Velocity of Ultrasound in the Tibia - An Investigation

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1. Page 50 Para 2 Line 9
For "Andre 1981" read "Andre 1980"

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3. Page 180 Para 2 Line 3 and 4
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Summary of Thesis

Introduction

Osteoporosis is an increasingly common condition leading to fragility fractures of bone. It may be age related, or have a secondary cause. Over the past decade, new preventative treatments have arisen, which need to be targeted to the population at highest risk of fracture. To achieve this, bone assessment by densitometry is performed, mainly using large, immobile and expensive scanners, which use a small dose of ionising radiation. New, ultrasound, devices have been designed to be portable, less costly and avoid radiation. The main potential roles of densitometry devices are initial assessment of risk for fracture, follow up to define natural history, to assess response to treatment, and as a surrogate end point to assess efficacy of new preventative treatments. It is not fully understood how ultrasound interacts with bone, nor how alterations to bone as a result of age, disease or treatment will effect ultrasonic measurements.

Aims

To investigate the interaction of transmitted ultrasound with bone. To investigate how alterations to bone as a result of age, disease or treatment will affect ultrasonic measurements. To assess the likely efficacy of an ultrasound device in any of the main roles of densitometry. (These being initial assessment of risk for fracture, follow up to define natural history, to assess response to treatment, and as a surrogate end point to assess efficacy of new preventative treatments.)

Methods

The device used for these investigations was the "SoundScan 2000" (Myriad, Rehovot, Israel). This is a handheld device, which uses one transmitter and two receivers at a fixed distance to make measurements, from the tibia of a human subject, of the velocity of transmission of a 250kHz pulse over a fixed 5-centimetre distance. To assess interactions with bone, an in-vitro model was developed using bovine ulna. This model was assessed for reproducibility, and then subjected to manipulations of temperature, interface thickness and decancellation.

To assess behaviour of the measurement in humans, volunteers were used. Initial measures were made of reproducibility, and variance with repositioning and temperature. A dataset of normal individuals was then made to compare anthropometric and demographic measures with both existing densitometric, and ultrasound measures.

Volunteers with specific disease states were then assessed (Paget's disease of bone, hyperparathyroidism, glucocorticoid excess, soft tissue injury, bone injury and fracture). This allowed an assessment of the response of ultrasound to diseases known to affect bone. These subjects were compared with the normal volunteer subjects, or where appropriate, a comparison of the affected and unaffected limb was made. Discriminatory ability was tested using the data from 329 volunteers, of whom 72 had an existing osteoporotic vertebral fracture. Thresholds were derived for ultrasound and bone density measures using the normal dataset, and these were applied retrospectively.

Finally, as part of a larger, prospective study of the efficacy of a drug (clodronate) in prevention of osteoporotic fracture, volunteers had ultrasound and other densitometric measures made before randomisation to placebo or drug. During 3-year follow up, further measures were made. Thus it was possible to comment on the suitability of ultrasound transmission velocity measurement as a substitute for the main roles for densitometry.

Results

The in-vitro model confirms the good reproducibility of the device, but also its sensitivity to temperature change. The model of interface thickness suggests that this is potentially an important confounding variable. Decancellation of the bone made no difference to ultrasound transmission velocity.

In human volunteers, reproducibility was also good. Temperature variance did not measurably occur, and repositioning error was non significant. The dataset from normal volunteers showed that ultrasound velocity, like other densitometric measures, had an age related decline, and was systematically greater in males than in females. Normal ranges were established as the basis for standard deviation based Z and T scores.

Volunteers with specific disease states produced interesting results. In Paget's disease of bone, the affected leg was found to have greater bone mineral content and density than the unaffected leg, but a significantly lower ultrasound velocity. Neither volunteers with unilateral soft tissue injury, nor unilateral bone injury demonstrated any residual side-to-side differences after 1 year. The device proved to be impossible to use for the measurement of fracture healing. In both hyperparathyroid and hyperglucocorticoid states, ultrasound velocities were reduced compared to normal ranges. The reduction was relatively greater in hyperparathyroidism. The ability to discriminate between individuals with and without a vertebral fracture was as good as other densitometry. When combined models were made using logistic regression, ultrasound velocity maintained a degree of discrimination independent of bone density measurement.

In the 3-year study group, results were disappointing. In neither the placebo, nor the treatment group were any significant changes found at any time point. Other bone density measures declined in the placebo group and significantly increased in treatment group, even after one year.

Conclusions

This work has shown that ultrasound velocity is a stable and reproducible measure, which is systematically higher in males than females, declines in an age related manner and has the potential to discriminate between individuals with and without certain disease states. Specifically those states are osteoporosis, hyperparathyroidism, hyperglucocorticoidism, and Paget's disease of bone. This work has also shown that ultrasound velocity measurements remain stable over periods of at least three years, in spite of measurable decline of bone mineral density in the same time period. In addition, ultrasound velocity has not shown any change in response to treatment with the bisphosphonate, clodronate, in spite of a measurable rise in bone mineral density.

It can be concluded that ultrasonic measurement using the "SoundScan 2000" may be of some use for initial assessment of risk for fracture. The device has shown no potential for use in the other roles of densitometry.

Declaration

No portion of the work in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

The work presented in this thesis has been performed and written up by the candidate. Some aspects of the work have been performed in collaboration. In particular, the collection of the data on skeletal status in the normal population, and the prospective trial of the efficacy of bisphosphonate are the results of close collaboration with Dr McCloskey and Professor Kanis. The candidate was involved in planning, data acquisition and analysis. These studies both required commercial, and for the bisphosphonate study Medical Research Council, sponsorship. This was secured by Dr McCloskey and Professor Kanis.

Acknowledgements

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Background and Introduction

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Background and Introduction

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Introduction – The Importance Of Osteoporosis

We find ourselves, in a new millennium, facing an epidemic of fractures as a consequence of skeletal failure. Landmark studies have (Gallagher 1980, Kanis 1992, Boyce 1985) identified the exponential rise in fractures of the hip and vertebral body with age. The discovery that the Rochester community had rates of hip fracture as high as one in six elderly men and one in three elderly women generated renewed interest in the clinical management of osteoporosis (Gallagher 1980). In addition to the human costs the financial burden was enormous. It was estimated that in the United States of America alone 1.2 million fractures occurred annually. The costs of treatment were estimated in 1986 to be 6.1 billion dollars in the U S, and 0.614 billion pounds sterling in the UK (Kanis 1992). A more recent estimate from the UK put the total cost of treating osteoporotic fractures in the female population at 727 million pounds sterling (Dolan 1998).

In order to control a disease with a lag time of decades, it is necessary either to have a universal preventative measure, or to have a technique to allow timely identification of individuals at risk and thus target early treatment.

To date no preventative measure has shown itself to be in any useful way universal. Like so many interventions it would seem that any treatment with a low enough side effects profile has only limited efficacy. Those pharmaceuticals which have shown themselves to be efficacious suffer the disadvantage either of cost or of an excessive rate of adverse events.(Kanis 1994)

It would seem therefore that the best hope for disease control lies in targeting high risk groups. High risk relates to race, sex, family history, and general health, mobility and risk of falling as well as to the underlying fragility of the skeleton (Porter 1990). Measures are available for most of these aspects of risk (Kanis 1994). The last two decades have seen a proliferation of physical measurements of skeletal strength. Most of these have attempted to relate the calcium content of bone to the future risk of fracture. Because osteoporosis is a disease whose definition includes the loss of bone mass, this does seem to be an appropriate approach.

The two broad groups of measurement techniques are

1 The measurement of photon absorption by calcium in the skeleton and

2 the measurement of absorption of sound energy by the composite of water, fat, protein and calcium hydroxyapatite crystals that make up locomotor tissues.

Potential Advantages of Ultrasound

Both of these methodologies have considerable merit. There are however certain problems which may be addressed. It has been shown that both of these approaches produce results which are site specific in terms of fracture prediction (Cunningham 1996). More recently it has been apparent that some treatments which increase bone density measurements may in fact reduce the ability of the skeleton to resist fracture (eg Fluoride) (Riggs 1990). This paradox may be explained with reference to architecture; both micro-structure and gross structure. In the study of engineering it is recognised that a construct's strength is related to both the properties of the material used and to the structure or shape into which it is formed. For example, the material properties of eggshell are relatively poor, but when eggshell is formed into an egg shape it can resist large forces. Conversely, aluminium is thought of as being strong, but we are familiar in our daily lives with being able to shape and fold aluminium foil with ease because the structural properties of a thin sheet are poor.

In the same way, bone may suffer excessive fragility due to failings of either the material from which it is made or of the structure into which the material is formed. In primary osteoporosis the material is in fact normal in terms of collagen, mineral type and, at a cellular level, mineral density (Albright 1979). It is the structure which is deficient. There is less bone in total and this bone must fill either the same total volume or more usually a greater volume after endosteal resorption and periosteal expansion has taken place in old age. As a consequence, the network of trabecular bone becomes first thinner and eventually discontinuous. At the same time the cortical shell becomes thinner. The cortical thinning is offset to some extent by the improved structural feature of a larger diameter cylinder.

Because the skeletal fragility caused by osteoporosis is so complex, it is clear that a pure absorptiometric technique can only reflect the changes of osteoporosis indirectly. Ideally a technique for measuring or assessing fracture risk should be sensitive to architectural changes as well. Ultrasound absorption techniques have gone some way towards addressing this. Only os calcis ultrasound (Broadband Ultrasound Attenuation and speed of sound) is currently recognised as a useful and reliable ultrasound technique [Meunier 1996]. It has as the advantage of assessing a weight-bearing site which is easily accessible physically and is socially acceptable. Speed of sound is a relatively simple concept to understand. As the sound wave travels through the composite structure of the heel it will travel faster in material which is more dense. This is a physical characteristic of sound transmission. In the human body the major portion of all tissues is water. Bone is an exception due to the solid phase crystalline structure. Therefore the two main determinants of speed of sound are the proportion of bone to soft tissue and the directness of the pathway through the os calcis.

BUA is a significantly more complicated approach. It was originally observed (Langton 1984) during os calcis speed of sound experiments that attenuation of the ultrasound pulse became quite marked at frequencies greater than 1 Megahertz (Mhz). It was observed with samples containing a larger quantity of bone, that significant attenuation occurred at lower frequencies. From these observations it was extrapolated that a slope could be produced of attenuation over a range of frequencies and that this slope may quantify an important aspect of bone strength. Langton then showed that the slope of BUA correlated very well with bone density both in bovine and human samples.

Potential of Cortical Measurement

It is believed that the main source of attenuation is scattering and particularly back-scattering of sound energy due to reflection from trabecular bone. It would then follow that in healthy individuals more trabecular bone with greater connectivity will be present and that these individuals will therefore have higher attenuation and therefore a steeper slope.

It can be seen that although BUA would hopefully include some information regarding structure as well as mineral content, empirical experiments show a very good correlation with physically measured bone density (Langton 1996). It may be the case that BUA is not telling us anything different. Another potential problem is that BUA is measured at a trabecular site. It is important to remember that the cortical shell also contributes to fracture resistance. CT densitometry experiments and observations on the distal radius suggest that at this site the mineral content of the cortical shell is equivalent to the mineral content of the trabecular bone, and that it makes an equal or greater contribution to strength (Spadaro 1994). Similar observations have been made for other sites (Vesterby 1991, Rockoff 1969).

With current technology it seems that an ultrasound-based device is the more likely to give some reflection of skeletal architecture. In whichever mode ultrasound is used, the trabecular thickness and connectivity and the cortical bone envelope are integral determinants of the result. High-resolution QCT may be able to provide architectural information down to a resolution of millimetres. However, unless volumetric and finite element modelling analysis is used QCT suffers from variation from one axial section to the next and comparison between individuals is complicated by the decision of which axial section to analyse (Flynn 1993). One very recent cadaveric study with QCT demonstrated a 10% variation with only a 4mm displacement of the specimen (Nagele 2004). Another recent cadaveric study of QCT and DXA has examined failure load of the radius. DXA correlated very well with failure load, and combinations with QCT only improved on this when QCT cortical thickness was used (Hudelmaier 2004). A contemporary review has suggested that continued improvements in volumetric QCT and high resolution Magnetic Resonance Imaging are making non-invasive imaging of trabecular bone more feasible (Link 2002).

Os calcis ultrasound, whilst useful, has not shown itself to be any more effective than photon absorptiometric techniques in predicting fracture. It does have an advantage in terms of cost, portability, and reduction of ionising radiation.

As mentioned previously, some recent evidence supports the concept that although osteoporotic fractures tend to occur in areas of predominantly trabecular bone, the cortical envelope has a major role to play in resisting fracture. A 1977 study (Saha 1977) showed that the % area of cortical bone filled with secondary osteons had an inverse linear relationship to the ultimate strength of human cortical bone specimens. Further evidence of the importance of cortical bone relates to measurement of the ratio of cortical bone to total bone diameter in the metacarpals (Garn 1976). There are marked changes in cortical thickness through life, which may be detected as early as the sixth decade.

If we are to improve upon current methods for targeting those at risk of osteoporotic fracture, it would seem that there are several routes which deserve further exploration. It is appropriate to examine the use of ultrasound, as this seems the most likely technique to provide useful architectural information. The role of cortical bone in resistance to fracture should also be studied. Finally, the combination of ultrasound and absorptiometry at cortical and trabecular sites may be able to perform better than either would alone. The Soundscan 2000 (Myriad, Rehovot, Israel) is a device which measures ultrasound transmission velocities through cortical bone. It is the aim of this thesis to assess its performance, both alone and in combination with other techniques.

Chapter 2

The Assessment of Osteoporosis

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The Assessment of Osteoporosis

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Theory

If we are to believe that ultrasound has the ability to assess the strength of the skeleton, then we must have some support from physics, and some understanding of the relevant biology.

Sound is the transmission of energy through a medium as a pressure wave. Ultrasound denotes that the frequency of the wave is above the threshold for human hearing. A wave has the properties of wavelength, frequency and velocity. For a given velocity, the wavelength and frequency are inversely related.

The velocity is a vectorial measurement of distance/time. In biology, the pathway of the sound wave is often indirect, and measurement of the time travelled starts prior to the signal entering bone and ends at a point after the wave front has left the bone. For these reasons, the preferred terminology might better be *apparent* velocity or speed of sound (speed is a scalar measure), and the inherent inaccuracy should be recognised. For simplicity, this work uses the word velocity whilst recognising its limitations.

In physics, the velocity depends upon the density and elasticity of the medium, and these in turn are affected by temperature.

The equation $e=\rho c^2$

expresses this relationship, where e is the modulus of elasticity, ρ is density, and c is the velocity of the sound wave. This holds true for a solid in which the cross sectional dimensions are smaller than the wavelength. In cortical bone, the velocity (c) is between 3000 and $4000ms^{-1}$ and at a frequency (f) of 250KHz this would give a wavelength (λ) according to the equation $v = f\lambda$, of 0.012-0.016m or 1.2-1.6cm. If a whole bone is to be considered as one solid and is measured, then this is not acceptable. If however the cortical envelope or the individual trabeculae are the subject, then these are of significantly smaller dimension than the wavelength.

As $c^2 = e/\rho$

it can be seen that as modulus increases (i.e. the medium becomes more rigid) so does the velocity. Apparently, as density increases, so velocity decreases. This is a difficult starting point when trying to understand what speed of sound is actually measuring. It is necessary to understand that there exists a very strong relationship between elastic modulus and density. In bone, this has been directly measured (Lee 1997) and a correlation of $r^2 = 0.89$ was established for cortical bone from the human tibia.

Measurements of the modulus and the density from multiple samples of human cadaveric bone from spine and femur (Keller 1994) showed a wide range of values for both. Modulus varied in the range 0.1- 10 GigaPascal (Gpa) and density in the range 0.2 - 2.0 gcm^{-3} in femoral cortical bone. Overall, variation was from 0.001 - 10 GPa for modulus and 0.05 - 2.0 gcm^{-3} for density, when spinal trabecular and femoral cortical bone were included. The correlation between %mineral (ash weight/dry weight) and modulus in the femoral cortical bone was $r^2 = 0.849$. Between density (ash weight/volume) and modulus, again, $r^2 = 0.849$. A quadratic relationship was used for this correlation, but for a linear equation correlation was still very close; $r^2 = 0.707$.

This indicates the subtlety of the relationship between ultrasound velocity and the mechanical properties of bone. It is unlikely that an unmatched change would occur in density, as that very change would influence the modulus. Claims that ultrasound velocity reflects the density of bone directly seem to be unlikely to prove true, as e/ρ would seem to be very close to a constant. Even if we accept that the $e:\rho$ relationship is non-linear, and that at higher densities the modulus starts to predominate (leading to higher velocity), the velocity will be an unreliable measure; being a square root of the ratio of an exponential function. The role of the equation [$e=\rho c^2$] is almost certainly overstated. What it probably does is to predict the general velocity of sound in bone, whilst several other more important factors dictate the actual velocity measured. It is these factors which we will now consider.

Cortical Thickness

In a material of infinite size, a sound wave will travel without reflection and without shear waves. As the thickness of the medium decreases towards the wavelength of the sound wave, shear waves appear. These travel at lower velocity than longitudinal waves. If these are measured inadvertently then a falsely low estimate of velocity will be made. One of the main features of cortical bone in the ageing population is the change in cortical thickness due to endosteal resorption and (in men) periosteal modelling of new bone. (Martin 1977, Ruff 1988) It may be a feature of bone ageing which is actually worth measuring rather than an artefact to be ignored.

Cortical Mineralisation

Experiments where bone specimens have been deliberately demineralised (Tavakoli 1991) have shown large reductions in ultrasound transmission velocity of the order of 10 - 30%. Whilst it could be argued that this is not a feature of osteoporosis, there are situations where it may co-exist. Remodelling processes in the elderly are characterised by delayed mineralisation of new osteoid (Villanueva 1983). There is a rise in parathyroid hormone (PTH) with age (Benhamou1991). This is said to be secondary to poor calcium absorption and suboptimal vitamin D levels, particularly in northern countries such as the UK. In these circumstances, some mineralisation deficit may be expected. Histomorphometric evidence (Arnala 1997, Rapin 1982) from femoral bone samples retrieved at hip fracture surgery found just such a mineralisation defect in between 2 and 25%. In addition, if a situation of frank osteomalacia were to exist, it would be just as important to identify and treat.

Cortical Porosity

It has been noted in histological studies (Albright 1979) that there is an age dependent increase in secondary osteons. It has also been shown that the mechanical strength of cortical bone samples is inversely related to the %area of the cortex occupied by secondary osteons (Saha 1977). This study demonstrated a 50% decrease in ultimate stress as "porosity" rose from 10% to 70%.

A comparison was made (Katz 1984) of ultrasound transmission in primary osteonal and secondary osteonal bone and concluded that a difference does exist, and that primary osteonal bone has a higher modulus. In an in-vitro study of the equine 3rd metacarpal (McCarthy 1990), porosity-measured as the %area of osteon canals- was well correlated, inversely, to ultrasound velocity $r = 0.773$. However, physical measures of wet bone density were also closely inversely related to porosity, $r = 0.857$. A strong correlation also existed between ultrasound velocity and density; although this was graphed, no Pearson correlation "r " was documented. In a further refinement, the ultrasound velocity and bone densities were compared in a young and an elderly group with matched %porosity. Although porosity was matched, both ultrasound velocity and density were lower in the younger group. Unfortunately, the converse experiment - where age and density matched groups are compared for porosity and ultrasound velocity - was not performed.

It is not clear from these studies whether porosity has an effect on ultrasound transmission velocity. This is because, like so many elements of bone quality, variation in porosity occurs concomitant with other changes such as those in modulus, density, mineralisation and cortical thickness. A model of porosity based on a cross section of population for samples would find it difficult to control for these factors.

In addition, a manufactured model of porosity due to longitudinal tunnels in an otherwise homogeneous material does not exist for cortical bone - although models based on grain size do exist for trabecular bone (Hodgkinson 1996). Despite this, it is persuasive to believe that an increase in %area of secondary osteons will reduce the total surface area available for sound transmission. In much the same way as reducing cortical thickness, this would produce shear waves, and a lower ultrasound velocity.

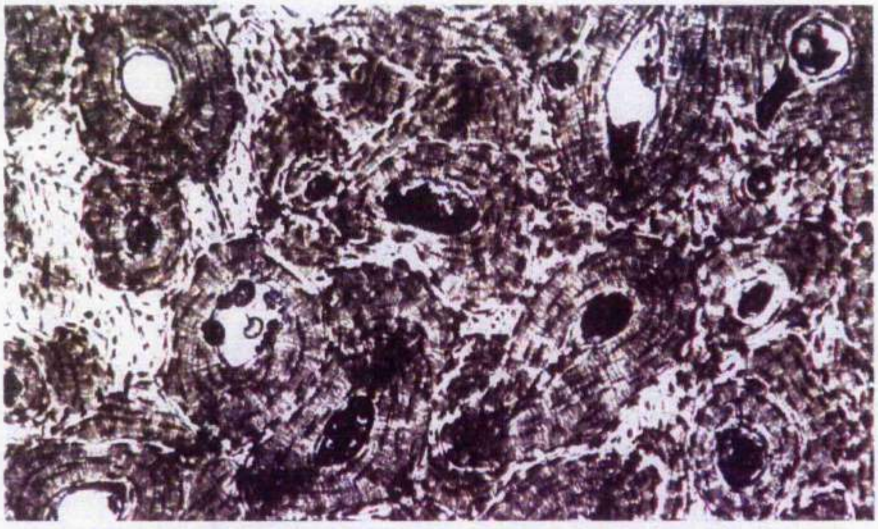

Figure 2.1 Cross sectional histology of cortical bone illustrating the variation in size and orientation of osteons

Haversian Discontinuity

At birth, cortical bone is made almost exclusively of primary osteons. These are narrow canals centred on vascular supply surrounded by a few layers of lamellar bone. Throughout life, remodelling occurs, replacing primary osteons with secondary osteons (Haversian systems). The secondary osteons remain centred on the vascular supply, but are wider and are surrounded by more layers of lamellar bone. The BMU (variously described as Basic Multicellular Unit [Frost 1973], Bone Modelling Unit and Bone Metabolic Unit) is the mechanism which is responsible. The control system is incompletely understood, but does seem to be triggered by local strain engendered by loading, and repair of fatigue damage. A cylinder of existing bone is removed lengthways by a group of osteoclasts - the cutting cone. The space is then filled in, usually incompletely, by an advancing group of osteoblasts. The osteons vary in length up to approximately 10mm. As more secondary osteons occupy the bone, the continuity of the longitudinal primary osteon system is disrupted. This presents a sound wave with a less direct pathway, and potentially a greater number of interfaces to cross; thus increasing transit time.

This is again difficult to prove, because osteon mapping is a time consuming activity, and insufficient data exist on actual osteon pathways, and much of this in dogs. (Cohen 1958, Enneking 1972).

It has certainly been shown that samples from the same bone with more primary osteonal structure than secondary osteonal structure have significantly higher tensile strength. (Vincentelli 1985).

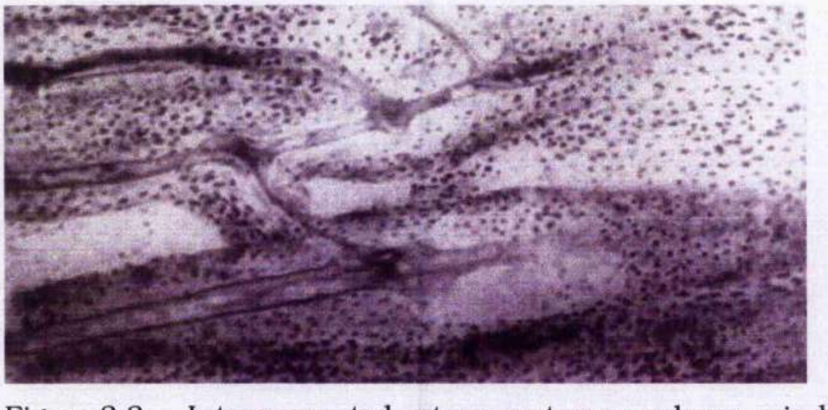

Figure 2.2 Interconnected osteon systems produce an indirect pathway for ultrasound

Fatigue Damage

It is known that fatigue damage may occur in any structure subject to repetitive loading. It has been shown that bone contains identifiable fatigue damage in the form of "microcracks" (Schaffler 1990,Burr 1998). It has also been shown that fatigue damage is associated with a reduction in elastic modulus and strength on mechanical testing. (Carter 1977, Carter 1985). Whilst there is no direct evidence that this reduces ultrasound transmission velocity in bone, the technique of ultrasound transmission has been used in industrial non-destructive testing, particularly to identify fatigue damage in engineering. The first such device was the "Supersonic Reflectoscope" patented in 1941 by Floyd A. Firestone of the University of Michigan, USA. There is every theoretical reason to believe that microfractures in bone will make the pathway for sound less direct and create extra interfaces for it to cross, therefore reducing velocity.

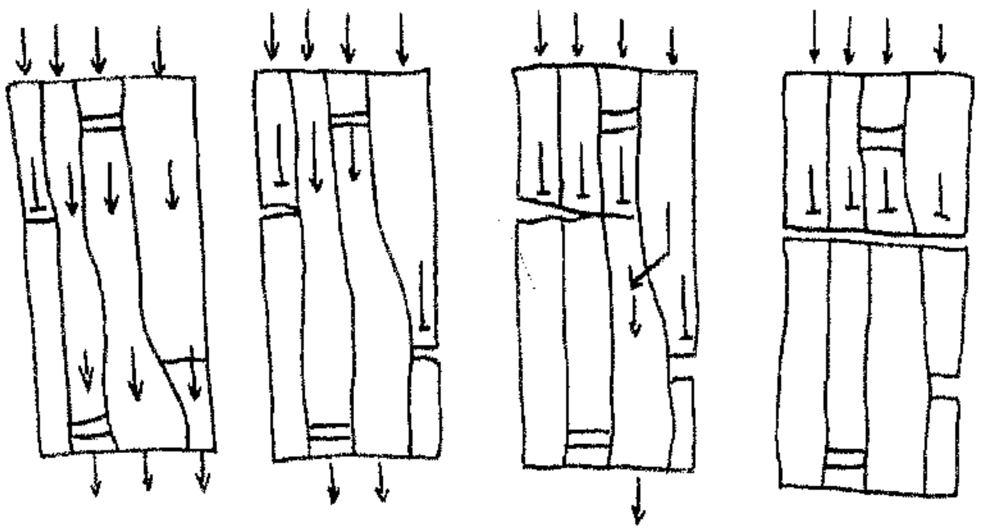

Figure 2.3 Demonstrating how the pathway and signal attenuation might be affected by propagating fatigue fracture

The figure gives a diagrammatic representation of interconnected osteons. It shows from left to right how a propagating crack might force a sound wave to take a less direct pathway, and eventually interrupt conduction altogether.

Haversian Orientation

It has been shown that ultrasound velocity is markedly affected by the direction of sound transmission in relation to the orientation of Haversian systems (Kann 1993). These investigators found a reduction in velocity of around 25% when transmission was perpendicular to rather than parallel to the longitudinal axis of the same bone samples. It seems unlikely that Haversian orientation will change in an individual during their lifetime, and this phenomenon has never been reported. It is possible, however, that differences exist between individuals and could introduce quite large errors. In addition, it must be kept in mind that the operator of an ultrasound-based device can introduce significant errors if longitudinal orientation is imprecise.

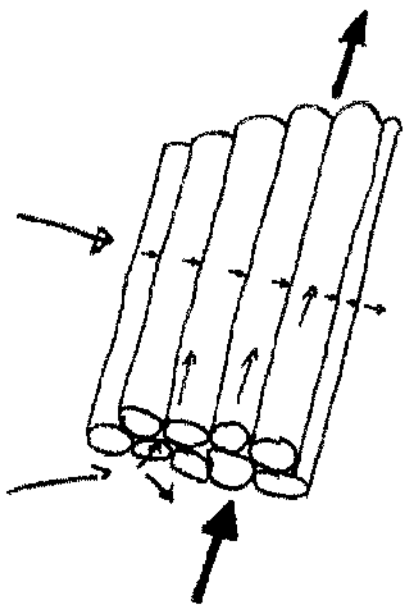

Figure 2.4 Demonstrating the proposed effect that the direction of an ultrasound wave might have on the number of interfaces that it would cross.

In conclusion, there do exist several theoretical reasons for a variety of properties of bone to influence the velocity of ultrasound transmission. Whereas those techniques measuring bone properties with photon absorptiometric techniques may say that they are measuring mineral content, it is not possible to say this about transmission ultrasound. Certainly, material density and modulus play a role, but it is vital to remember that theoretical physics deals with perfect, homogeneous solids, and that bone is not one of these - containing several levels of organisation and structure, from collagen fibre orientation, through Haversian systems up to cortical thickness and shape. Because of this, it is difficult to find a measure of "accuracy" against which to compare ultrasound in the same way, for instance, as ash weight/volume will give a gold standard mineral density against which to measure the accuracy of photon absorptiometry. There is no doubt that ultrasound transmission velocity is governed by several inter-related factors which have a bearing on bone strength, but it may be that the synthesis of these factors reflects "bone age" and may only be validated against prospective studies of fracture risk.

