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**Treatment of Osteoporosis with recombinant parathyroid
hormone, its effects on bone, total body muscle and fat
composition and factors determining response to this therapy**

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A thesis submitted in fulfilment of the requirements for the degree of Doctor
of Medicine

**UNIVERSITY OF DUBLIN
TRINITY COLLEGE**

April, 2015.

DECLARATION

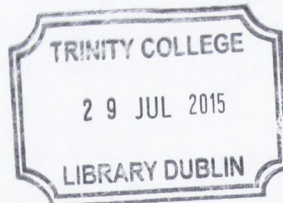
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Summary

Introduction

Total body bone mineral density (BMD) is relatively underutilised as an assessment tool for osteoporosis. Studies to date on Body composition have not clearly identified key factors which contribute to total body BMD, muscle mass and fat mass in osteoporotic patients. In addition, the effect of certain treatments for osteoporosis i.e., recombinant Parathyroid hormone (PTH) on these total body parameters remains under investigated. Furthermore clinical factors and assessment tools that determine optimal response to PTH have to be clarified. Lastly role of Quantitative heel ultrasound (QUS) in severely osteoporotic patients attending bone health clinic (BHC) remains to be evaluated.

Aims

This study aimed to

- Elucidate the role of total body composition in elderly osteoporotic population and to determine whether rPTH can potentially change the various total body DXA parameters such as muscle and fat after one year of treatment.
- Identify factors for no response to rPTH treatment.
- Role for Quantitative heel Ultrasound (QUS) as assessment in a severely osteoporotic population.

Methods

A total of 128 patients were recruited from Bone Health Clinic in St James's hospital who were elderly and osteoporotic and about to commence rPTH. This cohort was at first cross-sectionally examined with multiple laboratory and clinical assessments (including total body composition, biochemical markers and past history). A further subgroup of 60 patients had additional baseline QUS assessment performed and this was evaluated in relation to standard DXA and bone markers. Subsequently, 51 patients were followed longitudinally for one year whilst on regular rPTH, with total body DXA repeated at 1 year. This subgroup was finally divided into two groups i.e. responders versus non responders. The differences of various parameters between these two groups were then observed as determinants of no response to rPTH.

Results

The results of our study showed that both muscle and fat mass were independently predictive of BMD, while age was negatively associated with muscle mass and BMD on baseline total body DXA.

Exogenous PTH did not negatively affect the muscle and fat composition of the cohort after one year of treatment.

Pattern of biochemical bone marker response rate to rPTH could not distinguish between responders as shown on DXA over non responders.

Only 24 hour urinary calcium at 6 month could significantly separate responders from non responders.

Anabolic bone effects of rPTH remained unaffected by the past medical, fracture and drug history.

At baseline, bone turnover markers correlated only with QUS when these were markedly raised. Colles fracture significantly negatively correlated with QUS scores.

Conclusion

Total body DXA provides a reasonable estimate of body composition in people of different weights ranging from low body weight to obesity. It could therefore become a non-invasive method for assessment of muscle and fat in conjunction with assessment of osteoporosis in patients at high risk of fracture.

Fortunately, in contrast to elevated endogenous PTH exogenous rPTH is not detrimental to the body muscle and bone composition.

The response to rPTH is unaffected by prior medication use and existing clinical co morbidities.

Monitoring 24 hour urinary calcium excretion could distinguish the patients who are likely to respond to treatment with rPTH.

QUS can identify the patients with Colles fracture in severely osteoporotic population and it correlates well to bone turnover. Thus, it could be effectively used as a tool for aged based screening in osteoporosis with a high non-vertebral fracture risk in clinical settings where standard DXA is not available.

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ABBREVIATIONS

1,25(OH)₂D	1,25 dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
ADEs	Adverse Events
AI	Adequate Intake
ASPEN	American Society for Parenteral and Enteral Nutrition
BAPEN	British Association for Parenteral and Enteral Nutrition
BL	Baseline
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body mass index
BNR	Birmingham Nutrition Risk score
BUA	Broadband Ultrasonic Attenuation
Ca	Calcium
cAMP	cyclic Adenosine Mono Phosphate
CaSR	Calcium-sensing receptor
CI	Confidence Interval
CrCl	Creatinine Clearance
CRF	Chronic Renal Failure
CTX	Type I collagen C-telopeptide
DXA	Dual energy X-ray Absorptiometry
EAR	Estimated Average Requirements
EPAR	European public assessment report
ESPEN	European Society for Clinical Nutrition and Metabolism
FFM	Fat Free Mass
FPT	Fracture Prevention Trial
GPCRs	G Protein-Coupled Receptors
hPTH	Human Parathyroid Hormone
IGF	Insulin like Growth Factor
IOM	Institute of Medicine

IQR	Interquartile Range
IV	Intravenous
LS	Lumbar spine
MAG	Malnutrition Advisory Group
Mg	Magnesium
MNA	Mini Nutritional Assessment
MRI	Magnetic Resonant Imaging
mRNA	messenger Ribose Nucleic Acid
MUST	Malnutrition Universal Screening Tool
NHANES	National Health and Nutrition Examination Survey
NOF	National Osteoporosis Foundation
NOS	National Osteoporosis Society
NRS	Nutritional Risk Screening
OLES	Open Label Extension Study
ONS	Oral Nutrition Supplements
OPG	Osteoprotegerin
OR	Odds Ratio
PaTH	Parathyroid Hormone and. Alendronate study
PEM	Protein Energy Malnutrition
PINP	Procollagen type I N-terminal Propeptides
PO4	Phosphate
POWER	PTH for Osteoporotic Women on Estrogen Replacement
PTH	Parathyroid Hormone
PTH (1-34)	Teriparatide
PTH (1-84)	Full length PTH
PTHrP	Parathyroid Hormone-related Protein
QCT	Quantitative Computed Tomography
QUS	Quantitative Ultrasound
RANKL	Receptor Activator of Nuclear Factor-Kappa B Ligand
RCT	Randomised Controlled Trial
RDA	Recommended Daily Allowance
rPTH	Recombinant Parathyroid Hormone

SGA	Subjective global assessment
SI	Stiffness Index
SJH	St.James’s Hospital
SOS	Speed of Sound
TB DXA	Total Body DXA
TOP	Treatment of Osteoporosis with PTH study
TRES	Treatment Extension Study
US	Ultrasound
WHO	World Health Organisation

CHAPTER 1

Review of Parathyroid hormone, its physiology and therapeutic use in Osteoporosis: Section I

History, Physiology and mechanism of action of Parathyroid hormone

1.1 Historical Review

The early identification of Parathyroid hormone (PTH) goes back to the evolutionary history in amphibians with the transition from an aquatic to a terrestrial existence, where it was primarily responsible to uphold an ample concentration of extracellular calcium in body (Potts, Kronenberg et al. 2006, Potts 2013).

The role of the parathyroid gland in calcium metabolism was first identified in 1909 establishing that the tetany in animals post parathyroidectomy was associated with low blood calcium and this tetany could be cured by the administration of calcium (Maccallum and Voegtlin 1909). Literature review reveals that the physiological role and pathophysiological significance of PTH was later made possible by the observations and techniques of Collip, who prepared the first biologically active extracts of the parathyroid gland and developed a suitable assay for PTH (Collip 1925). This allowed further study of its role in calcium metabolism.

1.2 Anatomy of the parathyroid gland

The parathyroid glands are four small brownish red, beanlike bodies and these are located near the posterior aspect of the thyroid gland. The parathyroid glands develop from the third and fourth branchial pouches. Due to their embryonic origin, the inferior glands may be found at any point along the path of the thymus from the angle of the jaw to the anterior mediastinum. In humans, these usually weigh about 118 ± 48 mg in males and

131±45 mg in females (Gilmour and Martin 2005). The parathyroid glands have a distinct, encapsulated, smooth surface that differs from the thyroid gland. The colour of the parathyroid glands relates to their fat content, vascularity, and percentage of oxyphil cells within the glands (Harach 2010).

Histologically, the glands comprise of two types of cells: the chief cells which secrete PTH (secretory granules) and the oxyphil cells which are not present before puberty, show abundant pink cytoplasm (mitochondria) and whose function is unknown.

1.3 Biosynthesis and Structure of PTH

PTH is synthesized as a 115- amino acid polypeptide called pre-pro-PTH, secreted by the chief cells of the parathyroid glands. The pre-pro PTH is the primary transcriptional product of PTH mRNA. When its synthesis on the ribosome is complete, it is transported across the membrane of the endoplasmic reticulum with concomitant cleavage at the N-terminal portion first to pro-PTH (90 amino acids) and then to mature PTH (84 amino acids). The latter is the major storage, secreted, and biologically active form of the hormone (Murray, Rao et al. 2005). After synthesis PTH is stored in the chief cells, it may then be secreted or degraded intracellularly.

1.4 Regulation and Synthesis of PTH

Calcium

Calcium regulates both the release and the synthesis and degradation of PTH, in all its molecular forms. PTH 1-84 is secreted by exocytosis within seconds after induction of hypocalcaemia (D'Amour, Räkel et al. 2006) .

Calcium has long been reported to regulate PTH gene expression in vivo (Naveh-Many, Friedlaender et al. 1989). It has been demonstrated that small decreases in serum calcium from 2.6 to 2.1 mmol/l in rats led to large increases in parathyroid hormone messenger ribonucleic acid (PTH mRNA), reaching 3-5 fold that of control rats. In addition, these changes were rapid, occurring within 1 hour of the decrease in serum calcium and

persisted for 6 hours. High serum calcium had no effect on mRNA, even at concentrations as high as 6.0 mmol/l. Thus physiologically the parathyroid gland responds more promptly to falls in serum calcium, but not to increases in serum calcium.

