The use of quantitative ultrasound to investigate bone strength in the facial skeleton

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Declaration

I hereby declare that this is entirely my own work and that it has not been submitted as an exercise for a degree at this or any other University. I agree that the Library may lend or copy this thesis on request.

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Summary

Study background

Osteoporosis is the most common metabolic bone disease in the world, and is one of the leading causes of fragility fractures in elderly people, resulting in a number of comorbidities and loss of mobility. It is diagnosed by the use of Dual Energy X-Ray Absorptiometry (DXA), which is considered the gold standard in fragility fracture risk prediction. DXA scan provides a result based on bone mineral density (BMD). Quantitative ultrasound (QUS) is another method for detecting osteoporosis, which utilises propagation of an ultrasound wave through the tissue, providing a measurement based on signal attenuation and speed. Quantitative ultrasound of the calcaneus (heel bone) has been shown in a number of studies to have a similar ability to DXA scan to diagnose osteoporosis. More recently, multisite QUS (mQUS) has been developed for use on multiple peripheral sites throughout the body, including the metatarsal, radius, phalanx, and tibia. This device (Omnisense 8000, BeamMed, Israel) generates a Speed of Sound (SOS) reading, which correlates to bone strength. A recently published study has shown that the use of multisite QUS on the mandible can differentiate between osteoporosis sufferers, and healthy individuals. This study aims to investigate whether there are other facial sites that show this change, and determine if facial bone QUS can detect change in response to osteoporosis treatment.

Materials and methods

Two groups of subjects were recruited for this study: healthy premenopausal women, and women with DXA confirmed osteoporosis. Subjects were excluded from the control group if they had any significant risk factors for osteoporosis or a history of fracture not due to a high impact trauma. SOS readings were taken from the parasymphysis of the mandible, the frontal bone, and the zygomatic arch. To

compare results to already mQUS established sites. Readings were also taken from the radius and the metatarsal.

Readings in the osteoporotic group who were undergoing treatment were repeated after one year to determine if there was a response to treatment. Where available, the results were compared to DXA scans, bone biomarkers, and calcaneal ultrasound.

Results

A total of 170 subjects were enrolled in this study: 84 control subjects and 86 osteoporotic subjects. Mean SOS readings for the mandible were found to be 3,504 (SD 221) ms^{-1} for the control group and 3,298 (SD 239) ms^{-1} for the osteoporotic group. This difference was found to be statistically significant after controlling for the effect of age, height, weight, and number of missing teeth (p<0.001). No other significant differences were found between the other two facial sites. The zygomatic arch was found to be an unreliable site to successfully obtain readings. Difficulty in obtaining zygomatic arch readings was found to be significantly related to age (r_{pb} =-0.296, p<0.0001). No significant differences were found at any of the five measured sites in the osteoporotic group between SOS readings taken at recruitment and those taken at after one year.

Conclusions

This study has shown that there are changes in the mandible as a result of osteoporosis. Such changes are detectable by the use of multisite axial quantitative ultrasound device, and this may be utilised routinely in the future as part of a patient work up in a bone health clinic. The results from the zygomatic arch are inconclusive due to a high number of failures to obtain readings. However, the difficulty in obtaining readings increased significantly with age suggesting that there are morphological changes in the zygomatic arch related to age. No response to treatment was seen with SOS readings at any of the measured five sites over a one-year treatment course. However, of the subjects recruited who had a DXA scan at

baseline and the one year mark, no BMD response to treatment was detected either. Out of the five sites investigated in this study, the mandible demonstrated the strongest correlation between changes in SOS and changes in BMD over the observation period. This finding is significant considering that the radius and the metatarsal are two sites already established for use with mQUS, with a reasonable volume of evidence.

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Terms and abbreviations

AHA	Advanced Hip Assessment
ALP	Alkaline Phosphatase
ARONJ	Anti-Resorptive Osteonecrosis of the Jaw
BMD	Bone Mineral Density
BMU	Basic Multicellular Unit
BP	Bisphosphonate
BRONJ	Bisphosphonate Related Osteonecrosis of the Jaw
BSAP	Bone Specific Alkaline Phosphatase
BTM	Bone Turnover Marker
BUA	Broadband Ultrasound Attenuation
CSA	Cross Sectional Area
CSF	Colony Stimulating Factor
CT	Computerised Tomography
CTX-1	Carboxyterminal Cross-Linked Telopeptides of Type I Collagen
DPD	Deoxypyridinoline Crosslinks
DXA	Dual Energy X-Ray Absorptiometry
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GWAS	Genome Wide Association Studies
HAL	Hip Axis Length
HU	Hounsfield Unit
HSA	Hip Structural Analysis
IFCC	International Federation of Clinical Chemistry
IOF	International Osteoporosis Foundation
IQR	Interquartile Range
ISCD	International Society of Clinical Densitometry
MMP	Matrix Metalloproteinases
NSA	Neck Shaft Angle

NICE	National Institute for Health and Clinical Excellence
NTX-1	N-Terminal Cross-Linked Telopeptides of Type I Collagen
OC	Osteocalcin
ONJ	Osteonecrosis of the Jaw
OPG	Osteoprotegerin
PBM	Peak Bone Mass
PTH	Parathyroid Hormone
PYD	Pyridinoline Crosslinks
P1CP	Procollagen Type 1C Propeptide
P1NP	Procollagen Type 1 N Propeptide
qCT	Quantitative Computerised Tomography
QUS	Quantitative Ultrasound
Q1	1st Quartile
Q3	3rd Quartile
RANK(L)	Receptor Activator of Nuclear Kappa-B (Ligand)
rhPTH	Human Recombinant Parathyroid Hormone
SD	Standard Deviation
SI	Stiffness Index
SIGN	Scottish Intercollegiate Guidance Network
SOS	Speed of Sound
SRE	Skeletal Related Event
SSRI	Selective Serotonin Reuptake Inhibitor
TGF	Transforming Growth Factor
TRAP	Tartrate Resistant Acid Phosphatase
WHO	World Health Organisation
±	Standard Deviation

1 Introduction

Osteoporosis is the most common bone disease worldwide and is known to cause more than 9 million fractures a year.[1] Osteoporotic fragility fractures are associated with significant morbidity and are one of the leading causes of elderly patients becoming bed ridden with serious and potentially life threatening complications.[2] It is estimated that in developed countries the lifetime risk of a fracture of the hip, wrist or vertebral fracture is between 30 to 40%.[3] If the current trend of an ageing population continues, the incidence of osteoporosis related fragility fractures is set to significantly increase. It is important that osteoporotic changes in the skeleton are identified in those at risk of fragility fracture.[1]

There are a number of methods of diagnosing and monitoring osteoporosis, and assessing fracture risk. The most established of which is Dual Energy X-Ray Absorptiometry (DXA), which measures Bone Mineral Density (BMD) at the hip and lumbar spine.[4] This is generally regarded as the gold standard, however it is not without its limitations. It is costly, it produces ionizing radiation, and with an increasing elderly population, coupled with the fact that they tend to be performed at referral centres, there will be increased demand that providers, even at present, are not able to meet.

Research has emerged over the last few years of the use of quantitative ultrasound (QUS), which is used on peripheral bone sites, to diagnose osteoporosis. The use of QUS on the calcaneus (heel bone) is the most established form of QUS and has the highest volume of scientific evidence. Currently, it is the only form of QUS recognised by the International Society of Clinical Densitometry (ISCD) in the diagnosis of osteoporosis, however the ISCD still recommend that clinical decisions for the treatment of osteoporosis should be based on DXA.

The Omnisense 8000 (BeamMed Israel) is a multisite axial transmission quantitative ultrasound device that can be used to assess bone strength on multiple sites throughout the body. It features a probe that contains a number of transducers, some of which act as transmitters and others act as receivers. It generates pulsed acoustic waves, which pass through the soft tissue and propagate along the bone. As the distance between the transmitter and receiver are fixed, the speed of the first detectable signal can be measured, and this produces a Speed of Sound (SOS) reading, which reflects the properties of the bone at the site being assessed. There are a number of advantages with this technology: it does not produce ionising radiation, it can be used on multiple sites on the body, and it is portable which is of particular advantage to patients where mobility is an issue. It has been demonstrated in that this device can measure changes in bone in response to osteoporosis treatment.[4]

A study by Beattie *et al* in 2017 conducted in the Dublin Dental University Hospital with the co-operation of the Bone Health Clinic in St James's Hospital under Professor Stassen's supervision demonstrated that the use of this device on the mandible can differentiate between osteoporosis sufferers, and healthy individuals, as well as differentiate between patients with a recent history of fragility fracture and non-fractured individuals.[5] To our knowledge, that is the only in-vivo study that has used a quantitative ultrasound device on the mandible to investigate for osteoporosis.

This study aims to use the Omnisense to investigate other facial bones that may also show these osteoporosis related changes, and to measure any dynamic changes at these facial sites over a period of time on patients receiving either an anti-resorptive or anabolic treatment for osteoporosis. The facial sites investigated in this study are the parasymphysis of the mandible, the zygomatic arch, and the frontal bone.

Aims

- To investigate bone strength of the frontal bone, the zygomatic arch, and the mandible using a multisite axial quantitative ultrasound device.

- To determine whether any measurable QUS response to osteoporosis treatment over one year, can be detected in facial bones using multisite axial quantitative ultrasound.

Objectives

- To determine whether speed of sound measurements taken from the frontal bone, the zygomatic arch, and the mandible can differentiate between a healthy control group, and a DXA confirmed osteoporotic group.

- To assess long and short term precision measurements taken using the ultrasound device at each of the investigated sites.

- To investigate the capability of the ultrasound device to diagnose osteoporosis in other facial bone sites (frontal bone and zygomatic arch) and confirm our earlier findings on its use on the mandible.

- To correlate the facial ultrasound measurements to bone mineral density (BMD) measured by Dual Energy X-Ray Absorptiometry (DXA) scans.

- To compare the results from the three facial sites to the radius and metatarsal which are already established for use with the ultrasound device.

- To determine whether speed of sound measurements from each site can detect changes in response to osteoporosis treatment by comparing readings taken at baseline to those taken after one year of treatment.

2 Literature review

2.1 Bone

Bone is a complex connective tissue, composed of biocomposite materials, constituting the major component of skeletal systems in vertebrate animals.[6] It provides a rigid supportive framework for the body, allowing for protection of internal organs, with joints and attachment points for muscles, tendons and ligaments facilitating movement. It supports the dentition allowing for mastication. Red bone marrow is responsible for haemopoiesis. Yellow marrow stores energy in the form of lipids. Bone acts as a reservoir for growth factors and cytokines, as well as calcium and phosphate, essential for a number of cellular functions throughout the body, in addition to helping maintain acid-base balance.

2.1.1 Structure of bone

The structure of bone can be classified under the following hierarchal levels: - [6]

- Macro-structure (mm–µm): trabecular and cortical bone.
- Micro-structure (10–500µm): osteons and trabeculae.
- Sub-micro-structure (1–10µm): lamellae and single trabecula.
- Nano-structure (100 nm–1µm): fibrillar collagen and embedded mineral.
- Sub-nano-structure (<100nm): molecular structure of mineral, collagen, and non-collagenous organic proteins.

Bone is a two phase biomaterial consisting of an organic matrix that confers ductility and energy absorption ability, combined with a mineral phase providing stiffness.[7] Based on weight, bone is 60% inorganic, 30% organic, and 10 % water. Ninety per cent (by weight) of the organic component of bone consists of Type I collagen. Other

organic components of bone include other types of collagen (Type III and VI), osteocalcin, and osteonectin. The collagen molecules are organised parallel to eachother with a gap of 40nm between each molecule, and it is within these spaces that mineralisation occurs. The hardness of bone is due to its extracellular matrix being impregnated with a mineral phase formed primarily by hydroxyapatite crystals [$Ca_{10}(PO_4)_6OH_2$] combined with other forms of calcium and phosphate salts. The crystals grow parallel to the collagen fibrils, and the structure and orientation of the fibrils controls the size and orientation of the crystals.[7] The dimensions of the embedded hydroxyapatite crystals vary, and are 15nm wide, 2 to 5nm thick and 20 to 50 nm long.

Macroscopically, bone is formed of dense cortical bone surrounding the less dense trabecular bone. Cortical bone can be further divided into woven, and lamellar bone (Fig 2.1). Woven bone is found where there is fast bone growth, such as healing following trauma or during skeletal growth up to 16 years of age. It becomes replaced by lamellar bone. When in the midshaft of a long bone, lamellae are about 10 to 20 mm long, contributing to bone's anisotropic properties.

Haversian bone is organised in concentrically arranged laminae, which are approximately 7µm thick each, surrounding a central lumen approximately 50µm in diameter containing arterioles and venules. There are 10 to 15 lamellae in each Haversian system, otherwise known as an Osteon (Fig 2.1). Each osteon has a diameter of 200µm and a length of 1 to 3mm. In each osteon, no tissue is more than 100µm from its blood supply. There are approximately 15 to 20 Osteons per $1mm^2$ of cortical bone. Volkmann canals are channels that run transverse to the longitudinal axis of the haversian systems between the haversian canals. Like haversian canals, Volkmann canals allow for blood supply and have a similar diameter, however unlike haversian canals they are not surrounded by concentric lamellae.[8]

At the interface between the osteons and the extra-osteon bone matrix, the cement line is found (Fig 2.1). This is an area of reduced mineralisation and it is believed that this provides a ductile interface between the Osteons.[9]

Between the lamellae, the osteocyte containing lacunae are found. The osteocytes display dendritic processes that extend from the cells through extremely small canals 0.5µm wide and 3 to 7 µm long in the bone called Canaliculi, and meet at cellular gap junctions with the processes of other osteocytes. There are roughly 50 to 100 canaliculi per lacuna, and about 1 million per mm^3 of bone.[8] The collagen orientation between adjacent lamellae can be rotated by as much as 90°, increasing resistance to forces from different directions.[10]

The orientation of the Haversian systems is predominantly along the longitudinal axis of the bone, however they are not absolutely parallel to the long axis and are skewed up to 15° increasing fracture resistance.[11]

Trabecular or cancellous bone is composed of struts known as trabeculae (Fig 2.2). There are no vessels within the trabeculae, however there is an abundant blood supply within the spaces around them. Due to its structure, the surface area of trabecular bone is vast, for example, the pelvis has an average periosteal surface area of $80cm^2$ and a trabecular surface area of $1600cm^2$.[12] In trabecular bone the lamellae are less well organised and form a network of rods and plates, which are about 100 to 300 µm thick interspersed with marrow spaces. At peak bone density in youth, the trabecular plates predominate but with increasing age and diminishing bone density, the plates become more rod-like. The inter-trabecular spacing is in the region of 500 to 1500µm. Due to its high surface to volume ratio, trabecular bone is more susceptible than cortical bone to conditions of increased bone resorption and weakness such as osteoporosis.[8]

A feature of bone is its ability to adapt its tissue mass and architecture to the mechanical demand of applied forces; this phenomenon is known as Wolff's Law and was first described in 1892. It states "Each change in the form and function of a bone

or only its function is followed by certain definitive changes in its internal architecture, and secondary changes equally definitive in its external compliance, in accordance with mathematics law".[13]

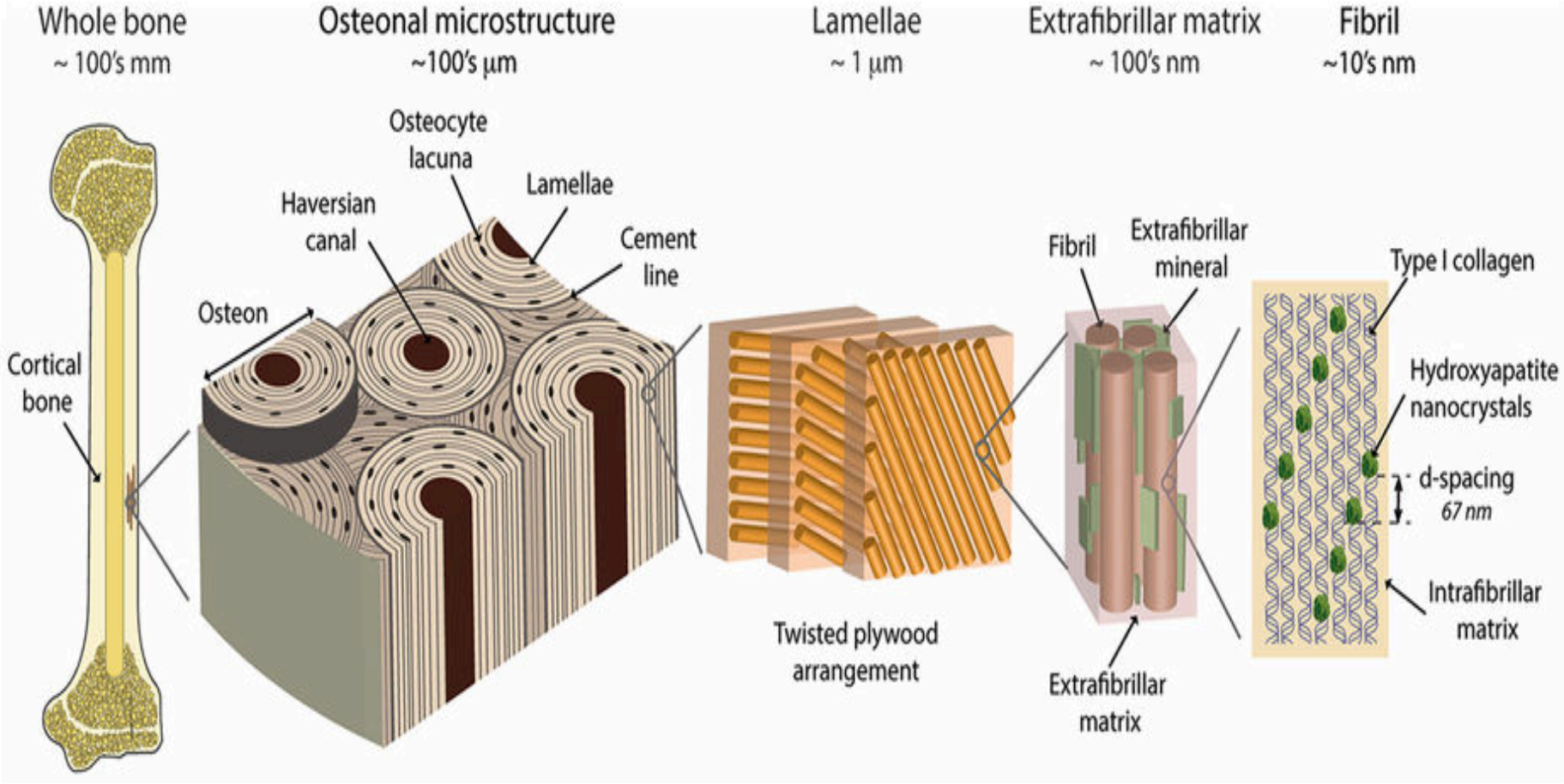


Figure 2. 1 Structure of cortical bone.[14]

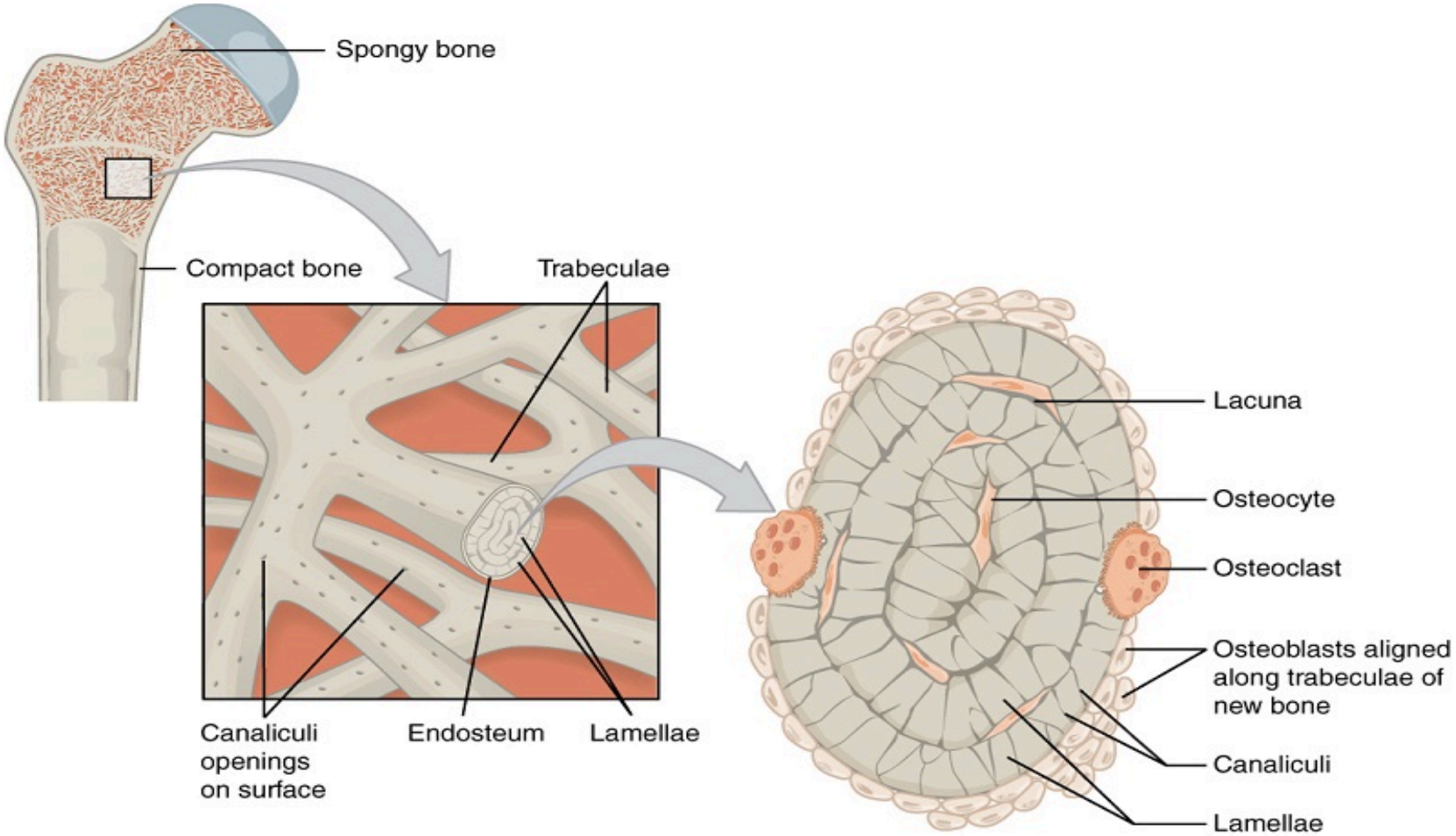


Figure 2. 2 Structure of trabecular bone.

2.1.2 Bone remodelling

Throughout its lifespan, the human skeleton is in a constant state of change due to continuous bone remodelling, and it is believed that 20% of bone tissue is replaced every year.[15] Bone remodelling involves the continuous removal of small pockets of bone in combination with replacement by new bone. This is performed by the action of osteoblasts, osteoclasts and osteocytes under the influence of mechanical and chemical stimuli.[16]

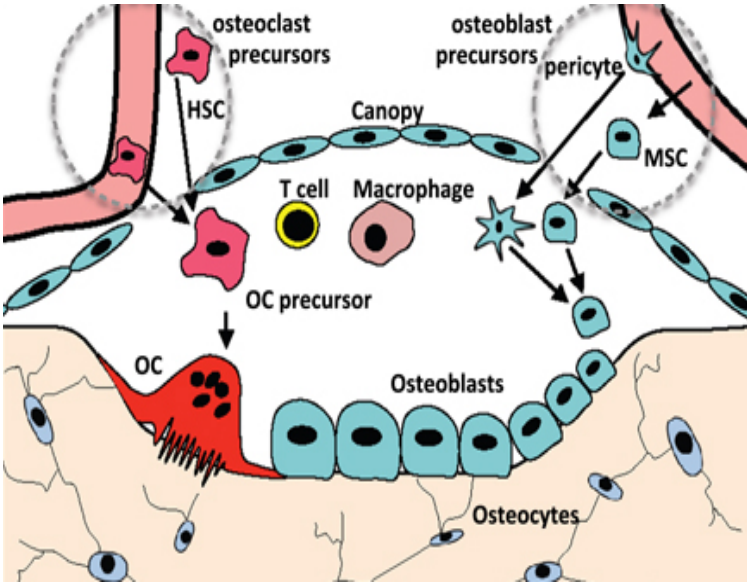
Osteoblasts are derived from mesenchymal stem cells. They are mononucleated cells responsible for deposition of bone matrix and regulation of osteoclasts. They possess a large number of receptors including receptors for Oestrogen and Parathyroid Hormone (PTH).[16]

Osteoclasts are multinucleated cells and are the only cells known have the ability to resorb bone (Fig 2.4). They are derived from mononuclear precursor cells of monocyte-macrophage lineage.[17]

Osteocytes are the most abundant cell type in bone forming 90 to 95% of all bone cells. They communicate with eachother through extensions of plasma membrane and are thought to act as mechanoreceptors, which may be a factor in initiation of remodelling, explaining why bone remodelling occurs as a result of mechanical stresses due to functional loading.[16, 18] Osteocytes are surrounded by a thin layer of extracellular fluid within small cavities known as lacunae. These lacunae, of which there are 25,000 per mm^3, are 5 to 8μm in diameter.

During the remodelling process, cells form a small temporary structure called a Basic Multicellular Unit (BMU) under a cover of cells of uncertain origin referred to as canopy cells (Fig 2.3). This creates a microenvironment that facilitates osteoclastic and osteoblastic activity. A BMU may be described as having a leading front of

osteoclasts, followed by what are referred to as “reversal cells”, and finally osteoblasts in the tail end.[19]



MSC – Mesenchymal Stem Cell

OC – Osteoclast

HSC – Haemopoietic Stem Cell

Figure 2. 3 Basic Multicellular Unit.

The action of remodelling can be broken up into a sequence of phases beginning with the Activation Phase, followed by the Resorption Phase, the Reversal Phase, the Formation Phase, and once bone formation is complete, the Quiescent Phase.

This process begins with detection of an initiator of remodelling, which may take the form of hormone action on the cells or direct mechanical strain resulting in structural damage.[18]

Parathyroid hormone acts as a chemical activator of remodelling and binds to osteoblasts resulting in the release of compounds that recruit osteoclast precursors and induce osteoclast differentiation and activation.[16]

Mechanical strain can cause damage to bone matrix that may result in osteocyte apoptosis. Osteocytes produce Transforming Growth Factor β (TGF-β), which inhibits osteoclast activity. Therefore when apoptosis occurs this osteoclastic inhibition is removed and remodelling is initated.[20]

During the Resorption Phase, in addition to recruitment of osteoclast precursors, there is osteoblast expression of the master osteoclastogenesis cytokines. Colony Stimulating Factor -1 (CSF-1) promotes proliferation and survival of osteoclast precursors. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) promotes proliferation of osteoclast precursors, and co-ordinates their differentiation into osteoclasts. It also promotes resorption activity, and prolongs the life of mature cells. Osteoprotegerin (OPG) is a negative regulator of osteoclastogenesis. The current belief is that RANKL/OPG ratio determines the degree of osteoclast differentiation and function.[21]

Matrix Metalloproteinases (MMPs), and in particular MMP-13, are secreted by osteoblasts. They degrade the unmineralised osteoid that lines the surface of the bone, which exposes adhesion sites allowing for osteoclast adhesion. The osteoclasts then adhere to the surface and create an acidic microenvironment by pumping hydrogen ions into this "Sealed Zone". This results in dissolution of mineralised matrix and produces what are referred to as "Howship's Resorption Lacunae". Low pH collagenolytic enzymes (Cathepsin K, tartrate resistant acid phosphatase, gelatinase, MMP) degrade the remaining organic matrix.[17, 22]

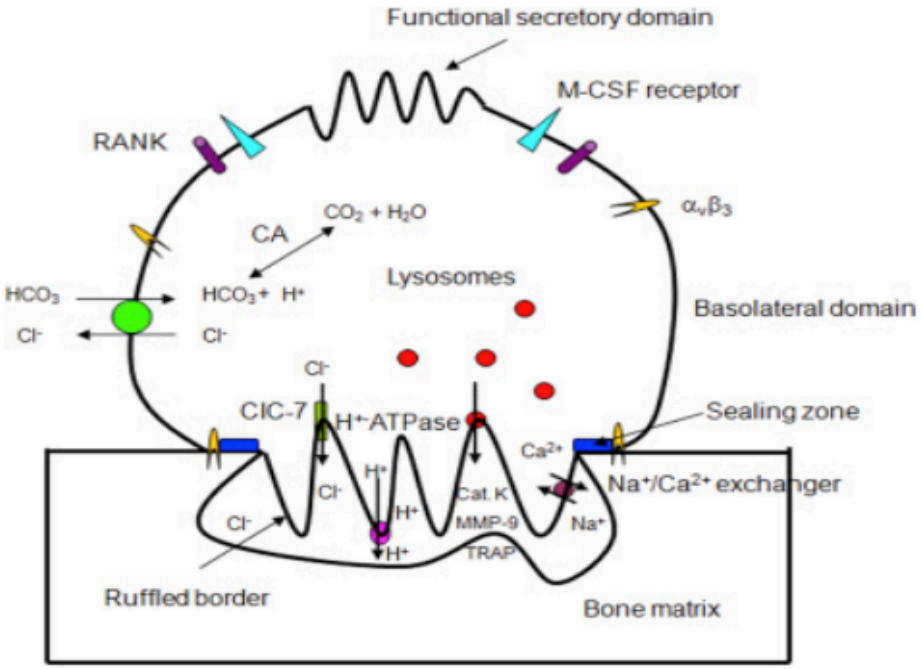


Figure 2. 4 Osteoclast showing ruffled border and resorption lacuna.[23]

The Reversal Phase is the transition phase between the Resorption phase and the Formation phase. At the start of this phase the Howship's lacunae remain covered in undigested collagen. A reversal cell removes these remnants and prepares the surface for the action of the osteoblasts. The origin of these reversal cells is not fully understood and has been the subject of much debate.[24]

In the Formation Phase, osteoid is laid down by osteoblasts. This is made up mainly of Type I collagen, and also contains non-collagenous proteins, including proteoglycans, lipids and osteocalcin. The osteoblasts release small, membrane bound vesicles containing concentrated calcium and phosphate, facilitating mineralisation of the osteoid with formation of hydroxyapatite.[25] Bone formation takes approximately 4 to 6 months.[26] Following remodelling 50 to 70% of the osteoblasts undergo apoptosis, and the remainder either revert to a bone lining phenotype or become embedded in bone and differentiate into osteocytes.[26]

2.1.3 Biomechanical properties of bone

Volume fraction is the ratio of the volume of actual bone tissue to the bulk volume of the specimen, and can be expressed by the following equation, where porosity (P) is expressed as a percentage.

$$\text{Volume Fraction } V_f = 1 - P$$

A major distinction between cortical and trabecular bone are their relative porosities. Cortical bone has a porosity (P) of less than 30%, or a volume fraction greater than 0.70. Porosity of trabecular bone is typically in the region of 50 to 90%. Porosity of human femoral cortical bone can be as low as 5% in a 20 year old, increasing to up to 30% in an 80 year old. In the elderly patient, porosity of vertebral trabecular bone may be as high as 95%.

Tissue density is defined as the ratio of the mass to actual bone volume, and is expressed as ρ_{tiss}. It is typically 1.8 to 2 g/cm^3 for both cortical and trabecular bone.

For tissue density there is little difference between trabecular and cortical bone. Apparent density ρ_{app} is defined as the ratio of the mass of the bone to the bulk volume of the specimen including the volume associated with the vascular channels and higher-level porosity (Haversian and Volkmann canals, and canaliculi are not taken into account).[8, 12]

$$\rho_{app} = \rho_{tiss} \times V_f$$

The apparent density of cortical bone is about 1.85 g/cm^3, with little variation between different skeletal sites. Greater variation between sites is seen in trabecular bone. Spinal trabeculae may be as low as 0.1 g/cm^3, the tibia is approximately 0.3 g/cm^3, and the proximal femur may be as high as 0.6 g/cm^3.[8]

Strain (ε) is the amount of dimensional change a body undergoes relative to its original size when a force is applied. In terms of length it is expressed by $\varepsilon = \Delta L/L$, where L is length. Strain itself is a dimensionless unit. Peak compressive strain in bone may be as high as 3,500 microstrain (με), or 0.35% strain.

Stress is the force per unit area, expressed in N/m^2, otherwise referred to as the Pascal (Pa).

Stiffness is the rigidity of an object, which may be described as the degree to which it will resist deformation when a force is applied. In bone, the relative proportions of collagen and hydroxyapatite, and their orientation determine stiffness.
When a stress is applied to bone and gradually increased, there is a linear increase in strain. This is known as the elastic region on the stress-strain curve, and if the force is relinquished the bone returns to its original size and shape. The slope of the curve at the elastic region is known as the Modulus of Elasticity (*E*), and is defined as:-

$$Modulus\ of\ Elasticity(E) = \frac{Stress(\partial)}{Strain\ (\varepsilon)}$$

A material with a higher stiffness will have a greater slope of the line. Reductions in mineralisation of bone lower the elastic modulus, and reduce the stiffness.[10]

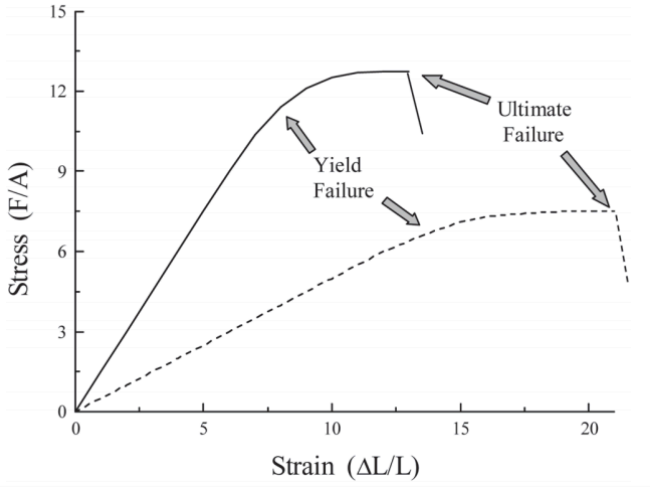


Figure 2. 5 Modulus of elasticity

Yield failure occurs when the load applied overcomes the elastic region and deforms the material to the point where it can no longer return to its original shape. In bone, the yield strain is approximately 6,800 με, and the yield stress is 130 GPa. At this point there are microcracks in the bone and significant disruption of collagen fibrils. Should the stress continue to increase, ultimate failure ensues and the bone will fracture.