CHAPTER 3

Transmission Ultrasound Velocity

A Review of the Literature

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Transmission Ultrasound Velocity

A Review of the Literature

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Introduction

There is a large literature on ultrasound and therefore references have been selected in order to enlighten the thought process behind the methodology. The section has been subdivided with this in mind.

Historical work is presented first. Early attempts to make use of ultrasound transmission velocity were made for an enormous variety of tissues from many species, and with differing methods of preparation. This demonstrated feasibility, and gave a general range for velocities in mammalian calcified tissue.

Early human work demonstrated that measurement was possible in-vivo, and that a general range of velocity also existed in humans. Studies were not able to show discrimination between broadly different groups such as males and females. However, the specific disease state of flourosis was associated with values different from a normal group. Variation was demonstrated according to site of measurement.

The pathway taken by the fastest ultrasound wave has been the focus of some investigators. It is clearly important when explaining some of the variations observed.

Another source of explanations of variance is the **physical properties** of the calcified tissue. Density, anisotropy, porosity and elastic modulus have been examined. Some authors report strong correlations, but others do not. There are always some limitations of methodology. It is clear that bone is anisotropic, and therefore, the direction of measurement will have consequences for measurements of both ultrasound velocity and modulus of elasticity.

Modern studies have used commercial devices, or a standardised experimental device to look more closely at the range of measurements in a population and how it relates to disease and fracture risk. Correlations between different methods of densitometry have been made in some populations. Most recently, attempts have been made to estimate fracture risk prospectively using ultrasound velocity.

Historical

The earliest reports on the use of ultrasound on skeletal tissue were observations made in the late 1940's and early 1950's (Goldman 1956), some in German language publications. In 1949, Theismann (quoted in Goldman) used a 0.8 MHz signal on fresh human skull at body temperature. A velocity of 3360ms^{-1} was recorded. No estimate of precision was made. Heuter (quoted in Goldman) measured attenuation of ultrasound in skull bone using frequencies in the range 0.6 - 3.5 MHz. The bone was of mixed origin. It was all human, but some was fixed and some was fresh. The data from both these studies were subsequently published in tabular form in Goldman's 1956 report.

A second tabulated report (Goss 1978) reviewed 144 publications of physical reference data reporting transmission ultrasound velocity in biological tissue. 84 were reporting on skeletal tissue, 30 mammalian and 54 human. 665 reference values for various tissues and species were given in all. 4 values for human tibia and 2 for mammalian tibia were given.

Early human studies

Millner (1975) reported on infant, adolescent, and adult male and female tibia SOS using a 1MHz probe in-vivo. Velocities ranged from 1875 ms^{-1} in female infants to 2200 ms^{-1} in female adolescent, up to 2750 ms^{-1} in males aged 70 - 95 years. No age-related decline in ultrasound velocity was found. Millner also studied male and female subjects with osteoporosis or fluorosis. Again, a 1MHz probe was used on the tibia in-vivo. The age range was 11 - 65 years. Recorded velocities ranged from 2000 ms^{-1} in children up to 2900 ms^{-1} in adults. Again, no age-related decline was found. In the 41 - 65 year old normal subjects a velocity range of 2200 - 2700 ms^{-1} was found for males and 2150 - 2750 ms^{-1} for females. In the osteoporotic group these values were 2150 - 2900 ms^{-1} and 2200 - 2900 ms^{-1} respectively. There was no clear relationship demonstrated between ultrasound velocity and ageing or osteoporosis. In fluorosis, 41 - 65 year olds were assessed. Ultrasound velocities were measured with a range of 2300 -3300ms^{-1} . Therefore, a higher ultrasound velocity was demonstrated with fluorosis.

Dubrov (1972) in a Russian language publication, made in-vivo measurements of human tibia ultrasound velocity using a 0.1MHz probe. Readings were taken from the tibia at 5cm intervals from the ankle joint towards the knee joint. Velocities rose from 3308 ms^{-1} 5cm from the ankle joint to 3625 ms^{-1} at the mid-tibia before falling to 3320 ms^{-1} at the proximal tibia metaphysis. This may indicate a relationship between ultrasound velocity and the thickness of the cortex of the bone, assuming that the main difference found at these sites is the greater thickness of cortical bone at the midshaft.

Pathway of Ultrasound

Anast (1958) published data for the in-vivo measurement of ultrasound velocity in the tibia of 20 subjects. Unusually, a relatively low frequency 20KHz transducer was used. Despite this, the average value was 3481 ms^{-1}, and the range 3260 - 3572 ms^{-1}. The method used both a handheld transmitting and a handheld receiving transducer, with longitudinal measurement of the tibia. In addition, 16 rabbit tibial fractures were measured daily and 26 human tibial fractures were measured at a variety of times during the course of healing (although a full data set up to union was not made for the humans). The rabbit tibias all returned to ultrasound velocities comparable with the normal side at the time of union. The 3 rabbit fractures which did not achieve union did not return to normal values. Because the human data was incomplete, it is difficult to comment on. There was, however, a reduced ultrasound velocity in the presence of fracture, and a normal velocity after union and rehabilitation of the fracture. Anast also assessed 11 paraplegics as a model of disuse osteoporosis. The results observed were not significantly different from the normal subjects. An estimated error of 5% was present throughout this study, which may have been higher because of the variability of positioning handheld probes.

Abendschein (1970) examined 51 cadaveric specimens taken from 4 individuals. 2 were considered normal and were 16 and 30 years old. One had peripheral vascular disease and was aged 58 years. The fourth had disuse osteoporosis and was aged 55 years. All samples were refrigerated in Ringer's solution. A 0.1MHz transducer was used. Mean physical density was measured as 1.97 gcm^{-3}, 1.85 gcm^{-3} and 1.66 gcm^{-3} in the normal, ischaemic and osteoporotic groups respectively. Ultrasound velocity was measured (as an average of 10 or more specimens from each individual) as 3526 ms^{-1}, 3147 ms^{-1}, and 2868 ms^{-1}. Thus a difference between normal and osteoporotic bone was demonstrated. There was no control for age and only four individuals were studied.

In a study spanning two species and two measurement sites, Ambardar (1976) used a 2MHz transducer to assess the femur and tibia of sheep and cattle in-vitro. Measurements were made both in the long axis and perpendicular to the long axis.

This showed a reduction in ultrasound velocity when the direction of transmission was transverse rather than longitudinal. This observation is preserved across species.

Adler and Cook (1975) made a similar observation in canine tibia. They performed an in-vitro study of thawed whole tibia. A transducer frequency is not recorded for this experiment, but the same paper reports using both 3 and 5MHz transducers in other experiments. Measurements were made in the long axis of the bone, and at 90° to the long axis. Velocities of 3325 ms^{-1} and 1966 ms^{-1} were recorded respectively.

	In Long Axis	90 deg to Long Axis
Bovine	3335 ms^{-1}	2824 ms^{-1}
Canine	3325 ms^{-1}	1966 ms^{-1}

Table 3.1 The effect of direction of ultrasound measurement in mammalian tissue

A number of studies have attempted to describe the pathway of ultrasound waves travelling transversely through a tubular bone. Jeffcott and McCartney (1985) used a 2.25 MHz handheld transducer pair to investigate in-vitro equine metacarpals. For separate specimens of medullary and cortical bone, ultrasound velocities were measured. The expected velocity for a "sandwich" of the two bone types was calculated. The actual velocity for a metacarpal measured transversely was substantially higher, but a second peak in the received sound pulse did match the expected velocity. It was suggested that the longer but higher velocity route around the circumference of the cortex would produce the first observed peak. Langton (1991) used a 1MHz calliper mounted transducer couple with in-vitro equine metacarpal and with a Perspex cylinder model. In this study, the opposite cortical faces of the bone and their equivalent in the Perspex were machined flat. This allowed direct comparison of the velocity direct through cortical bone and direct through the widest diameter of the bone. Thus a "sandwich of cortex-medulla-cortex" was compared to an equivalent thickness of cortex only. Ultrasound velocity was higher through cortex only. This was also found in the Perspex model. A pulse echo time between transducer and bone indicated that there was a significant water layer present, even though the experiment had tried to minimise this. It was concluded that a 10% error should be allowed for soft tissue in-vivo, but that the direct cortical pathway was responsible for the fastest transmission times. Kann (1995) used a 2MHz calliper mounted transducer pair with in-vitro porcine phalanges. They first measured the ultrasound velocity for a transverse direction through 17 intact phalanges. The trabecular bone was mechanically removed, and the specimens re-measured. The velocity before (1919 ms^{-1}) and after (1938 ms^{-1}) were not significantly different. They concluded that the circumference of the cortex was the fastest route.

Cadossi and Cane (1996) used a commercial device, the DBM Sonic 1200 (Igea, Carpi, Italy), which consists of a 1.25MHz calliper mounted transducer pair to assess in-vitro porcine phalanges. They found that mechanical removal of the medulla decreased velocity significantly, and that reconstitution of the defect with styrene polymer restored this. They also found that reducing the cortical thickness of the external surface of the bone increased apparent transmission velocity. The authors concluded that the main pathway is directly through the widest diameter of the bone. This is at variance with previous findings.

Relationship of bulk physical properties to transmission ultrasound velocity

A number of studies have compared ultrasound transmission velocity with mechanical measurements such as ultimate strength and modulus.

Bonfield (1982) used a 200KHz transducer pair with fresh in-vitro bovine cortical bone. All 10 specimens were bars machined from a single bovine femur. A parallel pair of transducer-receiver devices was used, in order that all tests could be controlled using a 10mm long control specimen. A physical density was calculated using a specific gravity technique with dehydrated specimens. The spread of densities was 1.897 - 2.107 $\times 10^3$ kgm^{-3}. Velocity ranged from 3210 - 3440 ms^{-1}. No correlation was drawn between these but the data are presented as a table and a correlation can be calculated from this as $r = -0.15$. If the one outlying specimen in density and velocity is excluded, this rises dramatically to $r = 0.88$. The authors do not discuss this. They did show that ultrasound velocity was inversely related to temperature when measured in the range 17 - 41°C. Ashman (1984) used a 2.25MHz transducer pair to measure 100 Human and 100 canine in-vitro specimens. These were machined polyhedrons modified to have either 18 or 8 faces respectively. A continuous wave rather than a pulsed wave was used, and the physical density was measured with a water immersion technique. Velocity was measured in all planes as a bulk wave. The elastic modulus was derived from the ultrasound velocity for the 9 independent elastic constants theoretically predicted for an orthotropic material. Although the authors found differences in all 9 derived constants, their conclusions are somewhat weakened by the fact that measured and derived modulus only correlated to $r = 0.531$ in their study of bovine cortical bone - upon which they rely for the validation of their use of ultrasound velocity to derive modulus. Despite this, it seems likely that adult human femoral cortex behaves as an orthotropic material. McCarthy (1990) used a pair of 2.25MHz transducers fixed 113mm apart in a water bath. Equine 3rd metacarpal was studied in-vitro. 40 cubes of cortical bone were prepared and had ultrasound velocity measured in 3 planes. Density was measured using a specific gravity method, and porosity was measured by histological section. A range of temperatures from 4 - 40°C was used. The correlation of velocity with

temperature was r = 0.99 ($-0.17\%^{o}C^{-1}$), although the sample number was low (n = 4). Porosity was found to correlate well with both density and velocity (r = 0.773 and r = 0.857 respectively). Velocity and density correlated well, but was direction sensitive (r = 0.75 longitudinal, r = 0.9 radial). Antich and Anderson (1991) compared the results of velocity due to transmission and velocity due to reflection on in-vitro human cortical bone cubes. They used 2.25MHz signal for transmission (calliper mounted pair) and 3.5MHz signal for reflection (water bath mounted on a rotating arm to adjust angle of transducers). 10 cortical cubes were prepared, but it is not clear whether these were all from one donor. A number of homogeneous plastic and metal cubes were also examined. The correlation between reflection and transmission velocity was r = 0.99 for homogeneous materials but was less good and not stated for the bone samples. The authors did convincingly show that bone anisotropy exists for both methods, and that velocities are highest for the longitudinal axis.
Turner (1991) examined cubes of bovine cancellous bone in-vitro using a 2.25MHz transducer pair. Velocity was compared with strength in mechanical testing. The yield strength was used as cancellous bone fails in a stepwise fashion with a prolonged plastic deformation phase. A correlation of r = 0.75 was observed. Rho and Ashman (1993) attempted to determine whether the behaviour of trabecular and cortical bone was comparable by testing individual trabeculae against prepared samples of cortical bone 0.3x0.3x2.0mm in size. Similarly sized steel and aluminium samples were also prepared. A 2.25MHz transducer pair was used in a complex testing jig. Twenty trabecular and 20 cortical samples were prepared from a single donor tibia. The mechanical test jig was also complex, and a tensile stress-strain curve was produced. The values of mechanically tested modulus and ultrasound velocity predicted modulus matched very well for the metal specimens (when density for the metals was taken from scientific tables). For the bone specimens, neither cortical nor trabecular predictions were well matched with measured modulus. This may have been a consequence of using average density values for bone which was sourced from a different individual. For both methods of measurement, however, trabecular modulus was significantly less than cortical bone modulus. This is attributed to the aligned nature of cortical architecture

and the more random pattern in trabecular bone. Njeh (1996) studied in-vitro cubes of bovine cancellous bone using a 1MHz calliper mounted transducer pair in a water bath. Velocity measurements were made along 3 axes, as was compressive modulus using an Instron testing machine. Density was calculated as the de-fatted, dried weight/measured volume of the cube. Modulus was around 30% greater testing longitudinally than antero-posteriorly. Velocity was around 10% greater for the same direction of testing. A correlation of r = 0.83 was observed for velocity and modulus overall, although velocity and density overall were less well correlated at r = 0.50. This may be because the density calculated was the gross density of the cube (i.e. bone plus air) rather than the actual density of the material involved (i.e. bone volume itself).

Modern studies

A sizeable literature exists regarding ultrasound transmission in cancellous bone, particularly the os calcis. I shall only examine a small number of these publications in order to give a general picture, as ultrasound transmission in cortical bone of the tibia and not the (mainly) trabecular bone of the os calcis is the subject of this thesis.

In a remarkably far sighted study, Porter (1990) measured the os calcis BUA of 1404 female, elderly nursing home residents. The mental state was also examined, as was mobility. On the basis of these data, a low-risk and a high-risk group were designated. In the 2-year prospective follow-up, there were 73 hip fractures. In comparison with the low risk group, the high risk group had a relative risk for fracture of 8.4.

Stewart (1996) in a prospective study showed that os calcis BUA discriminated for fracture with an odds ratios from 1.4 to 2.1. Bauer (1997) in a large prospective study showed that os calcis BUA predicts fractures strongly and independently of densitometry in older women. Each SD reduction in calcaneal BUA was associated with a doubling of the risk for hip fractures (relative risk [RR], 2.0; 95% confidence interval [CI], 1.5-2.7). A prospective study by Huang (1998) showed that os calcis BUA discriminated for fracture with an age-adjusted odds ratios of 1.50, 1.89, and 1.72 for vertebral fractures, non-spine fractures, and any fractures, respectively.

A number of studies have correlated ultrasound transmission velocity in a population with absorptiometric techniques. McCarthy (1992) measured the equine 3rd metacarpal in-vivo in 2 subject horses with a hand-held transducer couple (frequency not specified, although their other work uses a 2.25MHz system), and a Single energy Photon Absorptiometer (not specified). Short and long-term reproducibility estimates were made, as was inter-observer agreement. Reproducibility was good at <1% Coefficient of Variation and <3% CV respectively. The ultrasound result was found to be operator sensitive. Greenfield (1981) examined the proximal radius in-vivo in 72 normal volunteers, 12 patients with renal osteodystrophy and 66 other subjects, 18 of whom had radiographic evidence of existing low energy vertebral fracture. He used a 5MHz transducer in pulse-echo mode, a Norland-Cameron SPA, and a plain radiograph with a reference scale. No correlation was calculated, but it was stated that ultrasound and SPA disagreed in 9% of normal subjects. The ability of ultrasound to identify individuals with existing vertebral fracture was encouraging although not as sensitive nor as specific as SPA. Cunningham (1996) compared ultrasound velocity at 1 of 2 sites (tibia {89 subjects}, and os calcis {100 subjects},) with DXA at 4 sites (tibia {20 subjects only}, os calcis {20 subjects only}, femoral neck and lumbar spine {all subjects}). Correlations were calculated for these measures. For tibia ultrasound and tibial BMD $r = 0.71$. For os calcis ultrasound and os calcis BMD $r = 0.729$. For tibia ultrasound and BMD of the spine and femoral neck respectively $r = 0.299$ and 0.072. These correlations seem weak, but it should be realised that all measurements are surrogates for fracture risk and not for each other. Kann (1994) used a continuous wave technique in-vivo in the ulna of 21 young and 21 middle aged females. SPA measures were made of the same site using a commercial device, the ND 1100 (Nuclear Data [origin not specified]). An interesting feature of the study was that young and old individuals were paired on the BMD result and their ultrasound resonant frequency compared. This was significantly lower by around 20% in the older group. It was concluded that this technique was indeed measuring something which was related to ageing, other than density. Mazess (1989) assessed 40 normal and 36 osteoporotic (spine BMD<$1gcm^{-2}$) adult females. 68 had distal radius SPA measured using a commercial device

(SP2 Lunar Radiation, Madison, Wisconsin, USA) and all had os calcis ultrasound using a 0.4MHz fixed distance transducer pair in a water bath. Some also had DPA scans of femoral neck and lumbar spine. The correlations for ultrasound velocity with BMD were modest, being r = 0.66, r = 0.63 and r = 0.52 with radius, lumbar spine and femoral neck respectively. Wapniartz (1993) compared lumbar spine DXA BMD (QDR 1000, Hologic, Waltham, MA) with ultrasound velocity in the patella (Signet, Osteo-Technology, Framingham, MA) in 282 healthy volunteers. Correlation between UV and BMD was modest (r = 0.36). UV was higher in males than females by around 1% ($p < 0.05$), and showed an age related decline in men. Some doubt must be cast by the observation that there was no age-related decline in women. It may be that the volunteers were too healthy, or had an insufficient range of age.

Only a few studies have assessed fracture risk with ultrasound transmission velocity. Cross sectional fracture studies will be discussed first.

Heaney (1989) studied 293 females to compare lumbar spine BMC (by DPA), ultrasound velocity of the patella, and prevalent vertebral deformity on lateral spine radiographs. 2 cross-calibrated DPA devices were used (in different study centres Lunar DP4 (Lunar Radiation, Madison, Wisconsin, USA) and Nuclear data 2100 (origin not specified)) as was a single prototype mixed frequency calliper mounted transducer pair. The correlation of BMC with ultrasound velocity was poor, r = 0.05. However, both measurements discriminated equally well between fracture and non-fracture population on Receiver Operated Characteristic analysis (area under curve = 0.722 for ultrasound velocity and = 0.719 for DPA). The authors conclude that ultrasound velocity may be measuring something other than bone density which is nonetheless a useful discriminant. Heaney (1995) examined 371 subjects to assess ultrasound velocity of tibia and patella, SPA at the forearm and vertebral radiographs in those aged >40 years. Correlations between tibial and patellar ultrasound velocity were r = 0.46 and r = 0.27 in females and males respectively. Correlations between tibial ultrasound and forearm SPA BMD were r = 0.54 and r = 0.43 in females and males respectively. Using the patient's recollection of previous fractures, an odds ratio of 2 was calculated for fracture risk for a 1SD reduction of tibial ultrasound velocity. No such risk was identified when only vertebral fractures were considered. Fujii (1994) assessed the velocity of ultrasound through the patella in 160 Japanese women, and spine DXA in a subgroup of 39. It was noted that mean velocity was not different to previously reported Caucasian populations. It was also noted that ultrasound velocity was sensitive to age, and vertebral fracture - being significantly lower in older subjects and in those few with previous fracture. Stegman (1995) reported on the relationship of patella ultrasound velocity and forearm SPA to prevalence of fracture in the medical history of 1311 subjects over the age of 50 years. As part of a large, ongoing study, these subjects had had initial measurements of BMD at the distal radius and ultrasound velocity through the patella. A logistic regression analysis technique was used to derive for each measure an odds ratios per SD for a history of fracture. Neither technique was clearly better at differentiating the fracture from non-fracture population, although both were better than chance. Stegman and Heaney (1996) again reported on a cohort from their ongoing study (The Saunders County Bone Quality Study). Using essentially the same measurements in 1401 volunteers, they used a logistic regression model with vertebral deformity on the lateral spine radiograph as the categorical for calculating odds ratios. They found that both ultrasound velocity and forearm SPA had discriminant value in identifying fracture patients from the rest of the population, but that neither had as strong an effect as age.

Prospective studies

Prospective studies relating ultrasound velocity to fracture risk are few in number. Heaney and Avioli (1995), again using the same study population, identified 130 females with no vertebral deformity on their initial lateral lumbar spine radiographs. These had all had measurement of the ultrasound velocity through the patella on entry. After a two year follow up, 19 clinical fractures had occurred, of which 14 had concurrent radiographic changes. The odds ratio for an incident fracture per SD below the mean ultrasound value was 2.38 (95% CI 1.33 - 4.26).

Mele (1997) assessed the ultrasound velocity in the phalanges of the hands of 211 adult females using a commercial device (DBM Sonic 1200, IGEA, Italy). During a 3-year follow up 22 peripheral fractures were documented. The odds ratio for an incident fracture per sd below the mean was 1.5 (95% confidence intervals 1.1 - 1.7).

Hans (1996) assessed 5662 elderly females as part of the EPIDOS study. Os calcis ultrasound and femoral neck BMD were measured at entry to the study. At a 2-year follow-up, 115 hip fractures had occurred. Using the initial measurements, relative risk for hip fracture per sd below the mean value was calculated. The risk associated with ultrasound velocity was 1.7 (95%CI 1.4-2.1), with BUA was 2.0 (95% CI 1.6-2.4) and with BMD 1.9 (95%CI 1.6-2.4).

At the time when this thesis was initiated, these were the only three studies (Heaney 1995, Mele 1997, Hans 1996) which support the ability of *ultrasound velocity* to predict future fracture risk. None of these has examined the role of ultrasound transmission through the tibia, which, in distinction to other sites is both weight-bearing and a predominantly cortical bone.

CHAPTER 4

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Limitations of BUA

Whilst os calcis BUA has been shown to be of predictive value in assessing fracture risk, the precision error (the variation between consecutive measurements) has been reported as high as 10% (Rossman 1989). This introduces limitations in the utility of BUA in assessing individuals, rather than populations. With such a wide measurement error, it is impossible to be confident about an individual patient's risk of fracture, or to assess the effects of treatment. This is because natural drifts in skeletal measurements and changes due to treatment are normally of the order of a few percent per year and would be obscured by measurement error of 10%.

Ultrasound velocity precision error has not been widely reported in the literature. Where it has, there has been wide variation in error, probably due to the variety of devices, and the operator dependency - particularly with the un-mounted transducer systems. Rubin (1987) reported a 1.1% precision error for a calliper mounted 1MHz transducer pair used transversely on the tibia. Heaney and Avioli (1989) reported precision errors of 2.3% for a calliper mounted broadband transducer pair used transversely on the patella. Greenfield (1980), using a 5MHz transducer with a pulse echo technique on the femur reported a 4.3% error and (Andre 1981), using a 5MHz transducer with a pulse echo technique on the radius reported a 6% error. Siegel (1958) reported a 5% error using a handheld transducer pair of unspecified frequency longitudinally on the rabbit and human tibia. At least one early study does not report error at all (Gerlanc 1975). It can be seen despite this that the precision error for a variety of ultrasound transmission velocities is lower than for BUA, and could be low enough for use with individuals. A device with better reproducibility may be more clinically useful.

A second set of theoretical considerations concerns the site of measurement. BUA has almost exclusively been measured at the os calcis. This is a site of predominantly trabecular bone, which is weight bearing, and easily accessible. BMD has also been measured (using DXA, SXA, DPA and SPA) at sites of predominantly trabecular bone; the femoral neck, the lumbar spine and the

distal radius. These common sites of fracture have only a thin envelope of cortex. The obvious conclusion has been drawn that these sites fracture because they have mainly trabecular bone, and trabecular bone is weak. It may be that the converse is the case; that they are common sites of fracture because they *do not have much cortical bone, and cortical bone is strong*. This is supported by a consideration of the mechanics, and a few biomechanical studies (Spadaro 1994, Moseklide 1993, Lotz 1995).
Cortical bone has a higher modulus of elasticity (i.e. it is stiffer) and sustains much higher loads to failure (In Albright 1979, Carter 1976). Cortex is found - by definition - around the circumference of each bone. This allows the stiffer, stronger cortical bone the most advantageous lever arm to resist stress. In engineering terms this is described as the second moment of area. Cortical bone is known from work in orthopaedics to contribute enormously to resisting "hoop" stresses, which are the resultant lateral expansion forces produced by axial compression of a flexible or fluid, structure or material (Harrigan1991). This is easily visualised. If a volume of water is contained in a rubber cylinder and compressed by a piston, the rubber cylinder will simply balloon out under the hoop stresses. If the same water is placed in a metal cylinder and compressed by a piston, then the hoop stresses are better contained, and extremely high loads may be supported without deformation. In the same way, if trabecular bone is not supported, then under axial loading the force cannot be transferred to the supporting cortex, and like a cathedral wall without a flying buttress, collapse will ensue.

A further consideration is that remodelling rates may be 10-fold lower in cortical compared to trabecular bone (in Kanis 1994). This means that fatigue damage is more likely to accumulate in cortical bone. Therefore, the component which is relied upon for structural integrity, is most subject to fatigue failure in ageing.
Biomechanics studies support the role of the cortical bone, and probably underestimate it, as they have not studied the consequence of removal of the cortical shell. Spadaro (1994) assessed QCT measures of cadaveric distal radius prior to mechanical testing to failure. Area of cortical bone was a better

correlate of failure load than either trabecular area or density. Mosekilde (1990) studied cadaveric vertebrae. Whilst she did use a trabecular core for testing, this is not the same as testing the whole specimen with just the cortex removed. She found that the ash density of the whole vertebrae declined with age almost identically with trabecular ash density (i.e. the % contribution of the cortex to the total ash density was either small or did not change). Despite this, the load to failure was found to decline more in the trabecular cores than in the whole vertebrae over the same age range. The cortex was contributing 50% of the strength by the age of 70 years in both men and women. This is the age around which vertebral and proximal femoral fracture rates begin to climb exponentially (Gallagher 1980). This suggests that the contribution of the cortex is much larger than is generally realised and becomes more important as age advances.

Lotz (1995) used a finite element model of the proximal femur, which had been validated previously by predicting accurately the load to failure in cadaver studies. In this model both normal gait and a simulated fall were examined. In modelled normal gait and in a modelled fall, cortical bone carried 30% of the load at the subcapital region, 50% at the mid-neck, 96% at the base of the neck and 80% at the intertrochanteric region. During gait, the principal stresses were concentrated within the primary compressive system of trabeculae and in the cortical bone of the intertrochanteric region.

In conclusion, a cortical site could provide new information on bone toughness.

Theoretical and Design Aspects

The SoundScan 2000 has been designed to achieve these aims. A device designed to minimise the precision error, and allow longitudinal measurement of cortical bone in a load bearing site - the tibia- may offer advantages over the existing Os Calcis BUA.

The important aspects of design will now be discussed.