1, 25(OH) 2D3

1,25(OH)2D3, by binding to the vitamin D receptor, inhibits PTH gene expression and therefore PTH synthesis. 1,25(OH)2D3 also inhibits parathyroid-cell proliferation. Parathyroid cells have stereo specific, high affinity receptors for 1,25(OH)2D3. These receptors are similar to those found in classic target tissues for 1,25(OH)2D3, for example, in bone or intestine. Moreover after intravenous administration of radioactively labelled 1, 1,25(OH)2D3 to chicks, Stumpf (1980) note a marked accumulation of this labelled vitamin in the parathyroid nuclei. This data suggested that the parathyroid gland was a target organ for 1, 1,25(OH)2D3.

The effect of 1, 25(OH)2D3 on bovine parathyroid cells was studied by Silver in vitro (Silver, Russell et al. 1985). This study demonstrated a decrease in PTH mRNA levels to 50% of control values within 48-96 hours of administration. This effect was evident with extremely low concentrations. Furthermore this suppression of PTH secretion by 1,25(OH)2D3 was shown to be dose dependent.

Subsequently, it has been reported that 1, 25(OH)2D3 can directly affect synthesis and secretion of parathyroid hormone by inhibiting PTH gene transcription and synthesis of pre-pro parathyroid hormone messenger RNA (Justin Silver 1986).

Studies have demonstrated that when the stimuli of hypocalcaemia and 1,25(OH)2D3 were given simultaneously to rats a decrease in PTH mRNA was noted, suggesting that the effect of 1,25(OH)2D3 is dominant over the effect of hypocalcaemia (Naveh-Many, Friedlaender et al. 1989). In another study of human parathyroid adenoma cells, despite a normal concentration of 1,25(OH)2D3 receptors, there was no reduction in PTH mRNA or PTH secretion following addition of 1,25(OH)2D3 (Karmali, Farrow et al. 1989).

1,25(OH)₂D₃ may regulate the PTH gene at the level of the 1,25(OH)₂D₃receptor. The concentration of this receptor in the target tissues would allow modulation of the 1,25(OH)₂D₃ effect. An increased receptor count would amplify the 1,25(OH)₂D₃ effect, while a reduction in the number of receptors would reduce its effect.

Naveh-Many studied rats with experimental uraemia and showed that there was an increase in parathyroid cell proliferation compared with control rats. A high-Phosphate diet increased while a low-Phosphate diet dramatically decreased the number of proliferating Parathyroid cells (T Naveh-Many 1995).

Similar results were found by Yi et al. They showed that rats with experimental uraemia had an increase in serum PTH, PTH mRNA, and Parathyroid cell proliferation, all of which were prevented by mild dietary phosphorus restriction (Yi, Fukagawa et al. 1995).

1, 25(OH) 2D₃ may have a role in regulating PTH cell proliferation in chronic renal failure in addition to its role in decreasing PTH gene transcription. J.silver and Naveh-Maney summarise that small decreases in serum Ca and more prolonged increases in serum phosphate stimulate the parathyroid to secrete parathyroid hormone while 1,25(OH)₂D₃ decreases PTH synthesis and secretion. A prolonged decrease in serum Ca²⁺ and 1,25(OH)₂D₃, or an increase in serum Phosphate, such as in patients with chronic renal failure, leads to the appropriate secondary increase in serum PTH.(Silver, Kilav et al. 2002)

Recently, a study was performed in Spain comparing the normal rats to the uremic rats. Factors such as low serum calcium or high serum phosphate significantly induced parathyroid hyperplasia without the presence of uraemia. These findings emphasize the importance of normal serum Phosphate and Calcium in the prevention of Parathyroid cell hyperplasia (Canalejo, Canalejo et al. 2010).

Thus, Parathyroid cell proliferation is the final site of regulation and this occurs after the stimulus of prolonged hypocalcaemia, hyperphosphatemia and hypovitaminosis D. Nonetheless, reviews of epidemiological studies have also indicated the importance of

carefully controlling plasma phosphate, normalising and avoiding increases of plasma Ca^{2+} , and not to over suppress PTH during treatment (Brandi 2008).

1.5 Secretion of PTH

The normal reference range of PTH concentrations is $0.5\text{-}5.0 \text{ pmol}^{-1}$ in young adults (Hodsman and Steer 1993). The Dynamics of PTH secretion are summed up below:

Calcium

The role of calcium in PTH regulation is not limited to the release of PTH but also involved in synthesis and degradation. PTH secretion is increased in response to low calcium and decreased in response to high calcium.

PTH response to hypocalcaemia has been categorized as follows:

- Seconds to minutes — exocytosis of PTH from chief cells into the extracellular fluid.
- Minutes to one hour — reduction in the intracellular degradation of PTH.
- Hours to days — increase in PTH gene expression as a result of stabilization of PTH mRNA (also stimulated by low serum calcitriol concentrations, due to increased transcription of the PTH gene).
- Days to weeks — proliferation of parathyroid cells (also stimulated by low serum calcitriol concentrations).

Parathyroid hormone secretion under normal circumstances is only slightly higher than the maximally suppressed levels, which allows the gland to respond to hypocalcaemia with a marked increase in PTH secretory rate.

Calcium does not acutely alter the rate of synthesis of proPTH or its conversion to PTH, but affects hormone production by enhancing intracellular degradation of PTH within the parathyroid cell. Acute experiments (4-7h) on the effects of high extracellular calcium in bovine parathyroid cells revealed changes in PTH secretion with no detectable change in mRNA concentrations (Brookman, Farrow et al. 1986).

Experiments with longer incubation periods (16-24h) resulted in a decrease in mRNA in addition to a fall in secretion of PTH (J Russell 1983). Studies of human parathyroid adenomata in culture also confirmed this suppression of mRNA in response to high calcium although this was not accompanied by a fall in PTH secretion (Farrow, Karmali et al. 1988).

A two stage control of PTH synthesis has been proposed on the basis of these studies. Long term regulation would involve changes in mRNA concentrations within the cell, while acute control could involve alterations in post transcriptional events (Farrow, Karmali et al. 1988).

Both in vivo and in vitro studies of PTH secretion have demonstrated that there is an inverse sigmoidal relationship between calcium and parathyroid hormone secretion.

In studies, (Brown 1983, Haden, Brown et al. 2000) the set point of the calcium-PTH curve has been illustrated as a key controller of the serum ionized calcium concentration in vivo. In normal subjects, a decrease in serum ionized calcium of as little as 0.1 mg/dL (0.025mmol/L) results in a large increase in serum PTH concentration within minutes; conversely, a rise in calcium can rapidly suppress PTH within 2 to 3 minutes.

Prior to this, in 1982 the same researchers demonstrated an abnormal regulation of serum calcium in patients with secondary hyperparathyroidism due to chronic renal failure. Furthermore, in vitro parathyroid samples showed a shift in the set point to the right and a relative insensitivity to calcium.

The Calcium-Sensing Receptor CaSR

The CaSR is a member of subfamily C of G protein-coupled receptors (GPCRs). The calcium-sensing receptor (CaSR) represents the molecular mechanism by which parathyroid cells detect changes in serum ionized calcium concentration and regulate parathyroid hormone (PTH) secretion to maintain serum calcium levels within a narrow physiological range.

Evidence for the presence of a calcium receptor on the parathyroid cell surface first emerged from studies in 1983 which demonstrated that a number of divalent and trivalent cations depolarise the cell membrane and act to inhibit PTH secretion in cell culture (Jose Lopez-Barneo 1983).

Subsequent reviews have illustrated the recent developments in CaSR role. In addition to systemic mineral ion homeostasis, CaSR also contributes towards the regulation of hormonal secretion and the activities of various ion channels to the longer term control of gene expression, programmed cell death (apoptosis), and cellular proliferation (Brown and MacLeod 2001).

Subsequently, researchers have summarized the integral involvement of CaSR in each of three key components of parathyroid gland function i.e., hormone secretion, hormone synthesis, and cell proliferation. Research has linked CaSR's activation to a broad array of intracellular signalling cascades that mediate diverse physiological responses in parathyroid tissue (Chen and Goodman 2004). Most recently, CaSR has been shown to mediate the effects of dietary calcium on bone turnover and regulate parathyroid hormone (PTH)-induced bone turnover (Shu, Ji et al. 2011).

However, it is important to note that there are at least two major mechanisms that control the secretion of PTH. It is thought that alterations in extra cellular concentration are recognised by a plasma membrane receptor which then activates either the adenylate cyclase or inositol phosphate pathways through different guanine nucleotide-regulatory-proteins.

In situations of high extracellular calcium, Phosphoinositide C is activated and leads to the synthesis of triphosphoinositol and diacylglycerol. Triphosphoinositol causes a transient spike in intracellular calcium, derived from a release of intracellular calcium from the endoplasmic reticulum, and a more sustained increase due to the influx of calcium through voltage sensitive and insensitive channels. An increase in diacylglycerol concentration leads to an increase in protein kinase C activity which inhibits PTH secretion. In hypocalcaemia, this calcium receptor activates the cAMP pathway leading to

secretion of PTH. Thus CaSR has a fundamental value in parathyroid gland physiology as discussed in numerous scientific publications (Kumar and Thompson 2011).

Magnesium

The secretion of parathyroid hormone by the parathyroid gland is physiologically controlled by the serum calcium level but high magnesium concentrations modulate PTH secretion in a similar manner to calcium. However, PTH levels are influenced by several factors other than serum magnesium levels.

It is widely recognised that higher serum magnesium lowers PTH levels. Conversely, it has also been shown that low levels of magnesium stimulate parathyroid hormone secretion. However, very low serum magnesium concentrations could also lead to some degree of hypocalcaemia (Vetter and Lohse 2002). Researchers have described calcium mimicking effects of magnesium on parathyroid hormone secretion, and in turn PTH regulation of magnesium homeostasis by modulating renal magnesium reabsorption. It has been demonstrated that magnesium deficiency could also impair the acute regulation of PTH by serum ionized calcium (Iwasaki, Asai et al. 2007). Increases in serum magnesium can also potentially affect secretion of PTH but only at supraphysiological concentrations of two to three times the normal levels. A large number of the studies evaluating the influence between PTH and magnesium are either not sufficiently controlled or have methodological limitations to attain credible conclusions. Haemodialysis patients with inadequately low PTH levels have exhibited greater serum Mg concentrations and vice versa (Cunningham, Rodríguez et al. 2012). Magnesium is, therefore, essential for normal parathyroid hormone secretion.