Toughness is the amount of energy a material can absorb before fracture occurs. It is defined as the area under the curve up to the point of failure. Loading transmits energy into the bone, and if that energy exceeds what the bone is capable of absorbing, it will fracture.[10]

Bone Mineral Density (BMD) is correlated to strength and stiffness, however there is an inverse relationship between stiffness and ultimate strain. For example, a bone that is highly mineralised, such as in osteopetrosis will be stiffer and more brittle, and therefore may be easier to fracture. Conversely, bone that is poorly mineralised and weak, such as in a child, will be more ductile, resulting in increased work to

failure. The relationship of BMD to bone strength can be described as a U-shaped, meaning that too high or too low BMD can result in increased bone fragility.[27]

A material is classed as isotropic if its mechanical properties are the same in all directions. Bone is an anisotropic biomaterial, meaning its properties change depending on the direction of loading. Long bones have a greater capacity to resist heavy loads longitudinally than transversely across the bone surface. Bone is strongest against compression forces, and is weakest against tension and shear forces. A study by Reilly *et al* in 1975 demonstrated anisotropy in human femoral cortical bone by showing differences between longitudinal, transverse, and shear ultimate stresses, and longitudinal, transverse and shear elasticity.[28]

Ultimate Stresses[28]

Longitudinal	
• Tension	135 (± 15.6) MPa
• Compression	205 (± 17.3) MPa
Transverse	
• Tension	53 (± 10.7) MPa
• Compression	131 (± 20.7) MPa
Shear	65 (± 4.0) MPa

Table 2. 1 Anisotropic stress in bone

Elasticity[28]

Longitudinal modulus	17,900	(± 3,900) MPa
Transverse modulus	10,100	(± 2400) MPa
Shear modulus	3,300	(± 400) MPa
Longitudinal Poisson's Ratio	0.40	(± 0.16) MPa
Transverse Poisson's Ratio	0.62	(± 0.26) MPa

Table 2. 2 Elastic anisotropy in bone

When bone is deformed due to a tensile or compressive force, it becomes longer or shorter along its axis. This is referred to as the primary strain (ε_x). At the same time it becomes thinner or wider perpendicular to the direction of the force, and this is referred to as the secondary strain (ε_y). Poisson's Ratio (*v)* is a measure of the ability of a material or structure to resist deformation in a direction perpendicular to the applied force.[29]

$$\boldsymbol{Poisson's\ Ratio}\ (\boldsymbol{v}) = \frac{\boldsymbol{Primary\ Strain}\ (\boldsymbol{\varepsilon y})}{\boldsymbol{Secondary\ Strain}\ (\boldsymbol{\varepsilon x})}$$

2.1.4 Determinants of bone strength and fragility

BMD is an important determinant of bone strength and is measured by Dual Energy X-Ray Absorptiometry (DXA), which is the most commonly used method of assessing fracture risk in individuals vulnerable to fragility fracture. Mechanical cadaveric studies have found that BMD accounts for 60% to 80% of bone strength.[30] There are numerous factors that influence bone strength, including overall bone geometry, and morphology, hydroxyapatite crystal size and heterogeneity, collagen properties, osteocyte density, potential presence of microfractures, trabecular and cortical microarchitecture, composition of bone, and biophysical properties of the constituents of bone.[27]

Collagen fibres confer on bone its elasticity and ability to absorb energy. Alterations in collagen can affect the mechanical properties of bone, and increase vulnerability to fracture.[7] Inter- and intra- chain crosslinks between collagen chains maintains the chains in a close relationship allowing for a densely organised fibrillar structure. Abnormalities in cross-linking have been associated with increased fracture risk. Oxlund *et al* in 1995 in an animal control study reported that a 45% reduction in hydroxypyridinium crosslinks resulted in a reduction in mid-diaphyseal deflection until breaking, bending stress, and elastic stiffness (Young's modulus) of 21%, 26% and 30%, respectively, compared to a control group.[31]

Heterogeneity in the size and shape of hydroxyapatite crystals is believed to be favourable for bone strength. It has been observed in animal studies that in older animals crystal size tends to be larger with less variation compared to younger animals. This is believed to reduce the bone's resistance to loading, resulting in weakening of bone. In younger animals there is a wider range in crystal size.[32]

Changes in trabecular microarchitecture, particularly compromise bones in which cancellous bone predominates, such as the vertebrae and the periphery of long bones, independently to changes in cortical bone.[27] Khosla *et al* in 2006 investigating changes in the bone of the forearm (radius) in a sample of 602 subjects (324 female and 278 men) aged 21 to 97, found that while both young males and females have similar trabecular number and separation, males have a higher trabecular bone volume to tissue volume ratio (BV/TV), and greater trabecular thickness. With ageing, decreases in BV/TV are similar for both sexes, however women show a significant decrease in trabecular number (-13%) and an increase in trabecular spacing (+24%).[33]

2.1.5 Geometric properties of bone

The geometric properties of bone that influence strength include its size, shape, cortical bone thickness, and cross sectional area. The long bones of humans may be described as tubes with thick walls, imparting bones the ability to withstand heavy loading, whilst still remaining relatively light.[34] Cortical perimeter is an important geometric parameter of bone strength, because increasing the diameter of a hollow cylinder exponentially increases resistance to bending and torsion without increase in bone mass.[27]

Bones are subjected to a combination of forces. An example is the femoral neck, which is subjected to a bending moment, with a greater degree of compressional forces on the medial aspect of the femur and tensile forces on the lateral aspect.

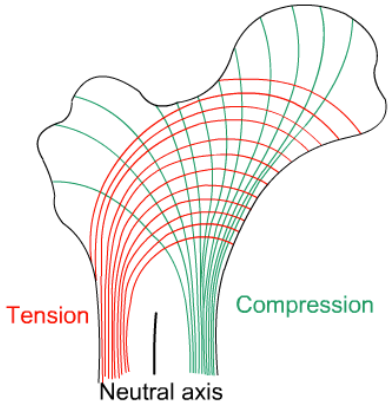


Figure 2. 6 Distribution of forces at the femoral head.

Hip geometry varies between people of different ethnicity. The rates of hip fracture are lower in African American women, and Japanese women when compared to Caucasian and Chinese women. It is thought that BMD alone cannot account for the difference in fracture rates, and it has been suggested that variation in the geometry of the femur varies the loading mechanics, and may account for the differences in rates of fracture.

Hip geometry can be assessed from images taken from DXA scans. There is software designed to carry out this task: Hip Structural Analysis (HSA) by Hologic, and Advanced Hip Assessment (AHA) from Lunar by General Electric are two examples. These programmes derive parameters known to be associated with bone strength and fracture risk, including Hip Axis Length (HAL), Cross Sectional Area (CSA) and Neck Shaft Angle (NSA).[30]

A study by Danielson *et al* in 2013 demonstrated the influence of geometry on bone strength and variation between different ethnicities by performing hip structure analysis on DXA scans performed on 1942 pre- and peri-menopausal women of four different races (50% Caucasian, 27% African American, 11% Chinese and 12% Japanese). They reported African American women had increased femoral neck BMD and while the narrowest part of the femoral neck was found to be narrower when compared to Caucasians, it had a greater cortical bone volume in cross section. The femoral neck length was similar in both groups, however Caucasian women had a

greater NSA. Japanese women were found to have a smaller NSA, and a longer femoral neck length compared to the other three ethnic groups, and a higher hip structure analysis BMD when compared to Caucasians. This is because of a higher cross sectional area and a wider outer diameter. The centroid position, which reveals asymmetry of the mass in cross section, was found to be more centrally positioned in Japanese women, and more medially positioned in Chinese women. This property confers increased section modulus when more centred. This study indicates a more favourable structural hip geometry in African American and Japanese women compared to Caucasian and Chinese women.[35]

Similarly, Bruno *et al in* 2014 demonstrated the effect of geometry on bone strength at the lumbar vertebrae. 981 male and female pairs were matched for age and areal BMD at the third lumbar vertebrae (L3). The authors found that men have larger cross sectional area, lower volumetric BMD, and higher vertebral compressive strength when compared to their age/areal BMD matched female counterparts. The larger cross sectional area compensated for the lower volumetric BMD, resulting in higher compressive strength, regardless of equal areal BMD.[36]

In 2015, an expert panel from the International Society of Clinical Densitometry evaluated the evidence for the use of hip geometry in hip fracture prediction, and acknowledged that geometry is critical to the mechanical strength of the hip and resistance to fracture under loading. Based on data from 27 studies, they concluded that HAL in multiple studies of postmenopausal women is a predictor of hip fractures, independently of BMD. However there is insufficient evidence that HAL is predictive in men. There is also good evidence that Neck Shaft Angle and Cross Sectional Area predict hip fracture in postmenopausal women and in men over 60 years of age, but not independently of BMD, bringing into question their clinical application.[30]

2.2 Osteoporosis

Osteoporosis is the most common metabolic bone disease. It is estimated to affect 200 million people worldwide. It is characterised by low bone mass and deterioration of bone microarchitecture leading to bone fragility with an increase in the risk of fracture.[37, 38]

The National Institutes of Health Consensus Development Panel on Osteoporosis in 2000 defined osteoporosis simply as 'A skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture'.[39] The World Health Organisation (WHO) defines osteoporosis based on bone mineral density (BMD) measured by Dual Energy X-ray Absorptiometry (DXA): 2.5 standard deviations or more below the mean of a young female Caucasian reference population is considered osteoporotic, and when combined with a history of fragility fracture, it is classed as severe osteoporosis. While this definition was described primarily for descriptive epidemiology, BMD measured by DXA has proven itself as one of the strongest predictors of fragility fracture, and is generally regarded as the gold standard in the assessment of osteoporosis.[40]

2.2.1 Epidemiology of osteoporosis

Osteoporosis is believed to cause 8.9 million fragility fractures a year worldwide.[1] In Europe in 2010, 22 million women and 5.5 million men were estimated to be osteoporotic, with 3.5 million fractures occurring. This consisted of 610,000 hip fractures, 520,000 vertebrae fractures, 560,000 forearm fractures and 1.8 million of other types of fractures.[41]

In Ireland it is believed that over 300,000 people over the age of 50 have osteoporosis, which represents over 25% of the over 50 population.[42 43] As there is an increasing demographic trend towards an older population, it is expected that the incidence of fragility fractures will increase. It is believed that in Caucasian

populations, 40 to 50% of women and 13 to 22% of men over the age over the age of 50 will experience an osteoporosis related fragility fracture in their lifetime.[44, 45]
In Ireland the cost of fractures related to falls in the elderly is approximately €402 million per year, and in the whole of Europe the total economic burden of osteoporosis in 2010 was estimated to be €39 billion.[46, 47]

Osteoporosis is usually asymptomatic and progresses undetected until a fragility fracture occurs. The bones most commonly associated with fragility fracture are the distal radius (Colles' fracture), vertebrae, and hips. Osteoporosis can also predispose people to fractures at other skeletal sites.

In 2010, there were an estimated 3,200 hip fractures in Ireland.[40] Hip fractures account for 14% of osteoporosis related fractures, and are a significant cause of morbidity and mortality.[48] The incidence of hip fractures is reported to be 7 per 1000 person years.[49] The outcome of a hip fracture can be devastating, and 12 month mortality rates related to hip fractures ranging from 12% to 35% have been reported.[50] This is likely to be related to hip fracture patients requiring a hospital stay, leading to chronic immobilisation, and an increased risk of a thromboembolic events or pneumonia.[51] Among those who survive a hip fracture, 67% will require long term care, and 20% will be admitted to a nursing home.[52, 53]

Vertebral fractures may occur without trauma, or with minimal trauma such as with bending or lifting. Symptoms include limited spine mobility, back pain, loss of height, and kyphosis. In cases of fracture in the mid-thoracic region, mild reduction of pulmonary function has been reported. Significantly reduced quality of life is associated with multiple vertebral fractures, and 20% of osteoporotic women with a recent vertebral fracture will sustain another vertebral fracture within 12 months.[54]
The prevalence of vertebral fractures is almost as high in men as in women. It has been suggested this is because occupation-associated trauma is higher in men when compared to women.[44] It is believed that vertebral fractures account for 27% of osteoporosis fractures, although the incidence of vertebrae fracture is difficult to accurately estimate as, despite associated co-morbidity, many vertebral fractures go

undiagnosed.[48] This is believed to be because up to two thirds do not cause symptoms, and fractures can go undetected on radiographs. Back pain due to osteoporosis may be incorrectly attributed to osteoarthritis and other causes of back pain.[55]

Distal radius fractures account for 19% of osteoporotic fractures, and their reported incidence is 8 to 10 per 1000 person years. [48, 56] This type of fracture tends to occur as a result of falls and many patients who suffer this trauma often lose their functional independence. There is an associated deterioration in quality of life: simple tasks such as preparing a meal can become challenging and for those in employment, it may result in days out of work. A prospective cohort study by Edwards *et al* in 2010 found that occurrence of a wrist fracture increased the odds of having a clinically important functional decline by 48% (odds ratio 1.48, 95% CI 1.04 to 2.12).[56] Wrist fractures can also be an indicator of osteoporotic changes, and patients who have experienced this type of fracture have a higher risk of other osteoporotic fractures. Cuddihy *et al* in 1999 reported that the risk of a hip fracture following a wrist fracture increased 1.4-fold in women, and 2.7-fold in men.[57]

2.2.2 Aetiology and pathogenesis of osteoporosis

The aetiology of osteoporosis is multifactorial. The strongest predictors of fragility fractures are age, history of a previous fracture, and low bone mineral density (BMD).[58]

It is thought that osteoporosis is a direct consequence of either insufficient bone mineral accruement in youth or excessive bone loss in later life. In many cases it is likely to be a consequence of both.[59]

Peak bone mass (PBM) in females is typically reached by 22 to 26 years of age.[60] Calcium uptake is one of the main determinants of PBM in adolescents and slows age related bone loss.[59] Physical activity during adolescence also plays an important role in the development of PBM. It has the ability to stimulate basic multicellular units (BMU) via mechanical and metabolic stimuli. A cohort study of 3,811

adolescents (1,866 boys and 1,945 girls), showed that physical activity was associated with a positive effect on lumbar spine and femoral neck bone mineral density.[61]

Chronic conditions with an associated increase in inflammatory cytokines such as Rheumatoid Arthritis, COPD, Crohn's Disease, and Ulcerative Colitis have been implicated in the pathogenesis of osteoporosis.[62]

Progressive loss of bone has long been recognised as a side effect of corticosteroid therapy, and there is an associated increase in fracture risk irrespective of BMD. A meta-analysis by Kanis *et al* in 2004, inclusive of seven prospective studies and totalling 42,542 patients found that for patients on steroids, when compared to those with no steroid exposure had risk ratios of 1.57 (95% CI 1.37 – 1.80) for any fracture, 1.66 (CI 1.42 – 1.92) for an osteoporotic fracture, and 2.25 (95% CI 1.60 – 3.15) for a hip fracture.[63]

Renal failure results in a disturbance of vitamin D metabolism, in turn leading to a decrease in calcium absorption from the intestine. It also causes an elevated serum phosphate level. Both the reduced calcium absorption and the increased phosphate cause a decrease in serum calcium, stimulating the parathyroid gland to produce PTH resulting in secondary hyperparathyroidism.[64]

2.2.2.1 Genetic influence on osteoporosis

Genetics play a significant role in the pathogenesis of osteoporosis. Children of people with a history of fragility fracture are likely to have low BMD themselves,[65] and a study by Krall *et al* in 1993 found that 46% to 62% of variance in bone density was attributable to inherited genes.[66] Osteoporosis is not inherited by one single gene, but rather a multitude of genes, which influence normal function such as those responsible for enzymes, bone matrix proteins, calciotropic hormones, cytokines and adhesion molecules.[67]

The GENOMOS (Genetic Markers for Osteoporosis) Consortium is an International group, whose aim is to research genetic factors contributing to osteoporosis, and improve clinical risk assessment. Much of this research is done through Genome Wide Association Studies (GWAS). To date over 60 genes have been identified as having an association with BMD, and 15 genes have been identified with an association with fracture risk. These include genes involved in the WNT/ß-catenin pathway, the RANK/OPG pathway, and the endochondral ossification pathway.[65] While there is necessity for further research, early identification of genetic risk factors should allow for early intervention to limit osteoporotic changes.

2.2.2.2 Menopause

The menopause is characterised by loss of oestrogen production in the ovaries. This is a result of normal ageing, or in some cases, it is due to surgical removal of the ovaries. Oestrogen maintains bone density by suppressing osteoclastogenic cytokine production in T-cells and osteoblasts, and by inducing osteoclast apoptosis. The menopause is associated with a rapid loss of BMD, in which the average cumulative loss in BMD from 1 year premenopause to 3 years postmenopause is about 9% to 10% in both spine and hips, which is equivalent to a T-score of about 0.7.[68] This bone loss may be prevented or reduced by hormone replacement therapy (HRT).

2.2.2.3 Social factors and lifestyle

Alcohol abuse is a cause of secondary osteoporosis, particularly in men. It has been suggested the mechanisms by which alcohol increases fracture risk include its direct effect on osteoblasts, increased release of endogenous calcitonin, and heavy drinkers are more likely to have poor nutrition. A meta-analysis by Kanis *et al* in 2005 showed that while there is no significant negative effect of 2 units or less a day, intake greater than this increases risk of fracture irrespective of BMD. Above 2 units a day, the risk ratio (RR) of any osteoporotic fracture is 1.38 (95% CI 1.16 to 1.65), and of a hip fracture is 1.68 (95% CI 1.19 to 2.36).[69]

The association between osteoporosis and smoking is well established. There have been a number of reasons postulated including direct toxic effects on bone, a possible reduction of absorption of calcium, and a temporary rise in endogenous cortisol. Law *et al* in 1997 found in a meta-analysis, that lifetime risk of hip fracture in smokers increases from 12% to 19% in women up to the age of 85, and increases from 22% to 37% up to the age of 90.[70]

There is evidence that lack of psychological well-being has a negative impact on osteoporosis. Stress triggers the release of glucocorticoids and in particular cortisol. Glucocorticoids inhibit osteoblast differentiation and function, and promote apoptosis of osteoblasts and osteocytes, whilst also inhibiting apoptosis of osteoclasts.[71]

Selective Serotonin Reuptake Inhibitors (SSRIs), which are prescribed as antidepressants, have been shown in a cohort study of 2,722 women to decrease BMD at the hip by 0.82% ($p<0.01$), while tricyclic antidepressants were not found to have an effect.[72]

2.3 Treatment of Osteoporosis

2.3.1 Teriparatide

Parathyroid hormone (PTH) is an 84 amino acid polypeptide hormone secreted by the parathyroid glands located behind the thyroid gland. It is an important agent of control of blood calcium levels, and remodelling of bone. PTH increases blood calcium levels by its effect on bone, the intestine, and the kidneys. Teriparatide is a recombinant human form of PTH and is made up of 1 to 34 of the amino acid sequence of the N-terminal end of PTH. It is abbreviated to rhPTH(1-34). In Europe, it is marketed under the brand name Forsteo (Eli Lilly). It is administered as a 20mcg daily dose given by subcutaneous injection, into the thigh or abdomen.

Increased levels of PTH in conditions, such as in hyperparathyroidism, can cause a reduction in BMD and an increase in risk of fragility fracture. However intermittent controlled low levels of added PTH have an anabolic effect on bone.[73, 74] This is believed to be related to the mechanism of action of PTH on osteoblasts. PTH does not exert a direct action on osteoclasts, but exerts its resorptive action on bone through the RANKL-OPG pathway mediated by osteoblasts. PTH has a short half-life of only 4 minutes, and shortly after its release, there is an increase in osteoblast formation and inhibition of osteoblast apoptosis.[75] With continuous release of PTH, the osteoblasts commence recruitment and maturation of osteoclast precursors, resulting in an increase in the population of osteoclasts leading to bone resorption. When intermittent low levels, rather than continuous levels of PTH are administered, it results in an increase in osteoblasts rather than osteoclasts, which in turn results in an overall net increase of bone formation, and an increase in hydroxyapatite crystal heterogeneity.[27, 74]

Its development is of particular importance as it is the first anabolic bone agent shown to stimulate bone formation. The Food and Drug Administration (FDA) in the US approved Teriparatide for treatment of osteoporosis in 2002. It is licensed for no more than 2 years use, as animal studies have shown an increased incidence of

osteosarcoma related to long-term use.[76-78] It is contraindicated in patients with a history of osteosarcoma or metastatic bone disease. In the US, there have been two reported cases of osteosarcoma occurring in patients on Teriparatide, however over 430,000 people have been safely treated.[78] Additional contraindications to the use of teriparatide include hypercalcaemia and hyperparathyroidism.

Teriparatide has been shown in multiple studies to be an effective treatment for low BMD. Neer *et al* in 2001 in a randomised placebo control trial of 1,637 participants with a history of vertebral fracture investigated the effect of teriparatide on BMD and fracture risk reduction. The cohort was divided into 3 groups based on treatment: placebo (n=544), 20µg teriparatide (n=541) and 40µg (n=552) daily. They reported that compared to placebo there was an improvement in bone mineral density (BMD) in the lumbar spine of 13.7% in the 40µg group, and 9.7% in the 20µg group over a 21 month period. The improvement in BMD in the total hip was 3.6% and 2.6% in the 40mcg, and 20mcg groups, respectively. Teriparatide also reduced the risk of new vertebral fractures compared to a placebo by 65% and 69% in the 20µg and 40µg groups, respectively. A weakness of this study was that it was cut short following the publication of the reports of increased incidence of osteosarcoma in animal studies.[76, 79]

Chen *et al* in 2006 also reported good results with teriparatide use in a study of 1637 postmenopausal women. Subjects were observed over an 18-month period, and the authors found that teriparatide mediated increases in spine BMD accounted for between 30% to 41% reduction in new vertebral fracture risk.[82]

The EUROFORS (European Study of Forsteo) study was a 2-year, prospective, randomised study, conducted in 95 centres throughout 10 European countries. The study had two research aims. The first was to investigate three different treatment regimens after 1 year of Teriparatide, and the second was to investigate the effect of prior antiresorptive treatment.[80, 81]

The three different treatments investigated were a further year of Teriparatide, a year of Raloxifene (a selective oestrogen receptor modulator) therapy, and a year of no active treatment. In the group receiving 2 years of continuous Teriparatide therapy (n=305) a mean improvement from baseline in spine BMD of 10.7% and hip BMD of 2.5% was observed. In the Raloxifene group (n=100) there was an increase of 7.9% in spine BMD in the first year and no change in the second year. In the no active treatment group (n=102) there was an increase of 6.3% in the first year, and a decrease of 2.5% in the second year.[81]

In the second arm of the study, 503 patients in the 2 year Teriparatide course were divided into three groups: treatment naïve (n=84), pre-treated with anti-resorptive (AR) medication (n=134), and pre-treated and showing no response to AR medication (n=285). The mean BMD gain in the spine and hip was greatest in the treatment naïve group at 13.1% and 3.8%, respectively. In the pre-treated AR group the mean gain in spine BMD was 10.2%, and 9.8% in the pre-treated "non-responder" group. Hip BMD gain for both of these groups was 2.3%.[80]

2.3.2 Denosumab

Denosumab is an antiresorptive agent used in the treatment of osteoporosis and bone metastases. It is marketed under the name Prolia (Amgen) for the treatment of osteoporosis, and Xgeva (Amgen) for prevention of skeletal related events (SRE) in patients with bone metastases.[83] They are both administered as a subcutaneous injection. Prolia is given as a 60mg dose every 6 months, and Xgeva is given as a higher dosage of 120mg every 4 weeks.[84] Denosumab was granted FDA approval in 2010. It is classed as a human monoclonal antibody (Immunoglobulin G2). They are highly specific antibodies produced by clones originating from a single cell line. This single cell line is produced by fusion of an antibody producing B cell and a myeloma cell, and is referred to as a hybridoma. These hybridomas lose their ability to display variation in new antibody chains, resulting in the ability to produce highly specific

antibodies, with little or no variation. These antibodies can be engineered to have highly specific therapeutic functions with few adverse effects.[85]

Denosumab inhibits osteoclastic activity via its inhibition of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL). RANKL is a member of the Tumour Necrosis Factor (TNF) cytokine group, and is present in many cells, including osteoblasts, T cells and B cells. RANKL attaches to the RANK receptor on osteoclasts and osteoclast precursors, stimulating differentiation, maturation, and activation.[86]

Denosumab mimics the action of Osteoprotegerin (OPG). OPG is described as a decoy receptor for RANKL. It competes with RANK to bind with RANKL, thereby neutralising RANKL, and inhibiting differentiation, and activation of osteoclasts.[21] As RANKL is expressed in T cells and B cells, it is thought that Denosumab has an immunosuppressive effect.[87] A systematic review has reported an increased incidence of urinary infections (p=0.012), and eczema (p<0.001) in patients on Denosumab, when compared to placebo.[88]

The efficacy of Denosumab to reduce fracture risk was investigated in the Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) Study, which was an International, randomised, placebo controlled trial. Osteoporotic (n=7868) women between the ages of 60 and 90 were enrolled, and half (n=3933) received Denosumab, and the remaining half (n=3935) received a placebo. Both were administered as a 6 monthly injection subcutaneous injection.

82% of participants completed the full 36 months of the study. The incidence of new radiographic vertebral fractures over the 36 months was 2.3% in the Denosumab group and 7.2% in the placebo group, representing a 68% reduction in relative risk of fracture (p<0.001). The cumulative incidence of hip fracture was 0.7% in the Denosumab group compared to 1.2% in the placebo group (hazard ratio, 0.60; 95% CI, 0.37 to 0.97; P=0.04) representing a 40% relative risk reduction. A relative increase in BMD of 9.2% (95% CI, 8.2 to 10.1) at the lumbar spine and 6.0% (95% CI, 5.2 to 6.7) at the total hip compared with placebo was observed.[89] To date this is the largest study investigating the effect of Denosumab.

The efficacy of Denosumab appears to be favourable in comparison to IV Zoledronate (bisphosphonate). Lipton *et al* in 2012 analysed data from 3 studies treating patients for bone metastases, and found that Denosumab was superior to Zoledronic acid in prevention of skeletal related events. The median time to the first on-study skeletal related event was 8.21 months longer with denosumab (27.66 months), than zoledronic acid (19.45 months).[90]

2.3.3 Zoledronic acid

Zoledronic acid is a third generation, nitrogen containing Bisphosphonate used for the treatment of osteoporosis, Paget's disease, tumour induced hypercalcaemia, and prevention of skeletal related events (SRE) in patients with advanced malignancy involving bone. It was approved for use by the FDA in 2002, and in Ireland it is marketed as Zometa (Novartis). It is administered as an IV infusion of 2, 4 or 5mg. For patients attending the Bone Health Clinic in St James's Hospital for osteoporosis treatment, it is administered as either a 4mg yearly infusion or a six monthly infusion of 2mg.

Bisphosphonates (BPs) are synthetic analogues of inorganic pyrophosphate, and are so named because they contain two phosphonate groups – ($PO(OH)_2$).[91] They have a high affinity for calcium giving them the property of binding selectively to their target organ, bone.[92] This allows them the opportunity to enter osteoclasts during bone resorption, and it is within the osteoclasts they carry out their effect.
There are two classes of bisphosphonate: Nitrogenous and non-nitrogenous, and they have a slightly different mechanism of action. Non-nitrogenous BPs are associated with a greater incidence of adverse effects, and so are less commonly prescribed.

Nitrogenous BPs bind to and block the enzyme Farnesyl Pyrophosphate Synthase (FPPS) in the mevalonate pathway (otherwise known as the HMG-CoA reductase pathway). This prevents post-translational modification (prenylation) of small

Guanosine Triphosphate (GTP) binding proteins, which are essential for cell function, maintenance of cytoskeleton, formation of the ruffled border necessary for bone resorption, and cell survival.[93, 94]

A major difference between Denosumab and bisphosphonates in their mechanism of action is that BPs are adsorbed to the hydroxyapatite bone surface, and then subsequently enter the osteoclast to perform their function, whereas Denosumab exerts it's effect external to the bone and bone cells.[92] Bisphosphonates display variation in their affinity for bone, explaining why different BPs have different potency, and may be cleared from the skeleton at different rates.[93] Zoledronate has been shown to increase BMD, and reduce bone turnover, for at least one year following infusion.[95]

The Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON) Pivotal Fracture Trial was a large, International, multicentre randomised placebo controlled trial investigating the use of a yearly infusion of 5mg IV Zoledronate over a 3 year period in post-menopausal osteoporotic women between the ages of 65 and 89. Out of 7,714 participants (3,889 received Zoledronate and 3,876 received placebo), 6517 remained under review for the full 3 year follow up period, and 6,260 received all three infusions. The 3 year incidence of vertebral fracture in the active treatment group was 3.3% versus 10.9% in the placebo group, representing a relative risk reduction of 70% (RR 0.30, 95% CI, 0.24 to 0.38). The 3 year incidence of hip fracture was 1.4% in the active treatment group versus 2.5% in the placebo group representing a relative risk reduction of 41% (HR 0.59; 95% CI, 0.42 to 0.83). BMD of the subjects on Zoledronate increased significantly at the total hip by 6.02% (95% CI, 5.77 to 6.28), 6.71% at the lumbar spine (95% CI, 5.69 to 7.74), and 5.06% at the femoral neck (95% CI, 4.76 to 5.36), when compared with the placebo group (p<0.001).[96]

A network meta-analysis by Jansen *et al* in 2011 looked at the efficiacy of Zoledronate compared to other treatments for osteoporosis in fracture risk reduction. Based on data from 8 randomized placebo controlled trials, they found

that those on Zoledronic acid showed for vertebral fractures, a relative risk (RR) of 0.30 (95% Credible Interval 0.23-0.37) relative to placebo, an RR of 0.55 (0.41-0.76) relative to alendronate, and a RR of 0.50 (0.36-0.70) relative to risedronate. Relative risk of hip fractures of zoledronic acid relative to placebo, alendronate, and risedronate were 0.58 (0.41-0.82), 0.95 (0.54-1.68), and 0.73 (0.37-1.44), respectively. The authors also reported that there was a 94% probability that Zoledronate showed the greatest overall reduction of any fracture.[97]

A similar network meta-analysis by Sanderson *et al* in 2016 inclusive of 46 randomized control trials conforming to PRISMA guidelines investigated the effect of alendronate, risedronate, ibandronate and zoledronic acid on hip and vertebral fracture risk and reported a similar result. They found that Zoledronic acid had the greatest effect on prevention of vertebral fractures (HR 0.41, 95% CrI: 0.28 , 0.56) and percentage increase in BMD (3.21, 95% CrI: 2.52, 3.86). The greatest treatment effect for hip fractures was given by Alendronate (HR 0.78, 95% CrI 0.44, 1.30), and for non-vertebral and wrist fractures, the greatest effect was found with risedronate (HR 0.72, 95% CrI 0.53, 0.89 for non-vertebral and HR 0.77, 95% CrI 0.44, 1.30 for wrist fractures).[98]

Both of these studies are well designed, adhere to strict guidelines when selecting studies, and their respective conclusions demonstrate the efficacy of zoledronate compared to other forms of bisphosphonate therapy.

There is evidence emerging of an adverse and almost paradoxical effect of long term bisphosphonate use (>5 years) on fracture risk: there is an association between bisphosphonate use and atypical femoral fractures. This was first described by Odvina *et al* in 2005 in a case series of nine patients on long term alendronic acid with atypical fractures of multiple sites (sacrum, rib, ischium, pubic rami), including the femoral shaft.[99] It is thought that long term (>5 years) suppression of bone turnover rate results in accumulation of microfractures, and a deterioration in bone quality. Interestingly, most atypical femoral fractures are associated with low dose BP therapy, such as weekly oral Alendronate, rather than IV Zoledronate.[100]

2.4 Bone turnover markers

Changes in bone turnover related to ageing are responsible for loss of bone and play a large role in the development of osteoporosis.[101] Bone turnover markers (BTM), also referred to as bone biomarkers, are bi-products of the continuous cycle of bone formation and resorption. They are classed as either markers of bone formation or resorption. However in most circumstances, these processes occur in unison and during osteoporotic related bone loss, levels of both are likely to be elevated. BTMs may be proteins such as those derived from bone matrix production and breakdown, or enzymes released by the action of osteoblasts and osteoclasts.[102]

Bone formation	Bone resorption
Bi-products of collagen synthesis • P1NP • P1CP	Collagen degradation products • Hydroxyproline Pyridinoline (PYD) • Deoxypyridinoline
Matrix protein • Osteocalcin	Cross-linked Telopeptides of Type I Collagen • CTX-1 • NTX-1
Osteoblast enzymes • Total Alkaline Phosphatase • Bone Specific Alkaline Phosphatase	Osteoclast enzymes • Tartrate Resistant Acid Phosphatase (TRAP) • Cathepsin K

Table 2. 3 Biomarkers of bone formation and resorption.[103]

The use of BMD alone provides an incomplete picture of bone remodelling, and response to treatment, and the development of tests to measure BTM levels in the serum and urine has led to a number of clinical applications providing a more complete profile of a patient's osteoporotic status, and fracture risk. In recent years, the use of biomarkers has increased due to the development of reliable, and cost effective assays with improved sensitivity, and specificity.[104]

The clinical applications of BTMs include observation of bone response to antiresorptive and anabolic treatment of osteoporosis, monitoring treatment of patients with bone metastases, and they provide an indication of the rate of bone remodelling. More severe forms of osteoporosis have been associated with high bone turnover rates. Garnero *et al* in 1996 showed an increase in BTMs of 37% to 52% for bone formation markers and 79% to 97% for bone resorption marker levels in peri-menopausal women.[101, 105] BTMs show a response to osteoporosis treatment before any change becomes apparent on DXA scans.[102, 106, 107]

The current evidence base for BTMs in osteoporosis related fracture risk assessment is limited, and to date, their use have not been validated for use in fracture prediction. It has been suggested that in conjunction with bone mineral density measured by DXA, biomarkers may improve predictability of fragility fractures, however the evidence is lacking and further research is necessary.[102, 108]
A number of BTMs can be measured from samples taken from both urine, and blood, however they do show more variability in urine, than in serum.[109, 110] Two major disadvantages of BTMs is that they are affected by food intake, and repeatability of assays may be unreliable due to urine and serum levels being subject to circadian rhythm, with values being at their highest in the morning. Therefore testing should be standardised, ideally performed in the morning, with the patient fasting.[104]

2.4.1 Procollagen type 1 N propeptide (P1NP)

P1NP is a biomarker produced as a result of new bone formation. Type 1 collagen accounts for 90% of organic bone matrix. It possesses a triple helical structure referred to as tropocollagen, consisting of three peptide chains wrapped around eachother.[111] Its precursor molecule, Type 1 Procollagen, is formed in osteoblasts and then secreted into new bone matrix. Type 1 Procollagen possesses N-terminal and C-terminal extensions, and in the bone matrix procollagen peptidases cleave P1NP from the amino (N) terminal end, and Procollagen Type 1C Propeptide (P1CP) from the carboxy (C) terminal end of the procollagen molecule to form the mature

collagen fibril.[102] These propeptides then enter the circulation where their concentrations can be measured.