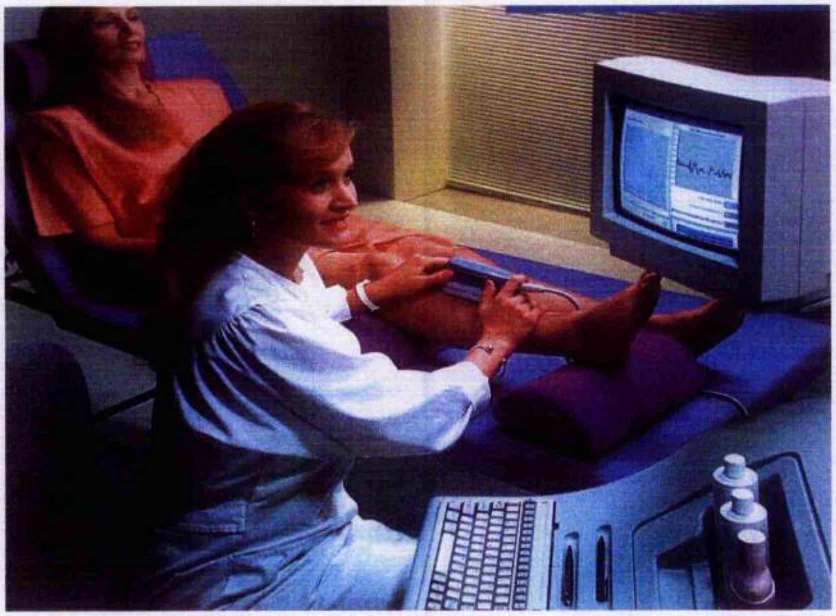


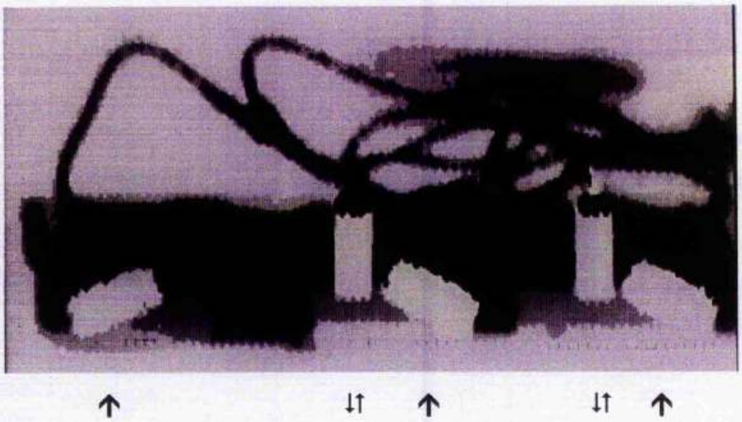
Figure 4.1 The Soundscan probe in use (Manufacturer's picture)

The layout of the device ultrasound transmitters is shown in figure 4.2. This device uses one transmitting and two receiving 250KHz transducers. They are arranged in an array inside a 150mm housing. Each transducer is acoustically insulated from the others in the array. Each transducer is housed within a gel filled neoprene-covered recess. The transmitter is located at one end of the array. The first receiver is fixed 65mm from it. The second receiver is fixed a further 50mm along the array. There is a 1MHz pulse-echo transducer located 10mm ahead of each receiving 250KHz transducer (that is, at 55mm and 105mm from the transmitter respectively). The time measured is that between the first signal reaching the first receiver, and the first signal to reach the second receiver. The known distance of 50mm allows conversion to ms^{-1} by the formula:

$$\text{Velocity} = \text{Distance} / \text{Time}$$

This is substituted with the known value to give:

$$\text{Velocity } (ms^{-1}) = 0.05(m) / \text{Time}(s)$$



250kHz 1 MHz

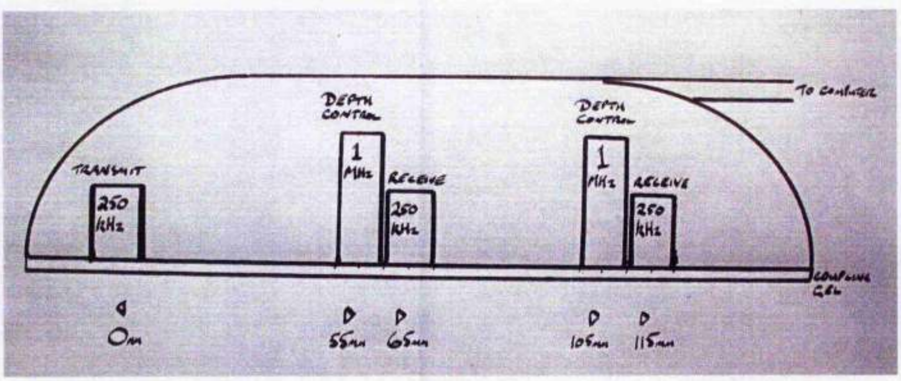


Figure 4.2 Radiographic image and explanatory line drawing of the SoundScan probe. This demonstrates on the left side a single transmitter, and on the right a pair of Receivers, each coupled with an adjacent smaller transducer.

The role of the 1MHz transducers is to measure the soft tissue thickness as close as possible to the receivers. The software will not allow measurement unless these measured distances of soft tissue thickness are within 1mm of each other. Because a signal must traverse a soft tissue layer of equivalent thickness to reach each receiver, there is no delay in the time taken for the signal to travel the distance between the two receivers.

Stated as a formula, the time taken for the 250KHz signal to travel from the bone surface to the first receiver can be expressed:

Time = Distance / velocity

or,

Time1 = Thickness of soft tissue / velocity in soft tissue

The time for the signal to travel to the second receiver is expressed:

Time2 = Thickness of soft tissue / velocity in soft tissue

+ Distance in bone / velocity in bone

The difference, i.e. the time actually measured, (Time2-Time1) cancels out the soft tissue effect, and it is seen that the time travelled in bone is the variable which remains.

Thus, the measured distance is fixed and the error from soft tissue thickness is controlled.

Two other potential sources of error are positioning error and sampling error. The array must be placed in a reproducible position. This is achieved by using bony landmarks (the distal pole of the patella and the tip of the medial malleolus) and measuring their midpoint using a tape measure. The array has a clear marking for positioning at the level of this line. It is positioned in line with the tibia with the transmitter most proximal. The software allows 150-300 sample velocities to be successively measured, whilst the array is gently slid from medial border of tibia to the lateral and back again. The mean of the 5 highest speeds is used to make it more likely that the "fastest" part of the tibia has been assessed. This, however, may not prove to be the best philosophy, as we may actually wish to measure the "weak link in the chain" rather than the strongest. These improvements should provide a low precision error.

Clinical Trials to Date

At the time of inception of the present thesis there was only a very limited literature on the Soundscan 2000. Only 3 published peer reviewed papers existed (Heaney 1995, Mele 1997, Hans 1996). Since that time, there has been a steady increase, and interest continues to grow. Chapter 13, Page 160 reviews these in more detail.

One of these three was (1995 Han) in a Korean language journal A translation is available through Myriad. Han examined 293 Korean females without known skeletal disease. Ultrasound velocity was measured at the mid-tibia using the described technique, and DXA BMD (XR-26, Norland, Fort Atkinson, USA) was measured at the hip and lumbar spine. The distribution of velocities in a cross section of female population was examined, and found to peak at age 40 years and decline thereafter. This mirrored changes in BMD at all sites. Correlations were calculated between DXA and ultrasound velocity, which varied from $r = 0.368$ to $r = 0.465$. The authors concluded that ultrasound may be useful as a technique for measuring bone mass. This conclusion is a little strong for the data presented. Perhaps it may be concluded that, in Korean women, ultrasound velocity, like so many physiological parameters, declines with ageing and that the correlation with BMD is modest.

Foldes (1995) performed a similar study involving 307 Caucasian females in Jerusalem. Tibial ultrasound velocity (tUV) was measured as described, and DXA BMD of the lumbar spine, femoral neck (XR-26, Norland, Fort Atkinson, USA), and forearm (2780, Norland, Fort Atkinson, USA) were also measured. Coefficient of variation for tUV was calculated as CV% = 0.25. Correlation between BMD measures and tUV were made and ranged from $r = 0.47$ to $r = 0.63$. When the distribution of tUV with age was examined in cross section, an age related decline in velocities was found, which was more marked in females. This mirrored the pattern in spine BMD, but was not as strong. Importantly, 83 women had bilateral tUV measurements. Dominant was compared to non-dominant side, and a very small non-significant difference of 0.3% was found. The authors conclude that the tUV's in their study were similar to those previously found using different devices. They note the high reproducibility, and the behaviour with age. They discuss the possible reasons for the modest correlations of tUV with BMD. They point out that tUV is probably sensitive to properties other than density, and that even with BMD different sites of measurement tend to correlate only moderately. Men were not included in the study, and although all subjects had been referred to an osteoporosis centre for assessment, no statement is made on the prevalence of skeletal disease or osteoporosis. Fracture and fracture risk was not considered.

Stegman and Heaney (1995) studied 183 men and 188 women in a population based study in Nebraska. At least 30 male and 30 female subjects were recruited in each decade of age from 30 to 80+ years. Measures were made of tUV, patella UV (Signet, Osteo-technology, Waltham, Mass), and SPA BMD (DT-100, Osteometer, Hoersholm, Denmark) of the distal radius. Lateral spine radiographs were made for all subjects over the age of 40 years. Men and women were compared as cross-sectional populations for the distribution of tUV. Mean values for each decade were higher for men. Data for both men and women described an age-related decline, which was more marked for women. Correlations of tUV with BMD measures ranged from r = 0.39 to r = 0.53. The subjects were categorised by "history" or "no history" of fracture since the age of 40 years and by the presence or absence of radiographic vertebral deformity. Odds ratios for prevalent fracture per SD reduction in tUV were produced by logistic regression models. A 1 SD reduction in tUV was found to be associated with an odds ratio significantly greater than one only for a "history" of *peripheral* fractures in men and women. No definite risk could be shown for vertebral deformity. Interestingly, in women, distal radius BMD was no more powerful. The authors suggested that tUV has potential in assessing fracture risk, but were guarded and wished to await prospective fracture trials. Perhaps the young mean age of the volunteers led to a low number of prevalent vertebral deformities and hence weakened the power of the study. It would also have been useful to compare the performance of axial rather than peripheral BMD with the other techniques which were used in the study.

Since 1996, and during the data collection for this thesis, a further eight have been published. Orgee (1996) examined a mixed group of 232 Caucasian women, of whom 73 were healthy volunteers and the remainder were patients referred for assessment of skeletal health. Reproducibility for tUV was good, CV% = 0.5, and was best when the maximum rather than minimum velocity was used. Measurement of the proximal, middle and distal thirds of the tibia in 29 women produced significantly different velocities for each site. No assessment was made of the potential error from small (1cm) differences in probe placement. In 78 women, dominant and non-dominant tibias were measured and no significant difference was found. In postmenopausal females, an age related decline in tUV was seen with cross sectional analysis. It was concluded that measuring the maximum velocity at a standardised site improves reproducibility of tibial ultrasound velocity.

Funck (1996) examined the tUV of 150 women without skeletal disease. In addition, he examined the tUV of 13 females who had had recent fractures of the hip. Reproducibility of tUV was good, CV% = 0.42. In postmenopausal females there was an age-related decline in tUV with cross-sectional analysis. This decline was not noted in those using Oestrogen replacement. Case-control comparison was made of women with fractures and without fractures. TUV was significantly lower in those with fractures (3837 vs. 3652 ms^{-1}). The authors conclude that, although the numbers are small, tUV is capable of discriminating patients with and without fractures. They make the points that reproducibility is good, and is unaffected by osteophytosis or vascular calcification - unlike axial BMD measurements.

Foldes (1996) examined the tUV of 71 patients dependent on renal dialysis. No measurement of densitometric BMD was made. "Time on dialysis" (0-18 years) had a wide range. Serum intact PTH, although used in analysis, is reported only as being > or < 34 pmol/l, and no local normal range is quoted for comparison. A previously reported database of 1686 male and female subjects from different authors (Liberman 1995) was used for comparison. Z score analysis was used. For tUV in dialysis patients the mean Z score in comparison to normals was -2.0, and ranged down to -7.0.

A significant inverse correlation was found for tUV with iPTH and with "time on dialysis". The authors conclude that renal dialysis and raised PTH levels are associated with low tUV measurements. Unfortunately, no control measurement such as BMD or bone biopsy or fracture history was made. It is not possible to say whether the low tUV is associated with low bone density, abnormal architecture or fracture risk. There is no doubt that this is a fascinating condition, the study of which may give useful insight into which aspects of skeletal health effect tUV.

Cunningham (1996) examined the relationships between tUV, os calcis speed of sound, os calcis BUA and DXA BMD of the hip and spine. 89 female subjects were used. TUV was not well correlated with either spine ($r = 0.3$) or hip BMD ($r = 0.07$). BUA of the os calcis was better correlated ($r = 0.41$ and $r = 0.41$

respectively). An interesting aspect of this study was the measurement of tibia DXA BMD in a small subgroup of 20. The correlation between tUV and tibial BMD was r = 0.71. This seems to be in conflict with correlations between tUV and both hip and spine BMD. It could be concluded that BMD measurement at one site is not a good predictor of BMD at another site. In any case, even with site matched measurements, BMD could account for only half of the variance in tUV.

Rosenthall (1996) examined the relationships between tUV, os calcis speed of sound, and DXA BMD of the hip and lumbar spine. 220 females were included, 28 of whom had an existing vertebral fracture. Reproducibility of tUV was good, CV% = 0.20. As other studies have also found, the correlations between tUV and DXA BMD of the spine and hip were modest. The difference in tUV for fracture and non-fracture groups did not achieve significance. The differences in BMD and os calcis ultrasound for fracture and non-fracture groups did achieve significance. The authors conclude that despite excellent reproducibility, the clinical value of tUV is not certain.

Foldes (1997) examined 27 young female dancers and 27 young female controls. TUV was measured, as was DXA BMD at the tibia and lumbar spine. It is of interest that the dancers had lower values for all measurements - although this was only statistically significant for tUV. It is suggested that dancers are more likely to be hypogonadal. It seems likely that the low CV% of tUV allowed a significant difference to be detected even with small numbers.

Leong (1997) examined 50 Asian females (Singapore) using tUV and DXA BMD of the hip and lumbar spine. He found the reproducibility of tUV to be good, CV% = 0.3. He also examined the tUV of left and right legs in 74 volunteers, and found no significant difference. The correlations between tUV and DXA BMD of the hip and lumbar spine were better than previously reported (r = 0.68 and r = 0.61 respectively). This may be a characteristic of the racial group studied.

A more recent publication was a basic science paper by Lee (1997). 26 fresh frozen cadaveric human limbs were examined. After thawing, tUV was measured using the Myriad Soundscan 2000 in the previously described fashion. A 40 x 4.5mm cylinder was then machined from the midpoint of the anterior crest of each tibial specimen. The 40 x 4.5mm cylinders had BMD measured using a peripheral QCT (Stratec XCT 960A, Norland corp, Fort Atkinson, WI). The 40 x 4.5mm cylinders then were machined to a diameter of 3mm over the central 8mm section. The cylinders were then tested to failure in tension using an Instron machine (Instron corp, Canton, MA) with an MTS gauge. Excellent correlations were found for tUV with qCT BMD ($r^2 = 0.74$), tUV with modulus ($r^2 = 0.84$), tUV with ultimate strength ($r^2 = 0.75$) and BMD with ultimate strength ($r^2 = 0.80$). Unfortunately, of the 26 original specimens, 5 were broken during the machining process, and a further 10 failed at the interface with the chuck rather than through the 3mm-diameter section being measured with the strain meter. Thus, of the original 26 samples, only 11 provided valid results for analysis. It is also unclear how representative a 4.5mm diameter sample is of the tibia, and although BMD was based on this sample, ultrasound velocity was measured in the whole bone. The ultrasound measurements were all made at "room temperature". The control of this temperature is not discussed. It is recognised that temperature effects ultrasound velocity substantially, and this will be examined later.

As discussed in the previous sections, it is clear that there is an extremely strong relationship between ultrasound velocity, and the approximately constant ratio of density and elasticity in bone. It is therefore not a surprise that extremely strong correlations exist between these variables in small, machined specimens. It may be that it is the aspects of bone other than density which are responsible for the potentially clinically useful variations in ultrasound velocity. The most recent publications regarding the Soundscan device will be addressed in a separate chapter.

Conclusion

To conclude, this novel device offers promise due to improved reproducibility and the use of a cortical bone measurement site.

This thesis aimed to systematically assess this new method of quantifying skeletal health, in several stages.

Reproducibility studies were performed. An in vitro model was developed to assess the pathway of ultrasound waves and test the effects of temperature, soft tissue thickness and positioning error. Temperature and positioning error were then tested in vivo. Several disease states with known effects on bone were then used as models to determine the effects on tUV and other methods.

A normal dataset was collected to allow gender and age effects to be assessed. This also allowed for normal ranges to be set for Z and T scores and thus thresholds for diagnosis of osteoporosis.

Fracture discrimination was tested retrospectively in an existing study population. Finally in a prospective trial, the natural history of tUV, as well as its response to bisphosphonates in comparison with BMD and BUA.

CHAPTER 5

Reproducibility and Reliability

CHAPTER 5

Reproducibility and Reliability

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Introduction

Clinical studies performed previously have indicated very low reproducibility error for the measurement of tibial ultrasound velocity (tUV)using the Soundscan (Myriad, Rehovot, Israel(Orgee 1996, Foldes 1995). CV% has ranged from 0.50% to 1.60% in the studies discussed. The validity of these studies is accepted. It remains desirable, however, to confirm these findings for the equipment and personnel locally. In addition, there were other sources of potential error which were important to examine, and an in-vitro model which required assessment. For this purpose, the recommendations of the American Food and Drugs Agency (FDA) were followed. These recommendations indicate that 10 subjects should have measurements made on two occasions to include repositioning error.

The two techniques to be used were tUV using the Soundscan, and BMD of the tibia using DXA with the Hologic QDR 2000plus (Hologic, Waltham, Massachusetts). As it was intended to assess these measurements in both a human in-vivo model and in a bovine in-vitro model, it was felt important that both methods should be tested in both models. The other potential sources of error which I wished to investigate for tUV were the effect of small variations in positioning of the probe, and the effect of temperature. Both of these subsequent studies are therefore included in this chapter.

Phantom

A plastic phantom is provided by the manufacturers of the Soundscan and was used on each day that the machine was in use. Before any clinical measures may be made using the Soundscan, the phantom must be measured. Without a velocity measurement made on the phantom matching the known value for it, the software will not allow further measurements to be made. This ensures a degree of intrinsic reliability through regular benchmark measures.

Human Subjects

Methods

10 adult volunteers were examined. In each case the non-dominant leg was used for measurement.

Measurement of tUV was made by a single operator on two occasions. A standard ultrasonic coupling gel was used, and a room with a thermostatically controlled temperature of 21°C. The non-dominant limb was exposed from knee to foot and the bony landmarks of the lower pole of patella and tip of the medial malleolus were identified. Using a measuring tape, the distance was measured between the two points. This distance was halved, and the halfway point was marked with a transverse line across the anterior face of the shin (and hence tibia). This is shown in Fig 4.1.

The Soundscan probe was placed on the shin. The markings on the probe (a line moulded into the plastic casing) and the shin were aligned. The transmitter end of the probe array was always proximal. Using the prompts on the display screen, the probe could be manipulated so that the soft tissue thickness was equal to within 1mm at each receiver. At least 150, and up to 300 measurements were made, whilst sliding the probe in a medio-lateral direction in the coronal plane. The aim of this was to ensure adequate sampling of the tibia, and thus make it likely that the part of the tibia with the highest ultrasound velocity had been measured.

The average of the 5 highest velocity measurements was used as the recorded velocity. It should be understood that between the highest and lowest velocity measured in the 5cm sample area of the tibia there is usually a difference of around 500ms^{-1}. A conceptualisation of this leads me to believe that what is measured with each signal is a "matchstick" shaped sample of bone. (This is not easy to prove in-vivo). As cortical bone is anisotropic, there may be wide variation between adjacent samples. In order to maximise reproducibility, several strategies may be applied.

Orgee (1996) tested these strategies. She examined the average of the maximum 5 readings, the average of the minimum 5 readings, the average of all 150-300 readings and a "trimmed" average of all readings between the 10th and 90th centiles. Reproducibility was CV% = 0.35 for the average of the maximum 5 readings and more for the other strategies (up to 1.17% for the average of the minimum 5). On this basis, it was decided to continue with the recording of the average of the 5 maximum readings.

The 10 adult volunteers were then examined using the Hologic QDR 2000plus. In each case the non-dominant leg was used for measurement of the BMD of the tibia. Again, the measurement was made twice on one day. Positioning was considered important to reduce errors. Each individual was positioned supine on the scanning area. The leg was exposed from knee to foot. The mortise of the ankle joint was used as the reference point for all scan measurements. In keeping with standard radiographic positioning practice, therefore, the foot was held in 45° of internal rotation by a plastic foot positioning frame during scanning. This was to bring the anteverted femoral neck parallel to the table to avoid foreshortening of the neck, which could falsely elevate measured BMD. A DXA scan was made using the pencil beam mode, in order to allow analysis using the software on a Hologic QDR1000 scanner. The software on the QDR1000 was designed for analysis of DXA scans without using automated protocols purpose designed for hip or spine, which would not be appropriate for a long bone such as the tibia. The scans were analysed with reference to the length of the tibia, measured during the ultrasound velocity measurement. As the tUV was measured in the 5cm distal to the midpoint, this was the site-matched area analysed for BMD and BMC. In addition, a global measure was made of the BMD of the tibia in the part from the ankle joint to the midpoint. All scans were analysed by a single observer.

The analysis used to assess reproducibility was made using the FDA recommended formula :

$$CV\% = \{SD_{POOLED}/X_{AVERAGE}\}\ x100$$

Where

$$SD_{POOLED} = \sqrt{\frac{\sum^{m}(X_{1i}-X_{2i})^2}{2m}}$$

Results

The result of short term reproducibility is displayed in table 5.1

It can be seen that both measures are capable of low reproducibility errors.

	Precision CV%
BMD - Global	1.77
BMD - Region of interest	2.25
TUV	1.35

Table 5.1 Precision of BMD and UV at the tibia in human volunteers

In-Vitro Specimens

Method

The model examined in-vitro used isolated bovine bone specimens. All samples were acquired from a commercial beef boning plant in Sheffield. The animals were all 12 months old beef cattle being prepared for human consumption, the bones being a worthless by-product from a commercial point of view. After slaughter elsewhere, the carcasses were chilled for transport and processing, but not frozen. No bovine bone from older or unsafe animals was used. The model used 6 bovine ulnae, which had been stored in formol saline prior to being embedded in polyester resin. The specifics of the preparation are best dealt with in the next chapter. For the purposes of confirming reproducibility, it is sufficient to state that specimen temperature was recorded for each ultrasound measurement.

For the ulnae, which were embedded in polyester resin, a different approach was used from the human volunteers. Two observers measured Ultrasound Velocity of a single sample repeatedly for a total of 18 measurements each. As before, measurements were made at room temperature (thermostatically controlled 21°C), and specimen temperature was measured for each scan. Analysis of variance was with one way ANOVA using the operator as the blocking factor. This can be expressed as the general formula :

$CV\% = (\sqrt{\text{Mean Square}_{WITHIN}})/\text{Average of all Measurements}$

To assess reproducibility of BMD, all 6 ulnar specimens were measured twice with repositioning. The analysis followed the same formula as was used for human volunteers (see above).

Results

The result is shown in table 5.2. It can be seen that the reproducibility error is low for both measures. The ultrasound velocity appears to have a higher error than in vivo. This may be an intrinsic failing of the model, or it may be due to the different methodology used to assess it. As there were not 10 specimens to repeat measures on, the repeated measures on a single specimen may be a limiting factor.

Bovine model	Precision - CV%
BMD	1.70
Ultrasound Velocity	2.46

Table 5.2 Precision of BMD and UV in in-vitro specimens

The polyester was subjected to DXA scanning as an isolated specimen on the QDR1000. It was measured as having a BMD of 0.0 gcm^{-3}, and it was therefore concluded that no significant effect on BMD would be attributable to the resin.

Temperature

In-Vitro

For the study of the effects of temperature, an in-vitro model has certain advantages. A wide range of temperatures may be studied without concern for safety or comfort of a volunteer. Temperature measurement may be confirmed by a thermometer placed inside the specimen. As there is no blood flow, the specimen temperature will rapidly conform to the surrounding medium, and will not correct itself towards 37°C as will a human volunteer.

Method

For this study, the 6 polyester embedded bovine ulnae were used. A thermostat controlled water-bath (designed for test-tube incubation) was used to control the temperature. Initially the water-bath, containing all 6 specimens and full of water, was cooled to 5°C. Measurement of the UV of each specimen was made using the Soundscan and repeated 3 times. The temperature was then increased in 5°C steps up to 40 °C using the thermostat. At each temperature, measurement of the UV of each specimen was made using the Soundscan and repeated 3 times. The temperature was confirmed at each stage using a scientific mercury thermometer, which was placed in a 4mm drill-hole made in the side of the specimen down to the level of the anterior cortex of the bone.

Analysis was made by plotting of UV against temperature, first as a scatter plot, and then as a plot of mean and SEM for the range of UV at each temperature point. A best fit line was plotted using Excel (Microsoft).

Results

There is an extremely strong inverse correlation between temperature and UV in-vitro over the range examined. A linear best fit line had $r^2 = 0.989$ (figs 5.1 and 5.2), and for a polynomial model the $r^2 = 0.996$. The variation with temperature was $-6.7ms^{-1}/{}^{o}C$. This matches very closely to the previously observed variation of –0.17% per degree centigrade, which gives a predicted variation of $6.63ms^{-1}/{}^{o}C$ for a velocity of $3900ms^{-1}$ (McCarthy 1990). In view of this result, it was important to test the behaviour of UV in-vivo.

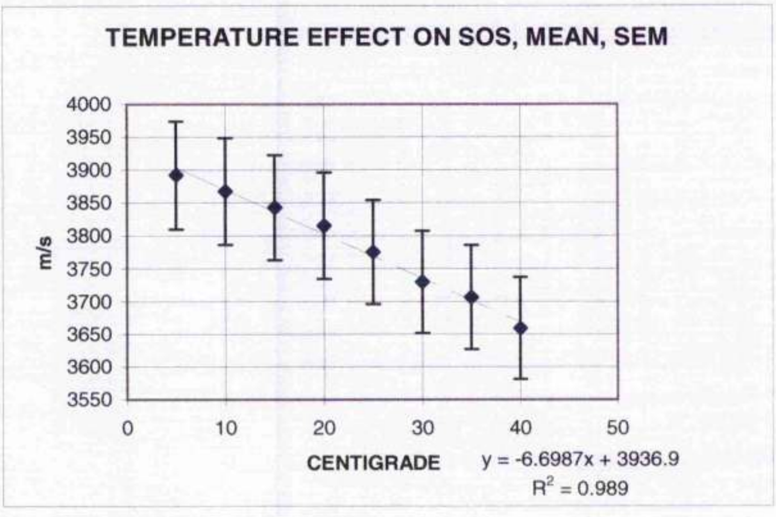

Figure 5.1 UV plotted against temperature for bovine specimens, mean of all specimens and SEM

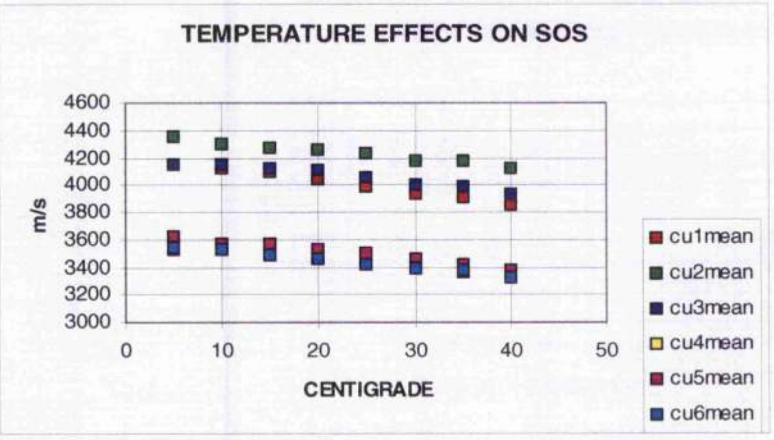

Figure 5.2 UV plotted against temperature, results shown for each individual bovine specimen

Temperature

In-Vivo

10 adult volunteers, 3 male and 7 female, were examined. It was recognised at the outset that actual measurement of the bone temperature would not be

possible, or ethically desirable. As there is no accurate method to convert a skin temperature to a bone temperature, It was decided that rather than attempt to measure an uncertain surrogate, it would be better to simply examine the most extreme effects of temperature likely to be encountered in clinical practice. This avoids the need to know the actual temperature, as it may be safely accepted that a clinician would never subject a patient to a temperature which goes beyond the threshold of comfort. Thus the effect on tUV of maximally chilling the subject's lower leg (within the limits of comfort) and re-warming it maximally, again within the limits of comfort was estimated.

Method

Each volunteer was examined supine, with the non-dominant leg exposed. In the previously described way, the midpoint of the tibia was marked. In order to chill the leg, an ice pack (temperature -20°C) was applied over a dry cloth (to protect the skin) at the marked midpoint of the tibia. This was held in place for 10 minutes. All volunteers reported a manageable degree of discomfort, although this was not quantified, and in all volunteers the skin over the tibia was cold to touch. TUV was then measured with the Soundscan probe in the manner previously described.

To re-warm the leg, a hot water bath was used. Each volunteer filled the bath for him or herself, with the water at as high a temperature as was comfortable. The chilled limb was immersed in the water for at least 10 minutes, and for as long after as the volunteer felt was necessary to feel fully warm. The tUV was then re-measured immediately in the described way. All the limbs felt hot to the touch after re-warming.

It is worth re-iterating that the scale of temperature measurement is not important. If there is no demonstrable difference in tUV between the extremes of cold and warmth met in real practice, then it is sufficient to say that in practice, cold and warmth do not effect the measurement of tUV.

The analyses of data were made with paired parametric and non parametric tests.

Results

First, a paired T test was performed. The difference was not significant at p = 0.33. In view of the small number of subjects, and the normality of distribution of measurements, a Wilcoxon test was performed. The difference was not significant at p = 0.17. The means of the tUV from the chilled and warmed limbs were 3962 and 3896 ms-1 respectively. The standard errors of the mean were 135 and 137ms-1 respectively. Fig 5.3 demonstrates the trend for higher measurements in the chilled limbs, but the confidence intervals indicate a non-significant difference.