1.6 Diurnal Variation in Circulating PTH

In healthy people there is a circadian rhythm for parathyroid hormone release. The hormone levels rise at night, after 8.00pm and fall during the day. Although the nocturnal rise is associated with a fall in serum calcium, the nocturnal rise in PTH has been shown to persist during continuous infusion of calcium (William Jubiz 1972). A large

component of PTH rhythm is endogenous and may play an important role in calcium balance (Fuleihan, Klerman et al. 1997). Any alteration in this rhythm may result in a catabolic calcium and bone-remodelling profile, thus contributing to the pathophysiology of osteoporosis (White, Ahmad et al. 2007).

1.7 Mechanism of Action of PTH

PTH/PTHrP receptor:

The classical effects of PTH on calcium and phosphate homeostasis have been the subject for debate since decades. It is well established that these effects are mediated through the PTH/PTHrP or PTH1R receptor. PTH and PTHrP bind to and activate a G protein-coupled receptor with 7 transmembrane spanning domains that is predominantly expressed in bone and kidney, but is also present in fetal tissue and growth plate chondrocytes (Lee, Deeds et al. 1995). It has the potential for alternate splicing and promoter usage that is characteristic of human, rat and mouse (Kong, Schipani et al. 1994). PTHrP, like PTH, is a distinct polypeptide and is synthesized and expressed by various tissues such as skin, blood vessels, smooth muscles, tooth buds, growth plate chondrocytes, bone, kidney and neuronal and glial tissues.

PTH/PTHrP receptor plays a main role in the action of PTH. Because of the complexity of cells in the osteoblast lineage this mechanism of action is not yet completely explained. The importance of PTH and PTHrP however, is evident by the variety of clinical syndromes caused by deficiency or excess production of either peptide. Mutations of the PTH/PTHrP receptor result in severe skeletal dysplasia i.e., Blomstrand chondrodysplasia, as well as severe abnormalities in breast and tooth development (Ogata 2010). PTH/PTHrP is recognized as an important regulator of bone remodelling, and has been demonstrated to enable paradoxical (catabolic and anabolic) functions *in vivo* and *in vitro* (Nabanita S. Datta 2009). Modulation of PTH/PTHrP signalling in osteoblastic lineage has the potential to open a new era towards anabolic therapeutic options in osteoporosis (Ono, Nakashima et al. 2007).

1.8 Metabolism of PTH

Intact PTH is degraded in the parathyroid gland by cathepsin like lysosomal enzymes, Cathepsin B which cleaves it between amino acid residues 36 and 37 yielding a COOH fragment and an amino-terminal (NH₂) fragment. A cathepsin D like enzyme cleaves PTH between 34 and 35 amino acids and yields more COOH and NH₂ fragments. The secretory products of the gland include intact PTH and mid and COOH-PTH fragments in approximately a 7:3 ratio. COOH-PTH fragments are further discussed below under 'heterogeneity of circulating PTH hormone'

1.9 Peripheral Clearance of PTH

Intact PTH is rapidly cleared from the circulation with a half life of approximately 2 minutes.(Kao, van Heerden et al. 2002). The extremely rapid peripheral clearance of intact PTH essentially limits the duration of its action and enables the secretory activity of parathyroid gland to determine the circulating concentration of biologically active PTH. PTH clearance occurs primarily (60-70%) in the liver and also in the kidneys (20-30%), and to a much lesser extent in the organs. Quantitatively less than 10-20% of secreted intact PTH is converted to circulating C-fragments by peripheral metabolism (John P. Bilezikian 2001).

Hepatic clearance

Research has shown that the liver contributes substantially to the clearance of circulating intact PTH (Fang and Tashjian 1972), and this has been confirmed by numerous subsequent analyses in several species, including humans(Oldham, Finck et al. 1978, Daugaard, Egfjord et al. 1990). Hepatic clearance exceeds renal clearance and is a complex process involving at least 3 different mechanisms:

- i) *Hepatic macrophages and Kupffer cells*: These take up most of the hormone to degrade it via cathepsin like enzymes into mid and COOH fragments as well as amino acids. These fragments may be added to the pool of circulating PTH fragments from the parathyroid gland.

ii) *Kupffer cells*: These are responsible for both the rapid clearance and extensive proteolysis of PTH that occur in the liver (Bringham, Segre et al. 2002)

iii) *Hepatocytes*: These take up small amounts of the hormone. Clearance by Kupffer cells is a high capacity system and it may not discriminate between active and inactive hormone, while uptake by hepatocytes is of low capacity, but specifically removes active hormone. The middle carboxy-terminal (C-fragments) generated and released by Kupffer cells reflect cleavage of the hormone between residues 33-34 and 36-37 of the intact hormone. C-fragments persist in the circulation for 5-10 times longer than intact PTH, principally because they are cleared only by glomerular filtration and not by hepatic uptake. The release by the Kupffer cells of C-fragments accounts for a substantial part of the heterogeneity of circulatory PTH.

Renal Clearance

Extensive proteolysis of PTH also occurs in the kidney. Renal clearance occurs almost entirely by the glomerular filtration. It has been established that impaired renal function leads to delayed renal clearance of C fragments, their accelerated generation both in the hyperplastic parathyroid glands and during peripheral metabolism of overproduced intact hormone, leading massive accumulation of C fragments vs. intact hormone in the circulation(John P. Bilezikian 2001).

Slow renal clearance of PTH in renal insufficiency is demonstrated in several other studies both in human and in animals (Slatopolsky, Martin et al. 1980, Lorenzo Sellares and Torregrosa 2008, Reinhardt W. 2011).

1.10 Heterogeneity of circulating Parathyroid hormone

Circulating PTH is immunoheterogeneous. The metabolism of PTH (1-84) within the parathyroid gland and the liver, kidney and bone all contribute to the final heterogeneity of PTH in circulation.

Berson et al first observed that different forms of PTH are present in blood (Solomon A. Berson 1963). Early evidence has revealed that hormonal fragments in plasma could arise either by direct secretion from the parathyroid glands, or by peripheral metabolism of the hormone. Thus, it consists of small amounts of intact PTH (1-84) which is biologically active and large amounts of middle (mid) and carboxyl terminal (COOH)-PTH fragments which are biologically inert. Both the parathyroid gland and peripheral degradation of secreted intact PTH constitute the major source of mid and COOH-PTH fragments.

In both healthy and control subjects with primary hyperparathyroidism the concentration of these fragments is 5 to 10 fold higher than the concentration of intact PTH. These fragments are known to accumulate in certain pathological conditions such as in patients with progressive degrees of renal failure since their metabolic clearance depends heavily on glomerular filtration.

Experiments confirm the presence of high levels of COOH fragments of significant molecular weight (> 4500 Daltons) in serum samples obtained from patients with end stage renal disease (Vieira, Kunii et al. 2009). The biological activity of PTH depends on the integrity of the amino-terminal sequence of the peptide, mainly its first four amino acids. However, the physiological role of the COOH-terminal PTH forms is yet to be determined.

There is convincing evidence that parathyroid gland secretion of PTH is stimulated by hypocalcaemia, whereas secretion of C-terminal fragments is promoted by hypercalcemia. Certain C-terminal PTH fragments inhibit osteoclast formation and bone resorption via direct effects on cells of the hematopoietic lineage through C-terminal fragment receptors, suggesting the presence of a negative feedback loop to restrain the release of calcium from bone into blood when it is not necessary (Murray, Rao et al. 2005) .

Conclusively, these C-terminal PTH receptors may protect the skeleton by limiting bone resorption and disturb the equilibrium in favour of net bone formation. It is evident that

these peptides have biological actions that are independent of activation or blocking of PTH1R (Murray, Rao et al. 2005)

1.11 PTH effects on bone

PTH enhances the release of calcium from the large reservoir contained in the bones. Bone resorption can be triggered by parathyroid hormone (PTH) in response to hypocalcaemia. PTH indirectly stimulates bone resorption by the generation of new osteoclasts (osteoclastogenesis). Receptors for PTH are located on osteoblasts; PTH binds to osteoblasts to increase their expression of RANKL (RANK Ligand) and inhibits their expression of a secreted factor called Osteoprotegerin (OPG). As its name implies, Osteoprotegerin "protects bone" by preventing bone resorption. Osteoprotegerin works as a decoy receptor for RANKL: it binds RANKL and therefore prevents binding to RANK (RANK stands for Receptor Activator of Nuclear factor-Kappa B) and stimulation of osteoclastogenesis. The ratio of Osteoprotegerin: RANKL produced by osteoblasts will determine the extent of bone resorption.

Biphasic bone effects of PTH:

The dual effects of PTH on bone are well supported in the literature which confirms that continuous treatment exerts catabolic and intermittent treatment exerts anabolic bone effects (Qin, Raggatt et al. 2004, Shinoda, Kawaguchi et al. 2010, Miyakoshi 2011). The PTH effects on bone turnover depend on the mode and dose of administration. Elevated PTH levels increase bone turnover, leading to either anabolic or catabolic skeletal effects involving multiple mechanisms dependent on the pattern and duration of elevation (Poole and Reeve 2005, Aslan, Andersen et al. 2012).