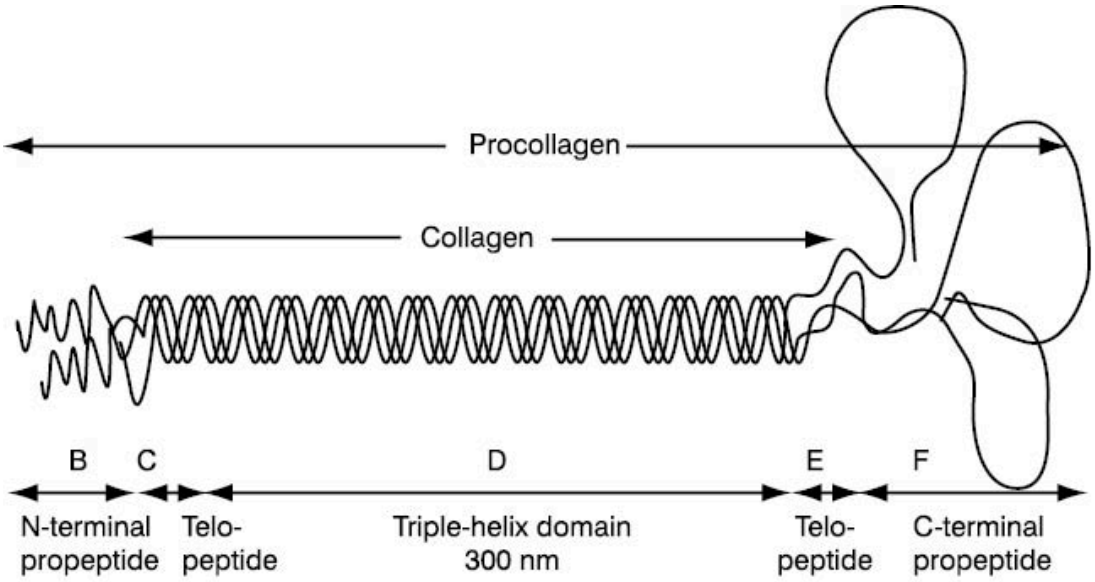


Figure 2. 7 Structure of collagen and procollagen showing propeptide terminals.

P1NP is usually released in its trimeric form, which is unstable at body temperature and prone to thermal degradation. It can be rapidly broken down to a monomeric form, and in the circulation exists in the trimeric, monomeric forms as well as several fragments of the degraded molecule. Antibodies of P1NP are used to detect the trimeric form by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). In contrast to P1NP, P1CP exists as a single protein, however it is not a commonly used marker of bone formation. A study has shown that following initiation of Teriparatide (recombinant Parathyroid Hormone) therapy, there is an initial increase in the value of P1CP at 1 month but this returns to baseline value at 3 months. The transience of P1CP elevation suggests that it is a less than an optimal treatment response marker.[112, 113]

P1NP also originates from other tissues such as skin, tendons, dentine, and ligaments among others. However these other tissues do not have a high turnover rate, and so do not contribute a great deal to the circulating serum P1NP, in comparison to bone turnover.[102] Serum P1NP has been shown to increase in response to treatment with Teriparatide. A biological response to anabolic osteoporotic therapy is considered to

be an increase in P1NP greater than 10mcg/L from baseline measurement.[113] A randomised control trial by Chen *et a*l in 2005 divided a cohort of 1,637 postmenopausal women into three groups based on their respective therapy: 20µg Teriparatide (n=541), 40µg Teriparatide (n=552), and placebo (n=544). Lumbar spine BMD was measured at baseline and 18 months, and femoral neck BMD was measured at baseline and 12 months. Increase in the levels of P1NP at 3 months, and P1CP at 1 month correlated significantly with increases in BMD of the lumbar spine at 18 months with correlation coefficients of 0.62 and 0.65, respectively. The correlation coefficients of femoral neck BMD with 3 month P1NP, and 1 month P1CP, were lower with reported values of 0.20 and 0.31, respectively.[112]

2.4.2 C-terminal cross-linked telopeptides of type I collagen (CTX-1)

CTX-1 is produced as a result of bone resorption. Metabolism of collagen during resorption results in the release of telopeptides at either end of the collagen molecule. CTX-1 is the telopeptide cleaved from a part of the non-helical region of type 1 collagen at the carboxy-terminal end of the collagen chain, during osteoclastic resorption of bone.[114]

Amino-terminal cross-linked telopeptide (NTX) may also be used as a bone biomarker, however CTX appears to be a more reliable and effective indicator of bone resorption.[110] However the CTX concentration is significantly affected by food intake. They are both small enough to be cleared by the kidneys and therefore may be measured in both serum and urine.[106]

Marx *et al* in 2007 proposed another clinical application for CTX. They suggested its use as a predictor of ARONJ as reduced levels of CTX reflect osteoclast suppression in patients on antiresorptive therapy. They found that while it was not a good predictor of ARONJ for individual patients, they did suggest a range of values could identify those at risk. Fasting values less than 100 pg/ml were considered high risk, 100 to 150 pg/ml was considered moderate risk, and greater than 150pg/ml was considered minimal risk. They also found a monthly increase in CTX levels of 26.4pg/ml following

cessation of oral Bisphosphonate therapy, which suggests a drug holiday would result in a return to the low risk range of values, and normal osteoclastic activity reducing risk of ARONJ.[115]

A number of recent studies have found that a low CTX blood level was not a good predictor of BRONJ,[116-120] A large study carried out by Hutcheson *et al* 2014 over a 6.5 year period with 950 patients, and 2,461 extractions reported a three fold increase in the risk of BRONJ in patients with a CTX level lower than 150pg/ml.[121] Currently, the American Association of Oral and Maxillofacial Surgeons (AAOMS) does not recognise the use of CTX as a predictor of ARONJ.[122]

2.4.3 Osteocalcin (OC)

Osteocalcin, a biomarker of bone formation also referred to as Bone Gla Protein (BGA), is the most abundant non-collagenous protein in bone and is also present in dentine. It is produced by osteoblasts and odontoblasts, and is involved in the regulation of the size and shape of the hydroxyapatite crystals, as well as signalling and recruitment of osteoblasts and osteoclasts.[102,123-125] It consists of a single chain of 46 to 50 amino acids and contains three Vitamin K dependent γ-carboxyglutamic acid (Gla) residues, which influence binding to calcium and hydroxyapatite.[125] While most of the osteocalcin becomes incorporated into the bone matrix, a small proportion is released into the circulation.[102]

A study by Singh *et al* in 2015 reported a significant difference between the mean osteocalcin levels in postmenopausal osteoporotic (22.28 ± 5.1 ng/ml), osteopenic (19.25 ± 5.1ng/ml), and non-osteoporotic (16.21 ± 2.8 ng/ml) women. This demonstrates an inverse relationship between BMD and osteocalcin levels, which is consistent with other research. A reason suggested for higher osteocalcin levels in osteoporotic individuals is that as osteocalcin has a high calcium affinity, when bone is resorbed as a result of osteoporotic change, the osteocalcin is released resulting in increased circulating osteocalcin levels.[126] Despite this, it has been shown that levels

increase in response to anabolic bone therapy. Sousa *et al* in 2010 reported a 165% increase in the level of osteocalcin following one month of treatment with Teriparatide, and a further increase of 11% after the third month.[127]

2.4.4 Bone specific alkaline phosphatase (BSAP)

Alkaline Phosphatase (ALP) is an enzyme found in tissues throughout the body. It is an enzyme present on the membrane of osteoblasts, and its presence in serum signifies bone formation. It exists in a number of isoforms depending on the tissue of origin. The two most common forms in serum are a bone isoform, and a liver isoform, both of which exist in approximately equal concentrations in those with normal liver function. Elevated levels of ALP may be an indicator of bone or liver metastases. BSAP assays have greater specificity for the action of osteoblasts. Its function in bone formation is unclear, however it is known that it is required for mineralisation.[102]

In a study investigating levels of ALP in post-menopausal osteoporotic women, it was found that ALP and BSAP levels are significantly higher in those in their 80s, compared to those in their 60s. Both ALP and BSAP decreased in response to bisphosphonate (Alendronate or Risedronate) therapy.[128]

2.4.5 Pyridinium crosslinks

Pyridinium crosslinks are similar to CTX, in that they are breakdown products of collagen and signify bone resorption. Part of the maturation process of Type I collagen occurs by stabilisation of the collagen fibril, by the formation of intra- and inter- molecular crosslinks and covalent bonds. Pyridinium crosslinks, divided into Pyridinoline (PYD) and Deoxypyridinoline (DPD) crosslinks, are located at both the N- and C- terminal ends of the collagen molecule, and stabilize the collagen fibril.[7] When collagen is broken down by the action of osteoclasts, they are released into the circulation. PYD can also be released from cartilage, ligaments and tendons, whereas DPD is released almost exclusively from bone.[102]

2.4.6 Osteoclast enzymes

Tartrate Resistant Acid Phosphatase (TRAP) is a metalloprotein enzyme expressed by osteoclasts, and an increase in bone resorption and osteoclast activity is accompanied by an increase in serum TRAP. Elevated levels are seen in Osteoporosis and other conditions associated with increased bone resorption such as hyperparathyroidism, Paget's disease and multiple myeloma. It is also used as a marker of disease in cancer cases with associated bone metastases.[129]

Cathepsin K is a cysteine protease involved in bone resorption that catabolizes elastin and collagen, allowing for breakdown of bone. It is secreted by osteoclasts at their ruffled border into resorptive pits where, in conjunction with TRAP, it degrades bone extracellular matrix. Cathepsin K inhibitors have been used in the treatment of cancer cases with bone metastases to slow down the destruction of bone.[130]

The International Osteoporosis Foundation (IOF) in conjunction with the International Federation of Chemical Chemistry (IFCC) Joint Working Group on Bone Marker Standards (WG-BMS) published a position paper in 2011, recommending the use of serum P1NP and serum CTX-1 as the primary reference markers for bone formation and bone resorption, respectively. The National Bone Health Alliance in the US also supports this recommendation.[131, 132]

2.5 Bone fracture risk assessment

2.5.1 Sensitivity and Specificity

Sensitivity and specificity are important statistical measures of the ability of a diagnostic test to correctly identify the presence of disease (true positive rate), and correctly identify the absence of disease (true negative rate), respectively.

	Disease Present	**Disease Absent**
Test Positive	True Positive (TP)	False Positive (FP)
Test Negative	False Negative (FN)	True Negative (TN)

Table 2. 4 Sensitivity and specificity

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}}$$

2.5.2 Fracture risk algorithms

Fracture risk algorithms are Internet based programmes designed for use in primary care settings to determine fracture risk based on clinical risk factors (CRFs). There are a number of examples including FRAX, QFracture, the Canadian Association of Radiologists and Osteoporosis Canada (CAROC) system, and the Garvan risk calculator in Australia.[40, 133-136]

2.5.2.1 FRAX

FRAX was developed by the WHO Collaborating Centre for Metabolic Bone Diseases at the University of Sheffield.[137] It was developed with calibration for different

countries because fracture risk shows marked variation between countries. It calculates fracture risk by means of models based on epidemiological data specific to each country. Since it was launched in 2008, it has become the most commonly used fracture prediction tool, and at the time of writing, it is available in 33 languages incorporating models from 63 countries including Ireland.[136] It is designed for easy to use information entry, and it can be used on an Internet browser page or as a smartphone app.

Information regarding the patient and their clinical risk factors (CRFs) is entered and from this data the algorithm calculates fracture risk, and gives two outputs, the 10 year probability of a hip fracture and the 10 year probability of a major osteoporotic fracture which includes hip, spine, wrist, and humerus. It links the result to the National Osteoporosis Guideline Group (NOGG) website which classes the result as either low, medium or high risk, and bases guidance on this output advising lifestyle advice, measurement of BMD or treatment, respectively. The CRFs taken into account include age, sex, height and weight, fracture history of the individual and their parents, smoking, corticosteroid use, rheumatoid arthritis, and alcohol intake (>3 units a day). Secondary osteoporosis is also taken into consideration and causes may include Type I diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition or malabsorption, and chronic liver disease.

An advantage of FRAX is that it can take femoral neck BMD into account. It is optional to enter BMD with a drop down menu to enter the type of scanner used, as there are variations between different brands of DXA scanners, thereby accommodating the variation between scanners. However, a number of studies have found that algorithms that don't take BMD into account are almost as good as those that do. A study of a population of 36,730 women and 2,873 men over the age of 50 found that including BMD in fracture risk is most beneficial in those considered at moderate risk of fracture, and risk assessment without BMD may be sufficient in those considered at either low or high risk.[138]

As femoral neck BMD is an optional entry, it has been suggested that FRAX can also be used as a potential screening method to determine necessity of DXA scan. Those without BMD measurement, and with a FRAX result of a low fracture probability may not require DXA; whereas there is indication for a DXA scan in those with an intermediate or high risk of fracture.

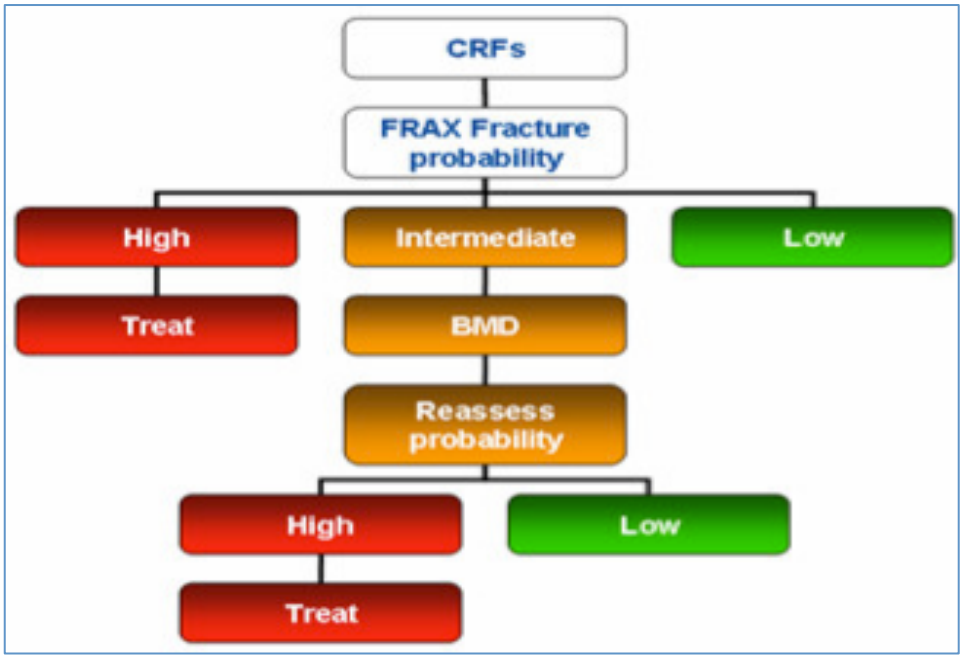


Figure 2. 8 NOGG Management algorithm of patients at risk of fracture.

FRAX is not without limitations. It is limited to people aged 40 to 90 and in cases where a patient is young, FRAX will default to a calculation based on 40 years of age. Weight, height, age and BMD are entered as continuous data, however the remaining risk factors can only be entered as a dichotomous entry with simply a yes or no answer, with the result that continuous information, and dose dependent effects are not taken into account. For example, tobacco and alcohol intake are not quantified, and therefore someone smokes 1 to 2 cigarettes a day would be classed in the same category as a heavy smoker. Likewise someone who drinks 21 units of alcohol a week is considered the same as someone with a history of heavy alcohol abuse. There is a large degree of variation in the causes of secondary osteoporosis and many of these will have varying effect on bone with subsequent variation in fracture risk, however FRAX assigns these an identical risk.[139, 140] Falls are a major

cause of non-vertebral fracture in post-menopausal women, however fall history is not one of the FRAX CRFs.

The algorithm was designed with data from patients who are treatment naïve, with no exposure to anti-resorptive therapy. This limits the use of FRAX to untreated patients, and the validity of results with patients who have been on anti-resorptive therapy is questionable.[141] Despite these limitations, there is a large volume of research supporting the use of FRAX, and it is recommended by NICE in the UK as a tool to identify those at risk.

2.5.2.2 QFracture

QFracture was developed in 2009, and bares a number of similarities to FRAX. It produces an output that gives a yearly probability of hip and major osteoporotic fractures as far as 10 years.[139] It is based on UK epidemiological data from general medical practices, and is suitable for use in patients aged 30 to 99. Its use is recommended by NICE guidelines and it is the preferred tool of the Scottish Intercollegiate Guidelines Network (SIGN).[140]

QFracture takes into account more CRFs than FRAX, and it allows for female related risk factors, including hormone replacement therapy and menopausal symptoms. The developers recognise that the effects of both smoking and alcohol are dose dependent and designed it without limiting the entry of these two variables to a binary option and instead gives the option of five different levels to enter for each. It is designed for use specifically in the UK, based on information recorded in UK patient notes using the Egton Medical Information Systems (EMIS) computer system used in 59% of general practices in England, whereas the data from FRAX is derived from cohort studies. BMD is not taken into account as it is rarely recorded in GP notes and is only likely to be recorded in selected high risk populations.[142] Qfracture is designed to be used prior to DXA scan.

2.5.2.3 Comparison of FRAX and QFracture

While both FRAX and QFracture take glucocorticoids as a CRF, neither takes dosage into account. FRAX advises a yes answer if the patient has been exposed to oral glucocorticoids for more than 3 months at a dosage of 5mg Prednisolone or higher. QFracture is less clear in its definition and advises a yes answer if 2 or more prescriptions were given in the last 6 months. Kanis *et al* in 2011 advises a dose related adjustment for FRAX: for low dose exposure (<2.5mg Prednisolone or equivalent) the probability of a major fracture can be reduced by 20%. For medium dose exposure (2.5 to 7.5mg Prednisolone), no adjustment is necessary. For high dose exposure (>7.5mg Prednisolone), fracture risk probability should be increased by 15%.[134]

There have been attempts to compare FRAX and QFracture. Hippisley-Cox *et al* in 2009 carried out a comparison and found that FRAX overestimated risk of hip fracture in comparison to QFracture.[139] It should be acknowledged that these authors were involved in the development of QFracture, so there may be increased risk of bias in their reporting. The same authors in 2011 found there were discrepancies between results entered in the 2008 FRAX and the results entered into the 2011 FRAX using the same CRFs. This was likely due to adjustments made in the FRAX algorithm or possibly bug fixes, making comparison between the two algorithms difficult.[133]

Collins *et al* in 2011 planned an independent comparative study, however the developers of FRAX did not take up the request to do a comparison. The authors did however continue with an independent evaluation of QFracture scores and reported that there was strong evidence to support the external validity of Qfracture scores in predicting 10-year risk of osteoporotic or hip fracture.[143]

The FRAX algorithm underestimates the 10-year fracture risk in older patients whereas QFracture has been shown to accurately predict fracture risk up to 85 years of age. It has been suggested this is because FRAX takes mortality into account whereas QFracture does not.[140]

A criticism directed at fracture risk algorithms is that as they are all based on epidemiological data, they underestimate the risk of vertebral fracture as up to two thirds of vertebral fractures go unreported.[55]

2.5.3 Dual energy x-ray absorptiometry

Dual Energy X-ray Absorptiometry (DXA) is the most widely used method of measuring bone mineral density (BMD) and was first introduced for clinical use in 1987. While it is used primarily for assessment of BMD, there is an increasing volume of research into its use to assess body composition.[144-147] DXA measures BMD at the hip and lumbar spine, and can also be used on the forearm in cases where the spine or hip cannot be measured.

Its mode of action is based on x-ray beam attenuation. Attenuation is the loss of energy of a wave or a beam as it passes through a medium, and the degree of attenuation of an x-ray beam depends on the thickness and density of the subject being scanned. Greater attenuation indicates denser and thicker bone and soft tissue. A single energy x-ray beam can produce a 2-dimensional image, which is suitable in diagnosis of a fracture, however while single energy x-ray absorptiometry can be used in BMD measurement, it cannot give a reading that compensates for soft tissue and is generally not used as soft tissue is responsible for a certain amount of attenuation. To overcome this problem, DXA produces a beam of two different energy peaks: typically the energy levels are 40 and 70 keV. The higher the energy of the beam, the lower the degree of attenuation. Bone is rich in attenuating minerals, and is readily distinguishable from soft tissue. By measuring attenuation based on both energy and subject thickness, the software algorithms can use two simultaneous equations to calculate the thickness and density of both bone and soft tissue. DXA can also differentiate between fat, and non-fat soft tissue. This allows for a more accurate measurement of energy absorption of soft tissue separately to that of bone, providing a more accurate result for BMD.[148]

The result from a DXA scan is given as an areal measurement, and expressed in grams per area (g/cm^2), rather than true density. True density expressed as grams per volume (g/cm^3) can be measured by quantitative computerised tomography (qCT).[149]

The radiation dose from a DXA scan is low in comparison to plain film x-ray and qCT, however there is variation between manufacturers. The radiation from a total DXA scan which includes three assessments: lateral vertebral assessment, an anterior posterior spine and femoral region (femoral neck and total hip) using the Hologic Horizon A used in St James's Hospital is 8.5µSv.

Short and long-term precision of BMD measurement using DXA has been shown to be reliable in multiple studies with reported co-efficients of variation, ranging from 0.5 to 2% at the spine, and 1.3 to 1.5% at the femoral neck.[150-153] However it has been demonstrated that obesity has a negative effect on precision,[146] and a recent study in rugby players suggests that high levels of lean mass may have a similar effect.[145]

DXA has a number of other limitations. It cannot take into account bone quality and microarchitecture. Two-dimensional areal BMD measurement of the spine is prone to artefacts from aortic stenosis and spinal degeneration. There is variation in scanners, software and scanning protocols between manufacturers meaning that inter-device comparisons are not always possible.[154] It may be prone to operator error resulting in reduced inter-operator and intra-operator precision. Incorrect positioning of the patient can affect the readings and as a result sequential scans may not be comparable if patient positioning has been shown to be inconsistent.[155] Despite these limitations, DXA has proven itself a reliable and cost effective method of identifying those at risk of fragility fracture, it has the highest volume of research validating its use, and after over 30 years, it is still considered the Gold Standard in clinical densitometry.

The World Health Organisation in a report published in 1994, proposed criteria for diagnosis of osteoporosis based on BMD. According to these criteria osteoporosis is defined as being 2.5 standard deviations or more below the mean of a young female population aged 20 to 40 years old, and is expressed as a T-score ≤ -2.5. Osteopenia is defined as between 1 to 2.5 standard deviations below the mean of this reference population and is expressed as a T-score of -2.5 to -1.0, and those with T-scores above -1 are considered as having normal bone density.[156]

T-scores are calculated by the following equation: -

$$\frac{\text{Patient's BMD - Mean BMD of the Reference Population}}{\text{Standard Deviation of the Reference Population}}$$

Prior to the WHO recommendations, Z-scores were used using an aged matched reference group, however the WHO found that the comparison against a healthy reference group would be more appropriate.

The T-score method of classifying osteoporosis is not without criticism. Multiple studies have found that the majority of fragility fractures occur in patients classified by this system as not being in the osteoporotic range (T-score > -2.5).[157, 158] Cranney *et al* in 2007 in a retrospective study of 16,505 women found that over half of fractures occurred in women with T-scores greater than -2.5, however a fracture rate of 21.6 (95% CI 19.7–23.4) per 1000 person-years was reported in those aged ≥ 65 versus 8.6 (95% CI 7.5–9.7) in those aged ≤ 65.[159]

A meta-analysis of data from 12 studies found that at the age of 65 years, the risk of osteoporotic fractures increased in men by 1.41 per standard decrease in BMD (95% CI = 1.33-1.51), and in women by 1.38 per standard deviation decrease (95% CI = 1.28-1.48).[160]

BMD is an important predictor of fragility fracture, however age is an important cofactor in fracture prediction, and needs to be taken into consideration when evaluating an individuals fracture risk.[156, 160] The use of BMD alone should be

approached with caution, as it may class patients as having a normal BMD when they are still at risk of a fragility fracture.

2.5.4 Quantitative computerised tomography

Shortly after the introduction of Computerised Tomography (CT) in 1973, its application in the measurement of volumetric bone mineral density (vBMD) was developed.[161, 162] Quantitative computerised tomography (QCT) is a 3-dimensional technique of measuring central BMD in the spine and hip, and peripheral BMD of the radius and tibia. It measures BMD as a volumetric value expressed in g/cm^3.

CT utilises x-rays and produces an image based on linear x-ray absorption coefficients of the tissues through which the x-rays pass. CT scanners are calibrated to the x-ray attenuation of water, resulting in CT numbers referred to as Hounsfield Units (HU).[163] The Hounsfield Unit scale expresses CT numbers in a standardised form of a scale where the radiodensity of distilled water at standard temperature and pressure is defined as being 0 HU, and air is - 1000 HU.[164] Examples of HU tissue values include fat at -50 to -100 HU, muscle at +40 HU and bone exhibits a range of values from 150 HU for trabecular bone to 2000 HU for dense cortical bone.

Lekholm and Zarb Classification of Bone [165, 166]	Description	Hounsfield Units
I	Dense Cortical Bone	> 1250 HU
II	Thick layer of dense cortical bone surrounding dense trabecular bone	850 to 1250 HU
III	Thin porous layer of cortical bone surrounding dense trabecular layer	350 to 850 HU
IV	Fine trabecular bone with thin or absent cortical bone	150 to 350 HU

Table 2. 5 Relationship between bone classification and Hounsfield Units.

To convert Hounsfield Units into bone mineral equivalents, a bone mineral calibration phantom is included in the scan field, when the patient is being scanned. These phantoms are made using concentrations of materials with similar attenuation characteristics of bone. The materials are usually either calcium hydroxyapatite or potassium phosphate. Results from different types of calibration phantoms are not interchangeable, however the European Spine Phantom (ESP) and the European Forearm Phantom (EFP) have been developed, to allow for such cross calibration and quality assurance testing on different manufacturers' scanners.[163]

There are a number of advantages of QCT over DXA. QCT gives a true measurement of bone mineral density unlike DXA scan, which gives a 2 dimensional areal BMD. Quantification of vBMD by CT scan is independent of bone size. Three-dimensional scanning allows for differentiation between cortical and trabecular bone. It allows for measuring of BMD in cases of severe scoliosis, which cannot be achieved with

DXA. QCT is of particular advantage in cases of spinal degenerative disease, disc space narrowing, aortic calcification and obesity where DXA may produce erroneously high results.

QCT is not without its disadvantages. Radiation dose is higher in comparison to DXA however it is still relatively low at 50μSv for a spinal QCT. Precision errors of 2 to 4% CV and accuracy errors of 5 to 15% have been reported.[163, 167] It is more costly than DXA. Faulkner *et al* in 1999 found discordance between T-scores measured by DXA and those measured by QCT.[168] The position of the International Society of Clinical Densitometry is that the WHO classification of osteoporosis in T-scores is only applicable to DXA.[169]

2.5.5 Ultrasound

Medical Ultrasound is a form of diagnostic technology that incorporates high frequency sound waves to characterize tissue. The sound frequency range detectable by the human ear is approximately 20 Hertz (Hz) to 20 Kilohertz (KHz). A sound wave with a frequency below the lower value of this range is referred to as infrasound, and above the upper value is referred to as ultrasound. The frequency range of sound waves utilised by medical ultrasound is typically in the range of 1 to 20 MHz, although due to increased attenuation of higher frequencies in bone, devices used for investigating bone strength operate at a lower frequency range of usually between 250 kHz and 1.25 MHz.

2.5.5.1 Principles of ultrasound

Ultrasound devices utilise a number of principles of sound waves including oscillation, refraction, reflection, and impedance. Ultrasound waves are generated by the conversion of electrical pulses into sound waves, by the use of piezoelectric transducers. Returning sound waves or are then converted back into electrical signals, which may be used to create an image of the tissues through which these

waves propagate, or in the case of Quantitative Ultrasound a numeric reading relating to bone strength.

Ultrasound waves cause disturbance of the particles of the conducting medium from their resting position, resulting in a transmitted pulse displacing the particles about their mean positions, but without an overall bodily movement of the medium.
Ultrasound waves propagate through solid material primarily by oscillation of longitudinal waves and shear waves.

Longitudinal waves sometimes referred to as compression waves, occur in the direction of the wave propagation with a combination of compression and rarefaction.

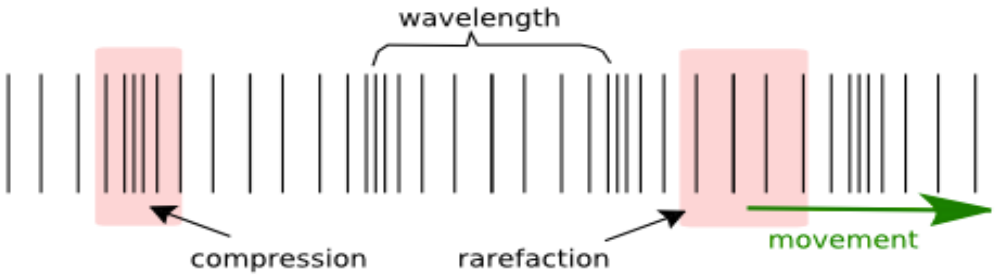


Figure 2. 9 Longitudinal wave.

In shear waves the particles oscillate transverse to the direction of propagation. They are relatively weak compared to longitudinal waves and are not effectively propagated in liquids and gases. They propagate through more solid media due to the strong binding of particles in a solid. Shear waves are usually generated in materials by the energy from longitudinal waves. In isotropic solids they occur perpendicular to the direction of propagation. In soft tissue, shear waves are highly attenuated at ultrasound frequencies and are usually neglected, however they must be taken into account in harder tissue like bone.[170]

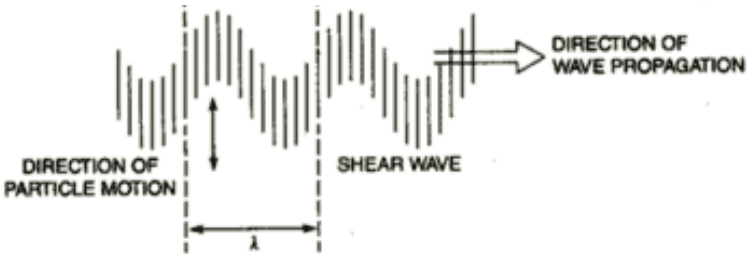


Figure 2. 10 Shear wave.

The speed of the ultrasound wave through a medium is referred to as the wave velocity. There are a number of mechanical tissue factors that influence the velocity including density of the tissue, and its modulus of elasticity. The modulus of elasticity relates to the compressibility of the tissue and its stiffness. In tissues with high density such as bone the velocity is higher, whereas in viscoelastic tissues of lower density, such as muscle and fat the velocity is lower.

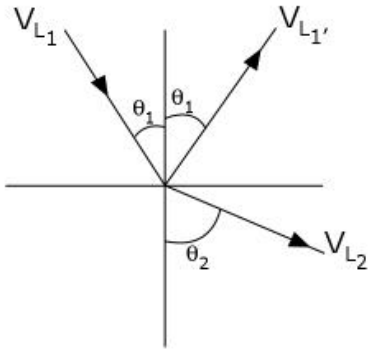
The velocity of a longitudinal wave is equal to $\sqrt{\frac{E(1-v)}{\rho(1+v)(1-2v)}}$

And the velocity of a shear wave is equal to $\sqrt{\frac{E}{\rho(1+v)}}$

Where E is the modulus of elasticity, ρ is the density of the medium, and v is Poisson's ratio, which is the ratio of transverse to axial strain.

Refraction of a sound wave occurs when a wave travels between more than one medium with differing densities. As a wave passes through tissues of different density and elasticity, the propagation speed and the direction of the wave change.

This is represented by Snell's Law.



$$\text{Sin } \theta_1 / VL_1 = \text{Sin } \theta_2 / VL_2$$

Where VL_1 and VL_2 are the longitudinal wave velocities in the 1st and 2nd medium, and θ_1 and θ_2 are the incident and refracted angles.

Figure 2. 11 Refraction of a wave.

Acoustic impedance, signified by Z, is the resistance an ultrasound wave encounters as it passes through tissue. It is dependent on the density of the tissue and the velocity of the wave. It has been found to be a predominant determinant of speed of sound (SOS) through tissue.[171]

$$Z = (\text{Density expressed in kg/m}^3) \times (\text{speed of the sound wave in ms}^{-1})$$

Therefore if the density of a material is high, it will have a high impedance value. The Standard International (SI) unit of acoustic impedance is the pascal second per cubic metre ($Pa{\cdot}s/m^3$) or the rayl per square metre ($rayl/m^2$). For example, bone has a high impedance value of 7.8×10^6 $rayl/m^2$ and muscle has a lower value of 1.7×10^6 $rayl/m^2$. The impedance value of water is 1.48×10^6 $rayl/m^2$.

When an ultrasound wave passes through two different tissues, it reflects off due to difference in density between the two different tissues. The amount of reflection that occurs is expressed as the following:

$$\text{Reflection Coefficient (R)} = \left(\frac{Z2 - Z1}{Z2 + Z1}\right)^2$$

Where Z_1 is the impedance of the first tissue encountered and Z_2 is the impedance of the second tissue. The greater the difference in the impedance values of the two tissues, the greater the amount of energy reflected.

The energy of the wave transmitted through the second tissue is expressed by the following:

$$\text{Transmission Coefficient (T)} = \frac{4(Z1 \times Z2)}{(Z1+Z2)^2}$$

The sum of the Reflection Coefficient and the Transmission Coefficient is equal to 1. Multiplying the reflection coefficient and the transmission coefficients by 100 gives the percentage of the energy reflected at the tissue interface and the percentage transmitted through the second tissue, from the incident energy, respectively.

Mode conversion is one of the properties of sound waves responsible for ultrasound attenuation. When a sound wave travels in a medium, one form of energy can be converted into another form. This occurs when a sound wave meets an interface between materials of varing acoustic impedances and the incident angle is not normal to the interface. An example is when a longitudinal wave hits an interface at an angle and some of the energy causes particulate movement in a transverse direction generating a shear wave.