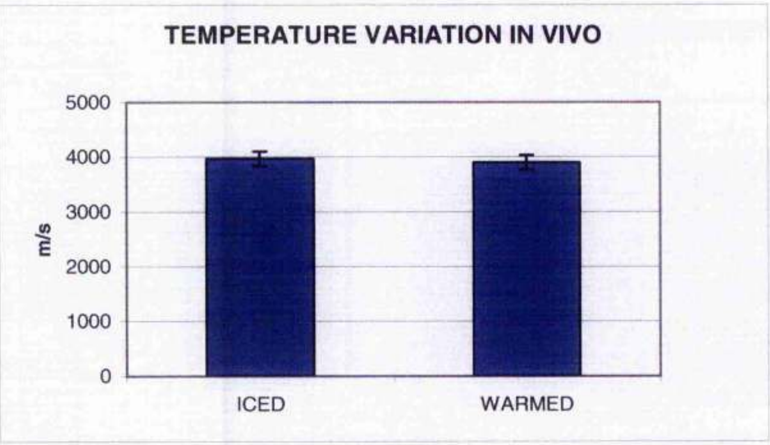

Figure 5.3 Mean tUV for volunteers with chilled and rewarmed legs

Finally a scatter plot was made (fig 5.4), and a Pearson correlation calculated. It can be seen from the plot that one individual had a reading which falls well outside the general pattern. If this outlier is retained, then the Pearson correlation is borderline significant with r = 0.45.

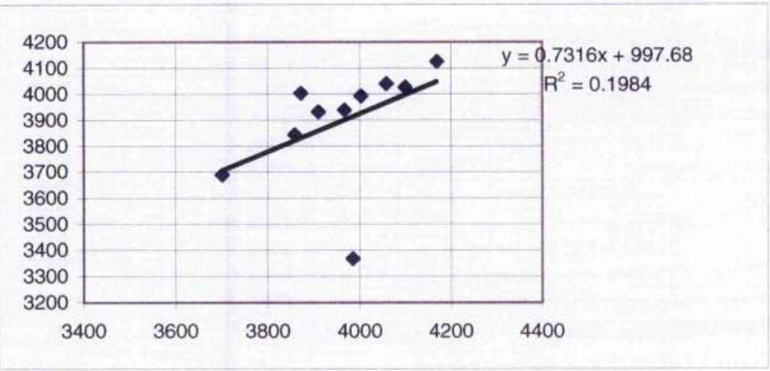

Figure 5.4 Scatter plot with regression line comparing warmed legs (y axis), with chilled (x axis)

If this outlier is excluded then the Pearson correlation is highly significant with r = 0.91. (fig 5.5)

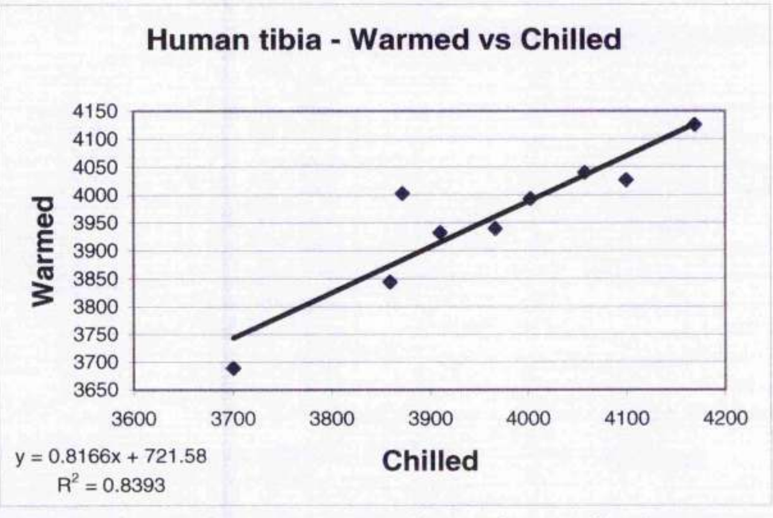

Figure 5.5 Scatter plot with regression line of tUV for human volunteers warmed legs (y axis), with chilled (x axis)

It can be seen that in this study, a non-significant trend towards higher readings of tUV was seen in chilled limbs. The exclusion of a single outlier almost eradicates this trend. There is no obvious reason for the outlier. User error remains most likely. We may conclude that in clinical practice, temperature does not cause a significant error.

Positioning variation of 1cm

In vitro

Method

For this study, 5 of the 6 bovine ulnae were used. The midline of the bone was marked as previously described. All specimens were warmed to room temperature, and had actual temperature measurement by mercury thermometer. The UV was measured in the described way using the Soundscan at the midpoint. In addition, UV was measured at 1cm distal to the midpoint and at 1cm proximal to the midpoint. Three readings were taken at each point. Analysis was with scatterplot and regression line, and non parametric testing.

Results

The graph demonstrates a scatterplot for each measurement point. The linear regression line shows a poor, but statistically significant Pearson correlation ($r^2 = 0.107$, $p=0.028$). It can be seen that up to 1cm positioning error may lead to a small but significant difference.

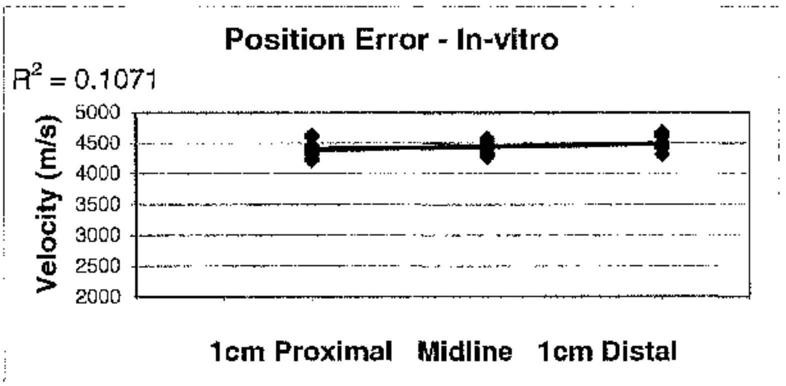

Figure 5.6 Plot showing error due to 1cm malpositioning in bovine specimens

This may translate into an important systematic error if large numbers of subjects are examined. It is clearly important to minimise errors of positioning in the measurement of UV. The mean values of UV at each position were 4402ms-1, 4406ms-1, and 4507ms-1 for 1cm proximal, midpoint and 1cm distal respectively. Kruskal-Wallis ANOVA gave P=0.0521 showing a non-significant trend for the malpositioned groups to be different from the index measurements.

Positioning variation of 1cm In vivo

Method

A group of 9 young adults and 10 elderly females (>75years old) had measurement of tUV in the previously described way. In order to test the effect of positioning error, a measurement was made at 1cm proximal and at 1cm distal to the mark for the midpoint. The means of the measurements at the three positions were compared.

Results

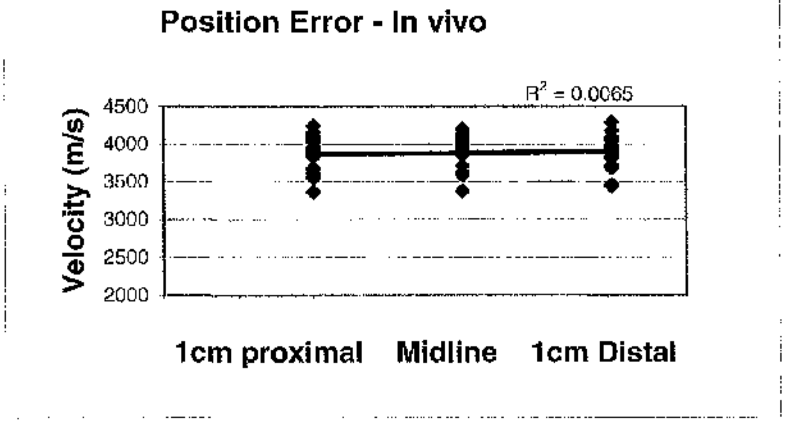

Figure 5.7 Plot showing error due to malpositioning 1cm in human volunteers measurements at the three sites.

The UV measured for each volunteer at each position is represented in figure 5.7 which shows the scatterplot and the regression line. The Pearson correlation is very weak and non-significant ($r2 = 0.0065$, $p=0.55$). The mean UV for each position was 3858ms-1, 3877ms-1 and 3901ms-1 for proximal, midpoint and distal respectively. Kruskal-Wallis ANOVA gave $P=0.779$, showing that there is unlikely to be a clinically important difference due to 1cm malposition compared with correct positioning.

It should be recognised that this still represents a small sample in epidemiological terms, and that care is required in positioning the probe in order to maximise reproducibility and reduce systematic error.

Discussion

The measures to be used throughout the rest of the study have been shown to have low reproducibility errors.

The coefficients of variation of these measurements are small and within the ranges previously reported for densitometric techniques. This range is acceptable for diagnosis. Whether the error with tUV is low enough to follow disease progress over the medium term is unproven. This is related to the rate of change in tUV exhibited by the target population. This is addressed in later chapters.

It is interesting to note that a 1cm error in proximal or distal placement of the ultrasound transducers did not produce a significant difference with the sample studied. It is also of note that there is a predictable linear relationship between UV and temperature, which is not demonstrably a problem in-vivo.

CHAPTER 6

In-Vitro Studies

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In-Vitro Studies

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Introduction

It is not clear which aspects of skeletal make-up are reflected by ultrasound transmission velocity. There are a number of experiments which can be performed in-vitro to clarify the answer, which would not be possible or acceptable for human subjects in-vivo. Preparations of human bone would be ideal for this purpose. Unfortunately, these are not easy or predictable to acquire. There may be wide variations in age, racial type, general health and skeletal health with post-mortem or amputation specimens. A model using animal bone material can overcome these problems. The age and breed are the same throughout each batch of animals processed, and the general health must reach a certain standard for slaughter for human consumption. There are some shortcomings inherent in a non-human model. In particular, it is recognised that the Haversian architecture prevalent in adult human cortical bone is not reproduced in 12 month old cattle, which have a predominantly primary osteon structure (Albright 1979). This may, however, be seen as an advantage in as much as porosity is unlikely to vary greatly and therefore influence the results.

In general, an in vitro model may be expected to answer questions concerning the effects of soft tissue, the effects of cortical thickness, the relationship with DXA BMD, and the pathway of the fastest ultrasound wave.

It is accepted that 6 samples may not be sufficient to robustly test all aims. However, a strongly significant finding using a small sample may lend support to forming a hypothesis of which bone features modify ultrasound velocity.

Specimen Preparation:

Materials And Methods Of Preparation Of Polyester Embedded Bone Specimens.

The Bone

Six specimens of bovine ulna were supplied by a commercial boning plant (Sheffield Cold Stores) in Sheffield between April and May 1996. These were a by-product of the preparation of high quality beef for human consumption in the local area and are of no intrinsic value. All source cattle were 12 months of age and had been slaughtered in a regulated abattoir prior to transport as chilled carcasses to the boning plant.

Following acquisition, the central 7 inches of each ulna was isolated by sectioning off the extremities at a measured mark using a power saw (Kress, West Germany). All soft tissue was removed using a sharp, hand held blade. The specimens were then submerged in Formol Saline 1% solution for 3 months to stabilise water content and ensure sterility.

The Resin

A commercially available polyester resin (Norpol) was chosen for embedding. The modulus of elasticity and density predicted a velocity of sound transmission close to that of soft tissue. Ease of use in previous casting trials was also an important factor, as was the good transparency of the fully set compound.

A manufacturer's data sheet is enclosed in the appendix.

The Formol Saline

The readily available 1% solution of Formol Saline as used in the pathology department of the Central Sheffield Hospitals was used to fully hydrate and sterilise the bone specimens. The surface of the bone specimens was wiped dry with a clean cloth immediately prior to casting, but the specimens remain saturated with the solution, as no time was given to allow drying.

Development of the in vitro model

In order to assess the effect of soft tissue thickness, it was planned to use a soft tissue substitute. This would be applied to the anterior surface of the bone specimen prior to measuring. It could then be made thinner and reapplied until there was direct contact between probe and bone. Initially, it seemed that water would be a simple solution, as the speed of sound in water approximates that in soft tissue. Initial attempts to do this however, all failed. For the most part, the Soundscan would read "no acoustic contact". This probably was caused by the pair of 1MHz transducers not receiving a sufficiently strong reflection from

Figure 6.1 Photographs of a prepared specimen of Bovine Ulna embedded in Norpol resin.

the water/bone interface to recognise it (personal communication from B. Wyshogrod, Myriad). In addition, it was clear that it was not practical to keep the probe array a set distance from the bone surface, and move it medio-laterally at the same time. A second attempt was made at a soft tissue substitute. Both fat (butter) and muscle (meat) were tried. It was immediately clear that both of these were highly deformable, and that the thickness could not be measured or altered with any confidence. As well as this there were marked problems with hygiene.

The ideal material would be a solid, transparent, non-deforming, machine-able substance, with a sound transmission velocity comparable with soft tissue seemed to be the ideal. The polyester "Norpol" - a cold curing plastic used for embedding objects (for example to make paperweights) - was chosen. It has the advantages of availability and ease of use. It is transparent, and sets at room temperature after the addition of a catalyst. The manufacturer's data sheet states that at $23^{o}C$ the density is 1.12 gcm^{-3} and the elastic modulus is 2.54GPa. This gives a calculated ultrasound transmission velocity at room temperature of $1506ms^{-1}$. This is within the range $1450\text{-}1550ms^{-1}$ quoted for human soft tissue. A single bovine ulna was embedded as a test model. The Soundscan had no difficulty identifying the bone specimen, and because the anterior surface was moulded flat, it proved very simple to move the probe in a medio-lateral direction in order to measure the full width of the bone. The velocities in bone measured were in the expected range of 4000-4500ms^{-1}. Because of the high transparency, the midpoint mark made as the probe positioning reference was easily visible throughout. The composite of bone and plastic was clean, and held the specimen stable during measurement. A further 6 specimens were therefore prepared. The thickness of polyester in front of the bone specimen was easily controlled. The solution was mixed with catalyst, and poured into a straight-sided tin to a depth of 12mm (measured with a ruler). This was allowed to set solid before the bone specimen was laid face down in the tray, with the "subcutaneous" flat surface lying on the solidified polyester. The remainder of the polyester was then poured in to cover the specimen (Figure 6.1). This, in fact, created two more advantages. The interface between bone and "soft tissue" was unchanged throughout the experiment, and the bone specimen was completely covered, thus preventing dehydration during the study.

Relationship of DEXA BMD to Ultrasound Velocity

Aims

To correlate the ultrasound velocity with a site matched BMD. This is not performed in clinical practice, as each device has been calibrated and assessed for its own site or sites of use. If UV is simply a reflection of bone mineral density, it can be hypothesised that a BMD measurement of the same site will correlate reasonably with UV.

Method

The specimens of bovine bone were subject to densitometry using a Hologic QDR 1000. This was performed prior to embedding in Norpol. A block of Norpol 5cm thickness with no bone was also subjected to densitometry with the same scanner. Using a custom "region of interest" program, two areas were assessed. Firstly, the entire specimen of bone was analysed. Using the same scan image, a second analysis was performed of the 50mm zone from the line used for ultrasound measurement. This was identified on the scan from an adjacent radio-opaque marker placed at the initial scan.

Comparison was made by scatterplot, and correlation coefficients.

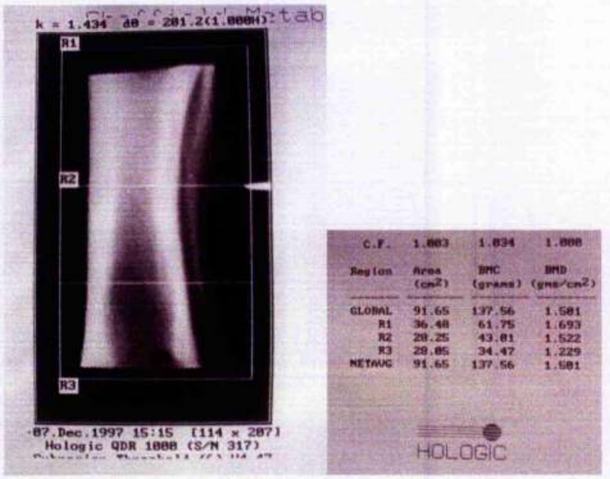

Figure 6.2 DXA scan of bovine specimen embedded, demonstrating region of interest.

Results

There was a highly significant and marked inverse relationship between Bone Mineral Density and Ultrasound Velocity at the matched site (r=-0.49). The correlation between BMD at the 50mm region of interest and BMD of the whole specimen was very close(r^2=0.90). The scatterplots (figs 6.3 and 6.4) illustrate these findings. Of interest, Norpol as a cast block had no detectable BMD, and was analysed at 0.000g/cm2.

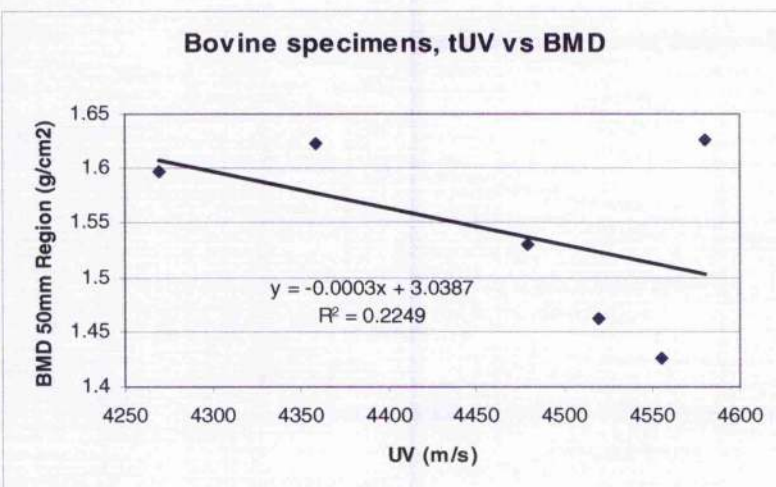

Figure 6.3 Scatter plot of ultrasound velocity against BMD for the bovine ulna specimens in their whole state. (P=0.64)

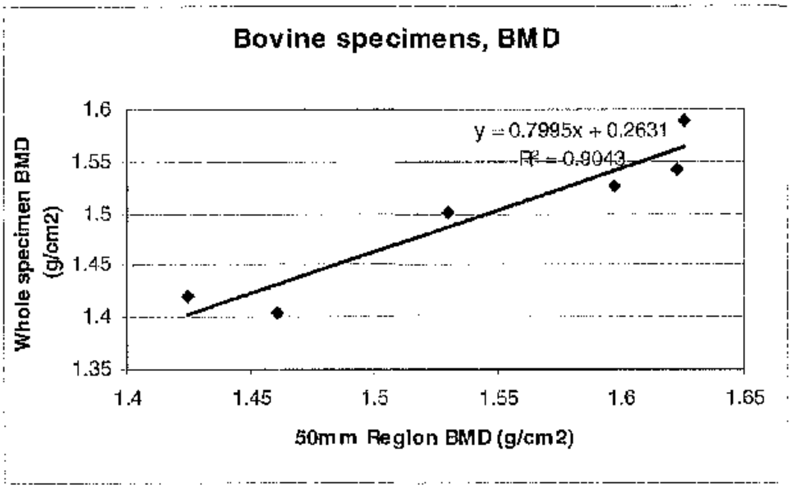

Figure 6.4 Scatter plot of BMD for the bovine ulna specimens, whole specimen against 50mm region of interest. (P=0.005)

Discussion

The inverse relationship is a surprise, even with a small sample. Even if this is an error, it suggests strongly that UV does not predict density, and needs to be treated with caution where densitometry is the clinical aim. It remains quite possible for an ultrasound "bone quality" measure to be useful, and to reflect general skeletal status without actually measuring density.

It is possible that inclusion of cancellous bone and the cortical bone of the far surface has systematically skewed the BMD measurements. However, given the extremely close correlation between regional and total BMD for these specimens, it does not seem likely that this error could be as large.

The Effects Of Soft Tissue Thickness

Aims

To investigate the effects of soft tissue thickness, and to test the manufacturer's claim that soft tissue thickness would be negated by the design of the probe array.

Method

At each measurement, the temperature was measured using a mercury thermometer in a drill-hole down to the depth of the bone specimen in the plastic. The drill-hole did not contact the bone, nor was it within any region measured either by ultrasound or DXA. The thermostatically controlled room was within 1°C of 21°C. Initially 3 measurements of Ultrasound Velocity (UV) were recorded for each specimen, as a starting point at soft tissue thickness of 12mm. The polyester was then machined off the anterior surface of the specimen in 2mm increments using a bench mounted power planer (Kress, West Germany). The thickness was confirmed by remeasuring with a calliper. After each reduction in the thickness of polyester, the UV was remeasured 3 times using the Soundscan at the marked midpoint.

Analysis was made using ANOVA to compare means of UV at each polyester thickness.

Results

A significant relationship between "soft tissue" thickness and ultrasound velocity was encountered. This is shown along with a best-fit line in fig. 6.5.

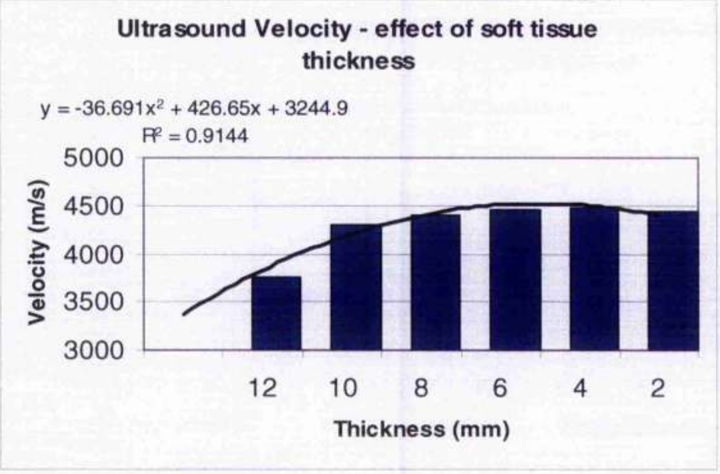

Figure 6.5 Ultrasound velocity plotted against thickness of polyester on the facing surface of the bovine ulna specimens

Measured UV tended to increase quite markedly as polyester thickness decreased. Even the initial 2mm decrement had a large effect.
Kruskal-Wallis ANOVA gave $P<0.00001$ when all thicknesses were compared.

Discussion

This is unexpected and difficult to explain. This could represent a false positive finding. The correlation is however, very strong and is repeated in each individual specimen. The manufacturer's claim may be mistaken, or this model may contain a systematic error. It may be that the interface between the probe array and the primary moulded surface of the polyester was somehow inferior to the interface with the machined surface. It is possible that even a small deviation from actual soft tissue UV is sufficient to cause software errors. From the design point of view, however, the UV in soft tissue should make no difference, as it is negated by the fact that there are two receivers. It may be possible that, if UV in the soft tissue is too high or the soft tissue is excessively thick, a signal may reach the first receiver direct through soft tissue from the transmitter, and the second receiver through bone and soft tissue as normal. This would overestimate the time lag between first and second receivers, and thus underestimate the UV in bone. The fixed distance between transmitter and first receiver is 65mm. The UV in bone is typically $4000ms^{-1}$. At a soft tissue thickness of 12mm, the UV in soft tissue would need to be in excess of $2050ms^{-1}$for this phenomenon to occur. This would indicate quite a large error in estimation of UV in polyester, and would not explain the continuing trend even at thinner polyester levels. It is possible that with a thinner layer of polyester the more lateral elements of the cortical cylinder come into range of the ultrasound signal. These would then be acting as "end on" rather than "face on" and may present a very much thicker cortical layer. If it is the case that thickness of cortical bone is related to the UV, then this may also explain the continued increase in UV seen with a thinner layer of "soft tissue". The skin and subcutaneous layer overlying the human tibia is typically 5 to 15mm in thickness at surgery. This is a personal observation but is not supported by any peer reviewed work. This raises concerns about the ability of the device to eliminate the effects of soft tissue thickness.

Decancellisation

One unresolved question relates to what portion of the specimen measured contributes to the ultrasound velocity. Previous animal studies have suggested that the cortical bone is the fastest route of transmission, but one has found that the direct route across cortical and cancellous bone is faster. None of these have examined longitudinal transmission.

Aims

To assess whether the bone deep to the facing cortical surface (ie cancellous bone and the far cortex) makes a measurable contribution to the maximum ultrasound transmission velocity.

Method

In order to answer this question, all the remaining embedded specimens were used. Using a hand saw, (to minimise vibration and heat damage), each specimen was cut in half in a plane parallel to the facing surface. The cancellous bone was then removed by hand. Because of damage to the surface interface, a total of 5 specimens were successfully prepared in this way.
The ultrasound velocity was re-measured three times for each specimen using the Soundscan on the original facing surface, at the original mark. This was then compared to the previous three measures made at the finish of the soft tissue thickness experiment, using a paired t test and a Spearman correlation.

Results

The mean velocity of the remaining 5 specimens was 4429m/s prior to decancellisation and 4406m/s immediately afterwards. This had a Spearman correlation of $r = 0.97$. A paired t test showed that there was no significant difference between the two sets of measurements ($P=0.29$).

A scatterplot with trendline fig. 6.6 is shown to illustrate this.

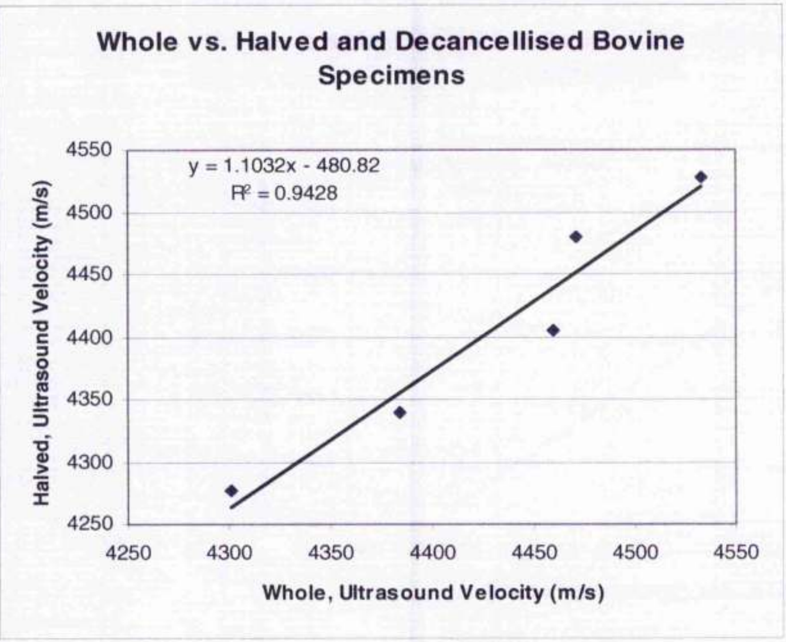

Figure 6.6 Scatter plot of ultrasound velocity of the bovine ulna specimens before and after decancellisation

Discussion

In this small sample, decancellation did not systematically change the measured UV. This strongly suggests that the bone deep to the facing cortical surface does not contribute to the measured UV. The smaller number of specimens available is related to the difficulty of preparation, and loss of integrity during the decancellation process.

Conclusion

It may be concluded that the surface thickness of soft tissue can have important effects on measurement of ultrasound velocity, despite the novel arrangement of depth testing ultrasound transducers. In particular, soft tissue thickness of greater than 6 mm has given measurements up to 12% lower than expected. The clinical relevance of this is that individuals with obesity or oedema may be categorised at a higher risk for osteoporosis than is correct. In addition, change in weight may have a confounding effect on repeated measurements from the same individual. Finally, normal values for populations may require to be compared for body mass index before interchangeable use.

These preliminary findings suggest that the maximum velocity of measured longitudinal ultrasound transmission in a long bone using the SoundScan does not rely upon either the cancellous bone, nor upon the cortical bone away from the facing surface. This may be an important finding as it would determine the area of interest to be a "plate" of cortical bone 5cm long, and as wide as the bone being measured, but only as thick as the cortical bone. This would be expected to affect predictions regarding how ultrasound transmission velocity in the tibia might be influenced by disease and its treatment.

Chapter 7

Paget's Disease of Bone - a Model of Architectural Change

Chapter 7

Paget's Disease of Bone - a Model of Architectural Change

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Introduction

One of the potential features of ultrasound transmission which may distinguish it from DXA BMD is that changes to architecture at the Haversian level of organisation might influence ultrasound transmission velocity independently of the bone density. There is some evidence (Kann 1993, Ambardar 1976) to suggest that an ultrasound wave transmitted transverse to the long axis of the Haversian system will travel slower than a wave transmitted longitudinally. This work was performed in mammalian bone specimens. No study exists in humans to confirm this. Because of the obligatory 55mm between the paired receiving transducers of the Soundscan, it was not practicable to simply measure the tibia of volunteers in a longitudinal and a transverse direction. In any case, if we accept that an animal model reflects behaviour in human tissue, this would only have shown that longitudinal transmission of ultrasound in cortical bone was faster than transverse. While this is important in interpreting results, and keeping error low by measuring in only one axis, it shows the dependence on direction of transmission, rather than the effect of a change in architecture. What I really wished to do was examine the effect of architectural *distortion* on velocity.