Catabolic effects of PTH

Continuous in vivo PTH infusion stimulates bone resorption. The response of bone to PTH is determined primarily by pharmacokinetics. The amount of time each day that serum teriparatide concentrations are above baseline levels of endogenous PTH in rats determines the anabolic or catabolic response, rather than the area under the curve of

teriparatide achieved (Frolik, Black et al. 2003). Subsequent studies have determined the mechanism of this PTH led bone resorption as PTH can result in an increase in receptor activator of nuclear factor- κ B Ligand (RANKL) expression and consequent osteoclastogenesis in culture, with an associated inhibitory effect on Osteoprotegerin expression (Locklin, Khosla et al. 2003). Earlier studies have also demonstrated that continuous infusion of PTH in female osteoporotic patients at a dose of 800 IU for 28 days may inhibit bone formation as observed by a significant decrease in the biochemical bone formation markers i.e., serum alkaline phosphatase, osteocalcin and the carboxy-terminal extension peptide of pro-collagen 1 (Hodsman and Steer 1993).

Several studies suggested that short-term 2-week continuous infusion of hPTH1–34 (40 ug/ kg/day for 10 weeks) exerts a catabolic effect with a significant decrease in trabecular connectivity density in old female mice (Iida-Klein, Lu et al. 2005)

There is evidence that continuous and excessive PTH secretion at sustained levels has catabolic effects on the skeleton, and certain pathological conditions are clinically responsible for this, such as chronic renal disease and hyperparathyroidism (Poole and Reeve 2005).

Thus, at higher concentrations of PTH, its catabolic effects on bone are increased. With primary hyperparathyroidism, the physiological stimulation of PTH on bone resorption and formation is exaggerated, thus leading to frequent bone loss with clinical and histomorphometric evidence for increased bone turnover (Aslan, Andersen et al. 2012).

Anabolic Effects of PTH

It was first discovered in 1932 that PTH could be anabolic when investigators observed that PTH caused an increase in bone mass in rats (Hans 1932). The anabolic responses of osteoblasts to PTH have now been studied abundantly and these studies have described the key regulatory components as increased recruitment, proliferation and differentiation and reduced osteoblast apoptosis.

To date, the use of PTH as anabolic agent for treatment of osteoporosis has been established intensively in human and animal literature. Hodsman has demonstrated benefits of intermittent low dose administration of PTH by a rapid increase in biochemical markers of bone formation, with a lesser and delayed increase in resorption markers following intact PTH (Hodsman, Hanley et al. 2003). This research has previously observed the same response following treatment with teriparatide, when it found histomorphometric evidence of osteoblast accumulation in response to teriparatide injections within six weeks (Hodsman and Steer 1993) and subsequently he observed increased cortical thickness than controls after 2 years of PTH 1-34 therapy (Hodsman, Kiesel et al. 2000).

Mechanisms have been established, by which intermittent PTH is anabolic, whereas continuous exposure to raised PTH is detrimental to the skeleton.. New techniques such as microarray analysis have confirmed the complexity of the mechanisms involved. However, it is well understood that PTH stimulates bone formation by acting directly through PTHR1 receptors on osteoblasts and it also indirectly stimulates differentiation and development of osteoclasts, leading to active bone resorption by increasing the receptor activator of nuclear factor Kappa-B ligand and its decoy receptor osteoprotegerin (RANKL/OPG) gene expression ratio (Stilgren, Rettmer et al. 2004, Potts 2005).

An emerging theory about the mechanism of teriparatide action suggests that its stimulatory effects are associated with bone formation before it affects the processes associated with bone resorption. Thus the anabolic effects are observed earlier than the catabolic action during treatment. Studies have named this “the anabolic window” in the first 3 months of treatment, where PTH stimulates bone formation to a greater extent than bone resorption, suggesting that teriparatide could initially induce bone apposition without prior bone resorption through modeling-based formation (Bilezikian 2007, Bilezikian 2008). Subsequent studies have further extended this “anabolic window” between 6 to 18 months of treatment (Aslan, Andersen et al. 2012). Further research on the anabolic effects of PTH and its analogues can result in an increased number of therapeutic targets.

Both animal and human studies have revealed the bone building properties of PTH when used intermittently.

In a study of 270 male Sprague-Dawley rats with femoral fractures, daily recombinant human PTH (PTH 1-34) injections 30 µg/kg for a period of 35 days enhanced fracture-healing by increasing bone mineral content, density and strength ($p < 0.05$). Further to this, several days after dosage discontinuation a sustained anabolic effect was observed throughout the remodelling phase of fracture-healing (Alkhiary, Gerstenfeld et al. 2005)

In an animal study, 18 rabbits underwent right tibia lengthening by callus distraction. Two groups of rabbits were treated with intermittent PTH (1-34) with a dose of 5 or 25 µg/kg/day for 20 days and the third group was given saline. PTH (1-34) treatment groups showed improved mineralization, and structural indices of regenerated distracted rabbits' tibia. Treatment at the higher dose of 25 µg/kg/day of PTH (1-34) was significantly more effective than 5 µg/kg/day of PTH (1-34) treatment when compared to the control group (Aleksyniene, Eckardt et al. 2006).

In a study of male and female patients with primary osteoporosis, intermittent subcutaneous administration of 1-34 N-terminal peptide of human parathyroid hormone (hPTH 1-34) (100 units/week during 1 year) increased the mean lumbar bone mineral density by 1.8%, 3.4%, and 4.6% after 12, 24, and 48 weeks of hPTH administration.

It is also noticed that intermittent weekly subcutaneous injections of hPTH (1-34) increase trabecular bone volume and improve microstructure, without causing the appearance of abnormal bone elements in primary osteoporosis (Miki, Nakatsuka et al. 2004). Emerging evidence has also shown benefits of teriparatide in the form of daily nasal sprays (Sato 2007) and injections at weekly intervals (Ishizuya 2011) in terms of increase in lumbar spine BMD.

It is proposed that intermittent PTH injection exerts its anabolic effects at three steps of bone formation: (1) stimulating the proliferation of preosteoblasts; (2) promoting the differentiation of preosteoblasts and osteoblasts; and (3) inhibiting osteoblast apoptosis (Qin, Raggatt et al. 2004).

The mechanism of anabolic effects of intermittent low dose PTH has recently been analysed in greater detail (Aslan, Andersen et al. 2012). Multiple factors are involved in

this mechanism such as: (1) PTH up regulation of c-fos expression in bone, (2) Role of IGF in exerting anabolic bone effects, (3) Role of bone lining cells in differentiation of osteoblasts, (4) Enhanced adhesion of mesenchymal stem cells to bone surface, (5) A direct antiapoptotic PTH effect on osteoblasts, (6) Over compensation of the osteoblasts upon PTH interference with remodelling, (7) Down regulation of Sclerostin by PTH, thereby removing inhibition of Wnt signalling which is required for PTH's anabolic actions (Aslan, Andersen et al. 2012)

1.12 Role of PTH in Calcium Homeostasis

It has been widely acknowledged in the literature that the Parathyroid gland and PTH plays a central role in regulation of Ca^{2+} homeostasis by modulating bone metabolism, the synthesis of $1\alpha,25\text{-dihydroxyvitamin D}$ ($1\alpha,25(\text{OH})_2\text{D}$) in proximal tubules, and the reabsorption of Ca^{2+} in the distal nephron (Kumar and Thompson 2011).

The association between calcium and PTH is bidirectional. Serum calcium controls the secretion of PTH as described above, while serum calcium is also regulated by PTH secretion. PTH responds rapidly to hypocalcaemia by:

- i) Stimulating the release of calcium and phosphate from bone.
- ii) Stimulating reabsorption of calcium and inhibiting phosphate reabsorption from the glomerular filtrate.
- iii) Stimulating the renal synthesis of $1,25\text{ dihydroxyvitamin D}$ and thereby increasing intestinal absorption of calcium and phosphate.

When the concentration of ionised calcium in the extracellular fluid rises it then inhibits PTH secretion completing the endocrine feedback loop (Felsenfeld, Rodriguez et al. 2007).

Conclusively, the physiological actions of PTH are in general directed towards raising the concentration of calcium in the ECF. The actions of PTH on the kidney and bone in effecting calcium transfer are direct whereas, the transfer of calcium from the gut to the ECF is indirect and secondary to the influence of PTH in enhancing the formation of $1,25$

dihydroxyvitamin D from 25 hydroxyvitamin D in the kidney. In addition, as previously stated, PTH plays a major role in the maintenance of bone turnover as it activates osteoclastic bone resorption which is thought to be the primary step in the initiation of bone remodelling.

CHAPTER 1

Review of Parathyroid hormone, its physiology and therapeutic use in Osteoporosis: Section II

Role and pharmaceutical forms of PTH in treatment for osteoporosis

1.1 Introduction

The anabolic effects of PTH have been discussed in section I above. These effects in cancellous bone are well recognised over the past two decades (Silverberg, Shane et al. 1989). Numerous animal and human based studies have found the basis of intermittent PTH use as anabolic bone agent in osteoporosis. (Dempster, Cosman et al. 1993, Alkhiary, Gerstenfeld et al. 2005, Aleksyniene, Eckardt et al. 2006, Aslan, Andersen et al. 2012) . The use of PTH in postmenopausal osteoporosis is relatively recent. While, a majority of therapies currently recommended for the management of osteoporosis act mainly to inhibit bone resorption and reduce bone remodelling, PTH and its analogs represent a new class of anabolic therapies for the treatment of severe osteoporosis, having the potential to improve skeletal microarchitecture.

Presently two forms of injectable PTH are being used for this:

- PTH (1-84), or full length PTH
- PTH (1-34), or teriparatide.

Both forms of PTH are strong anabolic bone agents. These drugs increase the cancellous bone mass and they have proven anti fracture efficacy in vertebral and non vertebral fractures in an osteoporotic population (Neer, Arnaud et al. 2001, Verhaar and Lems 2009).

Other forms of non full length or shortened PTH i.e., PTH (1-31), PTH (1-36), and PTH (1-38), have been studied as treatments for osteoporosis, and are yet to complete phase

III trials (Adami 2008). A number of trials have shown a teriparatide induced increase in spine and hip BMD, a reduction of fracture risk and changes in biochemical markers of bone turnover in postmenopausal women. However, considering the scope of this thesis, it is hereby focused mainly on the most recent literature on the clinical use of intact PTH (1-84) and to some degree on PTH (1-34) in terms of their use in osteoporosis.