2.5.5.2 Quantitative ultrasound

The clinical application of Quantitative Ultrasound (QUS) was introduced by Langton *et al* in 1984, demonstrating that attenuation of ultrasound waves over a frequency of 0.2 to 0.6 MHz was linear, and the slope of this relationship had the capability of differentiating between normal and osteoporotic subjects. This range of frequency was chosen as it possesses the highest sensitivity to osteoporotic change.[172, 173] Since its introduction, the interest in QUS and its application in fracture prediction has been investigated further, and there is an increasing volume of evidence supporting its use.

There are a number of advantages related to the use of QUS: it is a non invasve method of bone assessment, it does not expose the subject to ionising radiation, and as it is a portable system, it is of particular advantage in patients where mobility is an issue. QUS use requires comparatively little training and does not require a radiographer or sonographer to operate the device, and the cost is low compared to DXA. QUS devices are divided into two types based on mechanism of action: transverse transmission and axial transmission devices.

2.5.5.3 Transverse transmission ultrasound

Transverse transmission devices utilise a pair of transducers, one of which acts as a transmitter and the other acts as a receiver. These transducers are placed on either side of the skeletal site under investigation, and an ultrasound wave is produced which passes through the tissue.

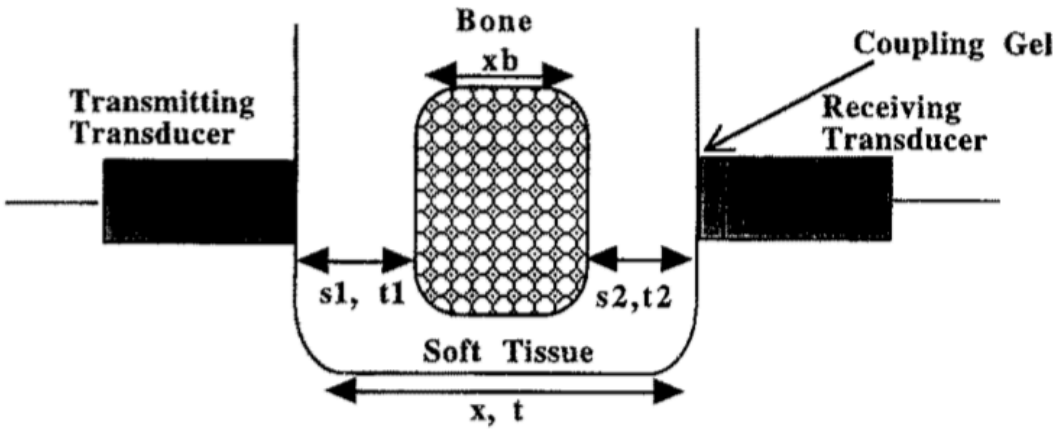


Figure 2. 12 Mechanism of action of calcaneal QUS.[174]

Transverse transmission QUS devices are capable of giving readings based on both Broadband Ultrasound Attenuation (BUA) and Speed of Sound (SOS). BUA is the degree to which the energy of an acoustic wave generated by an US device is lost as it passes through tissue. The factors influencing attenuation are diffraction of the wave, scattering, mode conversion and absorption. In cortical bone, absorption is

the predominant attenuation mechanism, whereas in trabecular bone the predominant mechanism is due to scatter.[173] Ultrasound absorption is not fully understood and the mechanisms thought to be responsible include thermal conductance effects, chemical effects, and viscous forces between neighbouring particles.[170]

SOS is the speed of the acoustic wave generated, as it propagates through a known length of tissue. Its unit is ms^{-1} and results vary depending on the properties of the tissue. The SOS measurement is higher in tissue of higher density. SOS may also be referred to as Velocity of Sound, apparent speed of Ultrasound, Apparent Velocity of Ultrasound, and Ultrasound Transmission Velocity.

Transverse transmission devices such as Calcaneal QUS can give a reading referred to as Stiffness Index (SI), which is a mathematical combination of both BUA and SOS and is calculated by the following equation.

$$SI = [(0.67 \times BUA) + (0.28 \times SoS) - 420]$$

A study using Calcaneal QUS found that SI was significantly better at identifying those at risk of fracture compared to either BUA or SOS alone.[175] The Calcaneal bone is the preferred bone for measurement using transverse QUS. This is because the calcaneal bone is composed of 90 to 95% trabecular bone, which has eight times the metabolic turnover of cortical bone due to its high surface to volume ratio, and the cortical bone on the medial and lateral sides are nearly parallel to eachother facilitating conduction of the sound wave. The Calcaneus is considered to be a bone that reflects the mechanical environment of the weight bearing sites, such as the hip, and the vertebrae. It possesses the added advantage of being an easily accessible site.[176]

2.5.5.4 Use of calcaneal QUS in fracture prediction

Calcaneal QUS is the most established form of QUS, and to date has the largest volume of clinical research than any other form of QUS. It is the only form of QUS that has been validated by the ISCD for clinical use in osteoporosis management. Whilst the ISCD recognises that calcaneal US predicts fragility fracture independently of central DXA BMD, it does recommend that spine and hip BMD measurements are preferred when making therapeutic decisions.[177]

One of the earlier studies investigating hip fracture prediction using Calcaneal QUS was conducted by Hans *et al* in 1996. 5,662 women with a mean age of 80.4 years were followed up for a mean of 1.99 (±0.57) years. The relative risk of a hip fracture occurring was found to be 2.0 (95% CI 1.6-2.4) and 1.7 (95% CI 1.4-2.1) for a 1 standard deviation decrease in BUA and SOS, respectively. A standard deviation decrease in BMD has a relative risk of 1.9 (95% CI 1.6-2.4) for a hip fracture, demonstrating that QUS has a similar efficiency to DXA in hip fracture prediction. The large sample size and prospective design are strengths of this study, but it does suffer from a short follow up period.[178]

Pluijm *et al* in 1999 reported a similar result in a prospective study. When adjusted for age and sex, the relative risk of hip fracture for each standard deviation reduction was 2.3 (95% CI, 1.4–3.7) for BUA and 1.6 (95% CI, 1.1–2.3) for SOS. The mean age of participants was 82.8 ± 5.9. Weaknesses of this study were a relatively small sample size with 710 participants (578 women and 132 men), and the short follow up time with a median of 2.8 years, and a maximum of 3.7 years.[179]

A larger study by Khaw *et al* in 2004 of 14,824 participants (6,485 men and 8,339 women) found that those in the lowest 10% of BUA measurements had a relative risk of fracture of 4.44 (95% CI 2.24 to 8.89, $p<0.001$), independent of age, sex, weight, height, cigarette smoking habit, and past history of fracture. Participants were followed up for a relatively short time period with a mean of 1.9 ± 0.7 years.[180]

Although these studies do suffer from relatively short follow up periods, they do demonstrate the application of calcaneal QUS in fracture risk assessment.

2.5.5.5 Axial transmission ultrasound

Axial QUS devices operate by a slightly different mechanism of action to transverse devices, and give a reading based on SOS. Similar to transverse devices they utilise transmitting and receiving transducers, however, unlike transverse transmission devices, the transducers on an axial QUS device remain on one side of the bone. An acoustic wave is generated by the transmitting transducer, which propagates through the cortical bone to a depth of 2mm to 6mm, along the long axis of the bone. The length of time this wave takes to reach the receiving transducer is measured and as the distance between the transducers is fixed, the device calculates the speed of the wave through the tissue, generating an SOS reading.

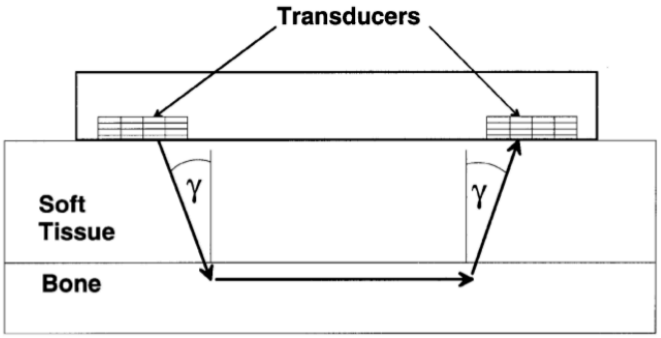


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Figure 2. 13 Mechanism of action of axial QUS.

Axial QUS devices have been developed for use on multiple sites with the most common sites being the tibia, the phalanges, the fifth metatarsal and the distal one third radius. They are referred to as multisite QUS devices, or mQUS. An advantage of mQUS devices is that, as they are used on multiple sites, they can be used directly on bones that may be prone to fracture such as the radius, whereas calcaneal QUS is limited to the calcaneal bone, which very rarely fractures.[182]

The most commonly used mQUS device used in clinical research is the Omnisense 8000, manufactured by BeamMed in Israel. In the US it is licensed for commercial and research purposes, but in Europe it is licensed for research purposes only.

2.5.5.6 Quantitative ultrasound and properties of bone

Bossy *et al* in 2004 investigated the relationship between SOS and properties of bone measured by Synchrotron Radiation Micro CT (SR-μCT). The properties under investigation were vBMD, porosity, cortical thickness, and mineralisation using 39 excised human radii from which the soft tissue had been removed. SOS was measured with a bi-directional mQUS device at a frequency of 1 MHz. They found that SOS readings are sensitive to both porosity and mineralisation in the 1mm thick periosteal region of the cortical bone, rather than the whole cortical thickness with R^2 values of 0.28 ($p<10^{-3}$) between SOS and porosity, 0.38 ($p < 10^{-4}$) between SOS and mineralisation, and 0.57 ($p < 10^{-3}$) between SOS and volumetric BMD. [183]

A related study by Raum *et al* in 2005 using SR-μCT and a Scanning Acoustic Microscope on 10 of the radii that were used in the study by Bossy, to examine the contributions of cortical structure, degree of mineralisation, and acoustic impedance on SOS readings. Cortical width was significantly correlated with SOS measured at 1 MHz (r=0.56, p=0.01), and acoustic impedance was found to account for 70% of variability of SOS. Acoustic impedance is a strong predictor of bone elasticity, therefore it can be inferred that elasticity is also a strong predictor of SOS.[171]

It is thought that as QUS readings are influenced by other properties of bone and not only BMD, it may provide information that DXA is not capable of taking into account. The combination of QUS and DXA may allow for better insight in determination of bone strength and fracture risk.

2.5.5.7 Use of mQUS in fracture prediction

Despite the availability of mQUS for close to 20 years, there is surprsingly little evidence relating to its use in fracture prediction. Olszynski *et al* in 2013 took baseline mQUS readings from the distal third of radius, midshaft tibia, and proximal phalanx using the Omnisense 8000 in a cohort of 2,633 women and 1,108 men. They prospectively followed them for 5 years. The authors found that in females, on

average a one standard deviation decrease in SOS at any of the three sites was associated with a 52% to 83% increase in the risk of any fracture, a 100% to 130% increase in risk of hip fracture, and 54% to 85% increase in vertebrae fracture risk over 5 years, however they did not find an association with fracture risk in males. This may be related to the fracture rate in males being particularly low at 0.7% resulting in the power of the study being too low to identify a significant effect.[182]

2.5.5.8 Comparison of mQUS measurements between sites

One of the earlier studies using an mQUS device (Omnisense) was conducted by Njeh *et al* in 2001. The aim of this study was to determine whether there was heterogeneity between SOS measured at multiple peripheral sites. Three hundred and thirty four adult women with a mean age of 48.8 (±17.4) and range of 20 to 89 years of age were recruited. Measurements were taken from the proximal third phalanx, distal one third radius, midshaft of the tibia and the fifth metatarsal, and T-scores were calculated for each site. They reported weak to moderate correlation between sites with Pearson correlation coefficients of 0.31 to 0.56 (p<0.001). The strongest correlation was found between between the radius and the phalanx (r=0.56), followed closely by the phalanx and metatarsal (0.52), and then the radius and metatarsal (r=0.51). Interestingly, the tibia displayed the lowest correlation co-efficient values, with values of 0.44, 0.38, and 0.31 with the radius, phalanx, and metatarsal,respectively. The authors also reported that SOS was significantly correlated to age with correlation coefficient values ranging from 0.33 to 0.55 (p<0.0001). The highest peak values for all sites were seen in those aged between 24 and 37. The authors found that when SOS readings are converted to T-scores, discordance between sites still exists. If a T-score of -2 is considered a cutoff value for diagnosis of osteoporosis, then prevalence of osteoporosis varies between sites with the tibia showing the lowest prevalence (22%), and the metatarsal, phalanx, and radius showing similar values of 32%, 36%, and 37%, respectively. It is unclear why the properties of the tibia appear to be significantly different from all the other measured sites.[184]

2.5.5.9 Comparison between QUS and DXA measurements

A number of studies have investigated the relationship between QUS and BMD, although the designs between different studies vary in their approach, making it difficult to compare findings. This evidence shows that a degree of discordance exists between QUS results and BMD. This is not surprising as QUS is used on peripheral sites, and DXA scans for the most part measure BMD at central sites. It should also be taken into consideration that DXA and QUS measure properties of bone based on different physical principles.

Töyräs *et al* in 2002 compared QUS and BMD of the calcaneus in 32 adults (17 males and 15 females). They found that BUA of the calcaneus correlated positively and linearly (r=0.849, p<0.01) with bone of low or moderate density BMD (< 1.0g/cm^2), but not with high density bone. There was no longer a linear relationship with high density bone. This study also investigated the attenuating properties of bovine bone as a number of previous studies had found different attenuation between human and bovine bone. They found a negative correlation between volumetric BMD and BUA (r = −0.540, n = 29, p < 0.01), although a strong positive correlation was found between volumetric BMD and SOS (r = 0.888, n = 29, p < 0.01).[185]

Larijani *et al* in 2005 comparing results from DXA scan and calcaneal QUS in 420 subjects found that the sensitivity of QUS to identify BMD diagnosed osteoporosis ranged from 83.9% to 87.5%. However they did find weak to moderate agreement between T-scores calculated by DXA, and those calculated by QUS. This is likely due to inaccuracies in each technique, as well as each method differing in parameters of bone density measurement.[186]

Cook *et al* in 2005 investigated correlation between three different assessment techniques in 246 subjects. BMD readings were taken using a Hologic QDR-4500C DXA scanner, SOS and BUA readings were taken using a Cuba Clinical Calcaneal QUS, and SOS readings from the distal radius, proximal phalanx, and the mid shaft tibia taken using an Omnisense 8000 mQUS. The strongest correlation between the

different devices was from BMD taken from the total hip and calcaneal BUA with a Pearson correlation coefficient of 0.661 (p<0.0001), followed by L1 to L4 BMD and calcaneal BUA with a coefficient of 0.588 (p<0.0001). The correlation between mQUS taken from the three peripheral sites and BMD was weak with coefficients ranging from 0.251 to 0.308 for lumbar spine BMD, and 0.171 to 0.315 for total hip.[187]

Moayyeri *et al* in 2009 conducted a prospective study with a long term follow with the aim of comparing fracture prediction of calcaneal QUS to DXA. 1,455 research subjects (703 males and 752 females) were followed up for a mean of 10.3 (±1.4) years. A one standard deviation decrease in total hip BMD was associated with a hazard ratio for fragility fracture of 2.26 (95% CI: 1.74–2.95). In a multivariable model with heel broadband ultrasound attenuation (BUA) in place of BMD, hazard ratio for a one standard deviation decrease in BUA was reported to be 2.04 (95% CI: 1.55–2.69). Harrell's C-index (equivalent to area under the ROC curve for survival data) values of 67.9% for BMD and 68.5% for BUA were reported indicating a small relative superiority of QUS in fracture prediction.[188]

2.5.5.10 Comparison between mQUS and DXA measurements

Knapp *et al* in 2001 compared fracture discrimination ability of mQUS to DXA. SOS readings taken using an Omnisense mQUS on the radius, tibia, phalanx and metatarsal, were compared to BMD measured at the spine, femoral neck and total hip using a Hologic QDR DXA scanner. Fracture discrimination ability was found to be stronger using DXA with reported odds ratios from 2.6 (95% CI 1.7 to 4.11) for the spine to 4.8 (95% CI 2.81 to 8.29) for the total hip. Fracture discrimination ability of mQUS was found to be quite poor in comparison. SOS from the tibia was unable to discriminate vertical fracture cases from controls (OR 1.2 95% CI 0.87 to 1.66), and while the phalanx showed the strongest fracture discrimination ability out of all the SOS sites, this was still quite modest with an odds ratio of 2.0 (95% CI 1.22 to 3.23). Correlations between SOS and BMD readings were also performed. The highest

correlation between SOS and BMD was reported to be between the phalanx and femoral neck with a correlation coefficient of 0.48 (p<0.001).[181]

2.5.5.11 Effect of age on SOS readings

A number of studies have used the Omnisense mQUS to collect data producing reference databases. The practical clinical application of these databases is to produce normative reference data with which readings can be compared to differentiate between normal, osteopenic and osteoporotic individuals in a similar manner to comparison of BMD measurements to a young reference population to produce T-scores.

Data was collected from a population of 1,521 healthy Israeli women aged between 20 and 90 by Weiss *et al* in 2000 to produce the initial reference database for the Omnisense ultrasound. SOS readings were taken from the distal radius, metatarsal, tibia, and third phalanx. SOS readings from the tibia peak earlier in life than all the other sites with a peak SOS of 3938 ms^{-1} at 20 years of age. The radius peaks at 38 years (4169 ms^{-1}), the metatarsal at 44 (3663 ms^{-1}) and the phalanx at 36 (4047 ms^{-1}). The largest rate of decline in the radius, 16 ms^{-1} per year, is observed almost 4 years past the mean age of menopause at 55 years of age. At older ages, 60 to 90 years, this decline appears to slow down to 6 ms^{-1} per year. A weakness of this study is that relatively few subjects in the older age groups had a DXA scan with the result that the osteoporotic status of many subjects was largely unknown. However, those who had a previous DXA were classed as either normal or osteopenic and the authors did apply strict exclusion criteria to reduce the possibility of subjects being osteoporotic.[189]

One of the largest of these reference database collection studies was recently published by Rivas-Ruiz *et al* in 2015 on a Mexican population of 9,308 (6,313 females and 2,995 males) subjects aged between 1 and 75 to show changes in SOS readings throughout life. The SOS readings were limited to the the radius and the

tibia on the non-dominant sides. Using a locally weighted regression smoothing scatterplot model, they found five different phases that constitute the biological development of bone over the course of life, from 1 to 6, 7 to 12, 12 to 25, 25 to 50, and 50 to 75 years of age (p<0.05). Similar to the study by Weiss in 2000, peak SOS was seen earlier in the tibia at 28 years in males (3896 ±120 ms^{-1}) and 30 in females (3873 ±126 ms^{-1}) when compared to the radius with a peak SOS reading at 41 years in males (4017 ± 129 ms^{-1}) and 39 years in females (4068 ± 127 ms^{-1}). Following peak SOS, the fall in readings between age of 40.1 and 50 in females is twice that of males. It is unclear why the authors chose only these two sites. They also performed DXA scans, and it is unclear whether there was an attempt to correlate BMD to SOS readings.[190]

Other reference databases have been collected using the Omnisense mQUS device from British, North American, and Asian populations. There seems to be considerable agreement in the results between these studies. Age related changes appear to be a consistent feature with SOS readings peaking between 20 and 40 years of age. An interesting feature in these studies is that SOS readings from the tibia peak in the 20s, approximately 10 years earlier when compared other sites.[181,191, 192]

2.5.5.12 Application of T-scores to QUS measurements

The WHO criteria for T-score classification of osteoporosis was developed to provide diagnostic thresholds for BMD measured by DXA at the hip and lumbar spine. A number of authors have investigated the clinical application of T-scores calculated with SOS and BUA readings taken at peripheral sites. Frost *et al* in 2000 compared BMD and calcaneal QUS taken using three different types of Calcaneal QUS, and found that the prevalence of osteoporosis in healthy postmenopausal women based on WHO criteria was 17%, 16% and 12% for lumbar spine, femoral neck and total hip BMD, respectively. When the same definition was used for cancaneal QUS measurements the prevalence of osteoporosis was lower and ranged from 2% to 8%

depending on the type of device used. They found that a T-score threshold of -1.8 applied to calcaneal QUS, would result in a similar percentage of post-menopausal women classified as osteoporotic by BMD.[193]

Knapp *et al* in 2004 compared three groups of women: healthy pre-menopausal women (n=278), healthy post-menopausal women (n=194), and post-menopausal women with atraumatic vertebral fractures (n=115). Healthy subjects were stratified into 10-year age groups and the mean T-score were calculated for each of the groups to investigate age related decline in T-scores, which ranged from -0.92 to -1.80 for SOS measurements in the 60 to 69 year age group, and -0.60 to -1.19 for BMD measurements in the same age group. They found the WHO T-score definition of osteoporosis is not applicable to SOS measurements at peripheral sites and suggested alternate values as thresholds for osteoporosis of -2.6, -3.0, -3.0 and -2.2 at the radius, tibia, phalanx and metatarsal, respectively. A reason suggested by the authors for these different values per site is that the tibia and metatarsal are both weight bearing sites, whereas the radius and phalanx are not.[194] It could also be argued that there would be an expected degree of discordance between SOS and BMD, as SOS is measured at peripheral sites and BMD is most commonly measured at central sites (spine and hip).

The ISCD does not recognise the use of T-scores derived from all forms of QUS for management of osteoporosis because the WHO T-score classification was established using central DXA, and the T-score threshold of -2.5 cannot be applied to QUS without the risk of discrepancies in the number of women diagnosed with osteoporosis.[169]

2.5.5.13 mQUS to monitor response to treatment

There are a limited number of studies investigating the use of QUS to monitor response to treatment, and of those available there are conflicting results. The current position of the ISCD is that QUS has not been validated for use of monitoring response to osteoporosis treatment.[177]

Drake *et al* in 2002 used an mQUS device (Omnisense 8000) on multiple sites (radius, tibia, metatarsal and phalanx) to monitor response to oral Alendronate treatment for osteoporosis in 81 female subjects over the course of 1 year. They found no statistically significant change in SOS readings as a response to treatment. A secondary aim of this study was to determine the efficacy and tolerability of two different weekly doses of Alendronate, 80mg and 160mg. The authors state that the data from the two groups were pooled. It is not apparent from the paper whether there was an effort to differentiate the response between the two doses, with the result that it is unclear whether there was a significant response in either of the two groups alone. Subjects in this study were not treatment naïve and were placed on 6 months of Selective Oestrogen Receptor Modulators prior to commencing therapy.[195]

In contrast to the results reported by Drake *et al*, Weiss *et al* in 2003 reported a positive response to osteoporosis treatment in 68 female patients on 10mg Alendronate daily, followed up over a 2 year period. After 12 months of treatment there was an increase in T-scores of SOS readings of 0.05, 0.01 and 0.21 in the Radius, Phalanx and Tibia, respectively. In those with a baseline T-score lower than -2 a greater response was seen, and an increase was observed in SOS T-scores of 0.25, 0.19 and 0.25 in the Radius, Phalanx and Tibia, respectively. While this study had a lower sample size than the study by Drake *et al*, a strength is that the enrolled patients were treatment naïve.[4]

Ingle *et al* in 2005 conducted a randomised control trial used a transverse transmission caliper style of mQUS device called the DBM Sonic Bone Profiler on the fingers to monitor Alendronate and Estradiol therapy in post-menopausal women. In the Alendronate group, they found an increase in SOS of 26 and 34 ms^{-1} at 12 and 24 months, respectively (p<0.001). This result is similar to the observed increase in BMD at the lumbar spine (LS) of 0.022 and 0.027 g/cm^2 at 12 and 24 months, respectively (p<0.05). In the Estradiol group an increase in SoS of 51 and 21 ms^{-1} was observed (p<0.01) at the 12-month point only, compared to an increase in LS BMD 0.096 and

0.082g/cm^2 (p<0.01) at 12 and 24 months, respectively. The authors conclude that changes in finger ultrasound are similar in clinical utility to DXA measurements at the femoral neck for monitoring of anti-resorptive treatment. A number of participants withdrew from the study leaving the Alendronate group with 14 participants and the Estradiol group with 8 participants. The resulting low sample size is a major study weakness making it difficult to draw definitive conclusions from this study.[196]

The evidence looking at the use of QUS to measure response to treatment is conflicted. There is a lack of good evidence showing an osteoporosis treatment response at peripheral sites using mQUS. However as the number of studies is rather limited, this makes a definitive conclusion difficult, and indicates there is necessity for further research.

2.6 Facial bones

2.6.1 Biomechanical properties of the mandible

The mandible undergoes deformation due to forces produced by muscles of mastication and reaction forces through the teeth and the temporomandibular joints. The distribution of stresses and strains depends on biomechanical properties of the mandibular bone, the nature of the applied forces, and the geometry of the mandible. It has been shown that the cortical bone of the mandible is stiffer at the lower border than at the alveolar bone, and the lingual cortex is stiffer than the buccal in the symphysis, and premolar regions. The anterior mandible has a greater elastic modulus and compressive strength compared to other areas. During mastication, the mandible bends in a sagittal direction resulting in compression on the lower border and tension on the upper border of the working side, and the reverse on the balancing side.[29]

2.6.2 Ex-vivo human biomechanical studies

A number of studies have been conducted investigating fracture thresholds of facial bones with the purpose of determining the mechanics and parameters of facial fractures. The importance of this research relates to its application in the design of protective equipment and vehicle safety. An in-vitro study by Hodgson *et al* in 1967 using human cadavers investigated fracture thresholds of the zygoma and zygomatic arch. They found that the force to fracture is time dependent. Forces up to 454kg can be tolerated for extremely short time periods of 3 milliseconds or less, and with impacts lasting beyond 4 milliseconds, the force to fracture is considerably less at approximately 90.7kg.[197] More recently, Unnewehr *et al* in 2003, using seven mandibles from which the soft tissue had been removed, looked at impact forces from a fronto-median and lateral direction. The fronto-median forces were directed to the most prominent point on the chin on four of the mandibles, and lateral forces were directed at right angles to the body of the mandible on the left side, adjacent to the first premolar and molar on the remaining three mandibles. They found that

the fracture threshold is between 2.5 and 3.1 kN for fronto-median impacts, which lead to mostly bilateral and always multiple fractures in the posterior area of the bone. The fracture threshold for lateral impacts was found to be between 0.6 and 0.8 kN, and for two out of three mandibles the fracture occurred at the body on the ipsilateral side.[198]

Peterson and Dechow in 2003, researching the density of the bones of the skull and the face, conducted a cadaver study with 15 dentate specimens (mean age 68.4 years and range 27 to 100). The sites under investigation were the frontal, parietal, occipital, temporal and the zygomatic bones. Cylindrical cortical bone specimens, 4mm in diameter, were harvested from multiple sites on each bone using a trephine bur. The temporal bone was found to have the highest density with a mean of 1.868 (SD 0.149) g/cm^2, the zygoma has the lowest density with a mean of 1.679g (SD 0.197) g/cm^2, and the mean density of the frontal bone was found to be 1.783 (SD 0.128) g/cm^2. Interestingly, while the zygoma had the lowest mean density, the density of the zygomatic process of the temporal bone, which forms a large portion of the zygomatic arch, was found to have a high value at 1.899 (SD 0.111) g/cm^2. [199]

2.6.3 Use of BMD to investigate facial bone strength

Shaw *et al* in 2012 investigated age related changes of BMD in the maxilla and the mandible, and compared it to lumbar spine BMD. 60 subjects (30 male and 30 female) were divided into three groups based on age with 20 subjects in each group. The age groups were 20 to 40, 41 to 60, and over 61 years of age. The maxillary BMD was calculated as an average of the left and right side of a region of interest 3mm x 6mm in the anterior maxilla. Similarly the BMD of the mandible was calculated as an average of the BMDs of an area 2mm x 10mm over the mandibular rami. They found that there is a statistically significant decrease in BMD in the midface between the young and middle-aged groups in both men ($t=3.660$, $p<0.001$), and women ($t=2.760$, $p<0.01$), and a similar decrease in the mandible between the young and middle aged groups in men ($t=3.015$, $p<0.05$) and women ($t=2.563$, $p<0.01$). Unlike

the maxilla and the mandible, the greatest change in the lumbar spine was between the middle aged and old group.[200]

Drage *et al* in 2007 conducted a similar study on 18 edentulous men (n=9) and women (n=9). They measured BMD in the hip and lumbar spines, as well as the anterior maxilla, and three sites on the mandible: the ramus, the body and the symphysis. Five subjects were found to be osteopenic and one was found to be osteoporotic. The authors reported Pearson correlation coefficients at the ramus of 0.59 and 0.5 with the femur and the lumbar spine, respectively (p<0.05). A correlation coefficient of -0.5 was reported between age and the ramus (p<0.05). The remaining jaw sites showed no statistically significant relationship with hip and lumbar spine BMD.[201]

Horner *et al* in 1996 investigated mandibular BMD as a predictor of osteoporosis. The ramus, the body and the symphysis were selected as the regions of interest of the mandible in 40 female subjects. Significant correlations were found between mandibular BMD and all other sites (p<0.02), with the highest correlation between the mandible and the proximal forearm with Pearson correlation coefficients of 0.52, 0.73, and 0.44 at the ramus, body and symphysis, respectively. The femoral neck was found to be the skeletal site with the lowest correlation to the mandible with co-efficients reported ranging from 0.38 to 0.45. It should be noted that this study was limited to edentulous women, as teeth within the regions of interest would introduce error into readings of BMD. This therefore makes the results and conclusions of this study difficult to apply to a dentate and partially dentate population.[202]

It is often difficult to compare studies investigating mandibular BMD as the techniques adopted to measure BMD vary widely. The study by Horner in 2006 conducted their study with intentional superimposition of contralateral sides of the mandible. A study by Çakur in 2009, which will be discussed later in this chapter, described their technique positioning the subject in such a way to avoid superimposition of the contralateral sides of the mandible.

2.6.4 Use of panoramic x-rays to assess osteoporosis in the mandible

There is a growing volume of research investigating the use of dental panoramic radiographs for assessment of osteoporotic change, and much of this research suggests that there are changes evident in the cortical bone at the lower border of the mandible related to osteoporosis. A dental practitioner is the most commonly visited healthcare practitioner for many people, and as there are 1.5 million panoramic radiographs taken yearly in England and Wales, and 17 million in the US, it stands to reason that there is interest in the use of dental radiographs as a potential screening tool for osteoporosis.[202 203]

A number of Indices have been described that categorise changes evident on OPGs. The majority of these indices are based on thickness of the cortical bone at the inferior border of the mandible. One of the earliest Indices is the Gonion Index (GI) described by Bras *et al* in 1982 as part of a two-part study published in two papers. This index is based on the thickness of the cortex at the angle of the mandible. 180 panoramic X-rays were randomly selected from patients of all age ranges and who were not known to have any form of metabolic bone disease. They found that a distinct cortical layer was not found in children up to 10 years of age and infrequently found in those up to 14 years of age. In the age group from 15 to 59, the cortex was relatively constant with a mean thickness of 1.56mm (range 1.0 to 2.5mm) and no significant differences between the sexes, and between dentate, partially dentate and edentulous patients. However the thickness was found to be lower in the female group aged 60 to 69 with a mean of 0.84mm (range 0.2 to 1.2m), while the same age group in men remained constant.[204] The second part of the study assessed the Gonion cortical thickness in 12 patients with chronic end stage liver failure diagnosed with severe (n=5), moderate (n=3), and mild (n=4) renal osteodystrophy. The mean thickness was found to be 0.22 ± 0.18mm, 0.58 ± 0.31mm, and 1.20 ± 0.47mm in the severe, moderate, and mild cases, respectively.[64] A criticism of this research is that it is unclear how the authors determined the point at the Gonion to measure and repeated this measurement for multiple radiographs. A number of authors identify the Gonion point by drawing two straight lines: one

along the lower border and another along the posterior border of the ramus. The angle these two lines make is bisected with line. Where this third meets the angle is the Gonion.

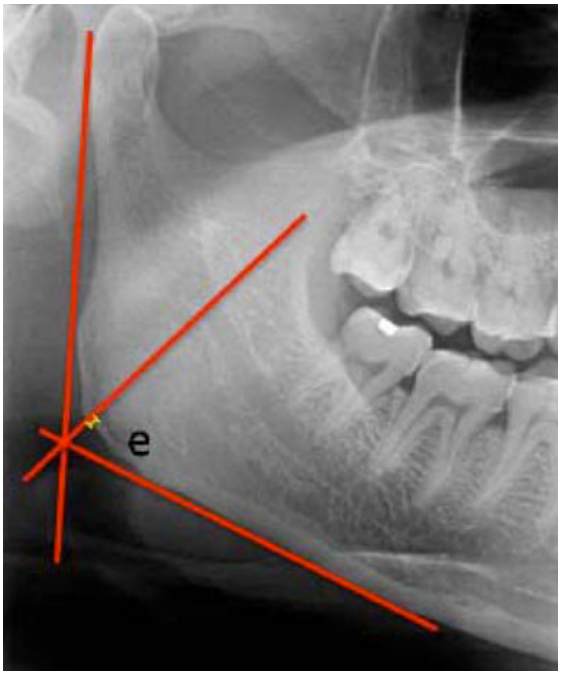
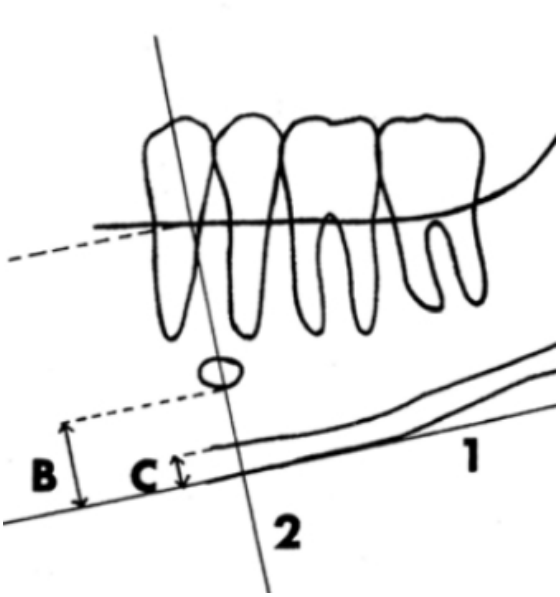


Figure 2. 14 Identification of the Gonion point

It is unclear whether Bras et al 1982 employed this technique when conducting this study.[64, 204]

The Gonion Index has been found to be unreliable for a number of likely reasons: panoramic radiographs are prone to horizontal magnification affecting the measurement, and as the masseter and medial pterygoid muscles insert at the mandibular angle, occlusal function may influence the GI measurement.[203]

The Panoramic Mandibular Index (PMI) described by Benson in 1991 is the ratio of the thickness of the mandibular cortex to the distance between the mental foremen and the inferior mandibular cortex.[205] A difficulty in using this index is that in some cases the borders of the mental foramen may not be evident on X-ray.[206]



PMI = C/B

Figure 2. 15 Panoramic mandibular index

Mandibular Cortical Width (MCW), also referred to as the Mental Index, is the cortical width measured below the mental foramen. Using this index a number of studies have found significant correlation with coefficient values between lumbar spine BMD and MCW ranging from 0.44 to 0.52. As the mental foramen provides a landmark for positioning of measurement of the cortical width it overcomes some of the limitations of the Gonion Index, however recognition of the position of the foramen may vary between examiners.[203]

Klemetti in 1994 in a study to determine whether qualitative changes apparent in the inferior border of the mandible can be used to predict BMD changes proposed the Mandibular Cortical Index (MCI). Rather than measure the thickness of the cortical bone, this index is based on changes related to porosity of the bone at the inferior border. It categorises the appearance of the inferior border of the mandible, posterior to the mental foramen, into three groups (C1 to C3).[207]

C1 – the endosteal margin of the cortex is even and sharp on both sides.