One model with potential for this is fracture healing. It could be proposed that a healing fracture would have distortion of architecture, and it seems likely that some of the early fracture studies with UV did show this effect (Anast 1958). It was not their intention to do so, and the results may have been underinterpreted. It is seen, however, in the human studies (Cunningham 1990), that even after clinical union the UV did not return to normal values. This could be interpreted as the effect of abnormal architecture due to the presence of unremodelled woven bone. It is now known that BMD declines rapidly following fracture and remains lower than normal long term (Eyres and Kanis 1995). It may be that the early studies were identifying this change with UV as well. It is likely that a repeat study with the Soundscan probe would not improve our knowledge, and would make the same observations. It would not then be possible to use the model to discriminate between changes caused by architectural distortion and post traumatic osteopenia.

A practical problem is that as stated previously, the majority of diaphyseal tibial fractures in Central Sheffield Hospital's orthopaedic department are treated by medullary nailing, and would therefore not be suitable for study with ultrasound or BMD.

There are few diseases of bone where architectural *distortion* is as marked and as well documented as Paget's disease. This disease is characterised on radiographic examination by thickened cortical bone, and coarse, thickened trabecular markings. The gross pathological changes match this, with marked cortical and trabecular expansion. One would expect, therefore that the BMC and possibly BMD would be elevated. Histological examination shows disordered architecture with woven bone and a mosaic "warp and weft" appearance in place of the highly organised Haversian systems. The behaviour of Pagetic bone is also different. It is prone to stress fracture and deformity.
Thus a natural model exists in which BMD, bone strength and architecture are discordant. The correlation for tUV between left and right legs has already been discussed (Leong 1997, Orgee 1996). If an individual were to suffer from Paget's disease of bone and have one affected and one unaffected tibia, then an internal control would exist and meaningful comment could be made.

Subjects and Methods

10 just such individuals were identified in the metabolic bone diseases clinic. None had any other significant skeletal disease. Each was interviewed during his or her normal clinic visit and gave consent to have DXA and tUV measurements made. Because the study design used an internal control, it was not felt necessary to collect demographic data other than age and sex, as anthropomorphic variation would not effect the analysis. Each patient had measurement of the tUV of both left and right tibia at its midpoint using the Soundscan. Each patient also had measurement of the DXA BMD/C of both the left and the right tibia from the midpoint down to the ankle joint using the QDR2000plus with the method previously described. Analysis of the DXA scans was made on the Hologic QDR1000 as before.

Results

10 individuals were studied, 4 male and 6 female. The mean age was 79.4 years with a range of 64 to 90 years. The left leg was affected in 4 cases and the right in the remainder. No individual had bilateral disease. There was no history of fracture in any limb. The time since diagnosis of Paget's disease was at least 5 years.

The results in terms of absolute values are shown in table 7.1.

The tUV for the normal and affected limb were analysed using a Wilcoxon test in view of the unknown normality of distribution. In order to allow graphic display of the results for comparison, the results were expressed as a percentage of the normal side. It can be seen in the graph of means expressed in terms of percentage of the normal side (fig 7.1)that the tUV was substantially and significantly lower in the affected compared to the unaffected leg. This is in contrast to the BMD, BMC and Area which are all markedly and significantly elevated.

	Normal		Pagets		
TOTAL	MEAN	SD	MEAN	SD	
US(m/s)	3840	51.9	3228	73.9	p=0.0069
BMC(g)	24.3	5.0	42.1	6.6	p=0.01
BMD(g/cm)	0.52	0.10	0.82	0.11	p=0.02
AREA(cm2)	45.9	1.11	50.2	1.90	p=0.01
5.5cm	MEAN	SD	MEAN	SD	
BMC	6.14	14.4	12.98	27.4	p=0.012
BMD	0.77	0.45	1.10	0.39	p=0.028
AREA	20.24	5.43	25.10	8.0	p=0.0021

Table 7.1 Comparing the mean values of measurements between the Pagetic and control limbs

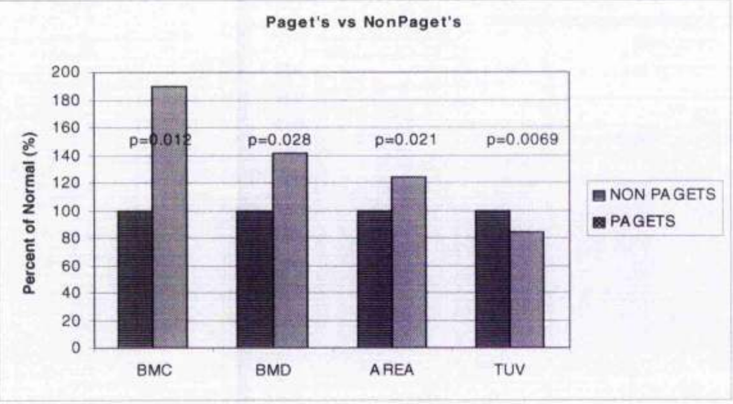

Figure 7.1 Comparing Pagetic and control limbs, with mean results expressed as a percentage of normal

Discussion

The marked difference in area, BMC and BMD is readily explained with reference to the known architectural features of Paget's disease. Expansion of the bone, increase in cortical thickness, and increase in trabecular thickness will all contribute. In fact, the BMC is disproportionately greater than the area, and thus BMD is also greater than in the normal limb. It may be that the disproportion arises because the area only rises as a square, whilst the BMC is related to the volume of the bone and rises as a cube.

UV can also be explained in terms of architectural features. The disorganisation of osteons in the Haversian system is a feature thought to reduce UV. Microfractures, should they exist, could disturb routes of ultrasound transmission and lead to lower velocities. None of these subjects had radiographic evidence of fracture, nor a history of fracture.

Paradoxically, although it may be expected that increased bone mineral or increased cortical thickness would contribute to a higher UV, this did not turn out to be the case.

Conclusion

This has demonstrated a model of diseased bone, where the structural properties are diminished due to abnormal microarchitecture. The BMD is markedly raised, yet despite this the bone remains prone to fracture and deformity. TUV is markedly reduced in the affected limb. The tUV concords with the fragility.

It may be concluded that a simple increase in bone mineral has a lesser effect on UV than does altered microarchitecture.

Chapter 8

Skeletal and Soft Tissue Injury: Models of Osteopenia

Chapter 8

Skeletal and Soft Tissue Injury:

Models of Osteopenia

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Introduction

One of the key functions of skeletal assessment is to assess changes in bone in response to both disease and treatment. Two studies (Eyres 1995, Kannus 1994) have shown that fracture of the tibia in adults is followed by a substantial and permanent reduction in BMD measured by DXA. A pair of Scandanavian studies, (Kannus 1992, Leppala 1999), have demonstrated the deleterious effects of Anterior Cruciate Ligament (ACL) rupture on BMD of the tibia using DXA. A further study of the response of the tibia to surgical osteotomy (Karlsson 2000) has provided post hoc support for the concept that surgical injury will also lead to reduction in tibial bone density. Therefore, both bone injury and soft tissue injury may provide a model with which to examine the sensitivity of tUV to post-traumatic osteopenia. The fact that there are neither differences in tUV between left and right, nor between dominant and non-dominant legs means that the un-injured limb may be used in each case as an internal control (Orgee 1996, Leong 1997).

It was hoped that the examination of a skeletal injury and a soft tissue injury would provide a model with marked changes and a model with more minor changes, and thus some comment on the sensitivity of tUV to osteopenia following injury.

Total Knee Replacement Arthroplasty (TKR) was used as a model of fracture for a number of reasons. The majority of tibial fractures in adults occur in young males due to violent injury. These are individuals who are of working age, and often default from follow up at the earliest opportunity, making recruitment difficult.. The location of the fractures is largely around the mid-shaft of the bone, or just distal to it, and the severity is highly variable. This leads to a site of locally disordered skeletal architecture. The aim of this part of the study was not to assess the effects of structural organisation - which will be addressed elsewhere - but to look at a model of rapid onset osteopenia. In any case, the majority of such fractures treated in the Central Sheffield Hospitals at the time of this study were stabilised by intramedullary nailing. The presence of a steel rod in the medulla of the bone would, unfortunately, make interpretation of results difficult.

Total Knee Replacement Arthroplasty involves osteotomy of the proximal tibia using a saw. The fracture therefore, is standardised and controlled in terms of the location and of the energy of injury. The age group of patients is usually associated with retirement, and because annual follow up in clinic is the normal practice, it is rather easier to recruit these individuals.

Those individuals with ACL injuries were all awaiting surgery for confirmed symptomatic ligament ruptures. The work of Kannus and Leppala suggests that a reliable reduction in BMD should be identifiable. Ultrasound has not previously been used to assess this injury group. The injury in this case is a soft tissue injury and one might hypothesise that it should therefore cause a less severe change in bone mineral density.

Methods

All patients who had had unilateral TKR for osteoarthritis between 1 and 2 years prior to this study were identified from the operation files in the Orthopaedic department of the Royal Hallamshire Hospital. Both males and females were considered eligible. 50 individuals were identified. All were contacted by a letter of invitation through their Orthopaedic consultant, and invited to attend for skeletal assessment. 13 volunteers responded that they would attend. Each of these was first interviewed, and gave informed consent..

All individuals awaiting a surgical repair for a symptomatic ACL injury were identified from the Orthopaedic department's waiting list. Both males and females were eligible. Again, only unilateral injury was eligible. 50 individuals were identified, and were contacted by letter of invitation through their consultant. 13 were willing to attend for skeletal assessment. These were all interviewed and gave informed consent. Clinical information was collected regarding age, sex, side of operation, date of operation and date of examination. Weight was measured on a balance scale and height using a Harpenden stadiometer. The tUV of both tibias was measured using the method previously described with the Soundscan probe. The DXA BMD was measured for each tibia using the same method as initially tested in the reproducibility study, using the Hologic QDR2000plus with internal rotation of 45°. The analysis was performed on the Hologic QDR1000 using the appropriate software. The area of 5cm distal to the midpoint was analysed as the region of interest to allow comparison of the matched site for ultrasound. The response rate to invitation was poor, but did not alter the diagnostic categories themselves. To some extent it reflects public willingness to take part in research if it is not convenient to them and no direct benefit is perceived. It is not likely to be related to disease severity, as a similar proportion of young and old invitees attended

Skeletal Injury Model

Results

Demographic and morphometric data are reviewed in the table 8.1. Correlations between weight, height, BMD and UV are shown (table 8.2) for those with TKR. Using data from both those subjects with ACL rupture and with TKR, age is plotted against tUV and BMD for the uninjured limbs in figures 8.1 and 8.2. Because those with TKR were older and predominantly female, it is not appropriate to make a statistical comparison directly between the TKR group and the ACL group for basic morphometric data.

	ACL	TKR
Age(years)	32.08	76.00
Height(cm)	174.23	157.46
Weight(kg)	78.15	78.31
M:F	11;2	2;11
R:L	11;2	8;5

Table 8.1 Demographic data for subjects with knee replacement or cruciate ligament injury

However, it is worth stating that those subjects with ACL rupture were taller and heavier than those with TKR, and were predominantly male. The difference of gender mix may substantially affect analysis of pooled results, and where these are shown caution must be used in interpretation.

Within the TKR group, moderate correlation exists between height, weight and tUV. BMD correlates modestly with weight, but only poorly with height or tUV.

TKR	Height	Weight	Age	BMD	UV
Height	1	*0.585	*-0.696	0.246	*0.621
Weight	*0.585	1	*-0.711	*0.685	*0.603
Age	*-0.696	*-0.711	1	-0.199	*-0.627
BMD	0.246	*0.685	-0.199	1	0.377
UV	*0.621	*0.603	*-0.627	0.377	1

Table 8.2 Correlations between densitometry and anthropomorphic measures in subjects with knee replacement (* P<0.05)

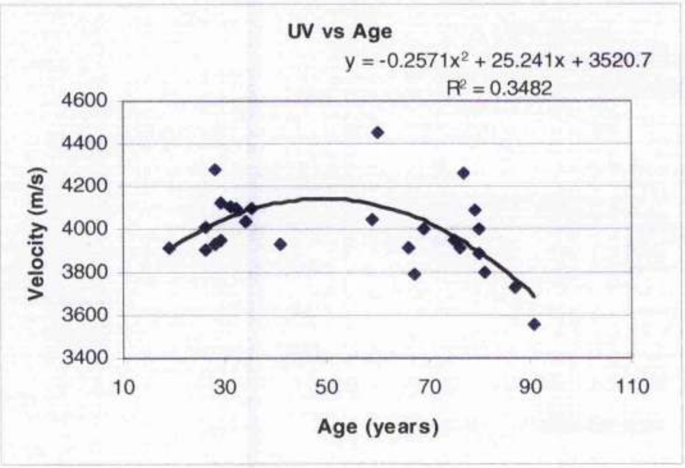


Figure 8.1 Scatter plot to demonstrate variation in the tUV with age for all subjects in this section of the study (P=0.036)

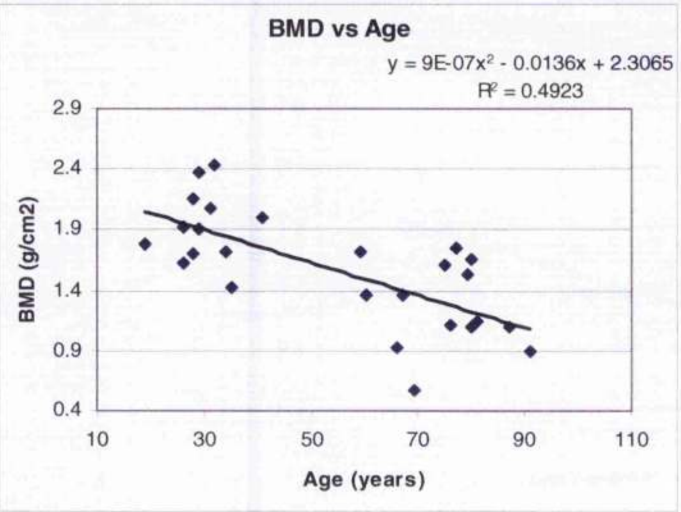


Figure 8.2 Scatter plot to demonstrate the variation in BMD of the tibia with age for all subjects in this section of the study (P=0.001)

The scatter plot Fig 8.1 shows all tUV results plotted against age, and Fig 8.2 shows all BMD results against age.

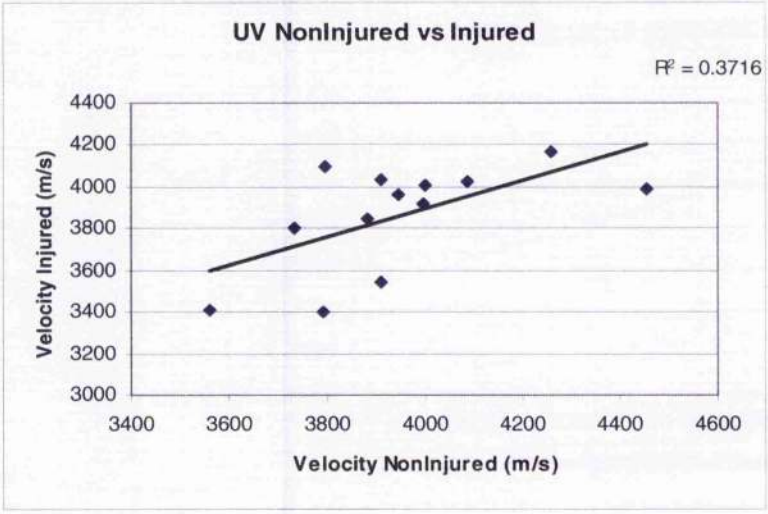
The quadratic regression lines are shown. In each case there appears to be a strong inverse relationship. This could just be as a result of the uneven male to female age distribution. Both measures behave similarly.

TKR	UV	BMD
NonInjury Mean	$3947.9ms^{-1}$	$0.862gcm^{-2}$
Injury Mean	$3862.4ms^{-1}$	$0.941gcm^{-2}$
Difference	$85.5ms^{-1}$	$-0.079gcm^{-2}$
% difference	2.17	-9.16
sd difference	0.37	-0.31
Wilcoxon	p=0.18	p=0.38

Table 8.3 Comparing the operated and non-operated limbs (mean values) BMD and tUV in subjects with knee replacement.

The first comparative analysis was of the means of tUV and BMD for the injured and uninjured limbs. In view of the small numbers and unknown normality of distribution, a Wilcoxon non-parametric test was performed. The differences between limbs in the population with TKR were non significant. (Table 8.3) This may represent a type I error.

Figure 8.3 Scatter plot comparing tUV for the operated and non-operated limbs in subjects with knee replacement (P=0.027)



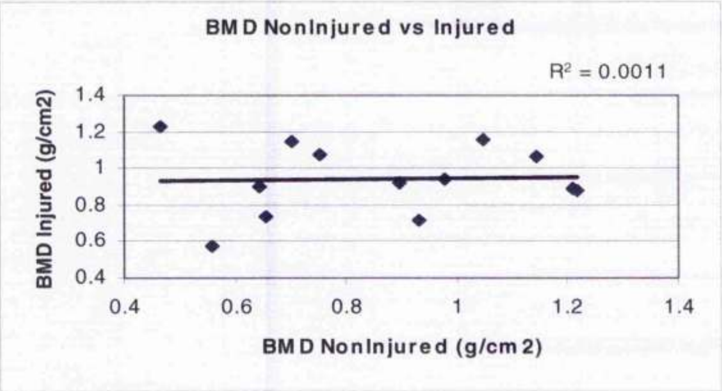


Figure 8.4 Scatter plot comparing BMD of the tibia for the operated and non-operated limbs in subjects with knee replacement (P=0.873)

While the ultrasound velocities for TKR and non-TKR limbs were of fair correlation, the BMD for paired limbs was effectively random. In small sample studies this observation could be explained by chance. It may be that BMD of the diaphysis is not affected in a predictable way by knee replacement. If the findings were correct, then it could be postulated that post injury soft tissue changes such as oedema, may be affecting measured UV.

Soft Tissue Injury Model

Results

Demographic data have been reviewed in table 8.1. Correlations between weight, height, BMD and UV are tabulated in table 8.4.

ACL	Height	Weight	Age	BMD	UV
Height	1	*0.799	*-0.561	0.045	0.004
Weight	*0.799	1	-0.261	0.021	0.078
Age	*-0.561	-0.261	1	-0.034	0.112
BMD	0.045	0.021	-0.034	1	0.232
UV	0.004	0.078	0.112	0.232	1

Table 8.4 Correlations between densitometry and morphometric measures in subjects with cruciate ligament injury (*P<0.05)

The correlation between height and weight is moderately strong. Neither BMD nor tUV correlate at all well with age, height or weight. The correlation between BMD and tUV was also very poor.

The first comparative analysis was of the means of tUV and BMD for the injured and uninjured limbs. In view of the small numbers and unknown normality of distribution, a Wilcoxon non-parametric test was used. The differences between affected and unaffected limbs were not significant. This could be type I error.

ACL	UV	BMD
NonInjury Mean	4032.2ms^{-1}	1.428gcm^{-2}
Injury Mean	4001.8ms^{-1}	1.424gcm^{-2}
Difference	30.4ms^{-1}	0.005gcm^{-2}
% difference	0.75	0.33
sd difference	0.28	0.017
Wilcoxon	p=0.19	p=0.92

Table 8.5 Comparing the injured and non-injured limbs (mean values) BMD and tUV in subjects with cruciate ligament injury

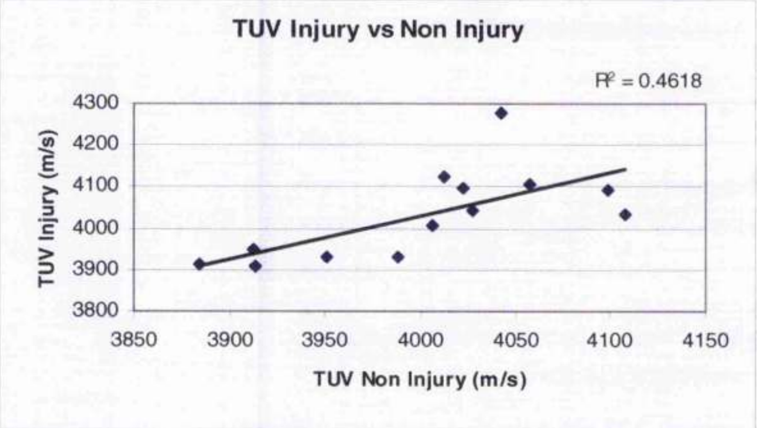


Figure 8.5 Scatter plot comparing tUV for the injured and non-injured limbs in subjects with cruciate ligament injury (P=0.01)

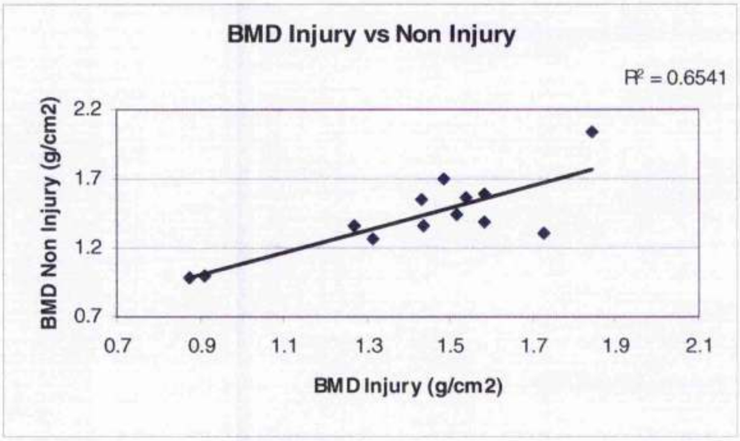


Figure 8.6 Scatter plot comparing BMD of the tibia for the injured and non-injured limbs in subjects with cruciate ligament injury (P=0.001)

Skeletal and Soft Tissue Injury - Combined Analysis

In order to compare the severity of osteopenia following a soft tissue as compared to a skeletal injury, tUV and BMD were considered separately. BMD was plotted for injured and uninjured limbs in subjects with TKR and with ACL rupture. This comparison is displayed in figure 8.7. It can be seen that there is a highly significant trend towards lower values of BMD in the TKR subjects compared to the ACL subjects (p=0.0015 Wilcoxon). There is no significant difference however, between injured and uninjured limbs.

The same comparison has been performed for tUV in figure 8.8. The results for BMD are mirrored by a non-significant trend towards lower UV values in TKR subjects compared to ACL subjects (p=0.22 Wilcoxon). Relatively, there is a more marked, although still nonsignificant, difference in UV than in BMD for both groups between the injured and noninjured limbs. BMD does not differ in the injured and uninjured limb in the ACL group. Paradoxically, in the TKR group, there is an apparently higher BMD in the injured limb. With the sample size available, it should be considered that no affect of injury could be demonstrated for either bone or soft tissue models.

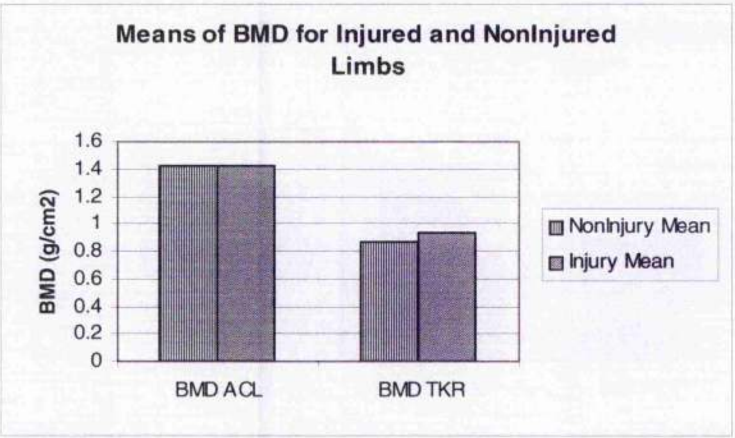


Figure 8.7 Graph demonstrating relative effect of ACL and of TKR on BMD, between affected and control limbs

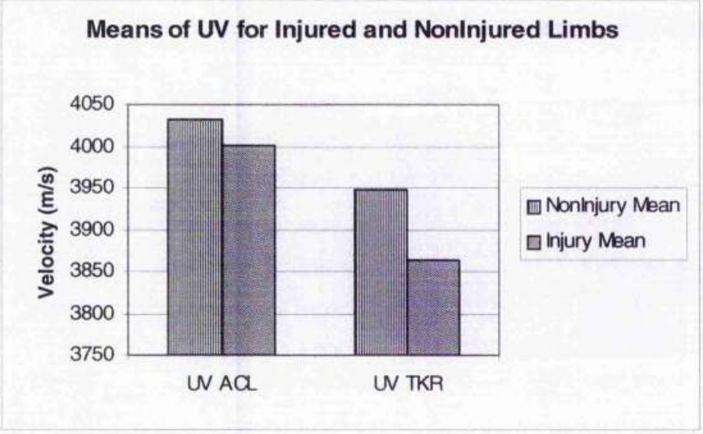


Figure 8.8 Graph demonstrating relative effect of ACL and of TKR on tUV, between affected and control limbs

In order to compare the sensitivity of tUV and BMD, both were expressed in terms of percentage of the normal result. This allows a qualitative, but not a statistical, comparison (Fig 8.9).

These results are graphically displayed, but are of uncertain value bearing in mind the relatively small sample.

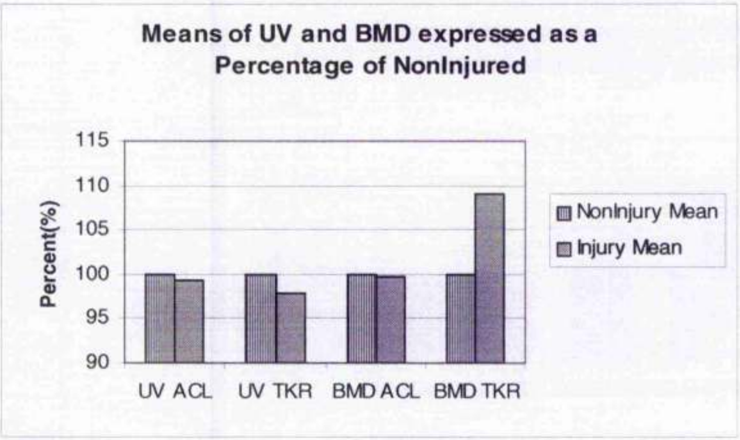


Figure 8.9 Graph comparing relative effects of bone and soft tissue injuries on BMD and UV of the tibia, the difference in bar height represents the effect size.

Conclusion

It was hypothesised that UV is able to detect a difference due to injury which displays a dose-response pattern; being greater for a bony than for a soft tissue injury. This was not demonstrated, neither for UV nor for BMD. There were non-significant trends in UV measures, and it is possible that a larger sample may lead to significant findings. BMD may not differ between injured and uninjured limbs in the diaphysis of the bone. Previous studies have examined BMD at the metaphysis of the tibia. Cancellous bone at the metaphysis would be expected to have a higher turnover rate, and this may explain the previous observations not being reproduced. In osteoarthritic patients, there may be an increase in BMD as part of an osteoarthritic process. Alternatively, it may be that weight bearing is increased after an operation which aims to relieve pain and correct limb alignment.

Chapter 9

The Effects of Systemic Disease

Chapter 9

The Effects of Systemic Disease

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Introduction

The previous chapters have examined the effects of localised disease of a single tibia. This has proven to be a useful technique because of the control limb. There are, however, disease states of particular interest because of their systemic effects on the skeleton. Specifically, these are osteoporosis secondary to parathyroid hormone excess, and osteoporosis secondary to corticosteroid exposure. Parathyroid hormone (PTH) excess is typically due to either primary disease of the parathyroid glands (hyperplasia, benign adenoma or malignant neoplasia), or the physiological response of the parathyroid glands to abnormal serum calcium and phosphate levels (commonly in renal impairment). This secondary phenomenon is complicated by terminology. When the serum levels of PTH are raised, but responsive to serum calcium levels, and a normal serum calcium level is maintained, the term Secondary Hyperparathyroidism is used. When the serum levels of PTH are raised (and relatively unresponsive) but a high serum calcium level is maintained, the term Tertiary Hyperparathyroidism is used.

The causation of osteoporosis by each disease will be discussed in the appropriate following section.

Because these are systemic diseases, there is no normal limb in each subject to allow comparison of normal and affected limbs. The analysis may be made, however, with reference to the normal population data discussed in chapter 10. The two possibilities for this approach are through a case/control methodology or by using the derived quadratic regression equation for the normal values and expressing the subject population values in standard deviation units as a Z score. In view of the size of the normal database, it was felt that it would be reasonable to derive regression equations. In addition, this technique allows the comparison of ultrasound and other densitometric methods. The regression equations for male and female normal data (and the equations for the matching SD) were calculated using the quadratic regression function on the statistics program (SPSS, Chicago). For each subject studied, it was possible to calculate the predicted mean for age and SD. This allows simple calculation of Z score by the formula :

Z score =

$$\frac{(Subjectvalue - \text{PredictedMEANvalue})}{(\text{PredictedMEANvalue} - \text{predictedSDvalue})}$$

It was intended that this section would, at least, test the hypothesis that tUV is sensitive to the changes caused by these systemic diseases. As each disease provides a model for a different type of histological osteoporosis, and therefore different architectural changes, it is hypothesised that differences in tUV and BMD between different disease states exists.