1.2 PTH (1-84)

European Public Assessment Report (EPAR), for PTH (1-84) Preotact, in 2012 has confirmed the description of the commercially available PTH (1-84) by European Medical Agency in 2007. In accordance with it, it is manufactured using a strain of *Escherichia coli* modified by recombinant DNA technology. The medicinal product is supplied in a dual-chamber cartridge: one chamber contains the PTH in powder, the other a sterile solvent for reconstitution. The cartridge is inserted into an injection pen, which injects subcutaneously a dose of 71.4 μL containing 100 μg PTH. Each cartridge contains 14 doses and, after reconstitution, has to be stored in a refrigerator (2 to 8 $^{\circ}\text{C}$).

1.3 Substantial Clinical trials involving PTH (1-84) and its effects on BMD and Fracture risk reduction

The pivotal large clinical studies warranting the use of PTH (1-84) in the treatment of postmenopausal osteoporosis are described below:

A large study of 2532 postmenopausal women, known as the TOP trial was a randomized, double-blinded, placebo-controlled, parallel-group study over a period of 18 months (Greenspan, Bone et al. 2007). The participants with aged 45-54 years were included if BMD was ≤ -3.0 at the lumbar spine, femoral neck or total hip, or if BMD was ≤ -2.5 with 1-4 prevalent vertebral fractures. Women with ages ≥ 55 -75 years) were included if BMD was ≤ -2.5 , or if BMD was ≤ -2.0 with 1- 4 prevalent vertebral fractures. Of the study participants 1286 women received 100 μg PTH (1-84) and 1246 women received placebo. All participants were supplemented with daily calcium citrate (700 mg/day) and vitamin D (400 U/day). The primary objective was the identification of new or worsening vertebral fractures by radiography. Secondary objectives were to track changes in BMD

at the spine, hip, distal radius, and total body and to observe the bone turnover markers. Changes in quantitative computed tomography (QCT) were also recorded. A total of 1701 women completed the study including 70% in the placebo group and 64% in the PTH group. Data analysis demonstrated increases at month 18 in trabecular BMD at the spine (37.6%, $P \leq 0.001$), total hip (4.7%, $P \leq 0.001$), femoral neck (4.5%, $P \leq 0.001$), and trochanter (4.3%, $P \leq 0.001$), but there were also increases in total hip cortical volume (9.0%, $P \leq 0.003$) and total hip cortical volumetric bone content (4.8%, $P \leq 0.040$). A decrease was seen in the total hip cortical volumetric BMD (4.7%, $P \leq 0.001$).

In this trial the incidence of new or worsened vertebral fractures was significantly ($P \leq 0.001$) lower in treated patients (1.4%) than in the placebo group (3.4%), with a 58% (95% confidence interval [CI] 0.24 to 0.72) reduction in relative risk (RR). The RR reduction for a first vertebral fracture was 68% (95% CI 0.14 to 0.75) ($P \leq 0.006$) in patients without a prevalent fracture at baseline. The number of patients that needed to be treated (NNT) for a median of 18 months to prevent a new or worsening vertebral fracture was 51. The NNT was 71 in patients without a baseline fracture, and 22 in those with a baseline fracture. Bone alkaline phosphatase (bALP) increased in this study, approximately 30% at month 1, 100% at months 6 and 12, and 80% at month 18 in the PTH group.

TOP study was followed by OLES study (Roux C 2007) in which 1681 women continued to participate. Its primary objective was to assess the safety and efficacy of prolonged use of PTH. From the PTH group, 781 patients and from the placebo group 900 patients continued the same treatment as the TOP for a further 6 months (on top of previous 18 months) and were monitored for 12 months after discontinuation. Changes in BMD, bone turnover markers, and adverse events were monitored.

Treatment extension for up to 24 months in OLES resulted in a continued increase in BMD from baseline, 6.8% in lumbar spine and 2.2% in femoral neck. The results had shown only a few new vertebral fractures which were counted as 1 (1.7%) in the group treated with PTH (1-84) for a further 6 months, 2 (1.9%) in the 12-month placebo-treatment group, and 4 worsened vertebral fractures in the former PTH (1-84)-treated

group. The low number of new fractures, during extended PTH treatment and after its cessation, confirmed the effect of PTH in the prevention of osteoporotic fractures.

A further follow up study named as TRES (Jodar-Gimeno E 2008) was done on 91 selected postmenopausal women from OLES. They were given about a 2 month break of PTH after OLES. These patients were treated with PTH (as in the TOP study) for a total of 36 months. Changes in BMD and bone turnover markers and adverse events were monitored. The patients treated in OLES and TRES for 36 months showed a progressive increase in lumbar spine and total hip BMD for the first 18 months (8.0% and 2.1% respectively). There was a small decrease (to 7.0% and 1.7%) during the approximate 2-month break without treatment. Lumbar spine BMD further increased until month 24 of PTH treatment (8.5%) remaining stable for the final 12 months. Total hip BMD increased progressively above OLES baseline by 3.4% at month 36.

The most recent 6 month open label extension trial on 782 subjects from the TOP study has shown continued increases in lumbar spine BMD by 6.8 % above baseline ($p<0.05$) when PTH (1-84) was continued over 24 months. An increase by 1.1 and 2.2 % above baseline of BMD hip and femoral neck respectively has also been observed. The PTH adherent group sustained significantly fewer fractures than the poorly adherent group (Black, Bilezikian et al. 2013) .

The PaTH study (Black, Greenspan et al. 2003) is a 1 year, randomized, double-blind clinical trial of 238 postmenopausal women. It has investigated whether the concurrent administration of PTH (1-84) and alendronate can increase bone density more than the use of either one alone. Postmenopausal women not on bisphosphonates with T score ≤ -2.5 at hip or spine or with a T score ≤ -2.0 along with an additional risk factor for osteoporosis, were randomly assigned to a daily treatment i.e., 119 women were treated with 100 μ g of PTH (1-84) , 60 women were treated with 10mg of alendronate , and 59 women were treated with both drugs . BMD changes at the lumbar spine, hip, and distal radius were primary endpoints and these were assessed by DXA. In a subset of 204 patients, QCT was also performed at the spine and the hip. Markers of bone turnover were measured; adherence and adverse events were assessed.

BMD spine was increased with all treatment regimes however it was substantial in the PTH group (6.3%) as compared with the combination therapy group (4.6%). Bone formation markers also increased markedly in the PTH group but not in the combination-therapy group. N-propeptide of type 1 collagen (P1NP) increased after 1, 3, and 12 months of PTH therapy (approximately 90%, 140%, and 150% respectively) and rapidly decreased after treatment suspension.

The above study was closely followed up by another trial (Black, Bilezikian et al. 2005) to investigate whether antiresorptive therapy should follow PTH therapy to maintain BMD gain. In this trial the monotherapy patients with PTH (1-84) for one year were randomly assigned to receive either placebo (n=60) or alendronate (n= 59). Meanwhile the patients on combination therapy or alendronate mono therapy during year one continued to receive alendronate. At the end of the following 1 year BMD in trabecular bone at the spine had increased by 13% in the PTH group followed by alendronate as seen on quantitative CT. Also at 2 years of PaTH study, 21 women (8.8%) had 1 or more clinical fractures, but no differences among groups were detected (Black, Bilezikian et al. 2005).

Several other studies even in the early stage have also demonstrated the benefits of the clinical use of PTH 1-84. The salient points of the relevant studies are outlined:

A phase II trial of 217 North American postmenopausal women aged between 50-75 years (Hodsman, Hanley et al. 2003) merits discussion. This double-blind, placebo-controlled study was done over a 12 month period objectively to investigate the safety and efficacy of PTH (1-84). All patients had a T score of ≤ -2.5 at lumbar spine, they had not received any recent osteoporotic treatment and they had no significant history of skeletal or vertebral issues.

The patients were randomly assigned to receive PTH (1-84) at doses of 50, 75, 100 μg and placebo. They were also supplemented daily with calcium carbonate (500 to 1000 mg) and vitamin D (400 IU). The primary objective was to assess the percentage change in vertebral BMD over the treatment period. BMD and bone mineral content (BMC) at

the femoral site, as well as bone marker values were also measured as secondary end points. Adverse events and compliance were also recorded.

Osteocalcin significantly increased dose-dependently in the PTH treated group in this study. Percentage changes ranged from 20% with 50 µg to 45% with 100 µg at month 1, from 60% to 120% at month 6, and from 75% to 150% at month 12.

The effects of PTH (1-84) as an adjunct to stable hormone replacement therapy (HRT) were evaluated in the POWER study (Fogelman, Fordham et al. 2008). It was a randomized, double-blind, placebo-controlled, parallel-group study conducted at 25 clinical centres. There were 187 postmenopausal study participants aged ≥ 45 years, at a minimum of 1 year after menopause with T score ≤ -2.0 . All women were on HRT ≥ 6 months. Subjects were randomized to receive 100 µg PTH (1-84) or placebo injections daily for 24 months ($n = 90/\text{group}$). Changes in lumbar spine BMD were tracked as a primary outcome.

At 18 months, the mean increase in lumbar spine BMD was 7.9% for PTH (1-84) subjects vs. 1.5% for those receiving HRT alone; between-group differences were significant at 6 months and persisted throughout the study. Lumbar spine BMD increased in 94% of women receiving PTH (1-84) compared to 59% for HRT alone. The PTH (1-84) subjects also showed significantly higher femoral neck BMD and bone turnover markers when compared to HRT mono therapy group. Bone alkaline phosphatase in the POWER study increased by 98% at month 12 and 115% at month 24 in the PTH with HRT group. However, a significant statistical analysis on fracture risk reduction could not be performed due to insufficient numbers of vertebral and clinical fractures.