C2 – the endosteal margin shows semilunar defects or seemed to form endosteal cortical indices on one or both sides

C3 – the endosteal margin shows cortical residues and is clearly porous

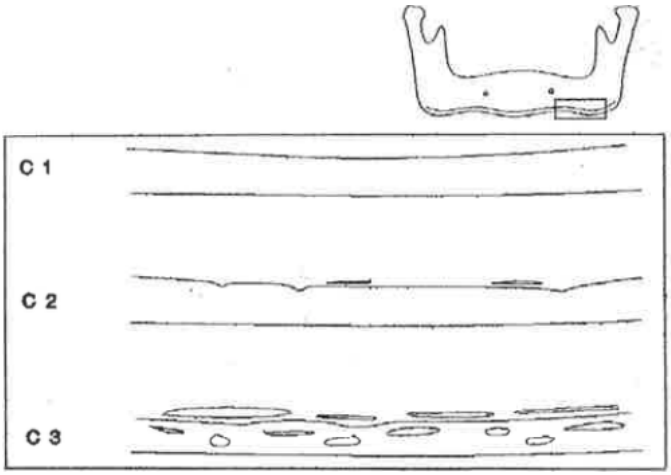


Figure 2. 16 Mandibular cortical index.

A major limitation of the MCI is that it does not involve a quantitative measurement and relies on visual inspection. It therefore introduces a degree of subjectivity that has the potential to create discordance in inter-observer agreement. Horner and Devlin in 1998 found MCI inter-observer agreement between two observers to be poor (Cohen's Kappa 0.3, standard error 0.12), however other studies have reported higher inter-observer agreement values including Drozdzowska *et al* in 2002, who reported a Cohen's Kappa of 0.70.[208] [209] Klemetti in 2004 reported that the sensitivity of this index in the diagnosis of fracture risk was found to be 13 to 16%, and specificity was 96 to 99% giving relatively little information relating to fracture risk. A possible reason for the low sensitivity is that they only included subjects aged 48 to 56, and it is likely a that only a small proportion within this age group would be at risk of fracture.[207] There are other limitations of this index. The cortex in young adults with normal BMD may give an appearance of an eroded cortex leading to incorrect diagnosis, and there are studies that show a high rate of an eroded cortical appearance on OPG in populations under 40 years of age.[203] Despite these limitations, there are a number of researchers who have adopted the MCI Index and have found significant correlation to BMD. Horner and Devlin in 1998 reported significant correlation between BMD and MCI, with R^2 values of 0.30 and 0.29 assessed by two observers.[208] Bollen *et al* in 2000 using MCI found that the odds ratio for a fragility fracture in C2 and C3 mandibular cortices was 2.0 (95% CI, 1.2 to 3.3) and 8.0 (95% CI, 2.0 to 28.9), respectively.[210]

Drozdzowska *et al* in 2002 in a study of 30 edentulous post-menopausal women compared MCI with PMI, BMD of the mandible and the hip, and QUS of the calcaneus and the phalanges. The group was divided into three subgroups based on MCI classification (C1 n=6, C2 n=16, C3 n=8). Following DXA, and based on WHO criteria, 2 participants were found to be osteoporotic and 12 were found to have osteopenia. MCI correlated significantly with mandibular BMD (r=-0.55, $p<0.002$), however all other correlations were not found to be significant. The authors concluded that while MCI may reflect mandibular BMD, it does not reflect skeletal status.[209] In a similar study, Çakur *et al* in 2009 compared the Mandibular Cortical Index and BMD measured at both sides of the mandible at a region of interest $10mm^2$ over the antegonial notch, with BMD of the lumbar spine and vertebrae in 80 untreated osteoporotic women. The mean BMD of the mandible was found to be 0.04 (±0.038) g/cm^2 compared to 0.737 (±0.095) g/cm^2 and 0.750 (±0.121) g/cm^2 taken at the vertebrae and femur, respectively. They reported a negative correlation between age and spine BMD with a coefficient of -0.358 ($p=0.001$). Vertebral BMD displayed a statistically significant correlation with femoral BMD (r=0.355 $p=0.001$), however there was no significant correlation between MCI and BMD from any sites including the mandible.[211] The largest study to date investigating the association between changes evident on Panoramic radiographs and osteoporosis was conducted as part of the OSTEODENT study, which was a multicentre collaborative study based at four research centres throughout Europe. The aim of this research was to investigate the use of radiographic and clinical indices for the diagnosis of osteoporosis applicable for use by dental practitioners.[212] DXA scans from the spine and hips were compared to panoramic radiographs of 653 post-menopausal women, aged 45 to 70 years of age. The indices under investigation were the Mandibular Cortical Width measured below the mental foramen and the Mandibular Cortical Index as described by Klemetti in 1994. They found that Mandibular Cortical Width demonstrates better efficacy in detecting osteoporosis compared to MCI, and those with a cortical width less than 3mm had a higher likelihood of being osteoporotic, and should be referred for further investigation.[213]

The OSIRIS Index (Osteoporosis Index of Risk) was developed to identify those in need of DXA scanning based on four variables: age, body weight, current HRT, and

history of fragility fracture. The index is calculated by adding the age of the patient multiplied by -2, double the weight in kg, -2 if the patient is a current HRT user, and -2 if there is a history of fragility fracture. A score of <-3 indicates high risk of low BMD, >-3 and <1 indicates intermediate risk and >1 indicates low risk.[214]

Karayianni *et al* in 2007, as part of the OSTEODENT study, investigated the diagnostic validity of MCW in combination with the OSIRIS index in the detection of osteoporosis in females by the use of Receiver Operating Characteristic (ROC) curve analysis. The area under the curve (AUC) for OSIRIS was found to be 0.838 (95% CI 0.808 to 0.866). Five observers, all of whom were dental radiologists, assessed the panoramic radiographs for MCW. AUC values for MCW ranged from 0.71 (95% CI 0.673 to 0.745) to 0.78 (95% CI 0.746 to 0.811). This suggests that clinical risk assessment is a slightly better predictor of osteoporosis than dental radiograph assessment.[212]

2.6.5 Use of cone beam CT of the mandible to assess osteoporotic change

Similar to the Index described by Klemetti, Koh and Kim in 2011 used an Index based on Cone Beam CT (CBCT) scans, referred to as the Computed Tomography Cortical Index (CTCI).

Like MCI, the authors described 3 levels of cortical bone porosity: -

- Type I – cortical endosteal margin is even and regular (A)
- Type II – the endosteal layer shows semilunar defects or 1 to 2 layers of cortical endosteal residues (B)
- Type III – the endosteal cortical layer is clearly porous (C)

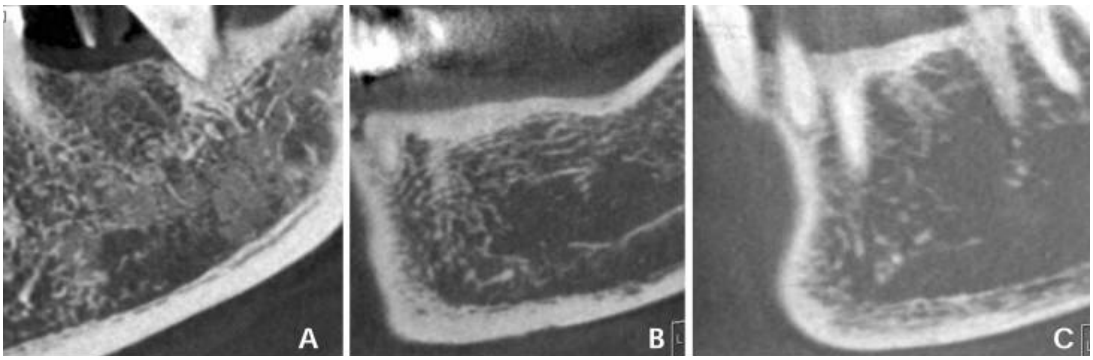


Figure 2. 17 Computerised Tomography Cortical Index

They compared two groups of post-menopausal female subjects: one group was confirmed as being osteoporotic (n=21), and the other group was confirmed as having normal BMD (n=21). All subjects had a CBCT of the unilateral mental region. It is unclear how the authors chose which side to scan. The authors reported that a higher proportion of the non-osteoporotic subjects were classed as Type I and in the osteoporotic group a higher proportion were classed as Type II and III. In the normal group 12 were classed as Type I, 7 were classed as Type II and 2 were classed as Type III. In the osteoporotic group 3, 11 and 7 subjects were classed as Types I, II and III respectively (p<0.001).[215] Mostafa *et al* in 2016 reported similar results in a study comparing osteoporotic subjects (n=25) to a control group (n=25) using the CTC Index. They reported a higher proportion of control subjects as Type I compared to the osteoporotic group: 18 subjects in the control group were classed as Type I, and 7 were classed as Type II. In the osteoporotic group 4 subjects were classed as Type I, 14 were classed as Type II and 7 were classed as Type III (p<0.001).[216] Both of these studies show that in osteoporotic groups, there is a higher proportion of mandibles with increased porosity at the lower border compared to control groups. This demonstrates that osteoporosis is related to increased porosity of cortical bone in the mandible.

These panoramic radiograph and CT scan indices appear to have limited clinical application in the diagnosis of osteoporosis. However, they do suggest that there are osteoporotic related changes in the cortical bone of the mandible in terms of both

quality and quantity. As discussed in a previous chapter, speed of sound measured by quantitative ultrasound is affected by properties of bone other than bone mineral density. As these devices are able to penetrate cortical bone up to a depth of 6mm, it is likely that they are capable of detecting changes in the mandible that other diagnostic methods may not.

2.6.6 Use of QUS on the mandible

There is a limited volume of research investigating the use of quantitative ultrasound on the mandible. The majority of these studies utilise a transverse transmission style device, and only one has been identified using an axial transmission device.

An ex-vivo study by Chan *et al* in 2014, using 23 dry human mandibles investigated whether there is a correlation between SOS measurements taken using a transverse transmission QUS device and Hounsfield Units measured using quantitative CT scan. Three regions of interest were selected in each mandible: the left canine region, the right canine region and the central incisor region. The authors reported a significant correlation between SOS and HU measurements with an R^2 value of 0.339 (p=0.004).[217] Al-Nawas *et al* in 2008 in an ex-vivo study using a transverse transmission device (DBM Sonic 1200) on 12 porcine mandibles reported a moderate correlation (r=0.54) between SOS readings and histological bone density measured by histomorphometry using image analysis software.[218]

There are only three studies that have used a QUS device on the mandible in an in-vivo setting. Rose *et al* in 2001 used a transverse transmission caliper style QUS device (Krautkramer USD 10) to investigate age related changes in the mandible. One hundred and eighty four subjects (93 females, 91 males) with a mean age of 17.1 (± 13.7) years were divided into four subgroups based on age: Infants (0 to <7 years), children (7 to <14), adolescents (14 to <20) and adults (≥20). SOS measurements were taken from the mandible at the basal bone between the canine and first premolar, and the mid-phalanx of the third finger. The mean SOS value of the mandible was 1777 (± 117) ms^{-1}, with the lowest mean SOS (1718 ± 108 ms^{-1}) in

the infant group and the highest mean SOS (1800 ± 105 ms^{-1}) in the adult group. The correlation co-efficient between the mandible and the phalanx was found to be 0.36. Significant differences between age groups were found (p<0.01), and there were no significant differences between sexes.[219] Similarly, Klein *et al* in 2008 in a pilot study used a caliper QUS device (DBMSonic 1200, IGEA, Carpi, Italy) on 108 partially dentate or edentulous patients (50 female, 58 male) to investigate the quality of bone at edentulous spaces prior to implant placement. Seven participants were confirmed as being osteoporotic, 14 received previous radiotherapy for cancer, and the remaining 87 were deemed healthy. Six intra-oral sites were chosen based on dividing the alveolar ridges into sextants. A major limitation of this style of device is that it requires a minimum height of 8mm of alveolar ridge to produce a measurement, and it is difficult to use in cases of limited opening. Due to restricted opening (< 30mm) in the radiotherapy group, or resorbed residual ridges, the authors were only able to measure 215 out of 522 potential sites. In the healthy subgroup they found the highest values at the posterior mandible with values of 1,713 (± 153) ms^{-1} in females and 1,734 (± 221) ms^{-1} in males, followed by the anterior maxilla with values of 1,648 (± 82) ms^{-1} in females and 1665 (± 189) ms^{-1} in males. The site with the lowest value was, as expected, the posterior maxilla with values of 1,538 (± 177) ms^{-1} in females and 1,583 (± 90) ms^{-1} in males. However, the authors were able to take readings of the anterior mandible from only three females and one male. Readings from the osteoporosis group appeared to be lower, however the authors did not perform statistical analysis due to lack of power.[220]

2.6.6.1 Use of axial transmission QUS on the mandible

A study by Beattie *et al* in 2017 has been the only in-vivo study to date to use an axial transmission QUS device (Omnisense 8000) on the mandible. The aim of this study was to determine whether differences in SOS readings taken at the parasymphysis of the mandible were evident in those with osteoporosis compared to two control groups. Three groups were compared: Healthy premenopausal women (n=26), healthy peri- and postmenopausal women (n=47), and women with

DXA confirmed osteoporosis (n=52). The authors reported statistically significant differences in the mean SOS readings taken at the parasymphysis of the lower border of the mandible between the three groups (p<0.0001). The mean SOS readings reported were 3,883 (±210) ms^{-1} in the premenopausal group, 3,514 (±221) ms^{-1} in the peri-menopausal group and 3,312 (±264) ms^{-1} in the osteoporotic group. Sensitivity of QUS on the mandible to identify osteoporosis was found to be 67%, and specificity was found to be 79%. This would suggest that the mandible is better at identifying absence of osteoporosis, rather than the diagnosis. However, a weakness of this study was that the BMD of the subjects in the healthy age matched control group was unknown, bringing into question any conclusions regarding the difference between this group and the osteoporotic group.[5]

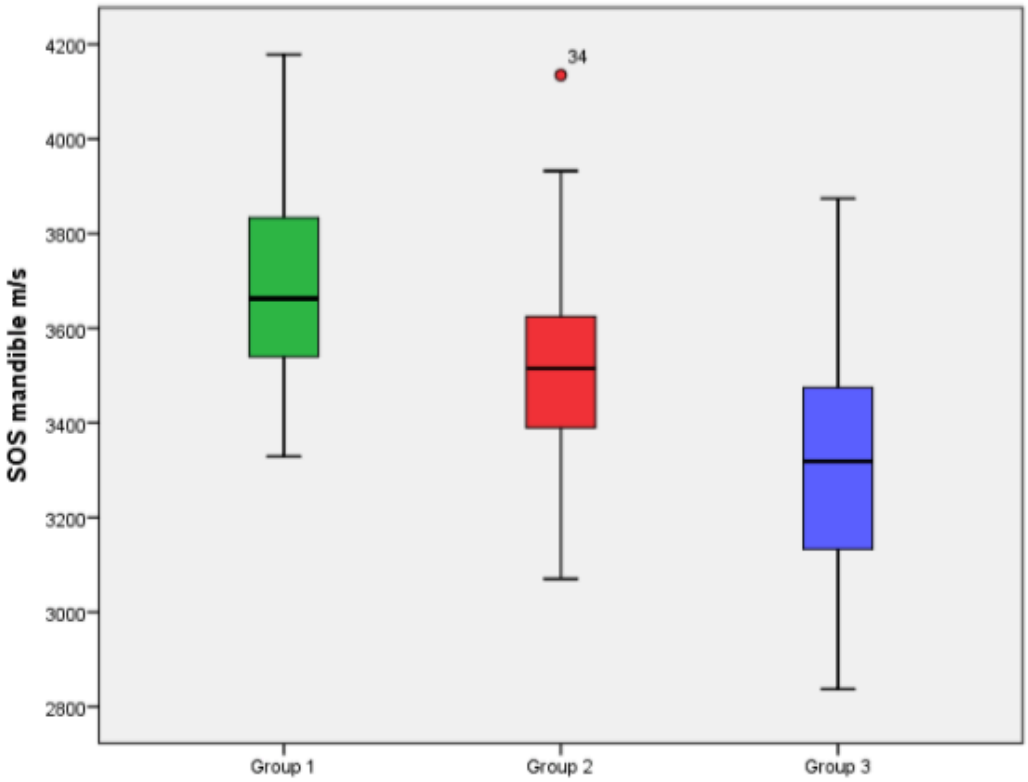


Figure 2. 18 Boxplot of mandibular SOS values from Beattie et al 2017.[5]

An exhaustive search of the literature has found no studies using quantitative ultrasound to investigate the frontal bone and the zygomatic arch.

3 Materials and Methods

3.1 Subjects

The study population consists of two groups: -

- Group 1 – Healthy premenopausal women
- Group 2 – Women with DXA confirmed osteoporosis according to WHO criteria

Group 1 were recruited from members of the general population, and members of the staff and students of the Dublin Dental University Hospital. Subjects were recruited by a member of staff, nominated to act as a gatekeeper.
Subjects for Group 2 were recruited on the Bone Health Clinic in St James's Hospital by one of the specialist bone health nurses, nominated as a gatekeeper.

Suitable subjects were provided with an information sheet explaining study aims and describing what participation involved (Appendix 2). Any questions were answered. If subjects agreed to participate, a consent form was signed. In Group 2, the consent form included permission to access their medical notes, to collect data on medical history, and the results of diagnostic tests.

3.1.1 Inclusion criteria

Subjects in this study were all Caucasian females over the age of 20, and capable of providing consent. The decision was made to limit inclusion to this group as the majority of research focuses on this population. A previous DDUH study using the same device (Omnisense 8000) on the mandible conducted in the bone health clinic used the same criteria. This would make for straightforward comparison between the results of both studies.

In Group 2 subjects were included if they were on one of three different treatments for osteoporosis: -

- Teriparatide 20mcg SC daily
- Zoledronate 4mg IV yearly
- Denosumab 60mg SC 6 monthly

3.1.2 Exclusion criteria

Subjects were excluded from both groups if they gave a history of: -

- Metabolic bone disease such as Paget's disease or osteomalacia,
- Lactose intolerance
- Eating disorders
- Hyperparathyroidism
- Chronic liver or kidney disease

Subjects were excluded from group 1 if they had a history of: -

- Fragility fracture
- Menopause prior to 45 years of age
- Amenorrhoea for ≥ 6months that is not related to pregnancy
- Drugs which may have an affect on bone metabolism such as corticosteroids

Exclusion Criteria for group 2

- Patients due to finish or change treatment during the one year study period
- Patients who are or have been on antiresorptive agents as part of ongoing cancer treatment or have completed cancer treatment within 5 years

3.2 Ethical approval

Tallaght Hospital/St James's Hospital Joint Research Ethics Committee granted ethical approval in March 2016 (Appendix 4).

3.3 Questionnaire

To gather subject data and determine suitability for the study, subjects were asked to complete a questionnaire with questions relating to medical history including fracture history, lifestyle, and clinical risk factors (Appendix 1). A number of questions were based on the FRAX fracture risk assessment tool.

3.3.1 Fractures

If subjects in Group 1 gave a history of fracture, the details of the injury were further discussed. If the fracture was caused by low impact trauma, or was considered a fragility fracture, then the subject was excluded from the study.

3.3.2 FRAX

The CRFs were entered into the FRAX algorithm for subjects over 40 in Group 1. If they were considered high risk of fragility they were excluded from the study. FRAX was chosen over QFracture, as there is a version of FRAX based on current Irish epidemiological data, and QFracture is based solely on UK data.

3.3.3 DXA scans

Subjects in Group 2 have had a DXA scan as part of their osteoporosis work up or as part of ongoing treatment. DXA scans of the lumbar spine, left femoral neck, and total hip were performed by a qualified radiographer in St James's Hospital. Potential subjects were invited to participate in the study only if they had a DXA T-score of -2.5 or less at one or more of these sites. The results from the DXA scans are recorded as part of the data collection.

Where possible and subject to availability, participants had a DXA scan after one year. Availability was limited for DXA scans due to staffing issues in the radiography department in St James's Hospital.

3.3.4 Calcaneal ultrasound

Calcaneal ultrasound is performed as part of a patient's initial workup on the specialist nurse led clinic. Calcaneal ultrasound measures Broadband Ultrasound Attenuation and Speed of Sound, which are used to calculate a T-score. Calcaneal ultrasound results are not available for all subjects; patients have a Calcaneal QUS when seen initially on the clinic. A number of subjects have been regular patients on the clinic for a number of years and as Calcaneal QUS is not validated for monitoring response to treatment, those subjects would not have had a repeat ultrasound.

3.3.5 Bone turnover markers

Subjects in Group 2 have blood tests including bone turnover markers taken as part of their monitoring, during their osteoporosis treatment. The results of these tests were recorded at recruitment and at the one-year review. This includes P1NP, CTX-1, and alkaline phosphatase.

3.4 Multisite quantitative ultrasound measurements

A Multisite Quantitative Ultrasound (mQUS) device was used to take SOS reading from multiple facial sites and the distal 1/3 radius of the arm and the fifth metatarsal. The mQUS device chosen for this study was the Omnisense 8000 (Sunlight BeamMed, Israel). The Omnisense was designed for use on the tibia, radius, phalanx and metatarsals and has three different probes available for measuring different sites. The CRB probe designed for use on the metatarsal, which was used in the study by Beattie *et al* in 2017, was selected for this study.[5]

The Omnisense probe contains piezoelectric transducers, acting as signal transmitters and receivers. The transducers are positioned at a fixed angle and at a fixed distance from eachother. It generates pulsed acoustic waves at a frequency of 1.25 MHz. Using the principles of Snell's law, the acoustic wave enters the bone and is refracted at a critical angle. It propagates along the cortical bone and emerges at

the same critical angle. As the transducers are a fixed distance from eachother the device calculates a Speed of Sound (SOS) reading in metres per second (ms^{-1}).

The device requires between three and five measurement cycles to produce an SOS reading. The measurements are produced and checked for consistency through an internal algorithm. For each measurement cycle, the 95^{th} percentile, the 25^{th} percentile and the mean are calculated and the coefficient of variation determined. If after three measurement cycles, the coefficients are below 1.2% then the three cycles are considered to be consistent. If they are not consistent then the device prompts a fourth cycle and if necessary then a fifth. If the device cannot obtain three consistent readings after five cycles then no reading is given.

If after five cycles an inconsistent result is given, then another attempt is made to achieve a successful reading. If the readings are still inconsistent after two attempts, then the site was recorded as a failure to measure.

Prior to any scanning session, quality verification was performed using a Perspex phantom allowing for calibration of the Omnisense mQUS.

Participants in Group 1 had SOS readings taken once only, except for a small number of participants who volunteered to have multiple readings as part of short and long term in vivo precision measurements.

Participants in Group 2 had readings taken on two occasions: on recruitment to the study and when they return after one year to the Bone Health Clinic for their follow up.

3.4.1 Measured sites

Subjects were positioned semi-supine on a bed to support their head for facial bone measurements.

3.4.1.1 Parasymphysis of the mandible

The lower border of the mandible at the parasymphysis on the right side was measured. This site was chosen, as this was the same site measured by Beattie *et al* making it possible to directly compare our results to the results from that study.[5] This site was determined by drawing a perpendicular vertical line bisecting the labial surface of the lower canine to the lower border of the mandible. This vertical line represented the anterior edge of the scanning point. In edentulous cases, this site was determined with the subject's lower denture in situ and using the same method of drawing a perpendicular line from the mid labial of the canine denture tooth to the lower border.

The CRB probe was moved in an arc around the lower border of the mandible.

Figure 3. 1 mQUS measuring point at the parasymphysis.

3.4.1.2 Frontal bone

Readings were taken from the frontal bone at a point in the midline 3cm above the nasofrontal junction. A point was marked in the midline and then a horizontal line was drawn representing the most inferior line at which the probe was positioned for taking a measurement. The CRB probe was positioned with its broadest edge at this

horizontal line. The probe has a marker at the midpoint allowing for accurate repositioning between cycles.

Figure 3. 2 Frontal bone measuring point 3cm above nasofrontal junction.

3.4.1.3 Zygomatic arch

Readings were taken from the right zygomatic arch. The point to read was marked 2cm anterior to the tragus over the arch. This mark represented the most posterior point on the arch to measure. Once a signal was detected the probe would not be moved until each cycle was completed.

Figure 3. 3 Zygomatic arch measured 2cm anterior to tragus.

3.4.1.4 Radius and fifth metatarsal

SOS readings were taken from the radius and metatarsal as described by the Omnisense protocol.

To take a reading from the radius, the length of the non-dominant arm was measured from the elbow to the tip of the middle finger, with the wrist straight and the fingers extended. The midpoint of this measurement was marked with a line and this represented the anterior point on the radius to take a reading. The forearm was positioned with the radius superiorly and the ulna inferiorly using an armrest that is supplied with the Omnisense. The CRB probe was positioned with its narrow end against the line marked on the arm and a reading was taken moving the probe in an arc over the bone.

Patients were positioned semi-supine with their leg laid flat supported on a bed. A line was drawn perpendicular to the long axis of the bone at the distal end of the fifth metatarsal. The narrow side of the probe was centred on the line, parallel to the fifth metatarsal and moved 1cm dorsally.

Figure 3. 4 Measuring points at the radius and metatarsal.

3.4.1.5 Measurement point on the zygoma and frontal bone

Ten study subjects from Group 1 volunteered to have five consecutive readings from the zygomatic arch and the frontal bone to determine the ideal position to take a

reading. The position that produced the most precise repeatable readings on each site was chosen as the point to take a reading.

On the frontal bone, readings were taken from the midline using the nasofrontal junction as a reference point. Attempts to take SOS readings at 1 cm increments from 1 to 4 cm above the reference point were performed.

On the zygomatic arch, attempts to take readings using the tragus as a reference point and were taken at 0.5cm increments from 1 to 3cm anterior to the tragus.

The positions that produced the most successful measurements relative to their respective reference points were identified and were used for the remainder of the study.

3.4.2 Precision measurements

3.4.2.1 In-vitro precision

Short-term in vitro precision was determined using the manufacturers' supplied Perspex phantom block and taking 10 consecutive measurements.

Long term in vitro precision was determined by taking weekly readings using the phantom over the course of a year.

3.4.2.2 In-vivo precision

Short term in vivo precision was determined by taking 5 consecutive measurements at each of the five sites in 10 subjects. The probe was repositioned between each measurement.

Long term in vivo precision was determined by taking monthly measurements at each of the five sites over the course of one year.

3.5 Statistical analysis

3.5.1 Power calculation

Advice from a statistician was sought when making the sample size power calculation. The results from the study by Beattie *et al* 2017 investigating SOS readings taken from the mandible were analyzed for determination of effect size.[5] The data collected by Beattie *et al* was used to determine an effect size (Cohen's d 1.55). It was determined that this effect size is too large and if used in a power calculation it would result in a very small sample size. Unless our findings were very similar to Beattie *et al* 2017, using such a small sample size would increase our risk of not achieving statistical significance. Therefore a more modest effect size of 0.2 was chosen. Based on an unpaired two-tailed T-test and a significance level of 0.05 with a power of 0.8, and an effect size of 0.2, a sample size of 82 was required in each group. This power calculation was based on data measured at the mandible. There are no studies looking at the use of mQUS on the zygomatic arch and the frontal bone. We accepted the power calculation for the mandible for both the zygomatic arch and the frontal bone knowing that while there was a possibility of not achieving statistical significance, the data we collected in this study could be used for power calculations for future research.

The osteoporotic group was further divided in three subgroups based on their respective treatment. Based on a Repeated Measures ANOVA with a significance level of 0.05, with a power of 0.8, an effect size of 0.2 and taking into account a 10% drop out rate over the course of the observation period, 24 subjects were required in each of the subgroups, totaling 72 subjects. The ISCD currently do not recommend using QUS to measure response to osteoporosis treatment due to lack of research in this area, and the studies looking at the use of mQUS measure response to treatment are conflicted in their results making it difficult to use them to determine sample size.[4, 177, 195, 196] We chose a small effect size of 0.2, with a power of 0.8, and a significance level of 0.05 knowing there was a possibility of not achieving statistical significance, but knowing that the data collected could be used for future research.

3.5.2 Descriptive statistics

Demographics of the study population were analyzed. Mean, standard deviation and subject numbers were reported where appropriate. Differences in age, height, weight and BMI between the two groups were analyzed using either an unpaired T-test or Mann Whitney U Test depending on outcome of tests of normality.

3.5.3 Precision measurements

Coefficients of variation for short and long term in-vivo precision for each research subject at each site were calculated using the following equation, and were expressed as a percentage.

$$CV\% = \frac{SD}{Mean} \times 100$$

The absolute precision results for each site, taking into account the multiple subjects, were presented as the root mean squared standard deviation (RMS SD), and the root mean squared coefficient of variation (RMS CV%).

They were calculated by the following equations: -

$$RMS\ SD = \sqrt{\sum \frac{(SD)^2}{n}}$$

$$RMS\ CV\% = \frac{\sqrt{\sum \frac{(SD)^2}{n}}}{Mean} \times 100$$

3.5.4 Statistical Tests

Tests of normality were performed for the SOS readings at all sites, and depending on whether the data were normally distributed, either an independent student T-

test or a Mann-Whitney U Test was performed to determine significance between the two groups at each of the sites measured.

A one-way ANCOVA was performed for any sites found to have significant difference between the two groups. The covariates taken into consideration were variables found to be statistically significant between both groups, including height, weight, and age.

Pearson correlation coefficients were calculated to explore the relationship between the SOS measurement taken at each of the five sites, and between the results from DXA scans.

Receiver Operator Characteristic curves were conducted for each site to determine their diagnostic ability.

To measure response to treatment, the SOS readings taken at baseline and after one year were analyzed. Either a paired Student T-test or a Wilcoxon Signed Ranks Test was used to determine significant differences at each site, depending on whether the data were normally distributed or not.

Change in SOS measurements, bone turnover markers and BMD measured by DXA scan by subtracting the baseline measurements from the one-year measurements. Pearson correlation coefficients were calculated with change in SOS to change in bone turnover markers, and to change in BMD.

Significance was taken as $p<0.05$ for all statistical tests.

Data analysis was performed using SPSS (IBM) version 23

4 Results

4.1 Subject Demographics

A total of 170 Caucasian females participated in this study. This consisted of 2 groups as follows: -

- Group 1 – Control: Healthy premenopausal women. n=84
- Group 2 – Osteoporotic study group: Women with osteoporosis confirmed by DXA scan. n=86
    - Participants in this group were subdivided based on the treatment they were receiving for osteoporosis: -
        - Teriparatide n=35
        - IV Zoledronate n=34
        - Denosumab n= 17

4.1.1 Age

The mean age of all the participants in this study was 50.35 (±22.65). The mean age in Group 1 was 29 (±7.47), and the mean age of participants in Group 2 was 71 (±9.38). A Mann Whitney U test found the difference in ages between these two groups to be statistically significant (p<0.001).

Table 4.1 shows a breakdown of the number of participants stratified into age groups. In Group 1, the 20 to 29 age group contained the highest number of participants making up 27.6% (n=47) of the total number of research subjects. The 70 to 79 age group contained the highest number of participants in Group 2 with 18.8% (n=32) of the total number of subjects.

		Group	
		Control n (%)	Osteoporosis n (%)
Age Group	20-29	47 (27.6)	
	30-39	28 (16.5)	
	40-49	8 (4.7)	2 (1.2)
	50-59	1 (0.6)	5 (2.9)
	60-69		30 (17.6)
	70-79		32 (18.8)
	80-89		14 (8.2)
	90-99		2 (1.2)

Table 4. 1 Numbers (percentages) in each group stratified into age groups

4.1.2 Height

The mean height in the control group was found to be higher than the osteoporotic group. The mean height was 1.66 (SD 0.08) metres in Group 1, 1.58 (SD 0.06) metres in Group 2. Tests of normality found a non-parametric distribution of height in both groups indicating the need to use non-parametric tests. A Mann-Whitney U Test found the difference between the groups to be statistically significant (p<0.001). This difference in height may be accounted for by spine kyphosis more commonly seen in elderly people, and loss of height related to vertebral fractures.

4.1.3 Weight

The mean weight in Group 1 was found to be 61.6 (SD 9.1) kg, and the mean weight in Group 2 was 57.5 (SD 9.2) kg. The distribution of weight values was found to be non-parametric. A Mann-Whitney U Test found that the difference between the two groups was statistically significant (p=0.019).

4.1.4 BMI

Despite the differences in Group 1 and Group 2 for both height and weight being statistically significant, a Mann-Whitney U Test found the difference in BMI was not statistically significant (p=0.195). The mean BMI in Group 1 was 22.4 (SD 3.2) and in Group 2 it was 23 (SD 3.5). Seven per cent of the study population were classed as underweight (BMI <18.5), 71.2% were classed as normal BMI (18.5 to < 25), 19.4% were overweight (25 to < 30), and 2.4% were obese (≥30).

	Group	
	1	2
	n	n
Underweight	5	7
Normal Weight	65	56
Overweight	12	21
Obese	2	2

Table 4. 2 Summary table of BMI counts in each group

	Group	
	Control n=84	Osteoporosis n=86
Age	29 (SD 7.47)	71 (SD 9.38)*
Height (m)	1.66 (SD 0.08)	1.58 (SD 0.06)*
Weight (kg)	61.6 (SD 9.1)	57.5 (SD 9.2)*
BMI	22.4 (SD 3.2)	23.0 (SD3.5)

Figures marked with * are significantly different at $p< 0.05$ in the two-sided test of equality for column means.