Hyperparathyroidism, A Model of Osteoporosis

In mild primary Hyperparathyroidism, it has been shown by Dempster (1999) that cancellous bone volume and its remodelling rate are greater than in normal or osteoporotic control subjects. This was demonstrated using iliac crest bone biopsy with double-labelled histomorphometry from 19 subjects who were mildly hyperparathyroid. Parisien (1990) found similar preservation of cancellous bone volume and architecture in 10 males and 17 females. Despite this, it was also found that cortical bone thickness was reduced, with increased endosteal porosity. Other reports have concurred that Hyperparathyroidism is associated with high bone turnover and increased porosity of cortical bone (Silverberg1989).

If tUV were to reflect in any significant way the status of cancellous bone, it would be expected that in hyperparathyroid states, velocity should be maintained or even increased. If tUV is relatively more dependent on cortical bone (as predicted), then we may expect a decrease in velocity. In addition to this, it would be useful to have an absorptiometric measure for comparison. BMD of the hip is made at a site where the skeleton is predominantly composed of cancellous bone, and where cancellous bone comprises 70% of the ultimate strength (Lotz 1995). It may be hypothesised that the model of Hyperparathyroid states will be reflected in different, and perhaps opposing, ways by tUV and BMD of the hip. Other absorptiometric sites such as distal radius may contain up to 90% cortical bone (Wishart 1990) and would not therefore be suitable to test for a divergence of behaviour between absorptiometry and UV in Hyperparathyroid states.

Primary Hyperparathyroidism

Subjects and Methods

With prior ethical committee approval, and informed consent, 16 female subjects with primary Hyperparathyroidism were recruited. 7 were assessed as being likely to benefit from surgery, while 9 had asymptomatic disease. Those with operable disease had their skeletal assessments made during their hospital stay for Parathyroid surgery. Those with asymptomatic disease had been identified from serum PTH measurements of asymptomatic volunteers who had consented to take part in an ethically approved osteoporosis prevention study. The same skeletal assessments were made for all subjects, but were made on an outpatient basis for the asymptomatic group.

All subjects had given consent for the measurements to be made.

All subjects had measurement of weight using a balance scale and of height using a Harpenden stadiometer. The serum PTH level was noted. Skeletal assessments were tUV of the non-dominant tibia with the Soundscan, and DXA BMD of the non-dominant proximal femur using the QDR4500 (which has purpose designed software for analysis of hip BMD, and has a faster scan time than its predecessing models).

Primary Hyperparathyroid

Results

It is clear that primary hyperparathyroid disease affects an ageing population. The mean age overall was in the mid seventies. It is also apparent that those with asymptomatic disease were on average 15 years older than those with symptoms.

	n	Age (yrs)	TUV (m/s)	BMDhip (g/cm2)	Zscore tUV	Zscore BMD
Symptc	**7**	**67.2**	**3711.5**	**0.640**	**-1.48**	**-1.62**
Asymptc	**9**	**82.3**	**3531.7**	**0.657**	**-1.97**	**-0.95**
All	**16**	**76.5**	**3616.2**	**0.648**	**-1.66**	**-1.22**
Mann Whitney U	P=	**0.001**	**0.33**	**0.8**	**0.78**	**0.06**

Table 9.1 Summarises the results for all subjects with primary hyperparathyroidism

Both BMD at the hip and tUV are lower than normal values both for the symptomatic and the asymptomatic subjects. The mean Z scores for both UV and hip BMD are substantially lower than predicted from the normal values (see Chapter 10). Z score for tUV is non significantly lower than for hip BMD (Wilcoxon P=0.10). It was not the intention to make comparisons between symptomatic and asymptomatic states, as this would not directly test hypotheses relating to UV measurement. It can be seen from fig. 9.1 that only three individuals had a Z score for hip BMD greater than zero and only one individual had a Z score for UV greater than zero. This seems to confirm the hypothesis that tUV will be sensitive to skeletal changes induced by primary hyperparathyroidism. This also confirms observations that BMD measurements are sensitive to skeletal changes in hyperparathyroid states.

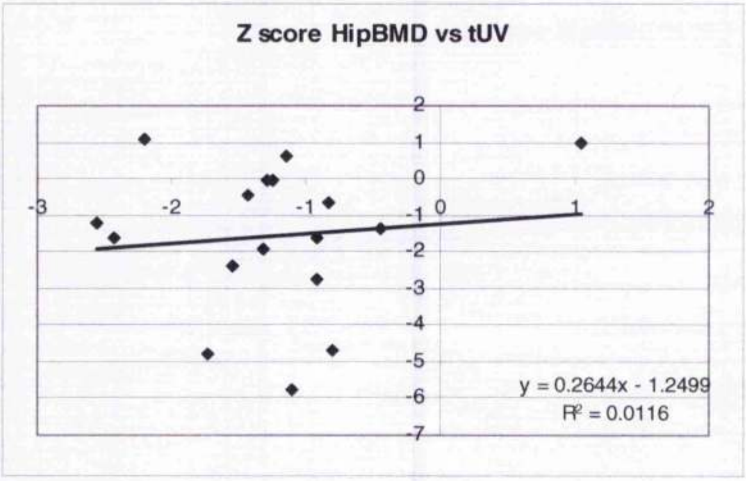

Figure 9.1 Scatter plot of BMD at the hip against tUV (both expressed as Z scores), in subjects with primary hyperparathyroidism (P=0.62)

This graph uses Z scores to compare the tUV with hip BMD in the primary hyperparathyroid population sample. It can be seen that there is no useful correlation. R^2 is very close to zero, suggesting a truly random relationship. This reinforces the hypothesis that UV is not a measure of or surrogate for BMD at the hip.

Renal Hyperparathyroidism

Subjects and methods

Those patents with renal Hyperparathyroidism were all identified and invited by the Sheffield Kidney Institute and its dialysis unit. All were stable and dependent on regular haemodialysis. Although a total of 60 patients were recruited, these fell into 2 distinct groups. 10 individuals were selected to form a group of special interest, in which all subjects had extreme high serum PTH levels (>1000pg/l, local range 15-68pg/l). The remainder had more modestly raised PTH (up to 1000pg/l).

All subjects had given consent for the measurements to be made.

All subjects had measurement of weight using a balance scale and of height using a Harpenden stadiometer. The serum PTH level was noted. Skeletal assessments were tUV of the non-dominant tibia with the Soundscan, and DXA BMD of the non-dominant proximal femur using the Hologic QDR4500. Initial comparison was made between the extreme and moderate groups.

Renal Hyperparathyroid disease

Results

		n	PTH	Age	tUV	Hip BMD	Zscore tUV	Zscore HipBMD
Female	Mean	29	568.9	50.3	3828.2	0.81	-1.20	-1.04
	SD		696.4	13.5	313.2	0.16	2.40	1.18
Male	Mean	31	595.4	52.9	3787.7	0.83	-2.03	-1.23
	SD		645.3	12.1	227.9	0.15	2.13	0.91
P=			0.87	0.42	0.57	0.51	0.17	0.47

Table 9.2 Summary of data for subjects with renal hyperparathyroidism

Table 9.2 shows summary data comparing all males with to all females with raised PTH as a result of renal disease. It can be seen that there is an equal prevalence in males and females. The majority of subjects are in the 5th or 6th decades. Males and females are well matched for age. The mean PTH measured was over 500 pg/l, but there was a wide spread, with large standard deviations. The mean tUV was, surprisingly, higher for females than for males, although this was not statistically significant. The Z scores for tUV indicate that in both males and females, the tUV was less than the expected age matched mean. The males had a non significantly higher BMD at the hip than the females. The Z scores for hip BMD show that both male and female subjects are well below the expected age matched mean. It appears that renal hyperparathyroid disease has similar effects on bone in both males and females, and that this is detectable with both BMD at the hip, and tUV. Further analysis treats males separate from females in order to allow meaningful comparison of relative effects of disease on BMD and tUV. For females the mean Z score for tUV was –1.20 and for hip BMD –1.04 (P=0.66 Wilcoxon). For males the mean Z score for tUV was -2.03 and for hip BMD -1.23 (P=0.043 Wilcoxon). This is suggestive that in males at least, there may be greater sensitivity to parathyroid related changes in TUV compared to hip BMD.

To look for a dose/response relationship, comparison was made of those with very high PTH (>1000) to the remainder.

		n	PTH	Age	tUV	HipBMD
Female	Mean	22	214	51.9	3882.2	0.83
Pth<1000	SD		234	13.8	292.1	0.15
Female	Mean	7	1683	45.1	3666.0	0.72
Pth>1000	SD		400	11.9	340.7	0.17
P=			0.00002	0.22	0.166	0.15

Table 9.3 Comparing females with high and low PTH

Table 9.3 shows comparative data for females with PTH measured over 1000 and under 1000 pg/l. It can be seen that those with the highest PTH measures were younger, although not significantly so. Although both tUV and hip BMD were lower in the high PTH group than in the low PTH group (by 5.7% and 13.2% respectively) this did not achieve significance with Mann-Whitney U test. (P=0.16 and P=0.15 respectively).

		n	PTH	Age	tUV	HipBMD
Male	Mean	23	257.2	52.8	3826.9	0.85
Pth<1000	SD		263.6	13.3	228.5	0.16
Male	Mean	8	1568.0	53.3	3675.1	0.78
Pth>1000	SD		330.5	8.4	197.1	0.15
P=			0.000001	0.9	0.09	0.3

Table 9.4 Comparing males with high and low PTH

Table 9.4 shows comparative data for males with PTH measured over 1000 and under 1000 pg/l. It can be seen that those with the highest PTH measures were of similar age. Although both tUV and hip BMD were lower in the high PTH group than in the low PTH group (by 3.9% and 8.2% respectively) this did not achieve significance with Mann-Whitney U test. (P=0.09 and P=0.30 respectively).

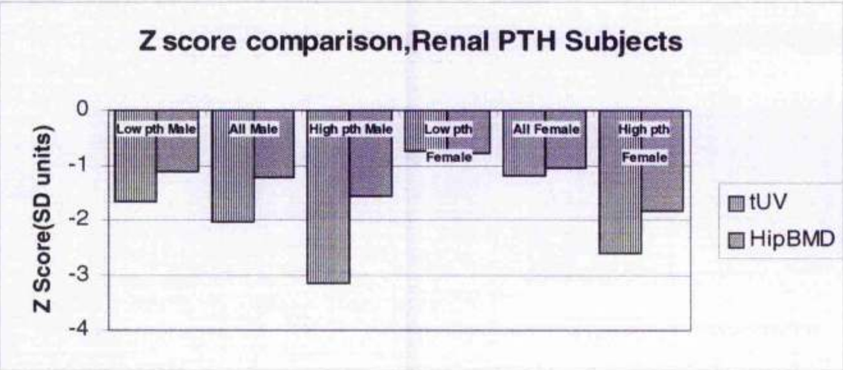

Figure 9.2 Relative effect size of PTH level on BMD and tUV (expressed as Z score)

This graph allows comparison of the "dose response" effect of PTH level on both tUV and hip BMD. All measures have been expressed as Z scores, to allow relative effects to be assessed. The Z scores were calculated using the local normal database (see Chapter 10). There appears to be a marked decrease in both BMD and tUV with an increase in PTH. In both males and females, and at all levels, the tUV appears to be more severely affected. Mean Z score for hip BMD in females with high PTH was –1.83 and in the remainder was –0.78 (P=0.018). Mean Z score for TUV in females with high PTH was –2.60 and in the remainder was –0.74 (P=0.17). Mean Z score for hip BMD in males with high PTH was –1.55 and in the remainder was –1.12 (P=0.4). Mean Z score for TUV in males with high PTH was –3.15 and in the remainder was –1.64 (P=0.21).

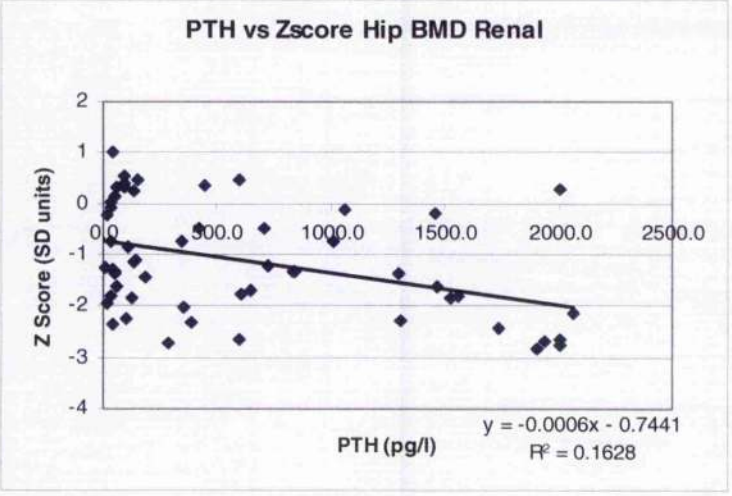

Figure 9.3 Scatter plot to demonstrate the relationship of BMD Z score to PTH. (P=0.07)

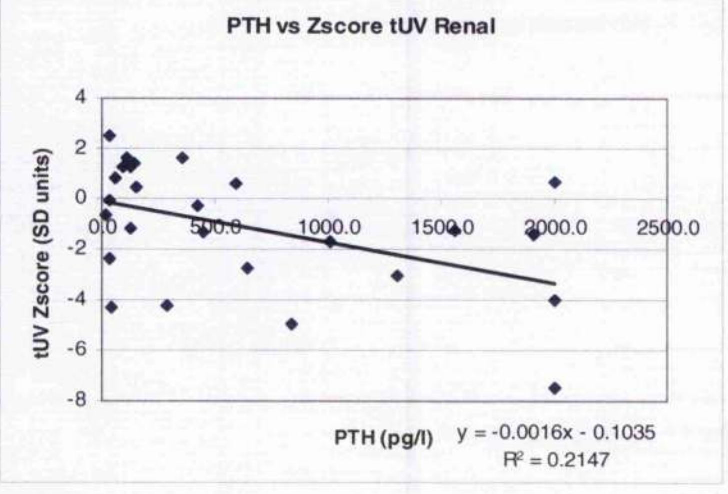

Figure 9.4 Scatter plot to demonstrate the relationship of tUV Zscore to PTH. (P=0.002)

Scatter plot of Z score against PTH for both BMD and tUV confirms the observation that bone quality is affected to a relatively greater degree at higher PTH levels.

Systemic Corticosteroid Exposure as A Model of Osteoporosis

It is clear from both human (Dempster 1983, Adinoff 1983) studies and observations (Toogood 1995), that supra-physiological exposure to glucocorticoid steroid hormones causes a secondary osteoporosis. This is associated with an increased fracture risk. (Adinoff 1983).
Studies of humans using absorptiometric methods have shown that sites containing predominantly trabecular bone (eg spine, proximal femur) are more affected than sites of predominantly cortical bone (eg mid forearm). (Gluck 1981). BMD has been shown to have similar predictive values to BUA at the Os Calcis in Crohn's disease (Javaid 2001) and to have equivalent discrimination to BUA at the Os Calcis in Rheumatoid arthritis (Sambrook 2001). The mainly cancellous nature of the Os Calcis may be well suited to allow ultrasonic assessment of fracture risk in corticosteroid excess.

Histological - histomorphometric - study of human bone from glucocorticoid exposed subjects (Frost 1973, Jowsey 1970, Dempster 1983, Jee 1970) has consistently shown reduction of the thickness of osteoid seams, a low mineral apposition rate and reduced wall thickness. It has been suggested that this is due to reduced protein synthesis. In addition, there is reduced calcium absorption from the gut (Hahn 1981) and increased renal calcium excretion (Suzuki 1983). Cancellous bone is affected to a greater extent than cortical bone, presumably because of its higher rate of turnover.

These features of glucocorticoid excess would lead us to expect that tUV would be a less sensitive measure of this form of osteoporosis than absorptiometry.

Subjects and Methods

It was intended to recruit 10 adult male volunteers with a history of chronic oral corticosteroid medication. Due to the chronic poor health of this population group, only 6 were able to attend for follow up. This of course limits the reliability of the results. These 6 were taking long term oral prednisolone for chronic bronchitis and emphysema.

All volunteers gave informed consent before assessment. All subjects had measurement of weight using a balance scale and of height using a Harpenden stadiometer. Skeletal assessments were tUV of the non-dominant tibia with the Soundscan, and DXA BMD of the non-dominant proximal femur using the QDR4500.

Results

Corticosteroid Exposure

Steroid Exposure	Age	TUV	Hip BMD	Zscore tUV	Zscore HipBMD
	68	4092	.97	1.08	-0.16
	37	3886	.88	-1.05	-1.47
	65	4023	.86	0.34	-0.85
	66	4105	.62	1.16	-2.27
	70	3901	.91	-0.72	-0.49
	21	4002	.70	0.17	-3.19
Mean	54	4001	.82	0.16	-1.40
SD	18.7	84.5	.12	0.83	1.05

Table 9.5 Result data for individuals with systemic steroid exposure.

Table 9.5 shows the actual densitometry results, alongside the derived Z scores. It is seen that the BMD is low. The Z score for BMD is quite varied, but in every case is below the age matched mean. The tUV is less varied, but in only 2 cases is below the age matched mean. (Wilcoxon P=0.046). TUV was not able to detect the bone changes in this small group. It may be that BMD is a more sensitive measure in steroid induced bone disease. This would be in keeping with the behaviours proposed for these measurements. These preliminary findings in only 6 subjects suggest that a larger study may produce interesting findings, however the exceptionally poor state of health in this population may preclude such a study.

Conclusions

It can be concluded that primary and renal hyperparathyroid states, and chronic oral glucocorticoid exposure all have effects on bone. These effects can be identified and assessed by the use of both BMD at the hip, and UV of the tibia. The results available suggest that tUV is more sensitive than BMD to changes related to PTH excess. This trend was apparent throughout the parathyroid analysis section, although this did not reach statistical significance in any one comparison. The scatter plots suggest a dose response relationship between tUV and PTH. In the oral corticosteroid exposure group, tUV was completely insensitive to bone changes which were consistently identified using BMD. This observation did achieve statistical significance despite the small sample size.

These observations serve to strengthen the hypothesis that tUV is assessing an aspect of cortical bone rather than cancellous bone. It could be explained by supposing that the hip is more affected than the tibia by hyperparathyroid states, but both are weight bearing bones, and a differential effect seems unlikely on grounds of site alone. Existing knowledge of the composition of these regions of bone, and the relative effects of corticosteroids and PTH on cortical and cancellous bone fits the observations better.

In clinical use, tUV may be useful in the assessment of patients with parathyroid excess, but may not have a role in assessment or follow up of individuals with glucocorticoid excess.

Chapter 10

Tibial Ultrasound Velocity and Skeletal Status in the Normal Population

Chapter 10

Tibial Ultrasound Velocity and Skeletal Status in the Normal Population

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Introduction

In the assessment of any novel measurement technique, it is important to know what the normal ranges are in the population. Until it has been shown convincingly that there do not exist nutritional, racial or other unknown geographical variations, it is important that a local database and hence local definition of normal is acquired. The converse is also true; that without the collection of widespread local databases, it will never be possible to show the extent of geographical variation, or similarity.

A data set of measurements from a normal population also allows examination of the cross-sectional distribution with age. If both men and women are included, then comparisons can be made regarding sex differences, if they exist, and how great they are. Normal data also allows comparison with other measures of both skeletal health and general health. This can allow a greater understanding of what is being measured by the new device, and whether this is anything different from existing devices.

Accepted methods for predicting fracture risk from bone density measurements include Dual Energy X-ray Absorptiometry (DXA) at the hip and lumbar spine. These methods have shown themselves to have adequate accuracy (5%) in measuring Bone Mineral Density (BMD) and good reproducibility (1%) (Kanis 1994, p118). Cross sectional studies have shown an increased relative risk of fracture in individuals with lower BMD. There is, however, some concern that published reference data for hip and spine densitometry suffer from the bias of being gathered from a largely North American population. This may lead to a systematic error when used in a different population.

Theoretical considerations and some published data suggest that ultrasound velocity in cortical bone will capture aspects of resistance to fracture which are not assessed by DXA measurements. In the collection of some databases, exclusions have been made on the basis of impaired health. It seems likely that this practice would introduce significant bias into reference ranges. This database has been collected with no explicit exclusions in order to avoid this potential bias. By performing analysis separately for individuals with health problems (these are specified in the appendix), it should be possible to quantify the bias.

Subjects and Methods

237 women and 194 men recruited by letter of invitation from the local population through their general practitioner patient list took part in the study. There were no exclusion criteria as the aim was to recruit a representative sample. The age range was from 21 to 80 years. At a single visit, each subject completed a questionnaire to document general health and gynaecological and familial risk factors for osteoporosis. Weight was measured with a balance scale and height was measured with a Harpenden stadiometer. A number of skeletal assessments were made in a single visit. These were BMD at the lumbar spine and hip using the Hologic QDR4500, BMD of the distal forearm using the Osteometer DTX 200, and Ultrasound velocity at the mid tibia using the Myriad Soundscan 2000. Individuals were classified by the health questionnaire into two groups; those considered "healthy" by the criteria that had been applied by the manufacturers of one densitometer (Myriad) and those considered "unhealthy" (see appendix). In keeping with existing databases, a quadratic regression analysis (SPSS, Chicago) of densitometric measurement versus age was used to describe the variations in densitometric measurements observed. These were repeated including and excluding the "unhealthy" population, and repeated for both sexes and each densitometer.

Results

Tables 10.1 and 10.2 show the demographics of the sample population both overall and grouped by decade and sex. As expected, the men were significantly taller and heavier than the women were in each age group. Peak weight occurred in the fourth decade in men and the fifth decade in women.

Females	Count n=	Age years	Weight kg	Height cm
Decade				
3	26	25.9±2.8	61.5±11.6	162.7±7.4
4	23	36.7±2.6	68.6±19.0	162.0±7.1
5	51	46.1±2.7	69.3±11.6	163.5±6.1
6	58	56.4±2.9	68.9±13.6	161.5±5.5
7	48	65.7±3.1	66.6±8.3	159.2±5.0
8	31	74.1±2.8	65.1±9.9	157.5±5.4
Total	237	53.1±14.8	67.2±12.4	161.1±6.2

Table 10.1 Summary demographic data for normal females

Males	Count n=	Age years	Weight kg	Height cm
Decade				
3	19	26.9±2.9	77.0±9.1	178.4±4.5
4	21	35.7±3.4	83.4±7.8	178.9±6.4
5	28	46.1±3.3	82.1±12.1	176.2±5.3
6	40	55.6±2.7	81.6±12.0	172.5±6.3
7	49	65.5±2.9	78.0±10.6	171.8±6.3
8	37	75.1±2.8	76.6±11.1	171.0±6.3
Total	194	55.5±15.6	79.5±11.0	173.8±6.6

Table 10.2 Summary demographic data for normal males

One way analysis of variation (ANOVA) was used to compare densitometric measurements by decade. All measurements in women showed a significant age related decline ($p=0.00001$). In men only BMD at the distal forearm did so ($p=0.00001$): BMD at the hip ($p=0.1775$) and tUV ($p=0.1452$) failed to do so.
Pearson correlations between measurements at different sites were moderate at best. For women, correlations were marginally better than for men. The results are displayed in Table 10.3.

Pearson correlation coefficients	Female	Male
tUV with Hip BMD	0.378	0.2042
tUV with Forearm BMD	0.425	0.4056
Hip with Forearm BMD	0.6357	0.5245

All $p>0.005$

Table 10.3 Correlation coefficients among different measurements (males and females separately)

Scatter plots were made and quadratic regression lines were graphed against age for each measurement and for both sexes.
In addition, measurements were graphed against each other, and regression lines plotted in figures 10.1 to 10.12.

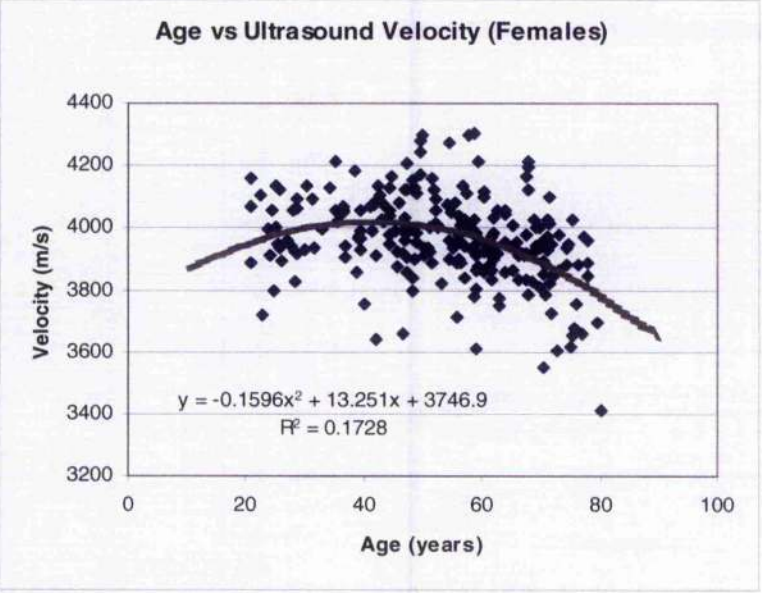

Figure 10.1 Scatter plot to show variation with age of tUV amongst females

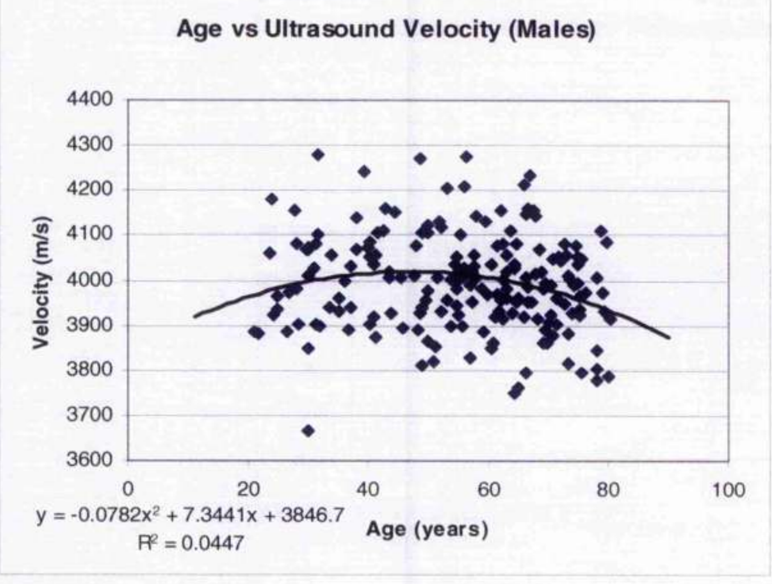

Figure 10.2 Scatter plot to show variation with age of tUV amongst males

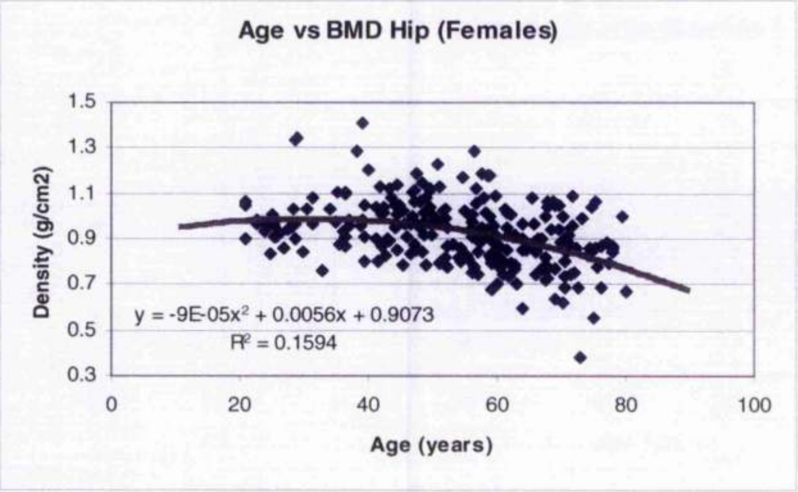

Figure 10.3 Scatter plot to show variation with age of BMD at the hip amongst females

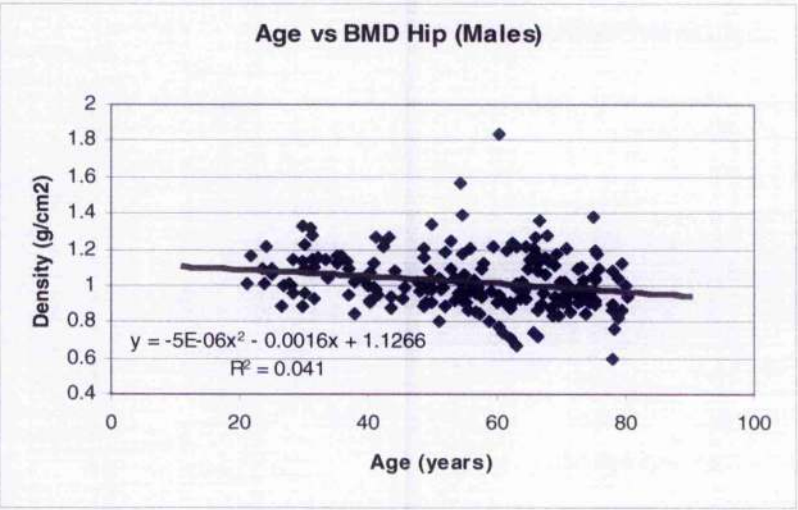

Figure 10.4. Scatter plot to show variation with age of BMD at the hip amongst males.