It is widely accepted that Parathyroid hormone (PTH) is the first bone anabolic drug approved for the treatment of osteoporosis and evidence proves its efficacy in terms of fracture healing at both cancellous and cortical sites, indicating that both osteoporotic and non osteoporotic fractures can be the target of PTH-induced healing. The data also suggests that PTH could partly prevent the delay in fracture healing caused by aging (Ellegaard, Jorgensen et al. 2010).

Animal studies have provided robust evidence that PTH (1-84) improves fracture healing and in a recent study of 65 elderly osteoporotic females it has been successfully used in pelvic ramus fracture healing (Einhorn 2011, Peichl, Holzer et al. 2011). PTH may therefore be a potential novel treatment option in humans with impaired healing (Jorgensen and Schwarz 2011); However, there is a need to do more extensive human studies in this area (Einhorn 2011).

1.4 PTH (1-34)

Parathyroid hormone is naturally an 84-amino-acid polypeptide. Teriparatide, rhPTH(1-34), produced in *E. coli*, using recombinant DNA technology, is identical to the 34 N-terminal amino acid sequence of endogenous human parathyroid hormone. PTH 1-34, teriparatide, at a dose of 20 mcg/day, is available in the United States and Europe for the treatment of severe osteoporosis in both men and women. The European Commission granted a licence valid throughout the European Union for PTH 1-34, teriparatide (Forsteo) to Eli Lilly Nederland B.V. on 10 June 2003. The marketing authorisation was renewed on 10 June 2008. European public assessment report (EPAR) describes Forsteo as a prefilled pen which contains the active substance teriparatide. Each 2.4-ml prefilled pen contains 600 micrograms of teriparatide. Each pen contains 28 doses of 20 micrograms (per 80 ml). Although the shelf life is 2 years, once opened the Chemical, physical and microbiological in-use stability has been demonstrated for 28 days at 2°C-8°C.

1.5 Substantial Clinical trials involving PTH (1-34) and its effects on BMD and Fracture risk reduction

The Fracture Prevention Trial (FPT) of PTH 1-34 (teriparatide) is a Pivotal study which ascertained the efficacy of teriparatide in osteoporosis treatment (Neer, Arnaud et al. 2001) . It involved 1,637 osteoporotic postmenopausal women (mean age 69.5 years) with previous vertebral fractures. These were randomly assigned to receive PTH either 20 or 40 µg /day subcutaneously or placebo. The primary indication of efficacy was the

prevalence of new vertebral fractures at the end of the study. The study also looked at non-vertebral fractures.

After 18 months of treatment, there was significant vertebral and non-vertebral fracture efficacy; the relative risks for vertebral fractures for the 20 and 40 mcg doses compared with placebo were 0.35 (95% CI 0.22-0.55) and 0.31 (95% CI 0.19-0.50) and for non-vertebral fractures the relative risks were 0.47 and 0.46, respectively (95% CI, 0.25-0.88 and 0.25-0.861).

It was observed that the fracture risk reduction did not differ by dose, even though BMD changes were significantly greater at a dose of 40 mcg/day (Neer, Arnaud et al. 2001). The safety and efficacy of teriparatide in postmenopausal women aged 75 and older was further assessed using Fracture Prevention Trial (FPT) data. Observations did not show any significant treatment-by-age interactions for the bone turnover markers, femoral neck BMD, vertebral and non vertebral fragility fractures and any important treatment-emergent adverse events. This analysis therefore established the safety and efficacy of teriparatide in relatively elderly population as well (Boonen, Marin et al. 2006).

Another recent analysis using Fracture Prevention Trial (FPT) data highlights that the risk reduction for non-vertebral fracture in teriparatide treated patients versus placebo depended on the set of non-vertebral fractures included in the analysis. The risk of non-vertebral fragility fracture was significantly reduced in the teriparatide group when all fracture sites were considered and lower risk reductions were observed when the outcomes were limited to fragility fractures (Krege and Wan 2012).

The European Forsteo Observational Study (EFOS) was a 36-month, prospective, observational study, the first of its type to evaluate prevalence of fractures in addition to back pain in severely osteoporotic postmenopausal women (Fahrleitner-Pammer, Langdahl et al. 2011). This study followed 1,649 postmenopausal osteoporotic women who were treated with teriparatide in the outpatient setting in eight European countries for duration of 18 months, followed by a post treatment period of a further 18 months. The primary study endpoint was clinical vertebral and non-vertebral fractures.

For all fractures including vertebral fractures, there was a significant reduction in the adjusted odds of fracture at 12 to <18 months of teriparatide treatment and during the post-teriparatide intervals compared with the first 6 months of teriparatide treatment.

In the total study cohort, the odds of fracture were reduced by 39% at 12 to 18 months of treatment ($p = 0.013$) compared with the first 6 months of treatment; this decreased further to 74% at 30 to <36 months ($p < 0.001$). The odds of having a non-vertebral fracture were significantly lower during the 24- to <30-month interval (OR 0.40, 95% CI 0.21 to 0.75) and 30- to <36-month interval (OR 0.41, 95% CI 0.22 to 0.76), compared with the first 6 months of teriparatide treatment. Similar results were observed for the main non-vertebral fractures.

Data from the post-teriparatide cohort showed there was no evidence of further change in the odds of fracture during the 18 months after stopping teriparatide (Fahrleitner-Pammer, Langdahl et al. 2011).

A subsequent predefined analysis of the same study (EFOS) also highlighted that both the frequency and severity of back pain decreased during teriparatide treatment in the elderly patients with aged 75 years and older and this was maintained after teriparatide was discontinued ($p < 0.001$) (Walsh, Lems et al. 2012).

This marked reduction in back pain during teriparatide therapy was consistent with a meta-analysis of five randomized controlled trials, which reported that the risk of new or worsening back pain was reduced by 34% after teriparatide treatment; taking account of teriparatide related reduced risk for any back pain [relative risk, 0.66 (95% CI, 0.55-0.80)], moderate or severe back pain [relative risk, 0.60 (95% CI, 0.48-0.75)] and severe back pain [relative risk, 0.44 (95% CI, 0.28-0.68)] (Nevitt, Chen et al. 2006)

Another double-blinded, double-dummy clinical trial was conducted in 428 men and women (ages 22-89yrs) with glucocorticoid induced osteoporosis (Saag, Shane et al. 2007). This study compared the effects of Forsteo and alendronate on BMD of spine over three years. The primary endpoint was lumbar spine BMD at 18months. A total of 214

patients received 20 µg of teriparatide once daily, and 214 received 10 mg of alendronate once daily.

A greater increase in the lumbar spine BMD was observed in the teriparatide group when compared to the alendronate group ($7.2 \pm 0.7\%$ vs. $3.4 \pm 0.7\%$, $P < 0.001$). A significant difference between the groups was reached by 6 months ($P < 0.001$).

Secondary outcomes of this trial included changes in bone mineral density at the total hip and in bone turnover markers, the time to changes in bone mineral density, the incidence of fractures, and safety. At 12 months, bone mineral density at the total hip had increased more in the teriparatide group. Fewer new vertebral fractures occurred in the teriparatide group than in the alendronate group (0.6% vs. 6.1% , $P = 0.004$); the incidence of non-vertebral fractures was similar in the two groups (5.6% vs. 3.7% , $P = 0.36$).

In summary, both teriparatide and alendronate increased the lumbar spine and hip BMD but this effect was significantly greater in the teriparatide group (Saag, Shane et al. 2007). A post hoc analysis studied the effect of baseline glucocorticoid dose on the 18-month BMD in 387 patients from this study. Lumbar spine BMD increases with teriparatide were greater (3.5%) in the low-dose category (≤ 0.05 mg/day) than in the high-dose category (≥ 15 mg/day) ($p = 0.012$), reflecting the different mechanisms of action of these agents (Devogelaer, Adler et al. 2010).

A recent metanalysis of the clinical trials further reinforces the effectiveness of teriparatide in osteoporosis treatment (Han and Wan 2012, Johnson 2012). The comparisons were made among the studies involving daily subcutaneous injections of teriparatide with durations ranging from 6 to 36 months, with a placebo in primarily postmenopausal women with osteoporosis, having no coexisting medical conditions or treatments that would affect bone or calcium metabolism. Primary objectives were to observe spine and hip BMD and vertebral and non-vertebral fractures.

In trials that measured BMD as an outcome, treatment was associated with an increase of bone mass. The meta-analysis of 8 trials ($n = 2206$) illustrated that teriparatide increased spine BMD by 8.1% (95% CI 6.72 to 9.55), and the meta-analysis of 7 trials ($n = 1303$)

demonstrated that teriparatide increased hip BMD by 2.5% (CI 1.67 to 3.29) compared with placebo; there was significant statistical heterogeneity ($P < 0.01$) across study results for BMD outcomes(Johnson 2012) .

In trials that reported fracture as an outcome, treatment was associated with a 70% risk reduction in vertebral fractures (risk ratio 0.30, 95% CI: 0.21-0.44; three trials, n = 1452) and 38% risk reduction in non-vertebral fractures (risk ratio 0.62, 95% CI: 0.44-0.87; three trials, n = 1842).(Han and Wan 2012)

1.6 Adverse rPTH effects

Since the anabolic bone effects of intermittent PTH have been established, both forms of peptide i.e., PTH (1-84) and PTH (1-34) have been well tolerated and effective treatments for osteoporosis. Of note, the incidence of adverse effects following rPTH treatment has been observed in animal and human based investigations. Although there is insufficient data to conclusively comment on side effects associated with intact PTH as yet, it is most likely similar to teriparatide.

Osteogenic sarcoma

Soon after the introduction of rPTH as a revolutionary drug in osteoporosis treatment, the process of development of PTH as osteoporosis medication abruptly came to a halt in a pivotal registration trial for teriparatide, when osteosarcoma was observed in an animal study on rats.

The study data suggested that these lesions resulted from the long duration of treatment and the exaggerated pharmacologic response of the rat skeleton to daily treatment with PTH(1-34). (Vahle, Sato et al. 2002). However, it turned out that this phenomenon was specific for a certain Fischer strain of rats, and only with lifelong treatment at extreme doses, starting at birth (Vahle, Long et al. 2004).