Table 4. 3 Age, height, and weight in each group

4.1.5 Social factors

Twice the number of subjects in the osteoporotic group smoked (26.7%) compared to the control group (13.1%) (χ^2 4.948, df 1, p=0.026).

There were nearly twice as many alcohol drinkers in the control group (84.5%) as the osteoporotic group (44.2%) (χ^2 30.053, df 1, p<0.0001).

The majority of subjects in both groups exercised regularly. As expected this number was higher in the control group as these were healthy young premenopausal women (χ^2 5.376, df 1, p=0.02), whereas in the osteoporotic group mobility for a number of participants would be compromised due to factors related to ageing, and fracture history.

	Group		Total
	Control	Osteoporotic	
	n (%)	n (%)	n (%)
Non Smoker	73 (86.9%)	63 (73.3%)	136 (80%)
Smoker	11 (13.1%)	23 (26.7%)	34 (20%)
No exercise	7 (8.3%)	18 (20.9%)	25 (14.7%)
Exercises	77 (91.7%)	68 (79.1%)	145 (85.3%)
Non Drinker	13 (15.5%)	48 (55.8%)	61 (35.9%)
Drinks alcohol	71 (84.5%)	38 (44.2%)	109 (64.1%)

Table 4. 4 Numbers (percentages) of participant habits.

4.2 Speed of sound measurements

4.2.1 Determination of optimal site at the zygoma and the frontal bone

At the zygoma, a point 2cm anterior to the tragus demonstrated the most successful site to take readings. In the 5 consecutive readings from 10 volunteers, a reading could be successfully taken from this site in 90% of attempts. This was followed by 1.5cm anterior to the tragus, which could give a successful reading in 72% of attempts. No readings could be taken from any other points on the zygoma.

Three centimetres above the nasofrontal junction proved to be the most successful site on the frontal bone to take a reading with 100% successful readings taken from the 10 volunteers. 3.5cm above the nasofrontal junction was 90% successful and 2.5cm above was found to be 82% successful. Beyond these points it proved difficult to successfully obtain a reading due to the probe requiring a flat surface to take a reading and the contour of the frontal bone.

4.2.2 In-vitro precision measurements

Repeated measurements of a Perspex phantom supplied by the manufacturer were used to determine short and long term in-vitro precision.

Ten consecutive measurements were taken for short-term precision. The standard deviation was 2.9 ms^{-1}, and the coefficient of variation (CV%) was 0.11%.

Fifty-two weekly measurements were taken over the course of 1 year for long-term precision. The standard deviation was 8.06ms^{-1}, and the coefficient of variation was 0.29%.

4.2.3 Short-term in-vivo precision measurements

Short-term precision was determined by taking 6 repeated scans with repositioning between each scan in 10 subjects from Group 1.

Subject	Coefficient of Variation (CV%)				
	Parasymphysis	Frontal Bone	Zygoma	Radius	Metatarsal
1	0.49	0.82	1.39	0.45	1.85
2	0.45	1.31	1.58	0.69	1.41
3	0.13	0.82	0.84	0.47	0.41
4	1.4	2.86	0.76	2.1	2.53
5	1.5	1.79	1.58	1.12	2.58
6	0.45	0.63	1.9	0.55	0.78
7	0.54	0.52	1.17	0.49	0.75
8	0.45	0.52	1.78	0.56	0.91
9	2.35	2.63	1.23	1.61	4.2
10	1.35	1.03	0.78	1.04	1.92
RMS SD	40.71ms^{-1}	44.63ms^{-1}	37.75ms^{-1}	42.92ms^{-1}	79.7ms^{-1}
RMS CV%	1.13%	1.59%	1.36%	1.05%	2.04%

Table 4. 5 Short term in-vivo precision measurements

4.2.4 Long-term in-vivo precision measurements

Long-term precision was determined by taking monthly measurements from each of the 5 sites, over 12 months in 6 research participants from Group 1.

Subject	Coefficient of Variation (CV%)				
	Parasymphysis	Frontal Bone	Zygoma	Radius	Metatarsal
1	0.46	0.93	1.25	0.69	1.74
2	0.95	1.47	2.39	1.01	0.88
3	2.45	0.91	3.06	2.45	2.05
4	1.72	2.69	3.02	1.99	4.07
5	3.33	1.26	1.57	0.88	2.5
6	0.47	0.53	1.45	0.51	0.81
RMS SD	67.68ms^{-1}	43.67ms^{-1}	63.64ms^{-1}	58.35ms^{-1}	88.33ms^{-1}
RMS CV%	1.88%	1.52%	2.29%	1.43%	2.29%

Table 4. 6 Long-term precision measurements

4.2.5 Mandible

SOS measurements from the parasymphysis of the mandible were taken from all research subjects (n=170). There were no failures to take a reading from this site in any participants.

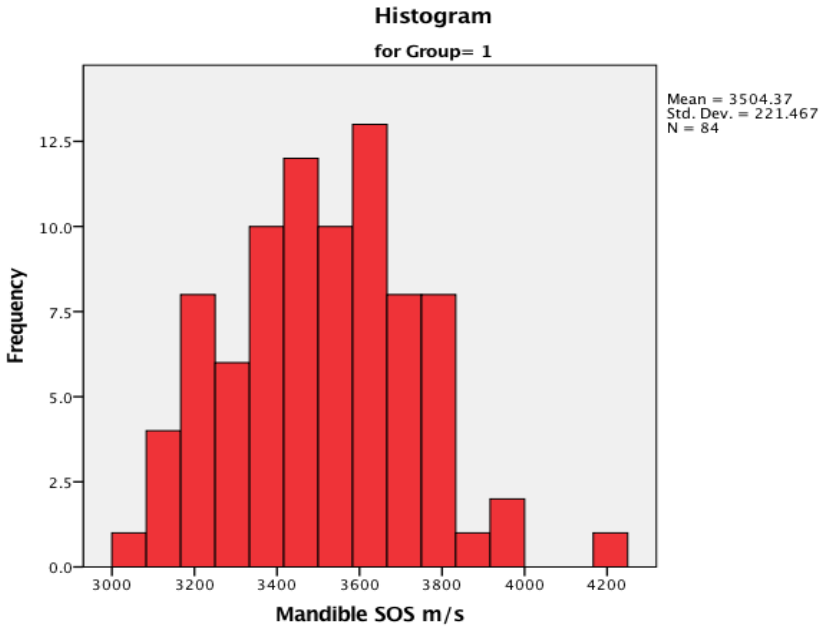


Figure 4. 1 Distribution of mandible SOS readings in Group 1.

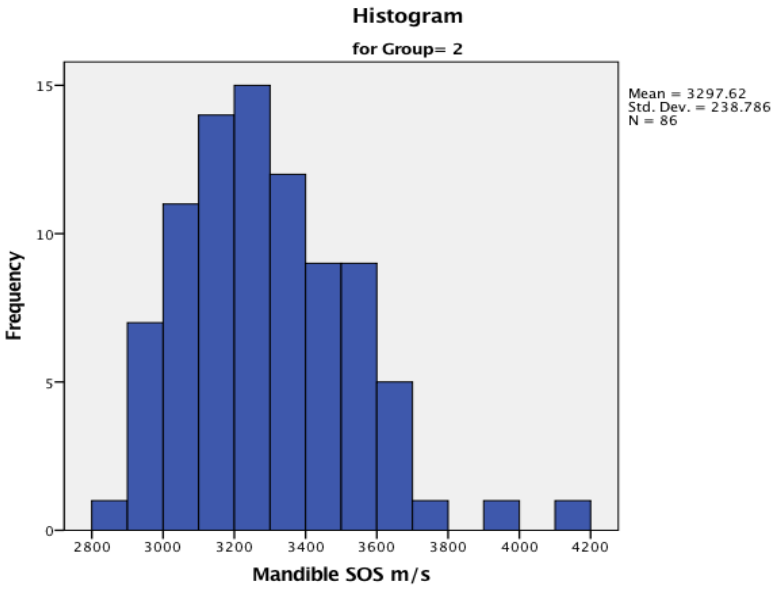


Figure 4. 2 Distribution of mandible SOS readings in Group 2.

Visual inspection of the distributions for normality and Kolmogorov-Smirnov tests (sample size >50) indicated the use of parametric tests.

Group		Kolmogorov-Smirnov		
		Statistic	df	Sig.
Mandible	1	.049	84	.200
	2	.062	86	.200

Table 4. 7 Tests of normality for mandibular SOS readings.

The mean of the SOS readings from the mandible were 3,504 (SD 221) ms^{-1} in Group 1 and 3,298 (SD 239) ms^{-1} in Group 2. An unpaired two-tailed T-test found that the difference in mandible SOS readings between the two groups was statistically significant (T 5.85, df 168, p<0.0001).

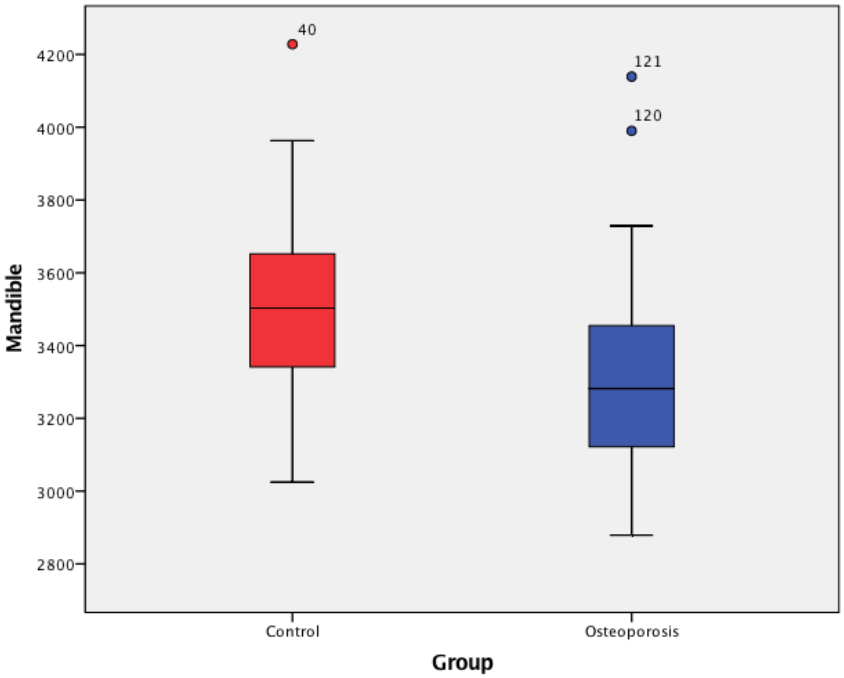


Figure 4. 3 Boxplot of mandible SOS readings.

4.2.6 Zygoma

SOS measurements were taken from the right zygomatic arch in 98 (57.6%) subjects. There were a high number of failures trying to obtain an SOS from the zygomatic arch, 70.2% of zygomatic arches could be read in Group 1 (59/84), and 45.3% in Group 2 (39/86).

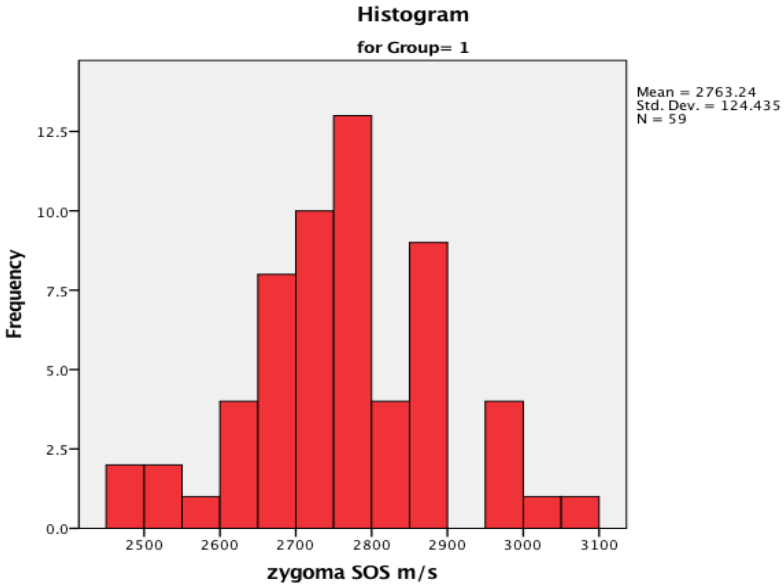


Figure 4. 4 Distribution of zygoma SOS readings in Group 1.

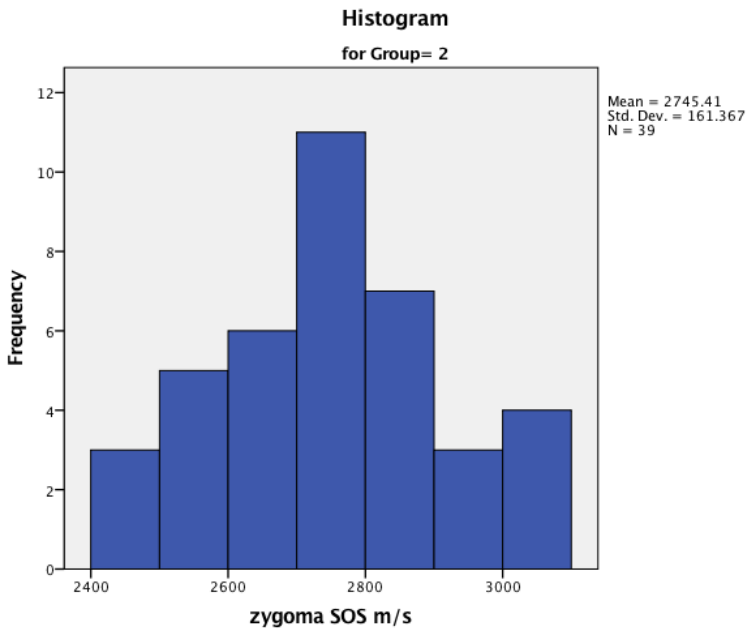


Figure 4. 5 Distribution of zygoma SOS readings in Group 2.

Visual inspection of the distribution and tests of normality found a normal distribution in both Groups for the SOS readings from the Zygomatic arch.

	Group	Kolmogorov-Smirnov		
		Statistic	df	Sig.
Zygoma	1	.070	59	.200
	2	.104	39	.200

Table 4. 8 Tests of normality for zygoma SOS readings.

The mean of the SOS readings was 2,763 (±124) ms^{-1} in Group 1 and 2,745 (±161) ms^{-1} in Group 2. A two-tailed independent sample T-test found there was no statistically significant difference between the two groups at the Zygomatic arch (p=0.539, df 96, T 0.616).

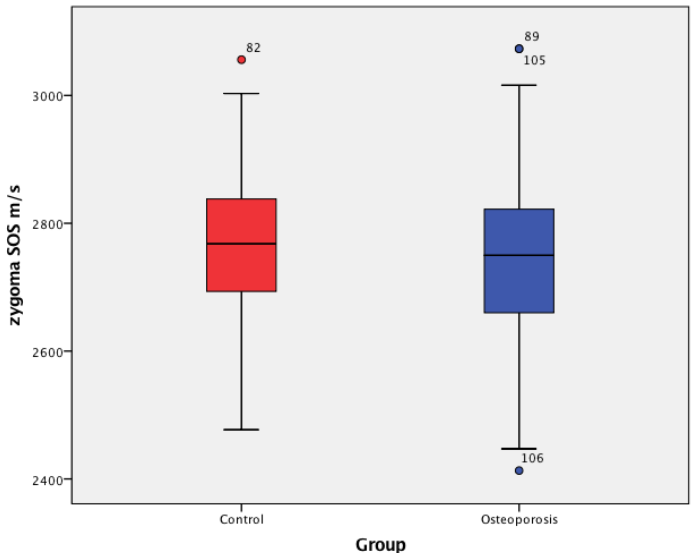


Figure 4. 6 Boxplot of SOS readings from the zygomatic arch in both groups.

4.2.7 Frontal bone

SOS measurements were taken from the frontal bone in 164 (96.5%) subjects. There were 6 failures to get a reading in Group 2, and no failures in Group 1.

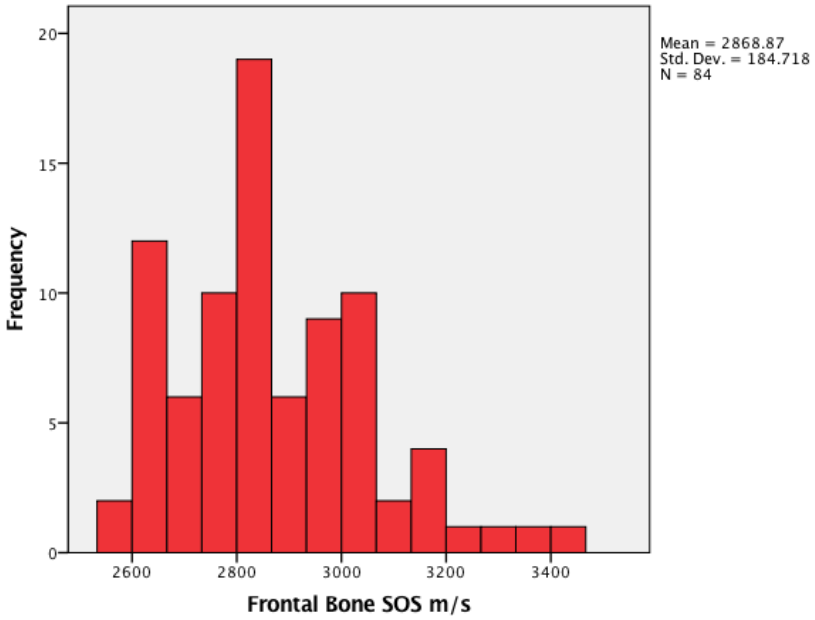


Figure 4. 7 Distribution of frontal bone SOS readings in group 1.

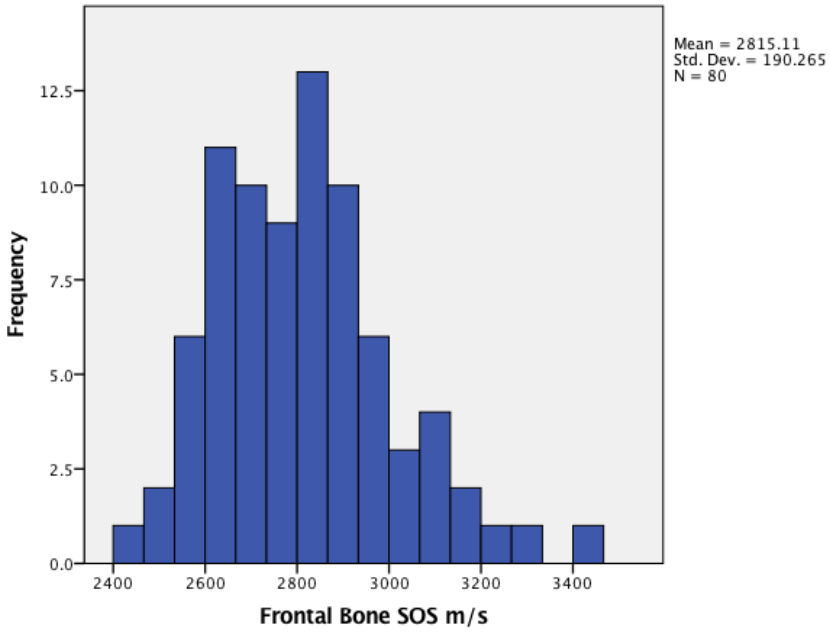


Figure 4. 8 Distribution of frontal bone SOS readings in group 2.

Tests of normality showed a non-parametric distribution of SOS readings in Group 1 indicating use of non-parametric tests.

	Group	Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Frontal Bone	1	.104	84	.026	.961	84	.013
	2	.078	80	.200	.973	80	.084

Table 4. 9 Tests of normality for frontal bone SOS readings

The mean of the frontal bone SOS readings was 2,869 (±185) ms^{-1} for Group 1 and 2,815 (±190) ms^{-1} for Group 2. The medians were 2,847 ms^{-1} for group 1 and 2,804 ms^{-1} for group 2. A Mann-Whitney U Test showed there was no significant difference between the two groups for the frontal bone (p=0.082).

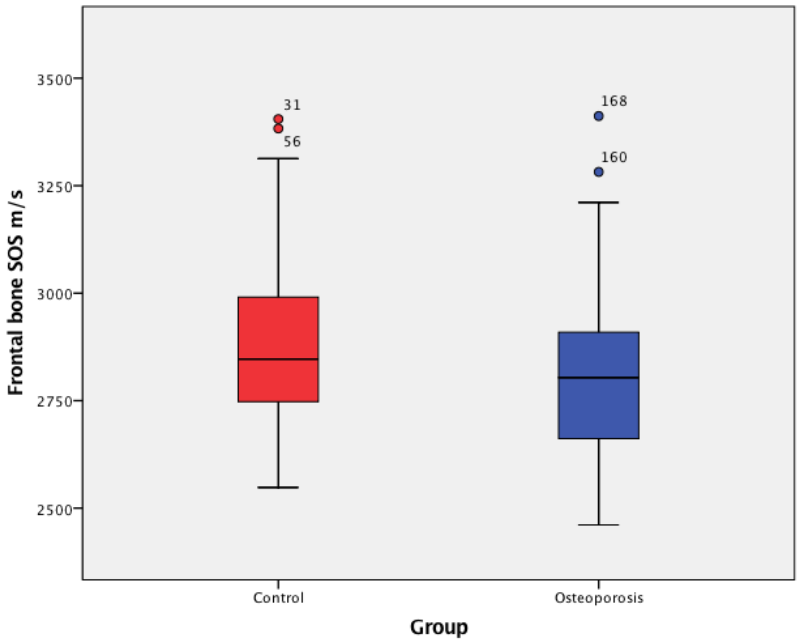


Figure 4. 9 Boxplot of frontal bone SOS readings in both groups.

4.2.8 Metatarsal and radius

SOS readings were successfully taken from the radius in 75 (89%) participants in Group 1 and 65 (75.5%) participants in Group 2. Difficulties were encountered due to soft tissue thickness preventing the ultrasound probe being close enough to the cortical bone of the radius to take a reading.

Readings were taken from the metatarsal in 76 (90.4%) participants in Group 1 and 61 (70.9%) in Group 2. Difficulties were encountered in a number of the osteoporotic participants due to swelling of the ankles and feet. In Group 1, a small number of participants declined having a metatarsal reading taken.

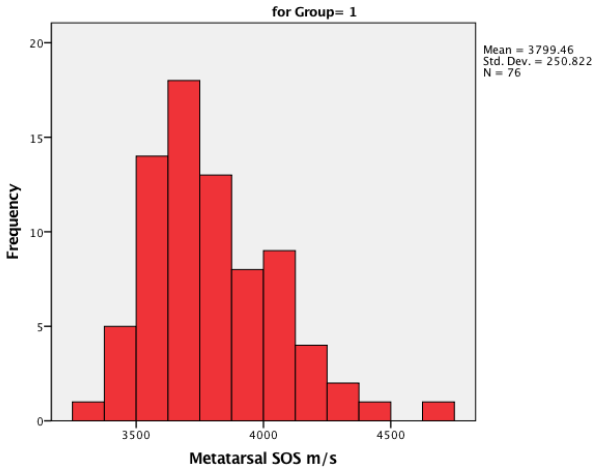


Figure 4. 10 Distribution of metatarsal SOS readings from both groups.

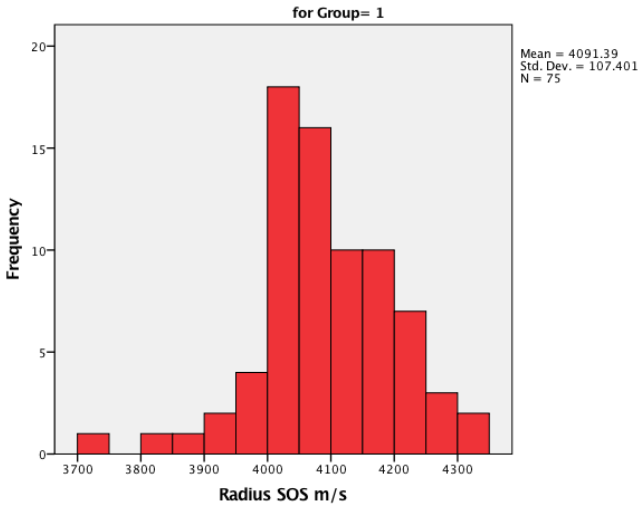


Figure 4. 11 Distribution of radius SOS readings from both groups.

	Group	Kolmogorov-Smirnov		
		Statistic	df	Sig.
Metatarsal	1	.091	76	.186
	2	.089	61	.200

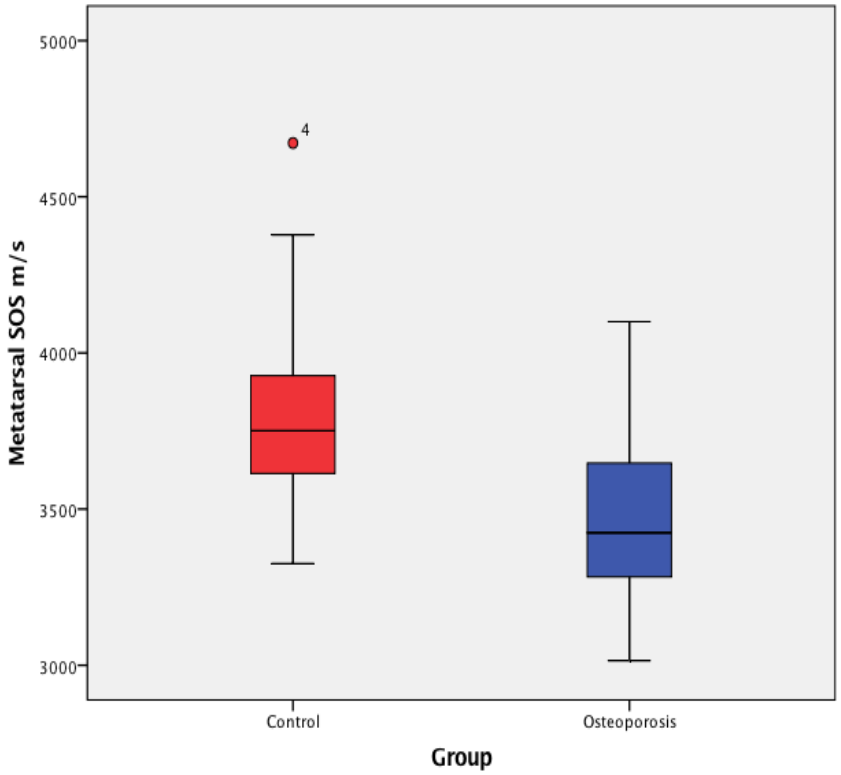
Table 4. 10 Tests of normality for metatarsal SOS readings

Tests of normality showed a non normal distribution in Group 1 indicating the use of non-parametric tests. A Mann-Whitney U Test found there was a statistically significant difference between Group 1 and Group 2 in SOS readings taken from the Metatarsal (p<0.001).

	Group	Kolmogorov-Smirnov		
		Statistic	df	Sig.
Radius	1	.080	75	.200
	2	.123	65	.016

Table 4. 11 Tests of normality for radius SOS readings

Tests of normality found non-normally distributed data for the radius SOS readings. A Mann Whitney U test found a significant difference in SOS readings between Group 1 and Group 2 (p<0.0001).



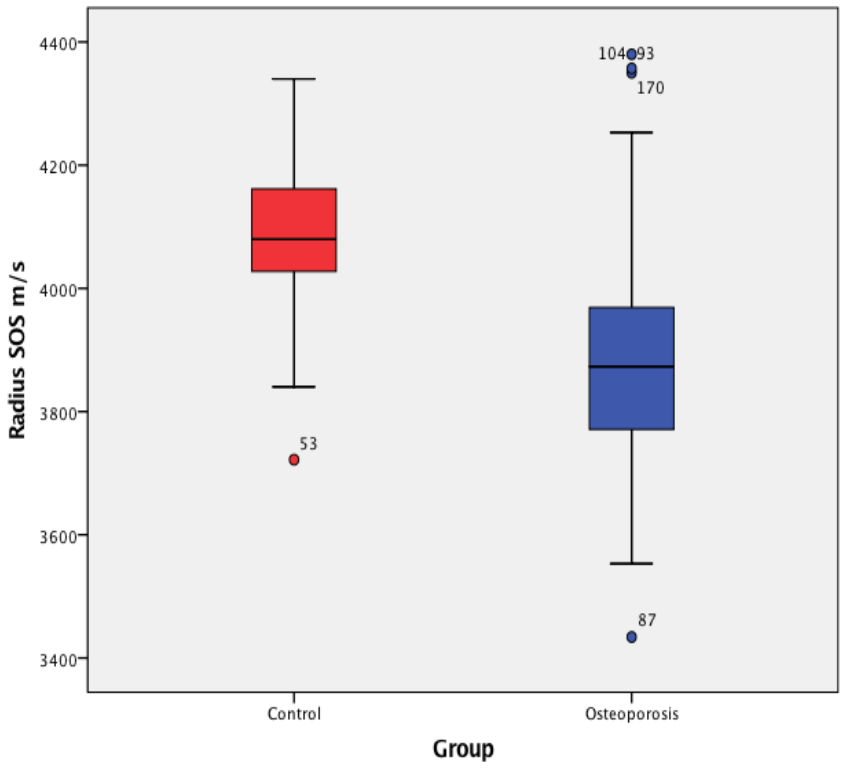


Figure 4. 12 Boxplots of SOS readings from the metatarsal and radius.

4.2.9 Mean SOS (ms^{-1}) values at each site

Site	Group		*p*-value
	Control ms^{-1} ±SD	Osteoporosis ms^{-1} ±SD	
Mandible	3504 ±221	3298 ±239	<0.0001
Zygoma	2763 ±124	2745 ±161	0.539
Frontal Bone	2869 ±185	2815 ±190	
Metatarsal	3799 ±251	3468 ±267	
Radius	4091 ±107	3884 ±197	

Table 4. 12 Summary of mean SOS readings taken at each site

4.2.10 Median SOS (ms^{-1}) values at each site

Site	Group		*p*-value
	Control ms^{-1} (Q1, Q2, IQR)	Osteoporosis ms^{-1} (Q1, Q2, IQR)	
Mandible	3503 (3342, 3652, 310)	3282 (3122, 3455, 333)	
Zygoma	2768 (2689, 2854, 165)	2750 (2655, 2824, 169)	
Frontal	2847 (2748, 2991, 243)	2804 (2662, 2909, 247)	0.082
Metatarsal	3751 (3614, 3927, 313)	3427 (3307, 3647, 340)	<0.0001
Radius	4080 (4027, 4164, 137)	3873 (3771, 3969, 198)	<0.0001

Table 4. 13 Summary of median SOS readings at each site

4.2.11 Measurement success at each site

The mandible was the most successful site to take an SOS measurement with readings taken from 170 (100%) study participants, followed by the frontal bone with successful readings from 164 (96.5%) participants. Difficulties were encountered taking measurements from the zygomatic arch. The ultrasound probe has a flat surface and in cases where the curve of the zygomatic arch was too pronounced, a reading could not be taken.

The zygoma had the lowest success rate with measurements taken from only 98 (57.6%) participants. There was a significant difference in the proportion of

successful readings taken between the control group (70.2%) and the osteoporotic group (45.3%) (χ^2 10.782, df 1, p=0.001).

A point-biserial correlation was run to determine the relationship between age and successful readings on the zygoma. There was a negative correlation between age and measurement success at the zygoma, which was found to be statistically significant (r_{pb} =-0.296, p<0.0001).

Site	Group		Total
	1	2	
	n (%)	n (%)	
Mandible	84 (100%)	86 (100%)	170 (100%)
Zygoma	59 (70.2%)	39 (45.3%)	98 (57.6%)
Frontal Bone	84 (100%)	80 (93%)	164 (96.5%)
Metatarsal	76 (90.5%)	61 (70.9%)	137 (80.6%)
Radius	75 (89.3%)	65 (75.6%)	140 (82.4%)

Table 4. 14 Measurement success rates at each site.

4.2.12 Effect of BMI on measurement success

For the radius, the mean BMI between those with who had successful readings was 26.4 (±3.5). The mean BMI for those in whom a reading could not be taken was 21.9 (±2.8). An unpaired T test found this difference to be statistically significant (T=7.569, df168, p<0.0001).

The mean BMI scores between those with successful readings and those with unsuccessful readings on the metatarsal were 22.3 (±3) and 24.2 (±4.4), respectively. The difference was found to be statistically significant (T=2.334, df 39.275, p=0.025).

There were no significant differences found in BMI with successful and unsuccessful SOS readings on any of the facial bones.

4.2.13 Relationship between age and SOS

An exploration of the relationship between age and mean SOS for each site was conducted. Subjects were stratified into groups based on decade of life and mean SOS values for each group were calculated.

The highest mean SOS readings for the mandible (3569 ±249 ms^{-1}) and the metatarsal (3872 ±247 ms^{-1}) were found in the 30 to 39 year old age group. The highest mean SOS readings for the radius (4170 ±196 ms^{-1}) and the frontal bone (3064 ±308 ms^{-1}) were found in the 50 to 59 age group, and the 70 to 79 age group had the highest readings in the zygoma (2807 ±170 ms^{-1}).

The over 90 age group demonstrated the lowest mean SOS values for the mandible (3177 ± 190 ms^{-1}, n=2), the radius (3815 ±35 ms^{-1}, n=2) and the metatarsal (3124 ms^{-1}, n=1). The lowest value for the zygoma was seen in the 50 to 59 age group (2620 ±177 ms^{-1}), and the lowest value for the frontal bone was found to be in the 70 to 79 age group (2761 ±145 ms^{-1}). It should be noted that while the 50 to 59 year old age group demonstrate the lowest zygoma and the highest frontal bone and radius SOS readings, the numbers in this group are relatively low.