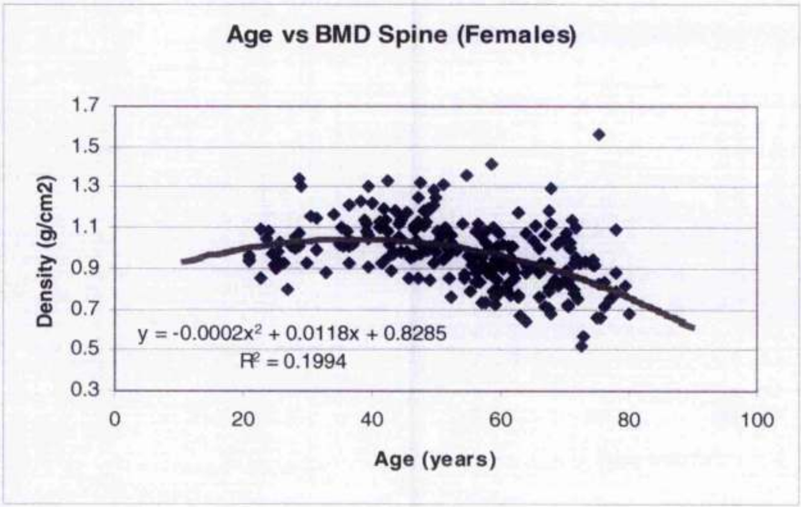

Figure 10.5 Scatter plot to show variation with age of BMD at the spine amongst females.

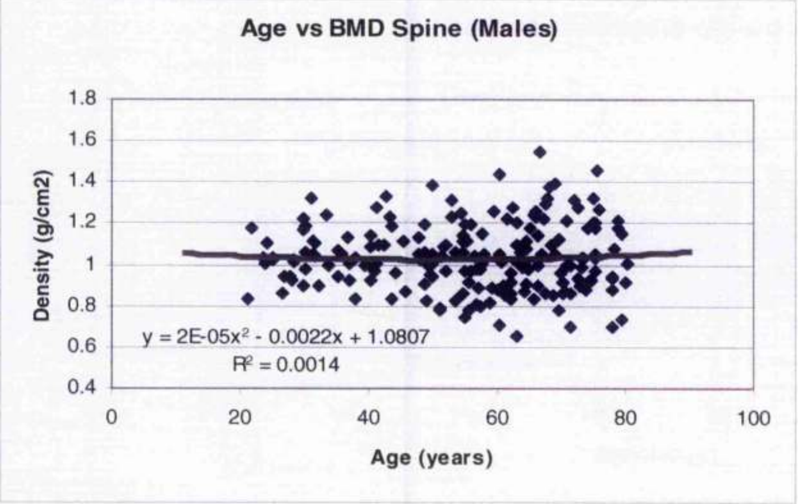

Figure 10.6 Scatter plot to show variation with age of BMD at the spine amongst males.

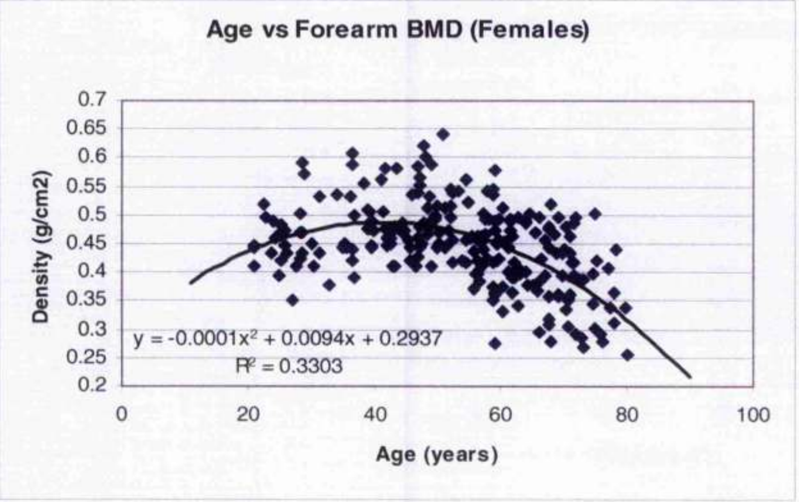

Figure 10.7 Scatter plot to show variation with age of BMD at the forearm amongst females.

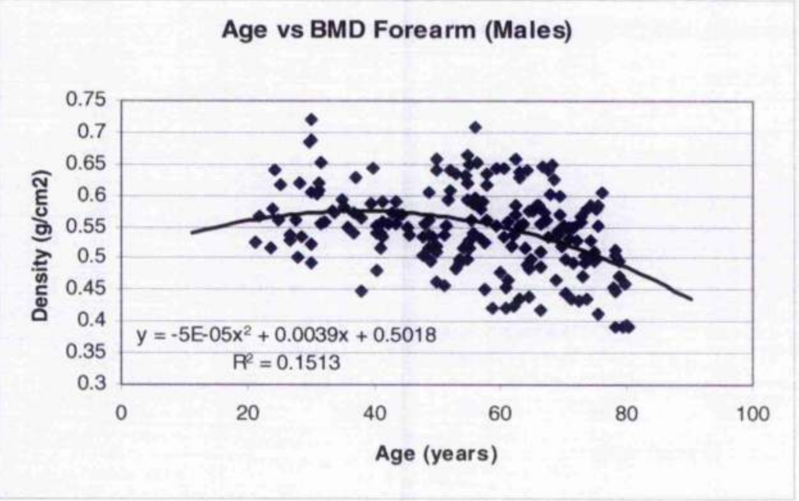

Figure 10.8 Scatter plot to show variation with age of BMD at the forearm amongst males.

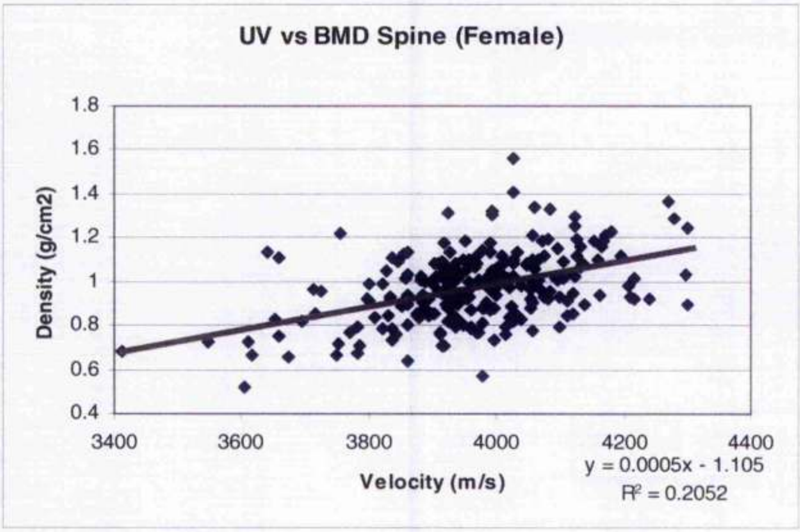

Figure 10.9 Scatter plot of tUV plotted against BMD at the Spine in females. The best fit regression line is shown with the correlation coefficient.

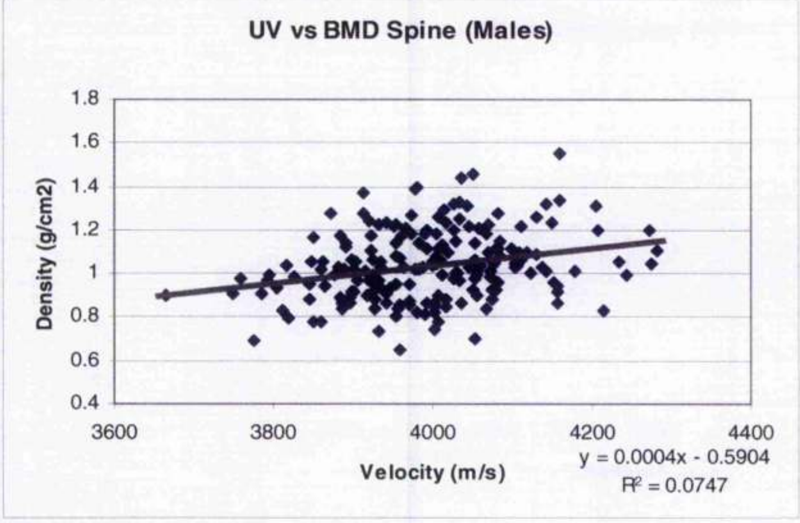

Figure 10.10 Scatter plot of tUV plotted against BMD at the Spine in males. The best fit regression line is shown with the correlation coefficient.

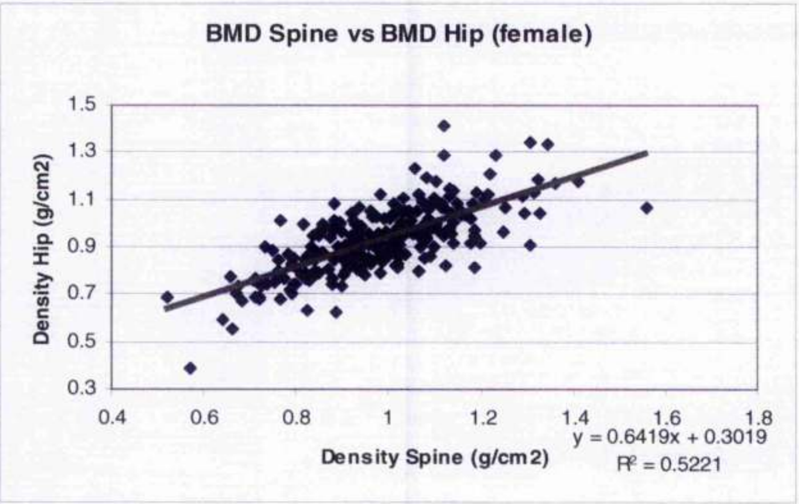

Figure 10.11 Scatter plot of BMD at the hip plotted against BMD at the Spine in females. The best fit regression line is shown with the correlation coefficient.

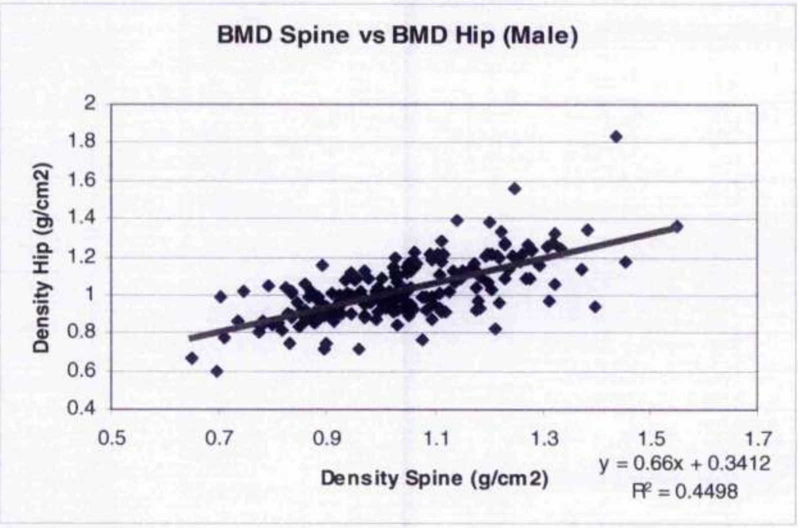

Figure 10.12 Scatter plot of BMD at the hip plotted against BMD at the Spine in males. The best fit regression line is shown with the correlation coefficient.

It is confirmed that for men only BMD forearm shows a definite variation with age. All other measures in men fail to achieve this. In females, by contrast, all measures decline with increasing age.

		Healthy	Unhealthy	
Females		Mean	Mean	P=
Height	cm	162	160.4	0.047
Weight	kg	67.5	66.9	0.72
Ultrasound	m/s	3968	3961.9	0.74
BMD Hip	g/cm2	0.94	0.92	0.38
BMD Spine	g/cm2	0.99	0.97	0.25
BMD Forearm	g/cm2	0.45	0.44	0.11
Grip	kg	25.6	24.7	0.26
		Healthy	Unhealthy	
Males		Mean	Mean	P=
Height	cm	175.4	172.4	0.0017
Weight	kg	81	78.2	0.069
Ultrasound	m/s	3995.8	3993.4	0.88
BMD Hip	g/cm2	1.04	1.01	0.19
BMD Spine	g/cm2	1.04	1.03	0.85
BMD Forearm	g/cm2	0.55	0.54	0.15
Grip	kg	44.9	39.9	0.001

Table 10.4 Comparing the results of separating measurements on the basis of health.

It can be seen that correlation between BMD hip and BMD spine is substantially closer than between tUV and either measure in both sexes. Even correlation between BMD hip and BMD spine are not good enough to make these measures interchangeable.

Mean results for each measurement are shown for those with normal health and those with poor health. In addition, Unpaired T-test was used to compare measurements of hip BMD, forearm BMD and tibial UV in the designated healthy and unhealthy volunteers. Males and females were analysed separately. Those designated as having poor health were shorter and lighter. Otherwise there were no statistically significant differences indicating that exclusion on grounds of health would have no significant effect on our normal ranges.

WHO thresholds of –2.5 SD from the young healthy mean were then applied to a group of 329 normal women over the age of 75yrs who had existing absorptiometric and ultrasound measurements. These women had been randomly recruited from the local population as part of an unrelated pilot study which intended to assess the feasibility of targeting treatment for osteoporosis by age (MRC Hip pilot study). Population rates of osteoporosis as defined by locally derived thresholds in this elderly group were as shown in table 10.5. There is a marked variation in sensitivity for diagnosing osteoporosis. This would have major effects on diagnosis and treatment decisions in clinical practice.

Site	% Below WHO threshold –2.5SD
Forearm BMD	66%
HIP BMD	51.4%
TUV	32.6%

Table 10.5 The prevalence of T score <-2.5 in 329 females, using the Sheffield local normal sample

Scatter plots were made to compare different techniques of measurement in all 329 females. They demonstrate the poor concordance between all techniques and show that simply altering the thresholds would not improve this. A second set of scatter plots was made, using data only from those 72 females in the group who had experienced an osteoporotic fracture. These demonstrate both the lack of concordance, and the variable sensitivity of different methods for diagnosis of osteoporosis.

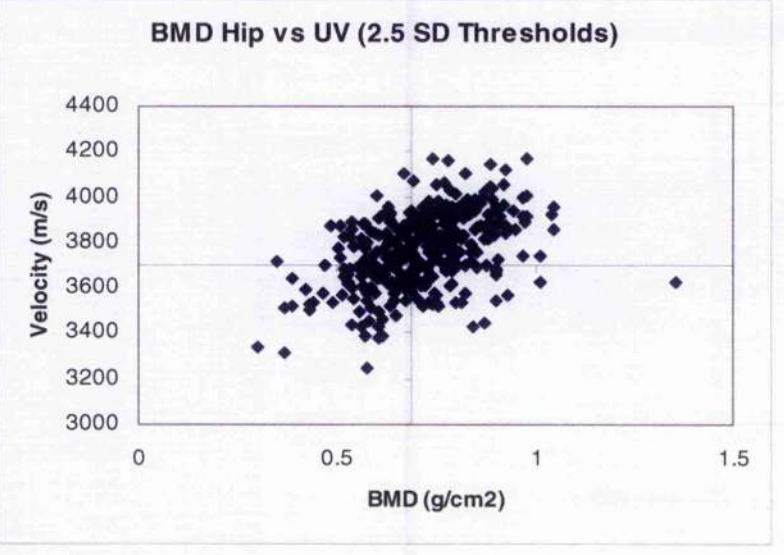

Figure 10.13 For 329 females recruited from the community, BMD at the Hip is plotted against tUV. The lines included demonstrate the T score of –2.5 for BMD hip and tUV calculated from the local normal sample.

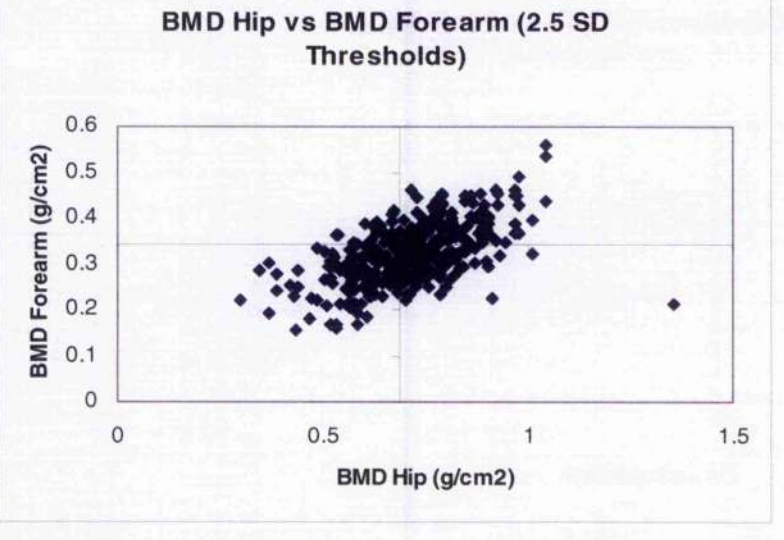

Figure 10.14 For 329 females recruited from the community, BMD at the Hip is plotted against BMD at the Forearm. The lines included demonstrate the T score of –2.5 for BMD hip and BMD forearm calculated from the local normal sample.

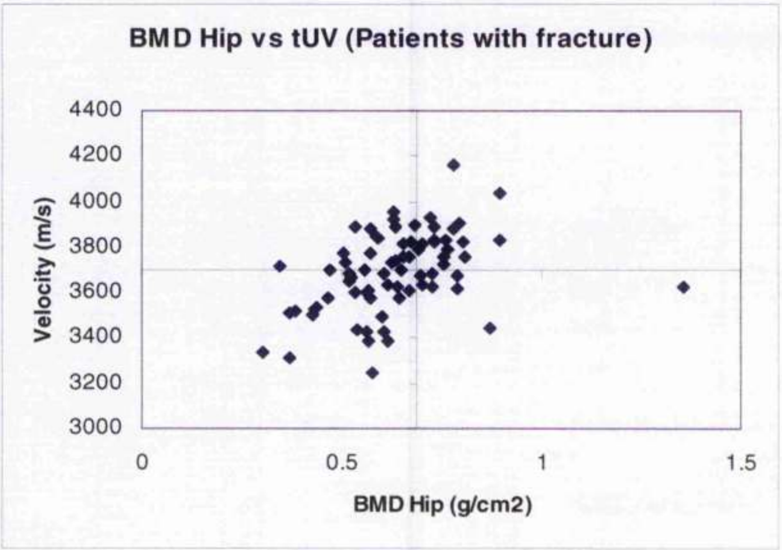

Figure 10.15 For 72 females known to have osteoporotic fracture, BMD at the Hip is plotted against tUV. The lines included demonstrate the T score of –2.5 for BMD hip and tUV calculated from the local normal sample.

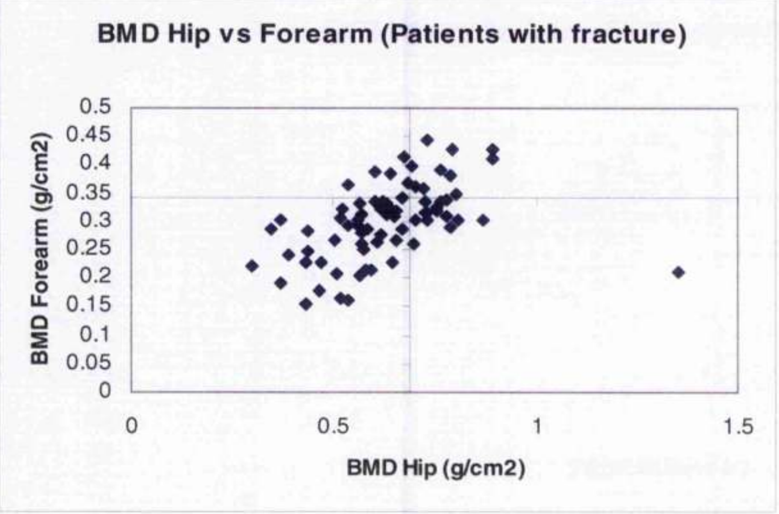

Figure 10.16 For 72 females known to have osteoporotic fracture, BMD at the Hip is plotted against BMD at the Forearm. The lines included demonstrate the T score of –2.5 for BMD hip and BMD forearm calculated from the local normal sample.

Finally, kappa scores were calculated to assess agreement between different techniques in identifying individuals whose measurements were lower than 2.5 sd from the young adult mean.

The kappa scores were poor, although they were better in women than men. The results are displayed in table 10.6.

kappa scores	Female	Male
hip BMD-forearm BMD	0.39	0.22
hip BMD-tUV	0.33	-0.009
forearm BMD-tUV	0.47	-0.009

Table 10.6 Inter measurement agreement on WHO thresholds in the local normal database, expressed as a kappa score

Conclusions

There were major differences in the cross sectional distribution of observations from male and female volunteers. Change of measurements with age, inter site correlations and kappa scores were all much weaker in male subjects. The value of densitometry in men is in question, and prospective follow up is required. The remaining conclusions relate only to women.

There was observed an age related decline in all measurements. This is consistent with previous studies of densitometry. Ultrasound velocity at the tibia was distributed similarly to the DXA measurements. In all combinations inter site correlation coefficients and kappa scores were weak. This indicates that reliance on different densitometric techniques or different sites of DXA measurement will identify -in general- different individuals from the population. This must have consequences for diagnosis, screening and treatment of skeletal disease.

In women tUV shows a similar pattern of age related decline to measurements with other techniques. In contrast to other measurements forearm BMD and tUV did show a significant decrease in elderly men. For elderly women, concordance between techniques in identifying osteoporosis is poor. Differences in prevalence of osteoporosis in elderly women suggest that unique thresholds may need to be developed for each technique.

In broad terms, this work shows that tUV has a distribution in the population that is similarly distributed to densitometric measures, and which is sensitive to ageing and established osteoporosis. Thus tUV remains a candidate technique worthy of further study.

The collection of this normal database will allow further study of tUV in disease states.

Chapter 11

Prevalent Vertebral Fracture

Chapter 11

Prevalent Vertebral Fracture

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Introduction

Quantitative ultrasound (QUS) techniques are increasingly used as indicators of skeletal fragility. Experimental studies have suggested that ultrasound measurements may give density-independent information relating to fracture risk (Gluer 1996, Lochmuller 1998, Bauer 1997, Han 1997, Bouxien1997). For this reason, it has been suggested that a combination of ultrasound and absorptiometric assessments might predict fracture risk more adequately than the use of a single technique alone. Two prospective clinical studies give conflicting information. In the EPIDOS study, hip fracture risk increased two-fold for each standard deviation decrease in hip bone mineral density (BMD) or heel BUA (Hans et al 1997) and the predictive value for QUS persisted after adjustment for hip BMD. In contrast, the Study of Osteoporotic Fractures was unable to confirm these findings and concluded that QUS at the heel offered no additional predictive value for fracture risk from that obtained by hip BMD alone (Bauer et al 1997).

The assessment of tibial Ultrasound Velocity is a technique which the previous chapters have shown to be reproducible and to correlate with age and bone density at other skeletal sites in young healthy subjects and postmenopausal women. Comparative estimates of fracture risk using different techniques can be derived from cross sectional studies. The aim of this study was to examine and compare the ability of tibial SOS, heel-based QUS and absorptiometric assessments at other skeletal sites to discriminate between patients with and without prevalent vertebral fractures. In addition, we wished to determine whether the discrimination could be enhanced by combining absorptiometric assessments at different skeletal sites or by the combined use of absorptiometric and ultrasound methods.

Subjects And Methods

The subjects were women aged at least 75 years of age who were recruited randomly from the local community by letter of invitation to take part in an MRC-funded pilot study of hip fracture risk and prevention. Women were included in this analysis if they had had measurements performed at all skeletal sites at entry to this pilot study and were not on concurrent treatment for malignancy or any treatment which would modify bone density (excluding thiazides). Women who had received bisphosphonate therapy within the previous year were excluded but calcium supplementation up to a maximum of 500 mg daily was allowed. Patients with marked renal failure (serum creatinine > 300mcg/l) , impaired liver function and/or biochemical evidence of osteomalacia were also excluded.

Each woman completed a self-administered and interview-based questionnaire to document past medical and gynaecological history and any history of low-trauma fractures. Standing height was measured using a Harpenden stadiometer and weight was measured using a balance scale. Span was measured from middle finger tip to middle finger tip across the chest using a flexible tape measure. Bone mineral density at the hip was measured using dual energy x-ray absorptiometry (QDR 2000plus, Hologic Inc., Massachusetts) and at the forearm using single energy x-ray absorptiometry (DTX-100, Osteometer, Hoersholm, Denmark). Broadband ultrasound attenuation and speed of sound were measured at the heel of the non dominant limb using the CUBAclinical (McCue Electronics, Compton, Winchester) and speed of sound at the midpoint of the non dominant tibia using the SoundScan 2000 (Myriad Ultrasound Systems Ltd, Rehovot, Israel). Finally, metacarpal cortical index (mCI) was measured at the mid-point of the second right metacarpal on plain radiographs of the hand using the Bonalyser densitometer (Teijin Ltd, Osaka, Japan).

In all women, lateral thoracic and lumbar spine radiographs were obtained under standardised conditions and examined for prevalent vertebral deformities using a semi-automated technique described previously (McCloskey et al 1993). Briefly, the anterior(A), middle(M), and posterior(P) vertebral heights of each vertebra from T4 to L5 were measured on a digitising tablet, and used to derive A/P, M/P and P/PP (where PP is the posterior height predicted from the adjacent vertebrae between these heights and those predicted from the adjacent vertebrae). These ratios were used to characterise the presence or absence of deformity using a threshold level of three standard deviations and the requirement of two criteria to be fulfilled for each type of deformity.

Statistical Method

Differences in mean values of the measurements in the populations with and without fracture were tested using analysis of variance. The correlations between the measurements and between anthropomorphic indices were derived using simple regression analysis in the whole sample and also in the women with and without vertebral fracture.

In order to examine the relationship between each assessment and prevalent vertebral deformity, the gradient of risk for prevalent vertebral deformity for each standard deviation (SD) decrement in each measurement was computed as an odds ratio using logistic regression analysis. For the purposes of this analysis, all values were expressed as SD units from the mean value in the women without prevalent deformity using the SD from the same population. The analysis was carried out prior to and following adjustment for age and weight. Finally, combined models were tested using forward conditional logistic regression to examine the hypothesis that a combination of measurements or of two sites could improve the gradient of risk.

Results

Prevalent vertebral deformities were identified in 72 of the women (21.9%). The women with deformities were marginally but significantly older and were of lower weight than women without vertebral deformity (Table 1). As might be expected, the women with deformity were shorter. Span was not significantly different between groups, suggesting that the difference in height was a result of vertebral fracture rather than a pre-existing constitutional difference (Table 1).

	NO FRACTURE n=257	FRACTURE n=72	P value
Age (years)	79.72±4.03	80.99± 5.11	0.03
Height (cm)	155.48± 5.84	153.28 ±6.49	0.006
Weight (kg)	63.79±10.48	59.83±11.08	0.008
Span (cm)	157.63±7.58	156.99± 7.01	NS
BMI (kg/m^2)	26.41±4.23	25.42±4.08	NS
Hip BMD (g/cm^2)	0.74 ± 0.12	0.63± 0.16	0.001
Tibial SOS (m/s)	3791 ± 152	3689 ± 176	0.001
Forearm BMD (g/cm^2)	0.330 ± 0.07	0.300 ± 0.07	0.001
Heel BUA (dB/MHz)	53.5 ± 15.7	46.72± 16.58	0.002
Heel SOS (m/s)	1537± 33	1526 ± 29	0.003
MCI	0.37 ± 0.06	0.34 ± 0.06	0.002

Table 11.1 Demographic and measurement details of women with and without prevalent vertebral fracture (means ± SD). P values relate to significance of differences between the two groups.

Regardless of the site of assessment, mean values for women with vertebral deformity were significantly lower than for those women without deformity. This was true for all of the absorptiometric, ultrasound and radiogrammetric techniques used in the study (Table 11.1). The differences between the women with fracture and controls, when expressed as a percentage, were greatest for hip BMD (14.8%) and lowest for tibial SOS (2.7%) but this largely reflects the marked differences in the magnitudes of the various mean values. When the differences were expressed as standard deviation units, the apparent differences between the groups were more uniform with hip BMD still showing the largest reduction in women with vertebral fracture (-0.91). In contrast to the percentage difference, tibial SOS (-0.67) showed a comparable value to hip BMD with the lowest values being observed with MCI (-0.50).

Correlation with age, height and weight and between sites

All measurements apart from heel SOS showed weak but significant negative correlations with age (r values -0.1 to -0.3)(Table 2). When expressed as annual rates of change (%), the rate of bone loss varied from 0.05% per year for heel SOS to 0.6% per year for total hip BMD, forearm BMD, heel BUA and mCI. For all measurements apart from tibial SOS the correlations and apparent rates of loss with age were similar in the women with and without deformity. In contrast, the apparent rate of decrease in tibial SOS with age was significantly greater in the subjects with vertebral deformity than in those without by a factor of fourfold. Body weight correlated significantly with total hip BMD, forearm BMD and heel BUA but the correlation was less marked with other measurements (Table 2).

