijn, want teveel "doctoren" aan een ziekbed kan fataal zijn. Helaas hoef ik hier niet bang meer voor te zijn, omdat je de euvele moed had om naar Maastricht te vertrekken.

Rick van Rijn: beste Rick, we hebben veel samen gedaan. Ik weet niet wat jij ervan vond, maar ik vond en vind het gezellig. Dit proefschrift heeft jou ook geen windeieren gelegd. We hebben samen een paar mooie trips gemaakt. Jammer dat Eilat niet meer door ging. Je was een onmisbare vervanger op de momenten dat ik afwezig was, met name in de tijd dat ik een half jaar in de States was. Ik bewonder je inzet en ook je snelheid van artikelen schrijven. Een ding moet me nog wel van het hart en dat is dat je mij altijd verweet dat het een chaos was, niet alleen op mijn kamer maar ook in mijn database. Dat laatste klopt wel maar dat eerste? Enige inside information brengt mij tot de conclusie: de pot verwijt de ketel.

Inge van de Sluis: beste Inge, zelfs een chaotisch iemand krijgt het voor elkaar. Voor jou dus een 'makkie'. Het is en was prettig om met je samen te werken. Alhoewel niet iedereen achter onze onderzoekslijn stond, hebben we er tot nu toe toch iets moois van gemaakt.

Gabriel Krestin: beste Gabriel, mijn dank voor je support aan dit proefschrift. Ik weet dat het onderwerp je niet na aan het hart lag, maar daarom des temeer respect voor jou dat je mij geholpen hebt om me verder te ontplooien en dit proefschrift te verwezenlijken.

Alle laboranten, maar met name Esther, Ingrid en Mieneke. Heel veel dank voor het invallen op onverwachte en op onmogelijke tijden om met veel energie die zogenaamd makkelijke tibia ultrasound metingen te doen. Zonder jullie was ik nog jaren langer aan het ploeteren om mijn proefschrift te voltooien. Ook veel dank aan de andere laboranten, die met tomeloze energie weer een handfoto moesten maken volgens Myriad of toch niet? Soms was het niet even duidelijk. Mijn excuses hiervoor.

Wiebeke van Leeuwen: beste Wiebeke, zoals ziet zijn niet veel van je inspanningen in dit proefschrift gekomen. Maar we hebben al een geaccepteerd artikel met jouw radiografische absorptiemetrie metingen. Als het aan mij ligt komen er nog meer artikelen uit.

De laboranten van het opleidingsinstituut in Haarlem en Eindhoven. Zonder jullie inzet, in het kader van jullie afstudeerproject, was mijn project nooit afgerond. Ik hoop dat jullie de onderzoeken leuk vonden en mijn begeleiding dan wel die van Rick hebben kunnen waarderen.

Miles Brennan: dear Miles, thank you for all your grammar and spelling advice. And so quick and accurate. The only explanation I can think of is your excellent scientific background. Reading the manuscripts, I hope I did not bore you too much with this thesis.

Esther van de Vosse: beste Esther jij was in de laatste fase van het proefschrift de "fax-dame" van over de plas, die het mogelijk maakte dat ik in een kort tijdbestek de Engelse en Nederlandse correcties kon doorvoeren. Hiervoor veel dank.

Andries Zwamborn en Teun Rijsdijk: beste Andries en Teun, wie op de afdeling kan nog zonder het gouden koppel? Andries, nogmaals bedankt voor je gastvrijheid in Chicago zodat ik daar een presentatie van een onderdeel van het proefschrift kon geven. Van je "snurken" heb ik in elk geval niets gemerkt. Dat zal wel de invloed zijn geweest van je overheerlijke fles whiskey.

Cees Entius: beste Cees, de momenten van het afzagen van een aantal onderbenen bij jouw op de afdeling brachten bij mij weer herinneringen naar boven aan mijn snijzaal practicum. Die geur alleen al, heerlijk. Ik hoop dat je nog even geduld hebt. Laat de vrieskist nog niet ontdooien. Ik durf het haast niet meer tegen je te zeggen maar misschien gaan we er toch nog wat mee doen.

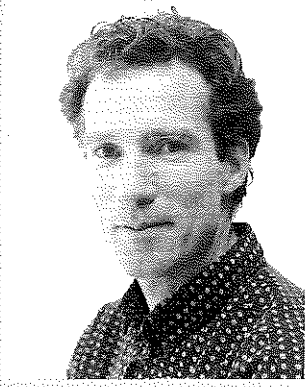
Wim Hop: beste Wim, net als bij Simon ging er voor mij een nieuwe, statistische wereld open. Jouw sessies duurde inderdaad altijd langer dan gepland. Een half uurtje werd vaak anderhalf uur. Je bakje koffie was op die momenten een noodzaak, om nog te kunnen volgen wat je allemaal aan het doen was. Het mooiste dat me bij zal blijven is de discussie die we hadden over de introductie van een standaard CV in plaats van een normale CV door Miller et al. Je vond het een prachtige vondst, zeker voor een niet "biomedisch statisticus".

De Belgenclub: Jonathan Verbeke, met dit schrijven ben je al weer een jaar weg. Toch wil ik je ook, net als Simon, bedanken voor je tomeloze inzet op de afdeling waardoor ook ik wat tijd voor wetenschap kon vrij maken. Annick Devos, het zonnetje in huis, bezorgt mij in elk geval extra werkvreugde. Ik hoop dat dit gevoel blijft.

Kees Ouwerkerk en de leerlingen van het Erasmiaans Gymnasium als ook de heer J.G. Radstake en de leerlingen van het Marnix Gymnasium te Rotterdam. Zonder jullie vrijwillige medewerking was dit proefschrift niet tot stand gekomen.

Ook de volgende instellingen wil ik bedanken voor de financiële steun die ik heb gekregen voor de verwezenlijking van mijn proefschrift: Schering, ATL, Nycomed, GE, Procter & Gamble, Acuson, Nutricia en de Stichting Anna Fonds te Leiden.

## CURRICULUM VITAE



Geboren in een van de koudste voorjaren van het vorige millennium (april 1963), als verjaardagscadeau voor zijn moeder.
Opgegroeid in een dorp wat niet meer bestaat, Dubbeldam, onder de rook van Dordrecht.
Na het lokale schooltje te zijn ontgroeid, gaat deze boerenjongen naar het Johan de Wit Gymnasium te Dordrecht.
In 1982 stort hij zich in het Leidse studenten leven. Gestart met de studie biologie maar al snel overgestapt naar "medicijnen". In 1983 gaat zijn geneeskundige carrière van start, die hem in november 1991 weer dichterbij zijn geboorteplaats brengt.
Tussendoor nog even een kleine adempauze, de militaire dienstplicht wordt vervuld bij de Koninklijke Luchtmacht.
Na vijf intensieve "licht therapie" jaren wordt hij losgelaten tussen de kinderen, waar hij nu sinds november 1996, tot zijn volle tevredenheid als kinderradioloog werkzaam is in het Sophia Kinderziekenhuis te Rotterdam.