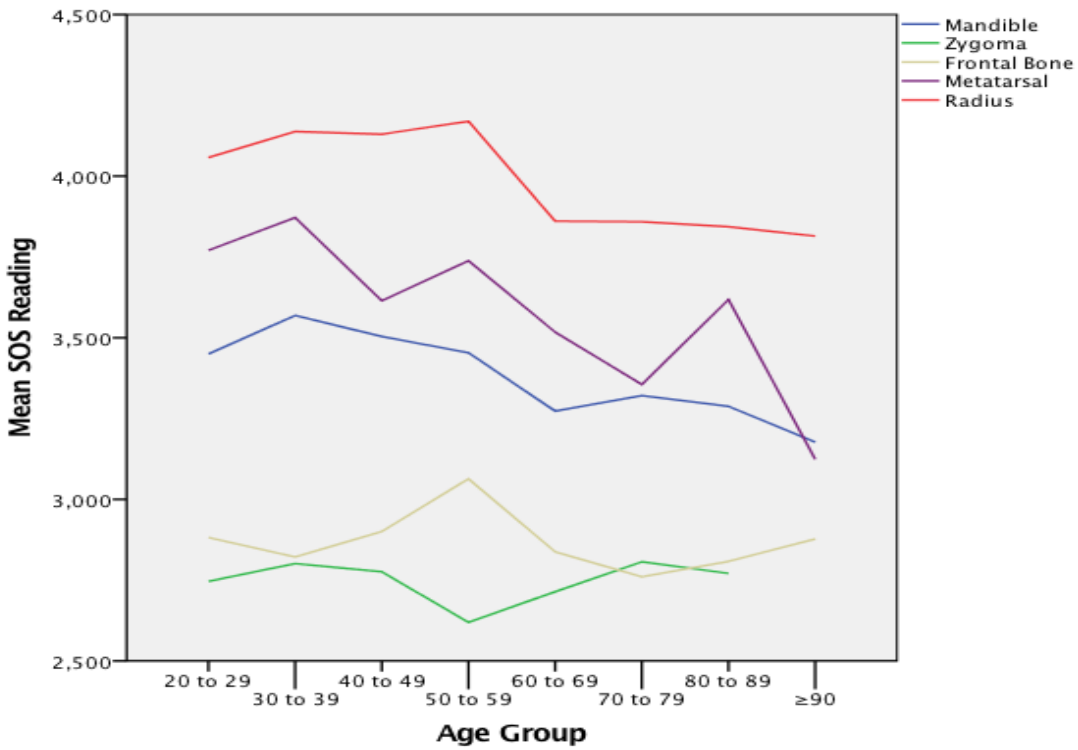


Figure 4. 13 Line graph showing mean SOS readings for each site stratified by age.

Age Group	Mandible			Zygoma			Frontal Bone			Metatarsal			Radius		
	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n
20 to 29	3450	±190	47	2746	±129	38	2882	±172	47	3770	±252	43	4058	±103	43
30 to 39	3569	±249	28	2801	±118	17	2822	±176	28	3872	±247	25	4138	±102	26
40 to 49	3504	±283	10	2776	±110	4	2901	±184	10	3615	±236	9	4130	±104	7
50 to 59	3454	±186	6	2620	±177	4	3064	±308	5	3738	±288	5	4170	±196	5
60 to 69	3273	±231	31	2714	±134	16	2838	±201	29	3517	±213	25	3861	±193	24
70 to 79	3321	±250	32	2807	±170	13	2761	±145	29	3356	±274	20	3859	±133	23
80 to 89	3288	±267	14	2771	±164	6	2808	±203	14	3618	±269	9	3844	±243	10
90 to 99	3177	±190	2	.	.	0	2878	±322	2	3124	.	1	3815	±35	2

Table 4. 15 Mean SOS values measured at each site stratified into groups by age.

4.2.14 Relationship between BMI and SOS measurements

The relationship between SOS and BMI was found to vary between sites. Subjects were stratified into four groups based on BMI: underweight <18.5, normal weight 18.5 to <25, overweight 25 to <30, and obese ≥30.

The highest mean SOS readings in the mandible were found in the overweight group (3,418 ±283 ms^{-1}) and the lowest values were found in the underweight group (3,289 ±220 ms^{-1}). Conversely, the highest mean SOS values from the zygoma were found in the underweight group (2,832 ±170ms^{-1}) and the lowest were found in the overweight group (2,715 ±160 ms^{-1}).

In both the radius and the frontal bone the highest SOS readings were found in the obese group with mean SOS readings of 4,076 ms^{-1} and 3,029 (±335)ms^{-1}, respectively. The lowest readings from both of these sites were found in the underweight group with readings of 3,985 (±205)ms^{-1} and 2,785 (±125)ms^{-1}, respectively.

No readings could be taken from the metatarsal in obese participants. The highest mean SOS readings were found in the normal BMI group (3,679 ±278 ms^{-1}) and the lowest were found in the underweight group (3,503 ±408 ms^{-1}).

Tests of normality indicated the use of parametric tests for the mandible, frontal bone and the zygoma, and non-parametric tests for the radius and metatarsal.

A one-way ANOVA was performed for each of the mandible ($F(3,166)=0.844$ $p=0.471$), frontal bone ($F(3,160)=1.746$, $p=0.16$) and zygoma ($F(3,94)=0.97$, $p=0.41$) with no significant differences found.

A Kruskal-Wallis Test was performed for the metatarsal ($p=0.426$) and radius ($p=0.963$) and also found no significant differences.

	Mandible			Zygoma			Frontal Bone			Metatarsal			Radius		
	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n	Mean	±SD	n
Underweight	3289	±220	12	2832	±170	7	2785	±125	12	3503	±408	10	3985	±205	11
Normal Weight	3406	±249	121	2754	±135	81	2839	±182	116	3679	±278	104	3994	±189	113
Overweight	3418	±283	33	2715	±160	9	2854	±206	32	3620	±342	23	4008	±173	15
Obese	3404	±151	4	2790	.	1	3029	±335	4	.	.	0	4076	.	1

Table 4. 16 Mean SOS readings at each site with BMI is stratified into four groups.

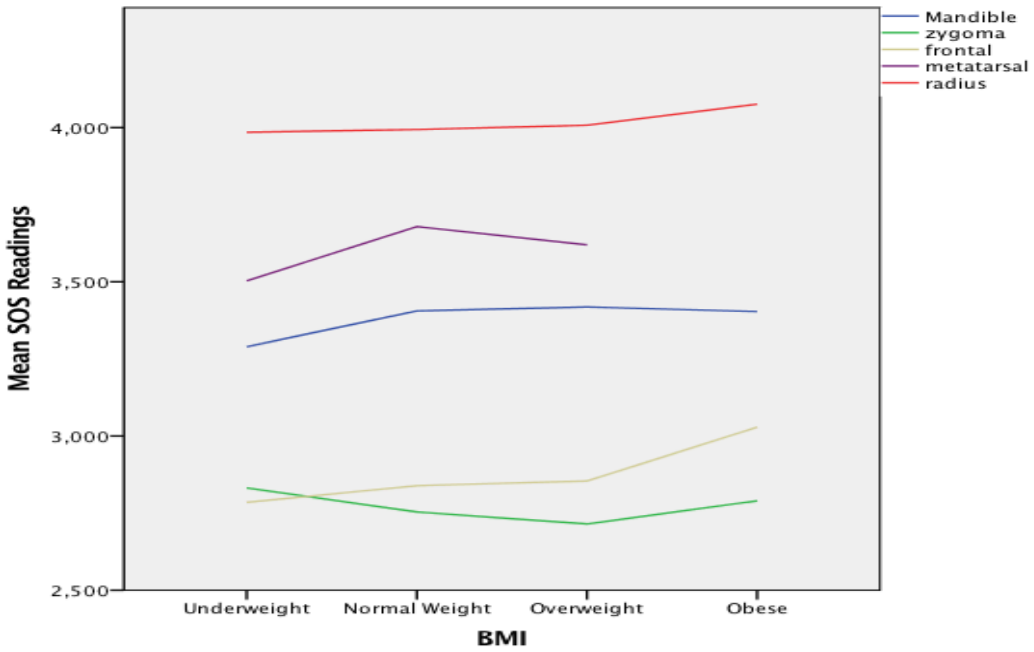


Figure 4. 14 Line graph showing mean SOS reading at each site stratified by BMI group.

4.2.15 Relationship between SOS readings and smoking

There were no statistically significant differences in SOS readings taken at all sites between smokers and non-smokers in both the control group and the osteoporosis group.

Site	Group			
	Control Group		Osteoporotic Group	
	Non Smokers n=73 ms^{-1} ±SD	Smokers n=11 ms^{-1} ±SD	Non Smokers n=63 ms^{-1} ±SD	Smokers n=23 ms^{-1} ±SD
Mandible	3496 ±220	3561 ±231	3288 ±232	3325 ±261
Zygoma	2760 ±122	2793 ±162	2716 ±156	2753 ±159
Frontal Bone	2865 ±181	2897 ±217	2820 ±180	2800 ±227
Metatarsal	3813 ±256	3711 ±202	3502 ±278	3371 ±214
Radius	4089 ±110	4113 ±88	3872 ±172	3916 ±255

No significant difference at the $p<0.05$ level between smokers and non smokers in each of the subgroups.

Table 4. 17 Mean SOS readings at each site in smokers and non-smokers.

4.2.16 Mean SOS and history of fragility fracture

In the Osteoporotic group, 64 (74.4%) of participants had a history of fragility fracture. There was no statistically significant difference in the mean SOS readings at all five sites between those with a fracture history and those without. The site with the largest difference between the fragility fracture subgroups was the metatarsal with a mean SOS of 3446 (±241) ms^{-1} in the fracture subgroup, and 3574 (±306) ms-1 in the non-fracture subgroup. The facial bone readings showed little variation between subgroups.

Site	Osteoporotic Group				*p*-value
	No Fracture History		History of Fragility Fracture		
	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n	
Mandible	3285 ±209	22	3302 ±250	64	0.775
Zygoma	2747 ±168	9	2745 ±162	30	0.974
Frontal Bone	2821 ±200	21	2813 ±188	59	0.87
Metatarsal	3574 ±306	15	3446 ±241	46	0.1
Radius	3907 ±189	18	3875 ±202	47	0.563

No significant difference at $p < .05$ level between those with and without fracture history

Table 4. 18 Mean SOS readings at each site based on fragility fracture history.

4.2.17 Relationship between SOS readings and exercise

Exploration of the relationship between exercise and SOS readings showed there was no statistically significant difference between readings taken from exercisers and from non-exercisers for both the control group and the osteoporotic group.

Site	Group							
	Control				Osteoporotic			
	No Exercise		Exercise		No Exercise		Exercise	
	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n
Mandible	3547 ±245	7	3501 ±221	77	3244 ±204	18	3312 ±246	68
Zygoma	2791 ±86	6	2760 ±128	53	2785 ±233	8	2735 ±141	31
Frontal	2894 ±247	7	2867 ±180	77	2813 ±222	16	2816 ±183	64
Metatarsal	3982 ±395	6	3784 ±232	70	3415 ±219	11	3491 ±270	50
Radius	4073 ±66	7	4093 ±111	68	3964 ±228	13	3864 ±186	52

Table 4. 19 Mean SOS in exercisers and non-exercisers.

4.2.18 Relationship between SOS readings and alcohol

Exploration of the relationship between alcohol and SOS readings showed there was no statistically significant difference between readings taken from alcohol drinkers and from non-drinkers for both the control group and the osteoporotic group.

	Group							
	Control				Osteoporotic			
	Non Drinkers		Alcohol Drinkers		Non drinkers		Alcohol Drinkers	
	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n	Mean ms^{-1} ±SD	n
Mandible	3531 ±196	13	3500 ±227	71	3286 ±257	48	3313 ±216	38
Zygoma	2815 ±148	9	2754 ±119	50	2765 ±162	21	2723 ±163	18
Frontal	2931 ±128	13	2858 ±192	71	2797 ±209	45	2839 ±162	35
Metatarsal	3891 ±272	9	3787 ±247	67	3497 ±272	35	3451 ±250	26
Radius	4072 ±78	11	4095 ±112	64	3844 ±198	37	3936 ±187	28

No significant difference at $p < .05$ in the two-sided test of equality for column means.

Table 4. 20 Mean SOS values in alcohol drinkers and non-drinkers for each group.

4.2.19 Relationship between missing teeth and mandibular SOS

A significant negative correlation (Pearson correlation r=-0.188, p=0.014) was found between number of missing teeth and mandibular SOS readings. The following table shows mean mandibular SOS when subjects are stratified into four groups based on numbers of missing teeth.

	Mandible	
Number of missing teeth	Mean ms^{-1} ±SD	n
0 to 1	3433 ±242	113
2 to 4	3377 ±271	28
5 to 7	3235 ±189	13
Edentulous	3341 ±285	16

Table 4. 21 Mean SOS values based on numbers of missing teeth.

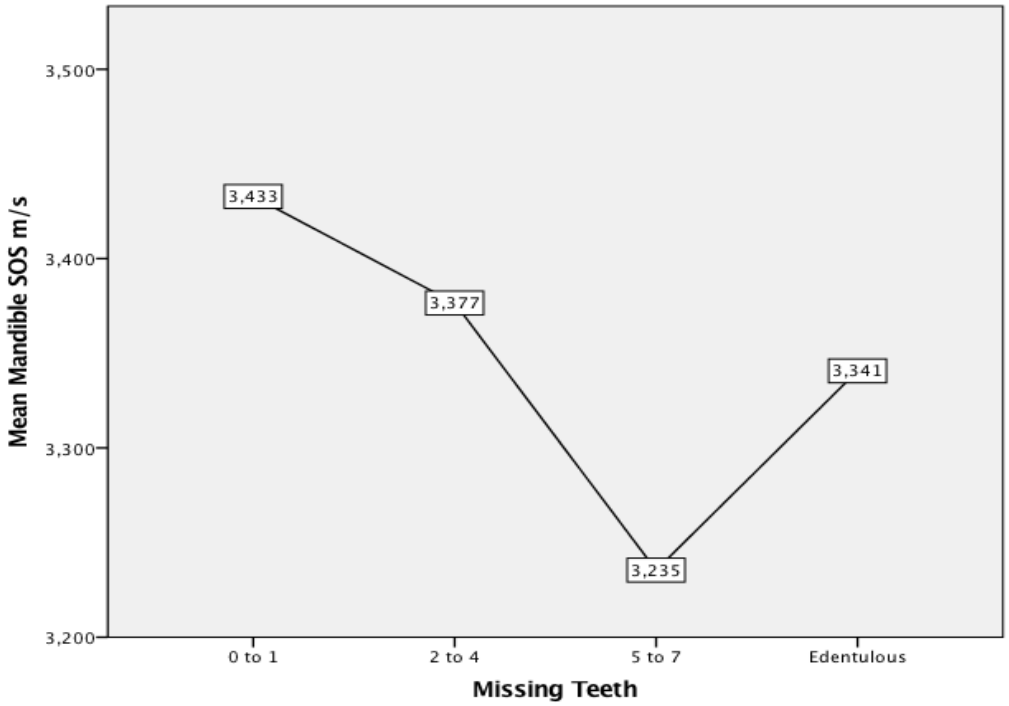


Figure 4. 15 Line graph of mean mandibular SOS and numbers of missing teeth.

Missing Teeth	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
0 to 1	.044	113	.200*	.989	113	.487
2 to 4	.086	28	.200*	.958	28	.318
5 to 7	.153	13	.200*	.940	13	.457
Edentulous	.166	16	.200*	.889	16	.054

Table 4. 22 Tests of normality based on numbers of missing teeth.

Tests of normality indicated use of parametric tests and a one-way ANOVA was conducted which found that there was a statistically significant difference between the four groups (F (3,166)=2.961, *p*=0.034). Post hoc tests were carried out using a Tukey's Test, which found there was a significant difference between subjects missing 0 to 1 teeth and subjects missing 5 to 7 teeth (*p*=0.036). No other significant differences were found.

4.2.20 Correlation Between SOS measured at Different Sites

Pearson correlation coefficients were calculated between all the sites measured. No significant correlations were found between SOS readings taken at the zygoma and any of the other measured sites. Significant correlations were found between the mandible and the radius (r=0.253, *p*=0.003), the mandible and the metatarsal (r=0.249, *p*=0.003), and the frontal bone and the radius (r=0.248, *p*=0.004) as shown in the table below. A moderate correlation was found between the Radius and Metatarsal (r=0.38, *p*<0.0001).

		Pearson Correlation	Sig. (2-tailed)	N
Radius	Radius	1		140
	Frontal Bone	.248**	.004	136
	Metatarsal	.380**	.000	121
	Mandible	.253**	.003	140
Frontal Bone	Radius	.248**	.004	136
	Frontal Bone	1		164
	Metatarsal	.101	.249	132
	Mandible	.011	.890	164
Metatarsal	Radius	.380**	.000	121
	Frontal Bone	.101	.249	132
	Metatarsal	1		137
	Mandible	.294**	.000	137
Mandible	Radius	.253**	.003	140
	Frontal Bone	.011	.890	164
	Metatarsal	.294**	.000	137
	Mandible	1		170

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. 23 Pearson correlation coefficients between sites measured with mQUS

4.2.21 Correlation between SOS measurements and DXA scans

No significant correlation was found between DXA scan results and SOS readings for any of the facial bones and the radius. The metatarsal was the only SOS site that demonstrated any significant correlation. A Spearman ρ correlation coefficient of 0.258 (p=0.05) was found between metatarsal SOS and total hip T scores, however when the DXA result is expressed BMD in g/cm^2 this result is no longer found to be significant (r=0.257, p=0.075).

4.2.22 Correlation between SOS measurements and calcaneal QUS

A moderate correlation was found between SOS measured at the frontal bone using the Omnisense and SOS measured at the calcaneus by a GE Lunar (Pearson correlation coefficient of 0.410, p=0.034). The calcaneal QUS generates a T-score using a combination of SOS and BUA. No significant correlations between calcaneal T-scores and SOS measured at all five sites were found. A significant moderate correlation was found between T-scores derived from calcaneal QUS and those derived from total hip DXA scans (Spearman's ρ = 0.522, p=0.006).

4.2.23 SOS readings controlling for age, height and weight

A one-way analysis of covariance (ANCOVA) test was conducted to determine if a statistically significant difference still exists between the control and osteoporotic groups for the SOS readings at the mandible, controlling for age, height, weight, and numbers of missing teeth.

An ANCOVA showed there was a statistically significant difference between the two groups at the mandible [F(1,164)=10.756, p=0.001].

4.2.24 Receiver operator characteristic (ROC) curves

ROC curves were conducted for the mandible, metatarsal, and radius SOS readings to determine their diagnostic ability to identify DXA confirmed osteoporosis patients. The area under the curve (AUC) for mandible SOS readings was found to be 0.747 (95% CI 0.674 to 0.82), $p<0.0001$. The AUC for the metatarsal SOS readings was 0.813 (95% CI 0.739 to 0.887) $p<0.0001$, and for the radius SOS readings it was 0.829 (95% CI 0.752 to 0.906) $p<0.0001$. This shows that all three sites have fair to good diagnostic utility in the identifying osteoporosis. These values indicate that the radius has the strongest diagnostic ability, followed by the metatarsal, and then the mandible. Based on the result from the AUC curve, if values below 3,390 ms^{-1} in the mandible were considered osteoporotic and above were considered non-osteoporotic, this cutoff figure would represent a sensitivity of 68.6%, and a specificity of 69%.

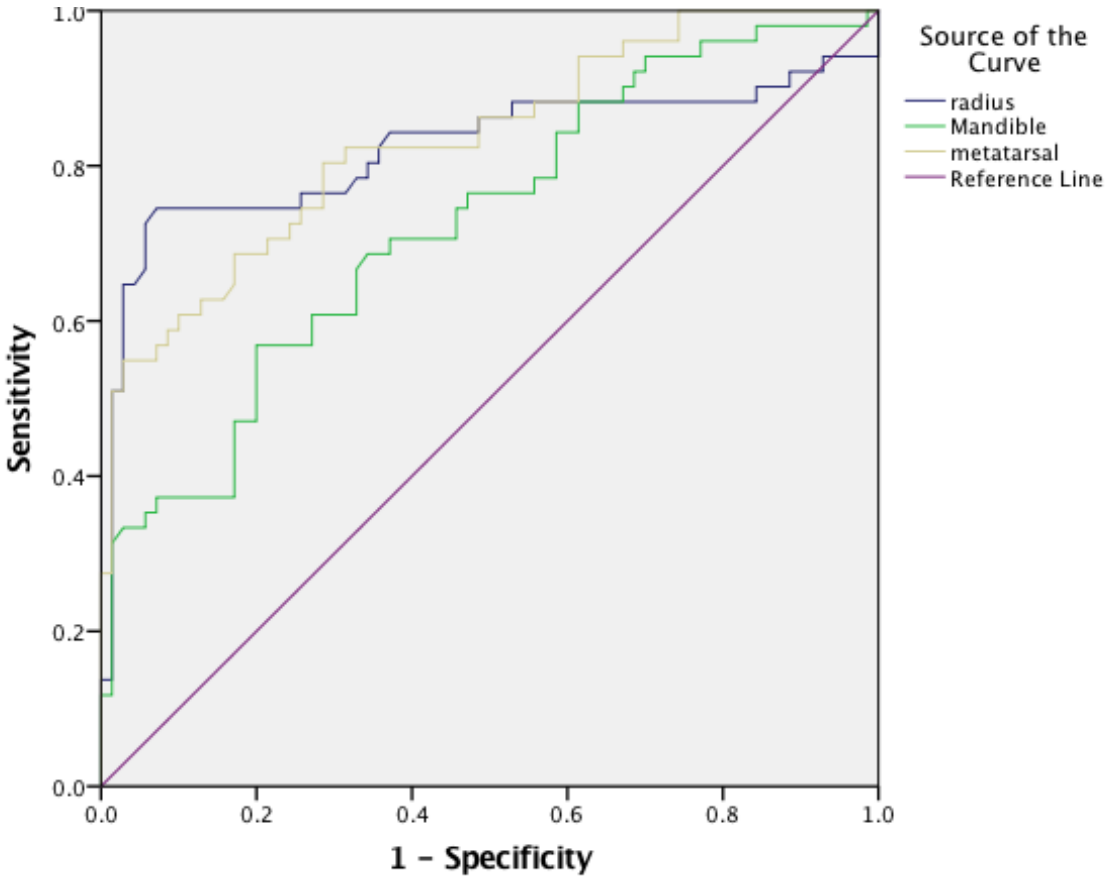


Figure 4. 16 ROC curves for the radius, mandible and metatarsal.

4.3 mQUS to measure response to osteoporosis treatment

Sixty-four out of the 86 (74.4%) participants in the osteoporosis group were followed up with a second set of scans after one year of osteoporosis treatment. This group of subjects consisted of the following numbers based on their respective treatment: -

- Teriparatide n=25
- IV Zoledronate n=26
- Denosumab n=13

Reasons for participant drop out included intolerance of the prescribed osteoporosis treatment, change of treatment during the observation period, development of a lung mass, development of breast cancer, non-compliance with the treatment regimen, age related cognition deterioration, and passing away.

4.3.1 Comparison of baseline and one year readings

Readings were taken at recruitment and after one year to measure response to treatment. Tests of normality indicated the continued use of parametric tests for the mandible and the zygoma. Two-tailed paired T-tests found that there was no significant difference between the baseline and one year SOS values taken at the mandible (T=1.182, df63, *p*=0.242), and those taken at the zygoma (T=-0.616, df29, *p*=0.543).

As the distribution of data for the baseline readings for the frontal bone, the metatarsal, and the radius has previously been to be non-parametric, the continued use of non-parametric tests were used to compare the baseline and one year readings at these sites. Wilcoxon Signed Ranks Tests found that there was no significant difference in baseline and one-year SOS values from the frontal bone (*p*=0.866), the metatarsal (*p*=0.874), and the radius (*p*=0.589).

Seventy participants (81.4%) had previously received treatment for their osteoporosis, and 16 (18.6%) were treatment naïve. No significant difference in SOS

values at baseline and one year were found at any of the sites between those with a history of previous osteoporosis treatment and those without.

	Mean ms^{-1} ±SD	Median (ms^{-1})	*p*-value
$Mandible_0$	3400 ±252	3391	0.242
$Mandible_1$	3301 ±242	3312	
$Zygoma_0$	2756 ±140	2761	0.543
$Zygoma_1$	2764 ±152	2755	
Frontal $Bone_0$	2843 ±189	12828	0.866
Frontal $Bone_1$	2810 ±191	2802	
$Metatarsal_0$	3656 ±301	3652	0.874
$Metatarsal_1$	3503 ±227	3471	
$Radius_0$	3995 ±187	4029	0.589
$Radius_1$	3877 ±170	3841	

$_0$ =baseline, $_1$ =one year follow up

Table 4. 24 Table showing SOS readings at baseline and at one year.

4.3.1.1 Baseline and one-year mandible SOS readings based on treatment

The baseline and one-year mandibular SOS readings were divided into groups based on osteoporosis treatment. Tests of normality indicated the use of parametric tests for the Teriparatide and Denosumab groups, and non-parametric tests for the IV Zoledronate group. A paired T-test was conducted for the Teriparatide and Denosumab groups, and a Wilcoxon Signed Ranks Test was conducted for the Zoledronate group. No significant differences were found in the baseline and one-year mandibular SOS values for each of the groups. P-values are shown in table 4.25.

Mean Mandibular SOS Readings (ms^{-1})					
			Baseline ms^{-1} ±SD	One year ms^{-1} ±SD	*p*-value
Treatment	Teriparatide	n=25	3322 ±234	3292 ±253	0.665
	Zoledronate	n=26	3353 ±271	3342 ±236	0.919*
	Denosumab	n=13	3275 ±209	3234 ±232	0.64

Baseline and one year mandibular SOS values divided into treatment groups. (Indicates non-parametric test)*

Table 4. 25 Mean SOS readings of the mandible based on osteoporosis treatment.

4.3.2 Change in BMD over the observation period

Twenty-four subjects had a DXA scan at baseline and at the end of the observation period. Not all participants were able to have a DXA scan at the one-year mark due to staffing issues in the radiology department in St James's Hospital resulting in increased demand.

A paired T-test found that there was no significant difference in total hip BMD at baseline and after one year (t=0.950, df 21, *p*=0.353). A Wilcoxon Signed Rank Test found no significant difference between baseline and one-year BMD at the left femoral neck (p=0.601), and at the lumbar spine (*p*=0.054).

4.3.3 Correlation between changes in SOS values and DXA scans

A significant moderate to high correlation was found between change in mandibular SOS and left femoral neck BMD over the observation period, with a Pearson correlation co-efficient of 0.696 (*p*=0.001). Moderate significant correlations were also found between mandibular SOS and lumbar spine BMD change (r=0.476, *p*=0.039), and mandibular SOS and total hip BMD change (r=0.534, *p*=0.009).

The metatarsal demonstrated a moderate significant correlation to BMD change at the left femoral neck (r=0.598, *p*=0.024). The other facial sites and the radius did not demonstrate any significant correlation to BMD change.

4.3.4 Correlation between changes in bone turnover markers and SOS

Significant negative correlations were found between SOS change at the frontal bone and change in the level of bone turnover markers over the observation period: P1NP (r=-0.443, *p*=0.001), Alkaline Phosphatase (r=-0.319, *p*=0.017), Osteocalcin (r=-0.413, *p*=0.002). No significant correlations to changes in biomarker levels were found with the other four sites.

4.4 Summary of key results

A significant difference between the osteoporotic group and the control group was found in SOS readings taken from the mandible, the radius, and the metatarsal. No significant difference was found in the readings taken from the zygomatic arch and the frontal bone. At the mandible, when adjusted for age, height, weight, and numbers of missing teeth, the difference between the two groups remains statistically significant.

Out of the five sites investigated in this study, the zygomatic arch proved to be the most unreliable from which to take SOS readings. There was a significant correlation between increasing age, and difficulty in taking readings from this site.

Weak to moderate correlations were found between SOS readings taken from the mandible, the radius, the metatarsal, and frontal bone. No significant correlations were found with the zygomatic arch and any other site.

Numbers of missing teeth had an effect on SOS readings from the mandible. ANOVA followed by post hoc tests found there was a statistically significant difference between subjects missing one or less teeth to those missing five to seven teeth on the right side of the mandible.

Receiver operator characteristic curves showed that the radius was the best site for diagnosis of osteoporosis, followed by the metatarsal, and then the mandible. The mandible showed reasonable diagnostic capability. The ability of SOS readings from the mandible to identify osteoporosis or absence of osteoporosis is approximately equal.

No significant change in SOS readings was found at any of the five sites over the one-year observation period. There was, however only two sites which showed a significant correlation between change in SOS and change in BMD over the observation period. These two sites were the mandible, which showed a moderate to strong correlation to lumbar spine, left femoral neck and total hip BMD, and the metatarsal, which showed a moderate correlation to left femoral neck BMD only.

5 Discussion

To our knowledge, this research is the first in-vivo study to use a multisite quantitative ultrasound device on the frontal bone and the zygomatic arch, and the second to use such a device on the mandible. This study aims to continue the research conducted by Beattie *et al* in 2017, who using the same mQUS device (Omnisense 8000) on the parasymphysis of the mandible, showed that it could be used to identify those with osteoporosis.[5] The primary aims of our study are to investigate other facial bones that may also show similar changes related to osteoporosis, confirming the mandible as a site in our investigation with a larger sample size, and to determine whether there are detectable changes over the course of one year of osteoporosis treatment.

The importance of this research is the potential practical application of the use of multisite quantitative ultrasound on facial bones to diagnose osteoporosis, and identify those at risk of a fragility fracture. A particular significance for oral and maxillofacial surgery relates to the fact that falls are a common cause of facial fractures in the elderly.[226] A study found that in elderly patients with facial injuries, falls accounted for over half of the cases, and that osteoporosis was found to be an independent risk factor for maxillofacial fractures. As the severity of osteoporosis worsened, patients were more likely to sustain a greater number of maxillofacial fractures (p=.01).[227] Where patients are identified as being at risk of facial fractures, as well as other common fragility fractures, fall prevention strategies can be implemented.

Osteoporosis has also been suggested as a risk factor for failure of dental implants, and while a review of the literature shows that the evidence is unclear at this time, this technology could potentially be developed to identify sites in dental implant case planning to identify sites of the alveolar bone affected by osteoporotic change.[228]

5.1.1 SOS readings from the facial bones

A mean SOS value of 3,298 (SD 239) ms^{-1} was found at the parasymphysis of the mandible in the osteoporotic group. This result is remarkably similar to the result reported by Beattie *et al* who found a mean SOS reading of 3,312 (SD 264) ms^{-1} in their osteoporotic group.[5] Both studies had good sample sizes in their respective osteoporotic groups; our study recruited 86 confirmed osteoporotic subjects, and Beattie *et al* recruited 52 subjects. This suggests that the mean SOS values from the parasymphysis of the mandible in the osteoporotic groups reported in both studies is close to the true value.[5] However, different mean SOS values were reported in the young control groups between the two studies: Beattie *et al* reported a mean SOS value of 3,683 (SD 210) ms^{-1}, and we found a mean SOS value of 3,504 (SD 221) ms^{-1}. In both studies the difference in mean SOS values between the osteoporotic group and the young control was found to be statistically significant. The result from our young control group was very similar to the result reported of the age matched control group by Beattie *et al* (3,514 SD 221ms^{-1}). The difference in the values reported in the young control groups between the two studies may be in part be accounted for by the relatively high long term coefficient of variation reported by Beattie *et al*, but it is most likely related to the relatively low sample size in the young control group in their study. Beattie *et al* had a relatively small sample size of only 26 subjects in this group, which would make their result more susceptible to bias. Our result is more robust due to the larger sample size, although it should also be acknowledged that there was a difference in the mean age of the young control group in both studies, which may also account for some of the difference. The mean age of the control group in our study was 29 (SD 7.47), and the mean age of the corresponding group in Beattie 2017 was 32 (SD 7.8) years. Multiple studies using the Omnisense have shown a peak SOS at multiple sites in patients in their 30s, and our study did find higher SOS values in subjects in their 30s than in those in their 20s.[5] The effect of age on SOS values will be discussed later.

Our results did not show a significant difference in mean mandibular SOS readings between those with and without a history of fragility fracture. This result is similar to

that reported by Knapp *et al* in 2004, who found that SOS readings from the tibia were unable to discriminate fragility fracture cases, although they did report that the radius, phalanx, and metatarsal displayed modest fragility fracture discrimination, with low area under the curve (AUC) values of 0.6, 0.6, and 0.61, respectively.

It is possible that our result may be related to undiagnosed vertebral fractures, when it is considered that up to two-thirds of vertebral fractures go undiagnosed.[55] This may result in a number of subjects without a history of fracture actually having had an undiagnosed fracture, but this is unlikely as all patients recruited had a DXA scan, and it is doubtful that any vertebral fractures would not have been picked up by the scans. Subjects in our study consented to allow the researchers access to their medical notes, and any history of fracture was confirmed and documented.

Both our study, and Beattie *et al* are in agreement that changes in bone strength related to osteoporosis affect the mandible.[5] The two studies demonstrated reasonable diagnostic utility of the parasymphysis to identify DXA confirmed osteoporosis, by the use of Receiver Operator Characteristic (ROC) curves. Beattie *et al* reported an area under the curve (AUC) value of 0.83, indicating good diagnostic ability, whereas our study found a lower AUC value of 0.747 indicating fair to good diagnostic ability. The ability of the ultrasound to identify osteoporosis from SOS readings from the mandible in both studies is the same with both studies showing a nearly equivalent result for sensitivity (68.6%, and 67%). However we found a lower value for specificity at 69%, compared to 79% found in that study.[5] Our results suggest that the ability of the mQUS to identify osteoporosis, and absence of osteoporosis is approximately equal. AUC values for the metatarsal and radius of 0.813 and 0.829, respectively, show that these two sites have slightly better diagnostic ability than the mandible, suggesting that the radius and metatarsal may be more prone to osteoporosis related changes than the mandible.

Investigation of the zygoma and the frontal bone found no significant difference between the osteoporotic patients and the control group. There were a number of failures to take readings in both of these sites, but these numbers were very small

for the frontal bone (3.5%). The presence of osteoporosis does not affect SOS readings in the frontal bone, and we can infer that the frontal bone is unaffected by osteoporosis and maintains its strength from early to late adulthood. This result is reassuring when it is considered that the frontal bone protects the brain, particularly as with age, there is an increased risk of falls.[226] It is unclear as to why the frontal bone does not demonstrate any change. The mandible, and to some extent, the zygomatic arch are subject to masticatory forces, and may be considered loaded bones. The frontal bone is not subjected to such loading, and perhaps bones that are subject to loading have more dynamic properties. While we did not find a significant difference in mean SOS values at the zygomatic arch, we cannot make this inference that the zygoma maintains its strength throughout life due to the high number of failures to take readings (42.4%). The CRB probe, which was used in this study, was designed primarily for the metatarsal. The Omnisense is available with three different probes, designed for use on the tibia, phalanx, radius and metatarsal. None of these probes were designed for use on facial bones. This study has shown that the CRB probe could be used successfully on the mandible and the frontal bone, but not predictably on the zygoma. It would be interesting to investigate the zygoma using a probe designed to accommodate the variations in the morphology of the zygomatic arch.