Osteogenic sarcoma was also observed in the PTH 1-84 preclinical carcinogenicity rat study over 2year. It showed a dose-responsive increased incidence of osteogenic sarcoma

(50- 100 µg/day). A non carcinogenic dose of PTH (1-84) was identified which was of 10 µg/kg/day at a systemic exposure to PTH and that was 4.6-fold higher than for a 100 µg dose in humans (Jolette, Wilker et al. 2006).

There is very little evidence of osteogenic sarcoma in humans followed by rPTH use. The only reported case of osteogenic sarcoma in humans treated with teriparatide, was made following the biopsy of a lung lesion in a female smoker and a particular bone source was not identified (Harper, Krege et al. 2007).

To date, a large number of the osteoporotic patients have completed the course of PTH (1-34) or PTH 1-84 without osteosarcoma cases being directly linked to PTH use.

Thus, rPTH doesn't raise any concerns about exerting any carcinogenic effects in humans, however, ongoing monitoring and direct side-by-side comparison of both forms of PTH is recommended.

Hypercalcaemia

The principal physiological role of PTH is to raise calcium during fasting periods, so transient hypercalcaemia is expected to occur 4 to 6 hours after PTH injection. However, PTH injections consistently increased serum calcium in a percentage of patients in all PTH trials (Miller 2008).

In FPT trial post dose serum calcium was above the upper limit of normal at least once in 11% of patients on teriparatide. Similar transient rises in serum calcium have been reported during other controlled trials with teriparatide (Black, Greenspan et al. 2003).

In the TOP trial the number of patients with elevated serum calcium concentration (>2.66 mmol/L or 10.7 mg/dL) in the PTH (1-84) treated group was 27.8% compared with 4.5% in the placebo group (Greenspan, Bone et al. 2007). This incidence is higher than reported in teriparatide trials and in the PaTH study i.e.12% (Black, Greenspan et al. 2003).

In an earlier study, 28 women taking teriparatide and two receiving alendronate had elevated four-hour to six-hour post-dose serum calcium levels at least once, and one

woman discontinued teriparatide treatment because of increased serum calcium levels after injection. The women with elevated serum calcium levels were asymptomatic, and these increases were not associated with clinically significant adverse outcomes (Body, Gaich et al. 2002).

While no clinical adverse events have been linked to any increments in serum or urine calcium, the most efficient means of identifying the small percentage of patients who require dose reduction has yet to be determined.

Hypercalciuria

In FPT, teriparatide treatment was followed by a small increase in 24 hour urinary calcium (24hrUrCa) excretion by a median of 0.75 mmol (30 mg)/day (Neer, Arnaud et al. 2001).

In the TOP study hypercalciuria occurred in 46% and 23% of women receiving PTH and placebo, respectively (Greenspan, Bone et al. 2007).

In the POWER trial hypercalciuria was detected in 43.3% of the HRT with PTH group and in 16.7% of the HRT group.

In the PaTH study, after year one, 9% of enrolled women developed hypercalciuria, 8% in the PTH-alone group, 11% in the combined therapy group, and none in the alendronate group. The frequency of episodic hypercalcemia or hypercalciuria in this trial was 21% in general. Episodes were generally mild and hypercalciuria resolved after discontinuing calcium and vitamin D in 12 patients (80%), after decreasing PTH injection frequency in 2 patients, and did not recur when daily frequency was resumed, and without intervention in the remainder (Antoniucci, Sellmeyer et al. 2007).

In the same trial, only 2 patients in the PTH-alone group had concurrent hypercalcemia and hypercalciuria. Mean urine calcium concentration significantly increased in both the PTH-alone and the PTH and alendronate groups, but there was no significant difference between the two groups. The risk of hypercalciuria increased by 50% with each

50 mg/day increase in baseline urinary calcium (relative risk 1.5; 95% CI 1.1 to 2.0) (Antoniucci, Sellmeyer et al. 2007).

Despite the increased risk of hypercalciuria on rPTH treatment, no serious adverse events have been reported to date.

Hyperuricemia

A significant increase in serum uric acid has been found in about 3% of patients after teriparatide therapy in the Fracture Prevention Trial (Neer, Arnaud et al. 2001). A significant increase in the mean serum uric acid concentration (61 mmol/L) has also been observed in patients treated with intact PTH in PaTH study (Black, Greenspan et al. 2003), in both the PTH with alendronate and the PTH-alone groups, and there were 2 patients with gout in the combination therapy group and 1 in the PTH group (Black, Greenspan et al. 2003) .

An increase in serum uric acid above the normal range was also observed in the PTH group in the TOP study at month 18, approximately 20% compared to 4.5% with placebo (Greenspan, Bone et al. 2007).

Other adverse events (ADEs)

The most commonly reported other adverse events in patients treated with rPTH include but are not limited to: nausea, headache, injection site complications, fatigue asthenia, neck pain, hypertension, angina pectoris, syncope, constipation, dizziness, depression, insomnia and vertigo. ADEs appear to increase with higher dosages. However in most of the cases rPTH is well tolerated and the side effects experienced by patients do not reach a significantly severe level.

Few studies mentioning ADEs related to PTH 1-84 use:

In the phase II trial of PTH (1-84) a non dose-related injection site reaction was the most common complaint in 134 of 217 women, but this event is not so widely reported in other studies.(Hodsman, Hanley et al. 2003) .

In the TOP study, a similar number of patients experienced adverse events in the PTH and placebo groups (94.0% versus 92.9%). However, PTH (1-84) treated patients complained more frequently of nausea and vomiting than the placebo group patients (22.6% versus 9.2%; $P \leq 0.001$ and 8.0% versus 4.4%, respectively). Headache frequency was higher in PTH treated patients in the TOP study, 28.5% versus 23% in the placebo group ($p < 0.001$).

The patients who discontinued the treatment in the TOP study because of adverse events comprised approximately 16% of the PTH group (nausea 4.4%, headache 1.4%, vomiting 1.1%, dizziness 0.8%, and elevated serum calcium 2.4% or urine calcium levels 0.5%) and 12% of the placebo group (only 2% for these combined events) (Greenspan, Bone et al. 2007) .

In the TOP study PTH induced a slight reduction in haematocrit, haemoglobin, and erythrocyte count at all time points. No serious alterations in cardiac rhythm, repolarization, or intraventricular conduction were reported (Greenspan, Bone et al. 2007).

In the POWER study, a similar number of patients experienced adverse events in all treatment groups. However, nausea was more frequent in the HRT and PTH group than in the HRT alone group (25.6% vs 3.3%) (Fogelman, Fordham et al. 2008).

Similarly, in the PaTH study almost the same number of patients reported adverse events in both treatment groups (96% PTH and HRT vs 93% HRT all events, 26.7% versus 17.8% serious adverse events). No differences in headache frequency between various groups were reported in the PaTH study (Fogelman, Fordham et al. 2008) .

Few studies mentioning ADEs related to PTH 1-34 use:

According to the study by Body et al, significantly fewer patients taking teriparatide (5.5%) had new or worsened back pain compared with patients taking alendronate (19.2%) although six patients taking teriparatide reported leg cramps (Body, Gaich et al. 2002).

In The Fracture Prevention Trial (FPT), 794 of 1,637 women withdrew because of ADEs. 18% of the women reported nausea, 13% headaches, 9% dizziness, and 3% leg cramps. The investigators also noticed an increase in circulating antibodies to PTH, which occur more often in higher doses of teriparatide (40 mcg) but these antibodies had no discernible effects on any of the measured clinical outcomes. Antibody formation was not found after intact PTH therapy (Hodsman, Hanley et al. 2003).

1.7 Adherence to treatment

Adherence to treatment and compliance in the patients treated with both forms of PTH in the clinical trials is high, when reported. In the phase II trial of PTH 1-84 compliance was very high, at 94.8% to 97.6% in all PTH groups, 14% of the patients discontinued the treatment, only 38% of these due to an adverse reaction and 48% due to study noncompliance (Hodsman, Hanley et al. 2003).

In the TOP study, 824 women out of 1286 (64%) in the PTH treated group completed the trial. Reasons for discontinuation when compared with the placebo group saw the number of drop-outs due to adverse events double, but serious adverse events, fractures, and confirmed bone loss were more frequent in the placebo group (Greenspan, Bone et al. 2007).

In the POWER study 62% in the PTH and HRT group and 93% in the HRT only group completed the study and overall compliance was high (96% and 97% respectively)(Fogelman, Fordham et al. 2008). In the PaTH study 95% of patients completed the first year visit, and 75% fully adhered to treatment by injection (Black, Greenspan et al. 2003).

1.8 Conclusions

In summary, rPTH is generally well tolerated and treatment compliance rates are favourable. An improvement in patient compliance and therapy costs could be achieved by a refraction of injection frequency.

A double-blind, randomized, placebo-controlled trial verified that less frequent PTH administration increased lumbar spine BMD, radial trabecular bone, and bone formation markers in 50 postmenopausal women aged 45 to 70 years with a femoral neck BMD T-score between -1.0 and -2.0. The administration regimen comprised of once daily injection of PTH (1-84) at a dose of 100 µg for one month, followed by weekly injections, same dose, for 11 months (Black, Bouxsein et al. 2008).

Recombinant PTH administration has been established as a safe and effective drug for treatment of osteoporosis in various regions of the world (Sethi, Chadha et al. 2008). Of note, large differences (up to 20 fold) still exist in the number of patients receiving PTH across different countries, due to varying accessibility to PTH as a result of a combination of restrictive schedules of reimbursement, high cost, and physicians' lack of awareness of the potential benefits of PTH (Hosking, Alonso et al. 2009).

Conclusively, current limitations on the length of treatment and the high acquisition cost mean that rPTH is best reserved for the treatment of patients with osteoporosis at high risk of fracture, or for patients with osteoporosis who have unsatisfactory responses to or intolerance of other osteoporosis therapies (Blick, Dhillon et al. 2009).