	Hip BMD	Forearm BMD	Heel BUA	Heel SOS		Tibial SOS
Age	-0.22**	-0.21**	-0.18**	-0.11	-0.31**	-0.15*
Height	0.26**	0.22**	0.18**	0.09	0.11*	0.19**
Weight	0.46**	0.34**	0.31**	0.15*	0.17*	0.13*

* p<0.05, ** p<0.002

Table 11.2 Pearson correlations of the measurements with age, height and weight.

The correlations were similar in women with and without vertebral fracture. There were significant correlations between measurements at all skeletal sites in the whole group of 329 women (Table 3), with coefficients ranging from r=0.31 (mCI vs. tibSOS) to r=0.64 (Heel BUA and SOS)(Table 3). The correlations were similar in women with and without prevalent vertebral deformity suggesting that the pattern of bone loss in women with vertebral deformity is similar at all measured sites.

	Hip BMD	Forear	Heel SOS	Heel BUA	MCI	Tibial
Hip BMD	-					
Forearm	0.58	-				
Heel SOS	0.51	0.51	-			
Heel BUA	0.46	0.44	0.64	-		
MCI	0.41	0.58	0.37	0.35	-	
Tibial	0.44	0.47	0.35	0.38	0.31	-

Table 11.3 Inter-site and inter-method correlation coefficients in all 329 women. All correlations are highly significant (p<0.001).

Gradient of risk for vertebral deformity

The gradients of risk for prevalent vertebral deformity expressed as odds-ratios are shown in Table 4. Hip BMD was the strongest predictor of prevalent fracture with an odds-ratio of 2.23 (95%CI 1.61 - 3.07) following adjustment for age and weight. However, decreases in all of the other measurements were also significantly associated with fracture risk with tibial SOS showing the second highest odds-ratio (1.75, 95%CI 1.34 - 2.30) and MCI the lowest (1.38, 95%CI 1.03 - 1.86)

To examine whether combinations of measurements could further enhance the gradient of risk for vertebral fracture, the forward conditional logistic regression analysis was repeated following further adjustment for hip BMD (Table 4). Decreases in Tibial SOS continued to be significantly associated with fracture following adjustment for hip BMD (1.38, 95%CI 1.02 - 1.87) but all of the other measurements were no longer associated with fracture risk with odds-ratios ranging from 1.01 to 1.05 and the 95%CI encompassing 1.0 (Table 4).

	OR (95% CI)[a]	OR (95% CI)[b]
Hip BMD	2.23 (1.61 - 3.07)	-
Tibial SOS	1.75 (1.34 - 2.30)	1.38 (1.02 - 1.87)
Forearm BMD	1.51 (1.12 - 2.03)	1.01 (0.71 - 1.44)
Heel SOS	1.42 (1.05 - 1.92)	1.04 (0.74 - 1.47)
Heel BUA	1.40 (1.05 - 1.86)	1.03 (0.74 - 1.42)
MCI	1.38 (1.03 - 1.86)	1.05 (0.76 - 1.44)

Table 11.4 Gradient of risk for prevalent vertebral deformities expressed as odds-ratios for a 1 standard deviation decrease in measurements at each site.
[a] Adjusted for age and weight. [b] Adjusted for age, weight and hip BMD.

Discussion and Conclusions

There has been a marked increase in the availability of ultrasound-based devices to assess fracture risk. The greatest experience has been obtained with water-based systems that measure the speed of sound or broadband ultrasound attenuation of the heel. A number of prospective studies indicate that such measurements may predict fracture risk, though with less sensitivity than that obtained by dual energy x-ray absorptiometry (DXA). This study compared DXA , QUS at the heel and tibial UV in a cross-sectional study of elderly women categorised according to the presence or absence of vertebral fractures.

The principal finding in this study is that all measurements, regardless of technique or site, were significantly lower in women with vertebral fractures than in those without. This concords with the systemic nature of bone loss in osteoporotic, elderly individuals. Spine BMD was not measured in this study due to the high prevalence of osteoarthritic changes in such an elderly group and the adverse influence these would have on BMD. The gradient of risk or odds-ratio is an indicator of the specificity of each technique for prevalent vertebral fractures and appears to be greatest for bone mineral density assessments at the hip. However, there was no significant difference between the odds ratios obtained by hip BMD and others.

There remains a great deal of uncertainty as to whether ultrasound assessment can add value to the assessment of BMD alone (Hans 1997, Bauer 1997). In vitro studies using mechanical testing of bone specimens suggest that BMD can account for between 60-80% of bone strength (Cheng 1995, Moro 1995, Tabensky 1996) with the remainder related to other material and structural properties of bone. The findings of this study suggest that there is little or nothing to be gained in predicting vertebral fractures by combining hip BMD with measurements of BMC at the forearm or BUA and SOS at the heel. In contrast, the data suggest that a combination of hip BMD and tibial UV may be of value, but it is unclear if the small increment in specificity will be of clinical value. It will be necessary to determine whether this combination can prove of benefit in longitudinal studies of vertebral fracture risk and to determine its association with fractures at other sites, most notably the hip.

The main limitation of this study is the cross sectional design, but the gradients of risk observed are comparable to those seen in prospective studies (Cummings 1993). It is important to remember that in studies of discriminant ability as a surrogate of predictive ability, measurements are obtained after the occurrence of the particular event in question. It is therefore necessary to assume that the event itself either does not influence subsequent measurements or , in comparative studies, affects all subsequent measurements equally. Low bone mass observed in fracture cases has been proposed to be an epi-phenomenon (Heaney 1992) and it is of interest that the apparent decrease in tibial UV with age is more marked in women with prevalent vertebral fractures. If true, this may have contributed to the ability of tibial UV to discriminate between fracture and non-fracture cases. This observation needs confirmation and further investigation in other study populations.

In conclusion, hip bone density and tibial UV appear to have the highest predictive value for prevalent vertebral fracture in elderly women. These data support the use of a single site, single technique measurement but the possible combination of hip bone density and tibial UV requires further evaluation.

Chapter 12

Prospective Data

Chapter 12

Prospective Data

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Introduction

The final verification of the performance of tUV can only come with prospective study. The data presented thus far suggest strongly that the technique is capable of assessing risk of fracture, and may be able to do so independently of BMD. It has not been demonstrated that tUV is responsive to changes in bone due to treatment of osteoporosis. Nor has it been demonstrated whether tUV is a sufficiently sensitive measure to detect age related decline in bone quality in an individual over a clinically relevant timescale. Both of these are fundamental to the range of clinical uses to which the measurement could be applied. As part of a prospective therapeutic intervention study, 143 subjects were available to help answer these questions.

Subjects and Methods

Subjects

143 subjects were recruited from patients attending the osteoporosis clinic at the WHO collaborating centre in Sheffield. The primary aim of the study was to assess the efficacy of the bisphosphonate clodronate in the treatment of symptomatic osteoporosis. Specifically, the primary end point was the incidence of new osteoporotic vertebral fracture. Secondary end points were the responses of a variety of densitometric and ultrasonic measures to treatment or placebo. Men and non fertile women were eligible to enter. The protocol was approved by the local Research Ethics Committee.

Inclusion criteria for women were either an osteoporotic vertebral crush fracture, or a BMD at the hip which was >2.5 standard deviations below the reference mean for young healthy adult women (WHO threshold for diagnosis). For men, inclusion criteria were either an osteoporotic vertebral crush fracture, or a BMD at the hip >3 standard deviations below the reference mean for young healthy adult males. Subjects were excluded on the following criteria: those receiving treatment for a malignancy; those currently taking medication likely to influence skeletal metabolism or the interpretation of results (i.e. >500 mg daily calcium supplements, oestrogens, progesterones, calcitonin, anabolic steroids and bisphosphonates); those who had received bisphosphonates within the year prior to enrolment; those with known malabsorption. The following criteria applied to baseline blood test results were used for post hoc exclusion: creatinine >300μmol/l; total white cell count <2 x 10^9/l; serum calcium adjusted for albumin <2.12 mmol/l with phosphate <0.7 mmol/l (suggesting hypovitaminosis D); non-parathyroid hypercalcaemia (serum calcium adjusted for albumin > 2.65 mmol/l); alanine amino transferase (ALT) > 64 U/l; serum alkaline phosphatase activity (S-ALP) >674 U/l with normal hepatic function, suggesting underlying bone disease.

Methods

Subjects were randomised to receive either clodronate as a single daily oral dose of two 400mg capsules (Bonefos ®, Leiras Oy, Finland) or an identical placebo to be taken at least 2 hours before breakfast and calcium containing liquids. Randomisation was administered by the company Leiras Oy, and the investigators were blinded to the allocation. Drug supply was also administered by the company who supplied stock to the hospital pharmacy prepackaged for each subject. All subjects had a daily calcium supplement of 500 mg.

All subjects had measurement of the BUA and Speed of Sound (SOS) of the os calcis of the non-dominant leg using the CubaClinical (McCue Electronics). This device uses a calliper mounted transducer pair, producing a broadband signal centred at 1MHz. Gel coupling is used rather than a water-bath.
Measurement was also made of spine BMD, hip BMD (total and subregions) at the non-dominant hip measured using dual energy X-ray absorptiometry (QDR2000+, Hologic Inc., Waltham, USA) and tUV at the mid-point of the non-dominant tibia (SoundScan2000, Myriad Ultrasound Ltd, Rehovot, Israel). Serum calcium (adjusted for albumin), serum phosphate, blood counts, liver enzymes and serum creatinine were followed for safety. Assessments were made at entry and at 6, 12, 24 and 36 months. Lateral spine radiographs were assessed for vertebral fracture at entry and annually. The subjects were stratified into 3 groups. Group 1 consisted of post menopausal women without a secondary cause of osteoporosis. Group 2 consisted of non fertile women with

a secondary cause of osteoporosis. Group 3 consisted of men with any cause of osteoporosis.

Results

The one-year results have been published (McCloskey et al 2001). Results beyond this await peer-reviewed publication. The trial is ongoing, and all data remains the property of the WHO collaborating centre in Sheffield. Only the data immediately relevant to the assessment of tibial ultrasound velocity, and the data from the one year interim analysis is available to me. For this reason, many interesting questions cannot be answered, although in due course the study results will enter the public domain.

ANOVA was used to compare tUV measurements at each time point, and for each subgroup, as well as for the entire group. No statistically significant differences were found at any time point, for any subgroup, nor for the entire group. No effect was found for either those randomised to clodronate nor for those receiving placebo.

This is displayed in figures 12.1 to 12.3.

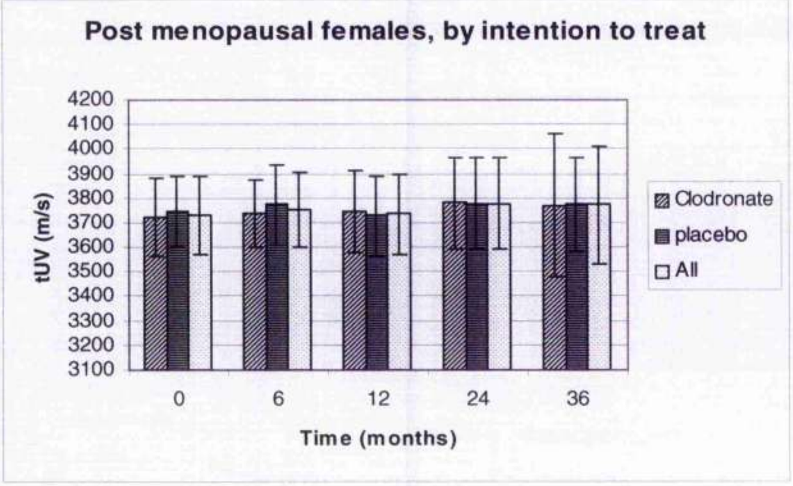

Figure 12.1 tUV during follow up for strata 1 Placebo and clodronate, Mean and SD

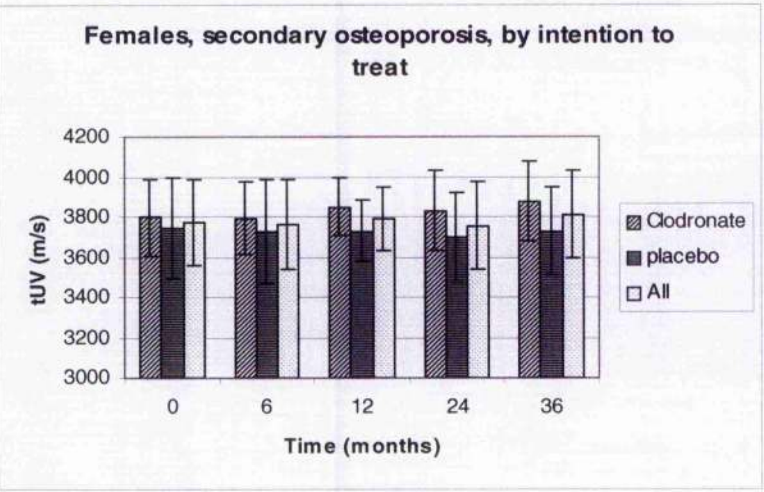

Figure 12.2 tUV during follow up strata 2 placebo and clodronate, Mean and SD

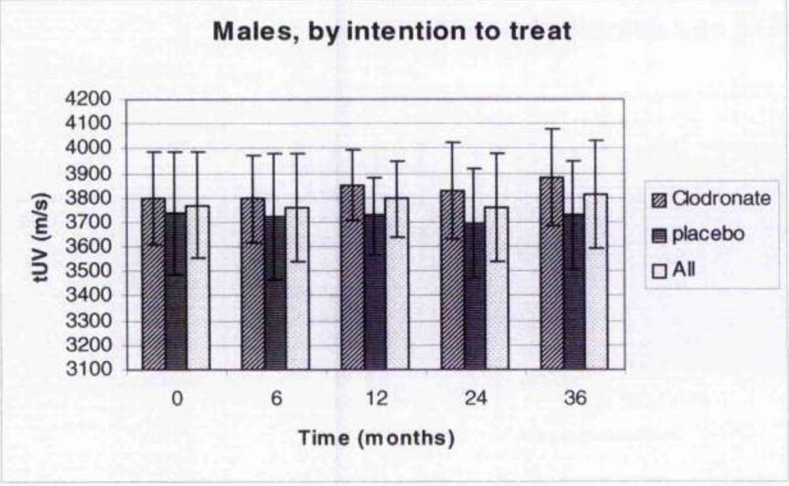

Figure 12.3 tUV during follow up for strata 3 placebo and clodronate, Mean and SD

For other measurement techniques, the observations were only available up to one year of follow up. The mean values are tabulated (12.1) below for the placebo and clodronate groups, expressed as a percentage change from the mean values at entry.

	BMD Hip	BMD Spine	Os Calcis BUA	Os Calcis SOS	Tibial UV
Placebo	-0.4± 0.3	+0.5± 0.3	+0.2± 1.2	+0.4± 0.4	-0.1± 0.3
Clodronate	+1.3± 0.3	+3.2± 0.3	+3.4± 1.2	+0.8± 0.4	+0.4± 0.3

Table 12.1 % Change at 1 year in placebo and clodronate groups mean $\bar{S}D$ (*P<0.05)

Discussion and Conclusion

It is clear that tUV has not been able to detect any changes during the follow up of this study group, even after 3 years. It is not clear why this should be so. It is possible that there were no changes. BMD measures at both hip and spine for those randomised to placebo also showed no significant change over one year (0.4%±0.3, 0.5%±0.3 respectively). In addition, other ultrasound measures (Os Calcis BUA and SOS) demonstrated no significant changes in the placebo group at one year. Further follow up will demonstrate whether these observations remain true. However, it is plausible to suggest that those subjects allocated placebo in this study did not experience any skeletal change during the period of observation. The calcium supplement may have influenced this.

By contrast, those receiving clodronate have shown significant increases in BMD at both the spine and the hip. Os Calcis BUA increased, and this trend was close to statistical significance (P=0.075). Neither ultrasound velocity at the Os Calcis, nor at the Tibia had a significant change. This suggests that clodronate specifically, and possibly bisphosphonates in general do not alter bone in a way that influences ultrasound transmission velocity. As bisphosphonates act by inhibition of osteoclast activity, it is hypothesised that they act to reduce the risk of osteoporotic fracture by prevention of further loss of bone mass. Whilst the osteoblastic completion of active remodelling cycles allows resorption pits to fill in, the prevention of further remodelling stops new bone loss. This adequately explains the observed increase in BMD. Ultrasound velocity might be influenced by the change in porosity. However, there will be no alterations in the Haversian (osteonal) architecture, the material density has not changed, cortical thickness has not changed, and the discontinuities due to accumulated fatigue can only increase once remodelling has ceased. Thus, it may be hypothesised that there is no reason to expect a measurable change in ultrasound velocity in response to bisphosphonate treatment.

It may be that the rate of change in the cortical bone of the tibia is much slower than in the cancellous bone of the hip or spine. As the rate of remodelling in cancellous bone is 10 times greater than that in cortical bone (Kanis 1994), one might expect that the actual rate of change in the bone in response to age or treatment should also be greater. This does not explain the lack of change in Os Calcis SOS.

The reproducibility of tUV may be too low to identify a trend over the 3 year time period. With further follow up a trend may become more apparent. The cross sectional data from normal volunteers would lead us to expect a rate of decline of up to 9 ms-1 /year, equivalent to 27ms-1 over a 3 year period. Even a 1% measurement error would obscure this.

In summary, tUV does not change rapidly enough to allow it to be used to follow the natural history of osteoporosis over a three-year period. In addition, tUV does not show any potential for assessing skeletal response to bisphosphonate treatment of osteoporosis, either in a trial or in a clinical setting.

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Review of Synchronous Literature

There have been four areas of research interest over the past few years.

Because of the absence of ionising radiation, the Soundscan has been used to assess bone quality in children and adolescents both with and without disease states.

It has also been used and assessed in adults, both to accumulate normal data, and to measure correlations with bone densitometry techniques.

It has been used to measure the progression of fracture healing.

It has been investigated to further elucidate which features of bone are associated with variation in ultrasound velocity.

Children and Adolescents

Falk (2000) studied the effects of exercise in two groups of 30 nine and ten year old boys. Two different exercise programs were used for an 8 month period. Although tUV rose, it did so by the same amount in each group. There was no control group, and so the change was not demonstrably for any reason other than growth.
Kaga (2002) examined a cohort of 862 male and 827 female normal Japanese children during growth. It was confirmed that tUV increases rapidly during growth, and is reasonably correlated with height, weight and tibial length.
Lequin (2000 and 2001) has examined 309 female and 287 male normal Dutch children. A strong correlation between age and tUV was confirmed for both males and females. The majority of the same cohort also had phalangeal radiogrammetric absorptiometry with an aluminium reference wedge. A moderately strong correlation was found between age and absorptiometry, and between tUV and absorptiometry. Lequin (2003) assessed a cohort of 25 male and 12 female patients who had acute lymphoblastic leukaemia. Over a three

year follow up, and largely within the first 6 months, a reduction in tUV was observed. It was concluded that ultrasound velocity was sensitive to disease states. No other objective measure of bone was made, and so it is not clear whether there was a change in bone (mineralisation, architecture, porosity), or in subcutaneous soft tissue.

Njeh (2000) has examined 22 caucasian children with juvenile idiopathic arthritis. Spine BMD and tibial UV were measured. Moderately good correlation was observed between these two measures. BMD was strongly correlated to age and weight. It was concluded that Ultrasound Velocity may prove useful in this population group.

Thus, it can be seen that uses in children have been practically limited to the production of normal data ranges. Whilst there have been observed variations in tUV related to health, it is unclear whether age, weight and growth represent such strong confounding factors that diagnostic value will be lost.

Adult Populations

Kim (2000) and Miyatake (2002) have examined tUV in adult populations. Miyake assessed 1176 normal Japanese subjects, of whom 804 were female. A very low coefficient of variation was established. Percentage body fat was inversely related to tUV. Age was negatively correlated to tUV. Kim assessed the tUV and BMD of hip and spine of 106 Korean women. Kappa values were calculated to assess agreement of categorisation as osteoporotic by tUV and BMD. A kappa of 0.35 between BMD spine and tUV was found, and a kappa of 0.48 between BMD hip and tUV was found. No gold standard such as fracture during follow up was assessed.

It can be seen that tUV cannot be used as a substitute for BMD measurements. These studies have not assessed the meaning or value of tUV as a measure of skeletal health.

Fracture Healing

Njeh (1998) used a plastic model to test the hypothesis that a discontinuity in a material could be identified and quantified by the reduction in tUV. The study showed that this hypothesis described the observed behaviour well.

Human studies are now required to assess the feasibility of using a limb which may be unstable due to a fracture. It will also be interesting to see if there is a threshold which determines fracture union, or whether initial healing is matched with a partial return of normal velocities which only corrects as remodelling is completed. Finally, the effect of surgical intervention on expected measurements needs assessment.

Basic Science

Finally two studies have examined the effect of specific aspects of bone on variation of tUV. One of these (Pande 2000) is the subject of Chapter 9 and will not be further discussed. The other (Prevrhal 2001) examined 22 normal and 23 postmenopausal osteoporotic women. Tibial UV was measured, as was a same site qCT. BMD was measured at the spine and the hip. The hypothesis tested was that cortical bone thickness and mineral content of the tibia would correlate most strongly with tUV and thus explain variation in tUV. There was in fact only modest correlation found between qCT measures and tUV ($r=0.67$ and $r=0.53$). This was barely a better correlation than that between tUV and BMD at the spine and the hip ($r=0.5$ for both).

It has been shown that many aspects of skeletal status may contribute to tUV, and these published studies suggest that both architecture and cortical thickness and cortical mineral content are involved. A complete understanding remains elusive.

Future Directions

Prospective clinical studies are required to clarify the sensitivity of tUV in predicting individuals at high risk of fracture. 3 year studies have now shown that tUV is not sensitive to changes in that time period related to osteoporosis or its treatment. It may be better with more rapidly progressive disease states in a short time period, or with osteoporosis over a much longer time scale. Again, only large prospective studies can answer these questions.

Use in paediatric and adolescent populations is a promising avenue. As previously stated, it is not clear whether the effects of growth will overwhelm those of disease. It is also not clear whether there are such populations at risk of fracture who could be usefully given preventative treatment. It may be that the diagnosis itself of a disease such as osteogenesis imperfecta is sufficient reason to start and maintain a treatment regime. Further studies will no doubt examine this.

Fracture management remains a difficult area. The certain declaration of fracture union is elusive and continues to be a decision based on pain, clinical stability, and radiographic findings. This is partly because it is the measurement of a process rather than an event. Previous ultrasound techniques have lacked reproducibility. The Soundscan has answered one problem, but others remain. A no radiation test for union is an attractive idea, but much further study will be needed.

A basic understanding of what ultrasound is measuring is still elusive. We do know that it rapidly changes during childhood growth, and changes slowly in old age. It is moderately correlated with BMD, and yet under certain conditions such as Paget's disease it is completely independent of BMD. It is affected by parathyroid disease and fracture but not necessarily by glucocorticoids or bisphosphonate drugs.

One expects that in vitro study of mammalian or human bone could elucidate the relationship with osteonal area, extent of fatigue damage, ash weight, cortical modulus and subregional density. Not least, it may be possible to explain the wide variations in ultrasound velocity of adjacent regions of the same bone which is observed in vitro and in vivo.

There remains much work to be done therefore before it is possible to say what ultrasound velocity can tell us about the skeleton, and whether it has a practical medical use.

As a final note, although the Myriad company gained FDA approval for diagnostic use, it failed to make a commercial success. The company has since ceased manufacture.

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Appendix

Glossary

Anisotropic- Having different material properties when stressed in different planes

Cortex- The compact lamellar bone which comprises the hard outer cylinder of mammalian long bones

Diaphysis- The mid shaft of a long bone- mainly cortical

Fluorosis- A bone disorder caused by excess dietary or medicinal fluoride. Radiologically sclerotic bone is formed.

Haversian- The compact bone architecture which is typically arranged in contiguous concentric layers around longitudinally oriented blood vessels. Characterises cortical bone. (After Havers)

Medulla- The spongy lamellar bone which forms the inner part of bone

Metaphysis- The end of a long bone – mainly cancellous

Modulus- A constant describing of the rigidity of a material calculated as stress/strain (Pa)

Osteomalacia- Bone fragility condition characterised by impaired mineralisation of osteoid

Osteon- A single Haversian unit. The basic remodelling mechanism in bone.

Osteoporosis-

The final common pathway of several conditions which is typified by bonc fragility, loss of bone mass, and uncoupling of apposition and resorption of bone in the remodelling cycle. Mineralisation should be normal.

Plastic Deformation-

Permanent change in shape brought about by stress

Strain- The deformation of a material caused by a load and expressed as ≡dimension/original dimension strain units

Stress- A deforming force or load (N/m2, or Pa)

Trabecular- The open network architecture which characterises medullary bone

Woven bone-

Bone typically seen in early fracture healing. The bone is laid down with random collagen orientation.

Yield strength-

In materials testing, the point at which deformation becomes irreversible (plastic)

List of Abbreviations

BMC	-Bone Mineral Content
BMD	-Bone Mineral Density
BUA	-Broadband Ultrasound Attenuation
Cm	-Centimetre
CV	-Coefficient of Variation
DPA	-Dual Energy Photon Absorptiometry
DXA	-Dual Energy X ray Absorptiometry
GPa	-GigaPascals
KHz	-KiloHertz
M	-Metre
MHz	-MegaHertz
Mm	-Millimetre
PTH	-Parathyroid Hormone
QCT	-Quantitative Computerised Tomography
R	-Correlation Coefficient
SPA	-Single energy Photon Absorptiometry
TUV	-Tibial Ultrasound Velocity
UV	-Ultrasound Velocity

Appendix – Data Sheet

Technical Information

JOTUN

Jotun Polymer

NORPOL 34-50

SOLD BY SCOTT BADER AS "RESIN C"

DESCRIPTION

Norpol 34-50 is a water clear speciality polyester specifically developed for all casting applications including buttons, encapsulation and clear and pigmented castings. The resin contains a trace level of Cobalt which is the minimum quantity required for curing and thus enables the excellent initial colour of the resin to be preserved. The exothermic heat of curing is developed over a longer period than with other resins, which reduces thermal shock and enables larger castings to be made.

PHYSICAL DATA IN LIQUID STATE AT 23oC

Properties	Value	Units	Test Method
Viscosity ICI Cone and Plate	280-330	cps	BS 2782: Part 7: Method 730B: 1993
Density	1.12	g/cm³	BS 3900: Part A12: 1975 (1991)
Acid Value	< 25	mg KOH/g	BS 2782: Part 4: Method 432B: 1976
Monomer	Styrene		--------------------------------
Monomer Content	35	% Weight	--------------------------------
Flash Point	31.5	oC	BS 3900: Part A9: 1986
Geltime: 2% Butanox M50	32-38	minutes	BS 2782: Part 8: Method 835C: 1980
Stability at 20oC from date of manufacture	6	months	--------------------------------

In common with other preaccelerated polyesters gel time drift occurs on storage. To compensate for this more catalyst may be required.

Curing Data

Formula No.	Catalyst MEKP 50%	Additional Accelerator 1 % Cobalt	Geltime at 20°C	Colour of Cured Castings
1	1.0%	None	90 mins	Water White
2	2.0%	None	50 mins	Water White
3	2.0%	0.1%	30 mins	Water White
4	1.0%	0.4%	15 mins	V. Pale Pink
5	1.0%	1.0%	8 mins	Pale Pink
6	0.5%	0.5%	22 mins	V. Pale Pink
7	0.5%	1.0%	11 mins	Pale Mauve

MECHANICAL DATA IN THE CURED STATE

Properties	Value	Units	Test Method
Tensile Strength	42	N/mm²	BS 2782: Part 3: Method 320C: 1976
Tensile Elongation	2	%	BS 2782: Part 3: Method 320C: 1976
Flexural Strength	92	N/mm²	BS 2782: Part 3: Method 335A: 1978
Flexural Modulus	2540	N/mm²	BS 2782: Part 3: Method 335A: 1978
Volume Shrinkage	6	%	BS 2782: Part 6: Method 644A: 1986
Heat Distortion Temp	50	oC	BS 2782: Part 1: Method 121A: 1991
Barcol Hardness	30-35	934-1	BS 2782: Part 10:Method 1001: 1989
Water Absorption - 7 day	45	mg	BS 2782: Part 4: Method 430A: 1983

Appendix – Criteria for "Unhealthy"

1. Current or previous fractures.
2. Receiving treatment for concurrent malignancy.
3. Medication influencing bone: (high dose calcium, oestrogens, progestins, calcitonin, anabolic steroids or bisphosphonates).
4. Malabsorptive states: (coeliac disease, Crohn's disease, etc).
5. Significantly impaired renal function: (serum creatinine >0.3mmol/l).
6. Leucopaenia: (white cell count $<2*10^9/l$).
7. Hypocalcaemia, hypophosphataemia or non parathyroid hypercalcaemia.
8. Impairment of hepatic function (Alanine Transaminase >2* upper limit).
9. Elevation of serum alkaline phosphatase activity and normal hepatic function suggesting metabolic bone disease: (ALP >2* upper limit).