An interesting finding is that, despite the mean SOS readings from the zygoma and frontal bone in the study groups not being significantly different, the mean SOS values of all five investigated sites were higher in the control group than the osteoporotic group.

5.1.2 Effect of age on SOS readings

A number of studies have been conducted to create reference databases for SOS readings at multiple sites.[192, 221] They report that most sites show peak mean SOS readings in the 30 to 39 age group, with the exception of the tibia, which peaks in the 20 to 29 age group. We found that mean SOS readings in the mandible peak in the 30 to 39 age group (3,569 SD 249 ms^{-1}), which is consistent with the study by

Beattie *et al* 2017.[5] We found that in the radius, SOS readings peak in the 50 to 59 age group (4,170 SD 196 ms^{-1}). While this does conflict with previous research, it is likely that due to the small sample size in this age group (n=5), this would result in our finding being of high risk of bias. We did, however, find that the mean SOS reading in the 30 to 39 age group (4,138 SD 102 ms^{-1}) followed closely, and this group did possess a much larger sample size (n=26). It is likely that with a larger sample size of those in their 50's, we would find a lower mean SOS reading more in line with the established literature. This underrepresentation in the 50 to 59 age group may also explain the result from the frontal bone, which also showed a peak SOS in this group (3,064 SD 308 ms^{-1}, n=5).

Our results show that in the mandible there is trend towards an age related decline in mean SOS readings. As age is considered a major risk factor for osteoporosis, and is one of the strongest predictors of fragility fracture, this result is not surprising.[58] When controlling for the effect of age, the difference in mean SOS readings between the control group and the osteoporotic group from the mandible was still found to be significant (p=0.001).

5.1.3 Precision measurements

The in-vivo precision measurements (RMS CV%) for the parasymphysis for both short and long term were shown to be reasonably low at 1.13% and 1.88%, respectively. Beattie *et al* in 2017 reported a lower root mean squared coefficient of variation of 0.74% for short-term precision at the parasymphysis, but a much higher long-term coefficient of 3.7%.[5] In that study, they do concede that there may have been a learning curve in use of the device at the beginning of the data collection, which may account for the relatively high value in their long-term precision measurements. We had the advantage of contact with Dr Beattie to advise on use of the ultrasound, and could anticipate any issues that may arise with its use.

Precision measurements were found to be the highest for the metatarsal for both short (RMS CV 2.04%) and long term precision measurements (RMS CV 2.29%). This

result is consistent with Knapp *et al* in 2001, who also used the Omnisense QUS on multiple sites, reported that the metatarsal demonstrated the highest short (RMS CV 1.48%), and long-term (RMS CV 3.69%).[181] The zygoma demonstrated the same high long-term precision as the metatarsal (RMS CV 2.29%), but a lower short-term precision (RMS CV% 1.36%). The radius demonstrated the best precision results overall for both short (RMS CV% 1.05%) and long term (RMS CV% 1.43%).

The precision measurements performed in this study were all conducted on subjects in the control group. No precision measurements were performed on those in the osteoporotic group. There are a number of reasons why we were unable to perform precision measurements in the osteoporotic group. Most of the patients attending the bone health clinic were elderly and a large number of them were attending escorted by family members. It would be unrealistic to expect them to stay for up to the three hours necessary to perform the short-term precision measurements or attend on a monthly basis for one year for the long-term precision measurements. While it would be ideal to perform short-term precisions on this group, it can be argued that the validity of long-term precision results is questionable, as we were also investigating this group for dynamic changes in response to osteoporosis treatment, although a comparison of long- term precision measurements between the control group and the osteoporotic group would be interesting.

This study demonstrates that precision measurements from the parasymphysis of the mandible compare favourably to other established sites that have been investigated in multiple studies using multisite quantitative ultrasound.

5.1.4 Difficulty in obtaining readings between sites

Studies using multisite quantitative ultrasound have reported difficulties in obtaining readings from some sites. We found that the most successful site from which to obtain a reading was the parasymphysis of the mandible with a 100% success rate in all subjects (n=170). Beattie *et al* 2017 reported only two failures out of 127 subjects to take a reading from the mandible. Both studies would agree that the

parasymphysis is a reliable site to successfully obtain a reading. The two subjects were noted to have a high BMI. They also reported that there was a failure to obtain readings from the radius in 24% (n=30) of cases. They found that high BMI was associated with difficulty to obtain a reading, and there was a statistically significant difference in BMI between those who they could successfully take a reading and those who they could not (p<0.0001). We found that we were unable to take readings at the radius in 17.6%, and in these subjects the BMI was found to be significantly higher. The BMI of subjects that we could not successfully obtain a reading was in the overweight group with a mean BMI of 26.4, compared to a mean BMI of 21.9 in those that were successful (p<0.0001).

We also found a similar result with the metatarsal and were unable to obtain readings in 19.4%. The mean BMI of those with successful readings was 22.3 and the mean of those with an unsuccessful reading was 24.2. Despite these two values being closer in value than the radius and both being within what is considered normal BMI, this difference was also found to be significant (p=0.025). In a number of subjects in the osteoporotic group who were within normal BMI range, oedema of the feet made it difficult to obtain a reading, which may account for this closer range of values.

The effect of BMI in creating difficulties to take readings has been noted in other studies using the Omnisense. Knapp *et al* in 2004 reported a failure to obtain readings in 3% at the radius and 8% at the metatarsal. They accounted for this difficulty to obtain readings due to obesity and in the case of lower limbs, oedema.[194] Weiss *et al* in 2000 also reported failures to obtain a reading at the radius, tibia, metatarsal, and phalanx in 6.4%, 3.6%, 15%, and 2.7% of cases, respectively. This was mainly attributed to soft tissue thickness, however the manufacturers were introducing different probes tailored to different sites during this study. It is likely that some failures may be attributed to the use of inappropriate probes at the time of attempted reading.[189]

Interestingly, BMI did not appear to have an influence on the ability to obtain a reading in the facial bones. The zygomatic arch proved to be the most unreliable site

with an overall success rate of only 57.6%. However, an interesting finding is that there was a much higher success rate in obtaining a reading from the control group (70.2%) compared to the osteoporotic group (45.3%), and this difference was found to be significant (p=0.001). The design of the probe relies on the probe being placed against a reasonably flat tissue surface, so that both transducers are in contact with the tissue allowing for both transmission and receiving of an acoustic signal. When this is not the case, such as in case of a bone with a relatively high degree of curvature, the ultrasound cannot generate a reading. This suggests there is a difference in the morphology of the zygomatic arch between young and older age groups. It is known that the maxilla retrudes with age, and there is continual remodelling of the facial bones throughout life.[222] We did find a negative correlation between increasing age and success in taking a reading from the zygoma. This result suggests that the zygomatic arch changes as part of the ageing process. There is no research in the literature on age related changes in the zygomatic arch. This may be a research question for future studies.

Following the mandible, the frontal bone (96.5% successful) was found to be the second most reliable site to successfully obtain a reading. In determining the most reliable position on the frontal bone to obtain a reading, we found that 3cm above the nasofrontal junction was a consistently reliable site. This point represents the most flat contour on the forehead, and we found difficulty in obtaining a reading once the probe is moved ≥ 0.5cm inferior or superior to this point, demonstrating the limitation of this probe on contoured surfaces.

5.1.5 Effect of BMI on SOS readings

While the effect of BMI was found to have a significant effect on measurement success, it did not appear to have an effect on the actual SOS readings themselves. Review of the literature is unclear of the effect of BMI on SOS readings, and the studies gathering population reference data have not shown attempts to correlate BMI and SOS readings.[181, 191, 192] There is evidence in the literature that there is an

association between BMI and BMD; the Framingham osteoporosis study reported that BMI accounted for 8.9 to 19.8% (p<0.01) of BMD variance in women, and 2.8 to 6.9% (p<0.01) in men.[229]

We found using a one-way ANOVA for each of the facial bones, and a Kruskal-Wallis test for the radius and metatarsal, that there was no significant difference between subjects of different BMI classifications. This may be due to the majority of subjects in this study being classed as having a normal BMI: in the control group 77.3% (n=65), and in the osteoporotic group 65% (n=56) were classed as normal BMI. There were only 2 (< 3%) subjects in each group classed as obese. This would result in a lack of statistical power in each of these groups, increasing the likelihood of failure to achieve statistical significance.

We found that for the mandible, the lowest mean SOS readings were found in the underweight group (3,289 SD 220ms^{-1}), following by the normal weight group (3,406 SD 249ms^{-1}), and the highest mean SOS readings were found in the overweight group (3,418 SD 283ms^{-1}). Beattie *et al* also found that BMI did not have a statistically significant effect on the SOS readings on the mandible, and that the lowest reading was found in the underweight group (3,319 SD 164ms-1). They did however find that the highest mean SOS was found in the obese group (3,539 SD 323ms^{-1}). They did have a higher number of subjects in this group (n=15), so it is likely this closer to the true value than our result.[5]

5.1.6 Effect of tooth loss on SOS readings from the mandible

There was a significant negative effect of tooth loss on SOS readings from the mandible (p=0.034), which would concur with Beattie *el al*.[5] As expected, the highest mean SOS readings were found in those missing one or no teeth (3,422 SD 242 ms^{-1}), however against expectations, the lowest value was not found in edentulous subjects, but in those missing 5 to 7 teeth (3,235 SD 189 ms-1). Subjects were stratified into four subgroups based on numbers of missing teeth and the difference between those missing 0 to 1 teeth and those missing 5 to 7 teeth was found to be

significant (p=0.036). While the edentulous group (n=16) did show a higher mean SOS (3,341 SD 285 ms^{-1}) than the group missing 5 to 7 teeth (n=13), it should be recognized that the edentulous group demonstrated the largest standard deviation, indicating that the edentulous group had the widest variation of SOS values of these four subgroups. It is well known that following extraction of teeth, progressive bone resorption occurs. This results in gradual loss of the alveolar bone, and if edentulous for a long enough period of time, resorption also affects the mandibular basal bone. It is possible that as an edentulous mandible loses volume, the thickness of the cortical bone remains constant, so that as progressive resorption of the mandible occurs, there is greater loss of the trabecular bone relative to the cortical bone. This would result in a higher ratio of cortical to trabecular bone, and this phenomenon has been demonstrated in histomorphometric studies. An ex-vivo study by Bertl *et* al in 2015 investigated the characteristics of the cortical bone component in edentulous mandibles with varying degrees of residual ridge resorption. They analysed 185 ground sections of edentulous mandibles with histomorphometric analysis, and found that with smaller total mandibular area (due to resorption), there was a greater percentage of cortical bone in the total area (ρ=-0.340, p<0.001).[223] Another ex-vivo study by Katranji *et al* in 2007, looked at buccal bone thickness in dentate and edentulous specimens, and reported quite an interesting finding. In the molar, premolar and anterior regions of the mandibles in the dentate specimens, the mean thicknesses were 1.99mm, 1.2mm, and 0.99, respectively. Surprisingly, in the edentulous specimens, the buccal cortical bone was found to be thicker with the corresponding values reported to be 2.06mm, 1.78mm, and 1.36mm, respectively.[224]

As there would be varying degrees of resorption in the edentulous subjects in our study, as well as variation in the number of edentulous years, this may explain the wide range of readings in this group. While we did record the number of missing teeth in the mandible, we did not note the extent of residual ridge resorption, or the number of edentulous years. It would be interesting to conduct a study in the future to investigate the correlation between these characteristics and SOS readings from the mandible.

Our result suggests that it may be possible to identify the type of bone as described by Lekholm and Zarb in 1985 using quantitative ultrasound.[165] This would be useful for planning the surgical approach for implant placement in edentulous mandibles, for example, selecting appropriate osteotomy drills and determining pre-operatively to use bone tapping.

5.1.7 Measuring response to osteoporosis treatment using mQUS

This study did not detect any significant changes in SOS measurements at any of the five sites in response to osteoporosis treatment in the whole osteoporosis group, and also in each subgroup based on their respective treatment. However, it is important to note that there was also no significant change in BMD detected in the number of subjects that had a DXA scan at recruitment and the one-year mark. There is conflict in the literature regarding the use of quantitative ultrasound to measure response to treatment. Weiss *et al* in 2003 reported a positive response oral Alendronate, and similar to the result of our study, Drake *et al* in 2002 reported no response to treatment. Both studies had a similar study design and sample size; however a major distinction between the two studies is the follow up period. Weiss *et al* followed patients up for two years, and similar to our study, Drake *et al* followed up subjects for only one year.[4, 195] This suggests that following initiation of treatment, a response is not detectable until at least after one year. The EUROFORS study looked at response to teriparatide treatment over 24 months, and reported that in the initial 6 months there is, in fact, a slight reduction in BMD at the femoral neck and total hip. The majority of gain in BMD was seen at 18, and 24 months.[225] It is likely, therefore that if we follow patients up for a similar time period, we should see gains in SOS readings in the facial bones in these timeframes.

The lack of response may also be related to previous osteoporosis treatment the patients may have received, and studies have shown that a greater response is seen in treatment naïve patients than in those previously exposed to anti-resorptive

therapy.[80, 81] The Bone Health Clinic in St James's Hospital is a specialist clinic with a secondary referral patient base. Many of the patients would have had treatment initiated by their own general practitioners, and if a poor response is detected or there are complications related to the treatment, they are then referred to the clinic. The majority of subjects in the osteoporotic group received previous osteoporosis therapy; only 16 subjects (18,6%) were treatment naïve, while 70 subjects (81.4%) had a prior history of osteoporosis therapy. This may partially account for the lack of response to treatment.

The ISCD guidelines do not recommend the use of QUS for monitoring response to osteoporosis therapy. This is because there is a lack of clear evidence that QUS is useful in this regard. It is unclear whether this is due to lack of response from peripheral sites, or perhaps may be related to precision issues.[177]

Despite the apparent lack of response to treatment, the use of facial bones to monitor response should not be dismissed. In fact, one of the most interesting findings of the study is that in those who had DXA scans at recruitment and after the one-year follow up, the site with the highest significant correlation between change in BMD and change in SOS measurements over the observation period was the mandible. Change in mandibular SOS resulted in Pearson correlation coefficients of 0.696, 0.476, and 0.534 when correlated with change in BMD at the left femoral neck, lumbar spine, and total hip, respectively. The only other site to show a significant correlation to BMD change was the metatarsal, with a correlation coefficient of 0.598 to left femoral neck BMD only. While it is not possible to make a definitive conclusion regarding the use of the mandible to monitor osteoporosis treatment response as the number of patients having a one-year DXA scan was too small (n=24), this result is encouraging and shows that this research certainly merits further investigation.

5.1.8 Study limitations

A limitation of this study is the lack of an age matched control group. The initial design of the study was to include three groups including an age matched group of control subjects for direct comparison to the confirmed osteoporotic group. During the design phase, we considered arranging DXA scans for an age matched control group, however because of a limited budget and requirement of a large sample size, this was not feasible. Another problem with this approach is that a certain amount of disease would be identified, resulting in the research team being responsible for arranging osteoporosis treatment. We also explored the possibility of recruiting subjects that had already been screened by DXA scan and confirmed as not being osteoporotic. This involved arranging meetings to discuss the research with medical staff at a number of health centres, and osteoporosis screening facilities. Few suitable candidates were found. Following exhausting all avenues, and after discussion, it was decided to omit the age matched control group from the study.
To recruit a suitable control group, we applied strict exclusion criteria to reduce the likelihood of subjects in this group being osteoporotic. However, it should be acknowledged that the BMD of this group was also unknown. While the risk factors for osteoporosis in this group were low, without BMD measurement, we cannot be certain that this group did not contain osteopenic or osteoporotic subjects. Ideally subjects in the control group should have BMD measurements, with T-scores within the normal range (> -1), excluding anyone with a T-score below -1.

We followed up 64 out of the 86 confirmed osteoporotic subjects recruited into the study. This represents a drop out rate of 25.6%. The subjects recruited to this study were keen to participate. An issue with recruiting subjects from this demographic is that they are of an age more prone to medical issues, with the result that it may be difficult to predict a drop out rate. Reasons for subject drop out included intolerance of the prescribed osteoporosis treatment, change of treatment during the observation period, non-compliance with the treatment regimen, age related cognition deterioration, and passing away. A history of cancer is a contraindication for Teriparatide, and one participant developed breast cancer shortly after commencing Teriparatide, and another who was planned for Teriparatide developed a lung mass, requiring further investigation and suspension of osteoporosis

treatment.[78] Our power calculation showed that we needed 24 subjects in each of the treatment subgroups, and fortunately we were able to follow up 25 subjects in the Teriparatide group and 26 in the IV Zoledronate group.

The primary researcher performed all the ultrasound measurements in this study, which introduces operator bias. Ideally the operator should be blinded to the aims of the study. This would have required the recruitment of another researcher who would be blinded to the aims of the study to carry out the scans. This would not have been realistic considering that the data collection for this study involved between five to ten hours a week, and took two years to carry out. Radiographers in St James's Hospital, who were not aware of the aims of this study, carried out measurement of BMD. This is the only form of blinding we were able to implement.

Subjects who participated in this study were not recruited by the primary researcher, they were recruited by two gatekeepers: one gatekeeper was a member of staff based in the Dublin Dental Hospital, and the other was one of the specialist bone health nurses based in St James's Hospital. The use of the gatekeepers in this study reduces the risk of selection bias.

The arm of this study looking at response to treatment suffers from attrition bias. We had a drop out rate of 25.6% in the osteoporotic group. These were elderly patients, and therefore have a higher risk of medical issues related to ageing. This would have had an effect on our results investigating response to osteoporosis treatment.

The difficulty in taking readings from a number of sites would have effectively resulted in lower than planned numbers required for a number of statistical tests. The mandible was the only site with a 100% success rate in taking a reading and therefore was the only site that reached the numbers required when comparing the two groups. The frontal bone did achieve the number of successful readings for the control group, but fell just short of the numbers required for the osteoporotic group. The numbers of successful readings on the zygoma fell well short of the power

calculation with the result that no meaningful conclusions regarding SOS readings taken from this site can be made.

It was planned that subjects in the osteoporosis group would have DXA scans performed at or prior to recruitment, and the one-year mark, when the second set of ultrasound readings would be taken. During the study period, there have been a number of infrastructure changes in St James's Hospital. This lead to staffing issues in the radiography department and increased waiting times to have DXA scans. As a result we were unable to arrange DXA scans for a large number of our osteoporotic cohort.

At the beginning of the study, DXA scans were performed with a GE Lunar Prodigy. This was replaced with a Hologic Horizon A after a number of months into recruitment for the study. It has been shown that there are variations between different brands of scanners and it is difficult to compare results.[154] The software from the Hologic does incorporate an algorithm, which adjusts the score from a previous scan, compensating for the variation.

The arm of the study looking at response to osteoporosis treatment did suffer from a limited time frame to carry out this research. Subjects were followed up for one year, however those on teriparatide were on a two-year treatment course. On the Bone Health Clinic in St James's Hospital, there is a clearly outlined protocol for teriparatide treatment. Patients deemed suitable for teriparatide, are seen by the specialist bone health nurses for education in the treatment regimen at initiation of treatment. They are then reviewed after three months to assess their tolerance to the treatment, and to have bloods to ensure there is no abnormality with calcium metabolism and to check biomarker response. They are then seen one year after initiation of treatment again to have blood tests. After two years, patients are then seen on the Bone Health Clinic to review their response to treatment with a DXA scan, and to discuss anti-resorptive treatment to ensure the anabolic effect of the teriparatide course is not lost. A treatment protocol such as this is highly conducive to research such as our study. It would be desirable to follow patients for the full duration of the teriparatide course, and to restrict the study to patients who are treatment naïve, so that the effect of previous anti-resorptive treatment does not

interfere with the remodelling effect of teriparatide. To conduct such a study, following patients on the full course of teriparatide, would need a longer timeframe to complete.

5.1.9 Potential future research

This study raises a number of research questions warranting further investigation. As already mentioned, these include potential dimensional changes in the zygomatic arch throughout life, and the impact of variations between edentulous residual ridge resorption on SOS measurement.

We were unable to find a significant difference in SOS readings between the two groups for the zygoma and the frontal bone. Based on the data we collected for this study we were able to calculate an effect size for each site, which may be used for future research. We found a Cohen's d of 0.134, 0.301, and 0.69 for the zygoma, the frontal bone and the mandible, respectively.

The potential for further use of quantitative ultrasound on the mandible and the maxilla looks promising. We found that this device could be used successfully to assess bone strength in the mandible, however this is only when used as an external device. There is the potential to develop an axial transmission QUS probe that can be used intra-orally, as currently there is no intra oral probe available that is compatible with the Omnisense.

A number of studies have used transverse transmission QUS devices on the mandible with an intra-oral approach, however these devices have caliper mounted probes, and have proved to be quite bulky making them difficult to use. They are suitable for use on edentulous sites, but when teeth are present within the ultrasound field, they can produce erroneous readings.[219, 220]

Such changes as we have shown in this study, may be detectable intra-orally, and if so, its clinical application in oral and maxillofacial surgery may be investigated further. Clinical applications of an intra-oral axial QUS may include assessment of bone as part of a work up for implant placement, and progression of healing of bone grafts. It would be interesting to conduct a long term cohort study using an intra oral device on sites planned for implant placement to determine its use in predicting implant success and failure.[229] It would also be particularly interesting to investigate the effect of osteonecrosis of the jaw on SOS measurements, both intra and extra-orally.

6 Conclusions

Due to a growing elderly population, the incidence of fragility fractures related to osteoporosis is set to increase becoming a significant public health issue. The impact of such fractures and their associated morbidity has a major negative impact on quality of life, and life expectancy, not to mention the financial strain this places on public health spending. It is important that those at risk of fragility fractures are identified, so that preventative measures can be implemented.

The most established methods of fracture risk assessment are evaluation of clinical risk factors, and measurement of bone mineral density at the hip and spine by dual energy x-ray absorptiometry. This forms only part of the overall picture and the use of other forms of diagnostic tests at peripheral sites in conjunction with DXA provide a more complete picture of patient fracture risk. There is an increasing volume of evidence supporting the use of quantitative ultrasound, and the use of calcaneal QUS for fracture risk assessment is recognised by the International Society of Clinical Densitometry. More recently, multisite QUS has been developed, however there is further research needed before its use can become more widely accepted.

This study, using a multisite quantitative ultrasound device, has shown that there are osteoporotic changes evident in the mandible, which may be evaluated as part of the overall work up in patient osteoporosis assessment. Speed of sound readings from all five sites did not show a response to osteoporosis treatment, however the mandible did show the best response out of all the sites investigated when change in SOS was correlated to change in BMD. This result is very promising for future research, considering that the radius and the metatarsal are two sites already established for mQUS use.

There are no identifiable osteoporosis related changes evident in the frontal bone, which suggest this bone retains its strength throughout life. The results from the

zygomatic arch are inconclusive, however they do add to the small body of evidence showing age related morphological changes in the facial skeleton.

7 Appendices

7.1 Appendix 1 – Patient questionnaires

Patient Questionnaire Group 1 ID number

Thank you for taking part in this study investigating the use of ultrasound to measure changes in the lower jaw compared with other sites on the body. The aim of this questionnaire is to anticipate any factors that may influence the results. Please note that all answers given will be kept in the strictest confidence.

Date of Birth: _____
Weight (Kg):_____
Height (cm):_____

Do you smoke?_____ If yes how many?_____
How many years have you smoked?_____

Do you exercise?_____
If yes how many hours a week of: -
Light exercise (e.g. walking)?_____
Cardiovascular exercise? _____
Weight bearing exercise? _____

Do you drink alcohol?_____
If yes how many units a week on average?_____
1 unit of alcohol is the equivalent of ½ pint of beer or a small wine or a single measure of a spirit

How often do you visit your dentist for check ups?_____
Do you suffer from any of the following? : -

Coeliac Disease _____
Asthma _____
Diabetes_____
Rheumatoid Arthritis_____
Chronic Liver Disease_____
Chronic Kidney Disease_____
Thyroid Disease_____
Lactose Intolerance_____
Metabolic Bone Disease (such as Osteomalacia, Paget's Disease)_____

Osteoporosis_____

Hyperparathyroidism_____

Please list your medications_____

_____

Do you or have you ever suffered from an eating disorder?_____

Do you have any dietary preferences/restrictions (eg vegan, vegetarian, health related diet restrictions)?_____

How long have you been on this diet? _____

Have you ever been on long term corticosteroids?_____

Are you currently undergoing treatment for cancer?_____

Have you received cancer treatment in the last 5 years?_____

Have you ever fractured a bone?_____

If yes which bone(s)?_____

When did this fracture occur?_____

Have you ever had a DXA scan (bone density scan)?_____

If yes what was the result?_____

Have either your parents ever fractured a hip?_____

Have you been through the menopause?_____

If yes when?_____

If no, is there any possibility you are pregnant?

If no has it been over 6 months since your last menstrual cycle?_____

Are you missing any teeth on the right side of your mouth?_____

Patient Questionnaire Group 2 ID number

Thank you for taking part in this study investigating the use of ultrasound to measure changes in the lower jaw compared with other sites on the body. The aim of this questionnaire is to anticipate any factors that may influence the results. Please note that all answers given will be kept in the strictest confidence.

Date of Birth: _____
Weight (Kg):_____
Height (cm):_____

Do you smoke?_____If yes how many?_____
How many years have you smoked?_____
Do you exercise?_____ If yes how many hours a week of: -
Light exercise (eg walking)?_____
Cardiovascular exercise? _____
Weight bearing exercise? _____

Do you drink alcohol?_____
If yes how many units a week on average?_____
1 unit of alcohol is equal to a small glass of wine or half a pint of beer or a single measure spirit

How often do you visit your dentist for check ups?_____
Do you suffer from any of the following? : -

- Diabetes_____
- Coeliac Disease_____
- Rheumatoid Arthritis_____
- Chronic Liver Disease_____
- Chronic Kidney Disease_____
- Thyroid Disease_____
- Lactose Intolerance_____
- Metabolic Bone Disease (such as Osteomalacia, Paget's Disease)_____
- Hyperparathyroidism_____

Please list your medications_____
_____
_____

Do you or have you ever suffered from an eating disorder?_____

Do you have any dietary preference/restrictions (eg vegan, vegetarian, health related diet restriction)?_____
How many years have you been on this diet? _____
Do you take Calcium Supplements?_____
Do you take Vitamin D supplements?_____

Have you ever been on long-term corticosteroids? _____
If yes, what steroid?_____
How many years?_____
What is you dosage?_____
What was your dosage?_____

Have you ever received treatment for osteoporosis? _____
What treatments/medications did you receive?_____
When did you receive this treatment?_____
For how long did you receive this treatment?_____

Are you currently undergoing treatment for cancer? _____
Have you received cancer treatment in the last 5 years?_____
Have you ever fractured a bone?_____
If yes which bone(s)?_____
Are you missing any teeth on the right side of your mouth?_____

7.2 Appendix 2 – Patient information leaflets

Patient Information Leaflet (group 1)

This study is a research project, which is being conducted here in St James' Hospital and in the Dublin Dental University Hospital. This information leaflet explains the aim of the study and what taking part will involve. If you would like to take part you will need to sign a consent form but it is important first that you fully understand what the study involves. You are under no obligation to take part in this study.

What is this study about?

Osteoporosis is the most common disease of the bones and believed to cause more than 9 million fractures a year worldwide. In Ireland it is estimated that over 300,000 over the age of 50 have osteoporosis and as we have an ageing population this number is likely to increase. Osteoporosis related fragility fractures are one of the leading causes of patients becoming bed ridden in recent years and in developed countries the lifetime risk of fracture of the wrist, hip or vertebrae is believed to be between 30 to 40%.

The most widely used method of assessing osteoporosis is a DXA scan, which is a form of X-ray. Ultrasound, which does not use ionizing radiation, can also be used to assess bone density however as the research on its use is quite limited it requires further investigation.

A recent study undertaken in the Dublin Dental Hospital and St James's Hospital showed that an ultrasound scan of the lower jaw was able to detect differences between osteoporosis sufferers and healthy individuals.

In this study we would like to investigate whether more sites of the face (forehead and cheekbone) would also detect these differences.

What will taking part in this study involve for me?

You will first be asked to fill out a questionnaire, which asks questions related to your medical history and lifestyle. If we have your consent you will then have an

ultrasound scan taken of your lower jaw, arm, leg, forehead and cheekbone.

What are risks of taking part in this study?

Ultrasound is completely safe. There are no risks associated with this study.

Who will be included in this study?

This study will concentrate on Caucasian females over the age of 18 years.

Will my details be kept confidential?

Yes. Any data collected will not be associated with your name and your name will not be published.

Can I withdraw from the study?

You will be free to withdraw from the study at any stage.

Will you provide details of the information gathered during this study to my medical practitioner?

At this stage the results of this study will be of no clinical value.

Patient Information Leaflet (group 2)

This study is a research project, which is being conducted here in St James' Hospital and in the Dublin Dental University Hospital. This information leaflet explains the aim of the study and what taking part will involve. If you would like to take part you will need to sign a consent form but it is important first that you fully understand what the study involves. You are under no obligation to take part in this study and declining to take part will not affect your treatment here in any way.

What is this study about?

Osteoporosis is the most common disease of the bones and believed to cause more than 9 million fractures a year worldwide. In Ireland it is estimated that over 300,000 over the age of 50 have osteoporosis and as we have an ageing population this number is likely to increase. Osteoporosis related fragility fractures are one of the leading causes of elderly patients becoming bed ridden in recent years. In developed countries the lifetime risk of fracture of the wrist, hip or vertebrae is believed to be between 30 to 40%. Early diagnosis is very important.

The most widely used method of assessing osteoporosis is a DXA scan, which is a form of X-ray. Ultrasound, which does not use ionizing radiation, can also be used to assess bone density however as the research on its use is quite limited it requires further investigation.

This study is trying to determine if ultrasound of the lower jaw, forehead and cheekbone can be used to assess changes in the strength of bone in patients receiving treatment for osteoporosis over the course of one year. In this study, ultrasound readings will be taken from the lower jaw, forehead, cheekbone, leg and arm. These readings will be repeated after one year. These readings will be analyzed to determine if there are any changes over one year. These ultrasound readings will also be compared with DXA scans, calcaneal scans, and blood tests that you have as part of your osteoporosis assessment.

What will taking part in this study involve for me?

You will first be asked to fill out a questionnaire, which asks questions related to your medical history and lifestyle. You will then have an ultrasound scan taken of your lower jaw, arm, leg, forehead and cheekbone. In the Osteoporosis Clinic in St James's Hospital it is routine to take DXA scans, calcaneal scans and blood tests to assess and monitor patients' osteoporosis status.

If we have your consent, we will perform these tests with the ultrasound scans at the beginning of the study and repeat them after one year.

Will taking part in this study affect my medical treatment?

No. Taking part in this study will not affect your medical treatment in any way.

What are risks of taking part in this study?

Ultrasound is completely safe. There is radiation associated with a DXA scan, as it is a form of x-ray, however this dose is very low and uses much less radiation than a standard x-ray. DXA scan is used as routine monitoring of your osteoporosis status in the osteoporosis clinic in St James's Hospital.

Who will be included in this study?

This study will concentrate on Caucasian females only because the risk of osteoporosis is unfortunately much higher in this group. You can only take part in this study if you are a Caucasian female and are a patient of the Osteoporosis Clinic in St James's Hospital.

Will my details be kept confidential?

Yes. Any data collected will not be associated with your name and your name will not be published.

Can I withdraw from the study?

You will be free to withdraw from the study at any stage, including in the middle of treatment. This will not affect your treatment in any way.

Will you provide details of the information gathered during this study to my medical practitioner?

At this stage the results of this study will be of no clinical value.

7.3 Appendix 3 –Consent forms

Consent Form (group 1)

Patient's Declaration

I have read, or had read to me, the patient information leaflet relating to this study. I understand its content. I have been offered the opportunity to ask questions and all questions have been answered to my satisfaction.

I understand what is involved in this study and have read this consent form. I freely and voluntarily agree to be part of this research study, without prejudice to my legal and ethical rights.

I understand that I may withdraw from this study at any time and that this will have no bearing on treatment received in this hospital.

I agree that data collected during this study may be published and I am aware that I will not be identified in any such publication. I have received a copy of this document. Participant's Name:

Participant's Signature:

Date:

Investigator's Declaration

I have explained the nature, purpose, procedures, benefits, risks of and alternatives to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Investigator's Signature:

Date:

Contact details: The principal investigators Prof Leo Stassen and Dr Robert Weld-Moore are contactable via the Dublin Dental University Hospital Switchboard on 01 6127200

Consent Form (group 2)

Study Title: The use of quantitative ultrasound to investigate changes in bone strength in the facial skeleton

Patient's Declaration

I have read, or had read to me, the patient information leaflet relating to this study. I understand its content. I have been offered the opportunity to ask questions and all questions have been answered to my satisfaction.

I understand what is involved in this study and have read this consent form. I freely and voluntarily agree to be part of this research study, without prejudice to my legal and ethical rights.

I understand that I may withdraw from this study at any time and that this will have no bearing on treatment received in this hospital.

I agree that the research team may access the results of any scans or blood tests that I have had performed as part of my osteoporosis assessment.

I agree that data collected during this study may be published and I am aware that I will not be identified in any such publication. I have received a copy of this document.

Participant's Name:

Participant's Signature:

Date:

Investigator's Declaration

I have explained the nature, purpose, procedures, benefits, risks of and alternatives to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Investigator's Signature:

Date:

Contact details: The principal investigators Prof Leo Stassen and Dr Robert Weld-Moore are contactable via the Dublin Dental University Hospital Switchboard on 01 6127200 or via email robert.weld-moore@dental.tcd.ie

7.4 Appendix 4 – Ethical approval

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hartin Ph: 4142199
email: claire.hartin@amnch.ie

Dr. Robert Weld-Moore
Dublin Dental University Hospital
Lincoln Place
Dublin 2

9th March 2016

the facial skeleton

REC Reference: 2015-03 Chairman's Action (7)
(Please quote REC reference on all correspondence)

Dear Dr. Weld-Moore,

Thank you for your recent application to SJH/AMNCH Research Ethics Committee in which you requested approval for the above referenced study.

The Chairman, on behalf of the Research Ethics Committee, has reviewed this application and grants ethical approval.

Yours sincerely,

Claire Hartin
Secretary
SJH/AMNCH Research Ethics Committee

The SJH/AMNCH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.

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