Recently a panel of Swiss experts in osteoporosis treatment has established a consensus on the treatment indications of rPTH in osteoporotic women and men after reviewing the emerging evidence supporting its use. It has been suggested that rPTH should be used as a first line agent in severely osteoporotic patients with a high fracture risk. It will subsequently deposit new good quality bone instead of maintaining pre existing bone mass achieved with antiresorptive drugs (Rizzoli, Kraenzlin et al. 2011).

1.9 Other uses of rPTH:

Emerging evidence for both PTH (1-34) and PTH (1-84) use in non osteoporotic or hypoparathyroid population has shown a potential for improvement in abnormal bone-remodeling dynamics and return of bone metabolism toward normal euparathyroid levels. It has also been established that both forms of PTH lower body requirements for supplemental vitamin D and also increase bone turnover. Further detailed studies in relation to rPTH effects on quality of life measures are ongoing (Rubin, Dempster et al. 2011, Cusano, Rubin et al. 2012).

Chapter Two

Malnutrition and identification of malnutrition in elderly: Section I

Malnutrition in elderly

2.1 Introduction

Nutrition is crucial to skeletal health. A healthy balanced diet will ensure that all nutrients necessary for bone health are provided in the diet. This can be achieved by following the principals of the food pyramid. However, prevalence of malnutrition increases with advancing age. Many elderly patients have an increased risk for malnutrition compared with other adult populations.

2.2 Definition of Malnutrition

Nestlé Healthcare Nutrition defines malnutrition as : A state of nutrition (under or over nutrition) in which a lack of protein, energy and other nutrients causes measurable adverse effects on tissue and/or body form, composition, function or clinical outcome. As per the scope of this chapter, malnutrition will refer to the state of under-nutrition as this remains the common use throughout the published literature.

Malnutrition can be significant if a person has:

- a BMI of less than 18.5 kg/m^2
- had unintentional weight loss greater than 10% within the last 3-6 months
- a BMI less than 20 kg/m^2 and has had unintentional weight loss greater than 5% within the last 3-6 months.

2.3 Prevalence of Malnutrition in the elderly

The prevalence of malnutrition in older adults is dependent upon the population studied, varying by geography, age distribution, and living situation.

It has been projected in the past that between 2%–16% of community-dwelling elderly are nutritionally deficient in protein and calories (Whitehead and Finucane 1997). With further inclusion of vitamin and mineral deficiencies in this estimate, a subsequent calculation of percentage of malnourished elderly population exceeds by 35% (Chandra 2002).

It has been observed since decades that between 16% to 39% of housebound patients suffer from malnutrition (Cederholm and Hellström 1992).

The British Association for Parenteral and Enteral Nutrition- Nutritional Screening Week (BAPEN NSW) in 2007 revealed a high prevalence of malnutrition amongst the elderly population:

- 35% in adults over 80 years of age
- 25 – 35% in adults 60 – 80 years
- 25% in adults less than 60 years of age

An analysis of NHANES 2003-2004 concludes that above 8% of elderly women in United States are consuming protein at a level below their estimated average requirement (Fulgoni III 2008, III 2008).

A subsequent study also reports the prevalence of malnourishment to be 28% in community-dwelling and ambulatory elderly persons (Ülger Zekeriya 2010) .

In hospitalised patients, prevalence of malnutrition has been identified in between 20% and 65% of the elderly population (Bienia, Ratcliff et al. 1982, T 1992), (Elmstahl, Persson et al. 1997, Chandra 2002).

Other researchers also report that 60% of hospitalised older adults (aged 65 years and above) and 35% -85% of patients in long term care facilities experience malnutrition (Furman 2006). This high prevalence of malnutrition in hospitalised patients was reiterated in 2010 (Kaiser, Bauer et al. 2010), who reported that while a third of hospitalised older adults (38.7%) were malnourished, those in rehabilitation units were more likely to be undernourished (50.5%) .

The National Diet and Nutrition Survey carried out in the UK on the over 65 age group found that one in six residents of residential homes were malnourished (16 per cent of men and 15 per cent of women). This compared with 3 per cent of men and 6 per cent of women of similar age living at home in the community.

In summation, multiple studies note that malnutrition and being underweight are more common in the elderly than in adults of other ages and require attention (Han-Magnus Kvamme 2011).

2.4 Risk Factors and Causes of malnutrition in elderly

There may be several factors contributing towards poor nutritional status in this elderly population. These include difficulties eating (for e.g. badly fitting dentures, arthritis), (Hall & Brown, 2005), poor appetite, loneliness, social isolation, difficulties accessing and preparing food and ill health. Identifying and managing any problems can have a significant impact on nutritional status.

Older people are also prone to dehydration which can exacerbate dizziness and increase risk of falls and injury.

Profound malnutrition and serious illnesses often present concurrently, and each can accelerate the progression of the other. Multiple co morbidities i.e. Cardiovascular, pulmonary, and neurological diseases, as well as osteoarthritis and osteoporosis, in the older population mostly contribute to overall nutritional compromise.

In short, causes of malnutrition include:

- **Reduced intake:** Poor appetite due to illness, food aversion, nausea or pain when eating, depression, anxiety, side effects of medication or drug addiction
- **Inability to eat:** This can be due to reduced levels of consciousness; confusion; difficulty in feeding oneself due to weakness, arthritis or other conditions such as Parkinson's Disease, dysphagia, vomiting, painful mouth conditions, poor oral hygiene or dentition; restrictions imposed by surgery or investigations
- **Lack of food availability:** poverty; poor quality diet at home, in hospital or in care homes; problems with shopping and cooking
- **Impaired absorption:** This can be due to medical and surgical problems effecting digestion & stomach, intestine, pancreas and liver /or absorption
- **Altered metabolism:** Increased or changed metabolic demands requirements related to illness e.g. cancer; surgery, organ dysfunction, or treatment
- **Excess losses:** Vomiting; diarrhoea; nutrient fistulae; stomas; losses from nasogastric tube and other drains or skin exudates from burns.

Below is a list of Risk factors for malnutrition (Hickson 2006) :

Medical Factors

- Poor appetite
- Poor dentition, other oral problems and dysphagia
- Loss of taste and smell
- Respiratory disorders, for example, emphysema
- Gastrointestinal disorders, for example, malabsorption
- Endocrine disorders, for example, diabetes, thyrotoxicosis
- Neurological disorders, for example, cerebrovascular accident, Parkinson's disease
- Infections, for example, urinary tract infection, chest infection
- Physical disability, for example, arthritis, poor mobility
- Drug interactions, for example, digoxin, metformin, antibiotics, etc
- Other disease states, for example, cancer

Lifestyle and social factors

- Lack of knowledge about food, cooking, and nutrition
- Isolation/loneliness

- Poverty
- Inability to shop or prepare food

Psychological factors

- Confusion
- Dementia
- Depression
- Bereavement
- Anxiety

Additional risk factors in hospital

- Food service --- Sole nutritional supply is hospital food--- Limited choice--- Presentation may be poor
- Slow eating and limited time for meals
- Missing dentures
- Needs feeding/supervision
- Inability to reach food, use cutlery or open packages
- Unpleasant sights, sounds and smells
- Increased nutrient requirement, for example, because of infections, catabolic state, wound healing etc.
- Limited provision for religious or cultural dietary needs

2.5 Consequences of Malnutrition

Both animal and human based studies have established that malnutrition can adversely affect virtually every organ system. The adverse outcomes of malnutrition are chiefly related to the duration and the degree of nutritional compromise. A number of co morbidities in the elderly have been associated with malnutrition.

Amongst the serious consequences of malnutrition on health, the salient ones include:

- Increased risk to local and systemic infections
- Functional decline
- Delayed wound healing
- Cognitive decline

- Impaired respiratory function
- Muscle weakness and depression
- Delayed recovery from acute illness
- Increased mortality

Kaya et al in 2010 used Mini Nutritional Assessment (MNA) to evaluate nutritional status of 413 elderly patients and reported that poor nutritional status was associated with lower blood haemoglobin, serum total protein and albumin, and revealed more chronic diseases and geriatric syndromes (6 ± 2 vs. 3 ± 2 , $p < 0.0001$). It was also noted that depression, faecal incontinence, decreased cognitive function and functional dependence was also found to be prominent in the malnourished population (Saka, Kaya et al. 2010)

Studies have shown that the adverse outcomes of malnutrition are not merely due to the actual disease process rather, malnutrition is an independent risk factor impacting on higher complications and increased mortality, length of hospital stay and costs (Correia and Waitzberg 2003, Lim, Ong et al. 2012).

In a study malnourished participants were shown to have a 3 fold increase in mortality over a 12-month period post discharge (Middleton, Nazarenko et al. 2001). This increase in mortality was reiterated in a subsequent study (Correia and Waitzberg 2003) which identified a mortality rate of 12.4% in the malnourished compared to a 4.7% rate in those with normal nutritional status ($RR = 2.63$). Similarly, in a recent analysis, malnutrition was a significant predictor of overall mortality with adjusted hazard ratio (HR) of 4.4, (95% CI 3.3e6.0), $p < 0.001$ (Lim, Ong et al. 2012).

2.6 Economic impacts of malnourishment in osteoporotic population

Malnutrition in the elderly is often under diagnosed (Gariballa 2000) and when diagnosed is often undertreated (Furman 2006). The co-existing co morbid conditions could potentially alter energy requirements in the elderly either by increasing energy expenditure or reducing requirements through muscle loss related to inactivity. Which could consequently lead the actual energy needs to vary widely from calculated energy

needs (Compher, Kim et al. 1998, Houwing, Rozendaal et al. 2003). This makes the elderly a heterogeneous group and more difficult to assess nutritionally.

Studies have identified that the length of hospital stay of malnourished patients is 1.5-1.7 times longer than well-nourished patients (Middleton, Nazarenko et al. 2001, Correia and Waitzberg 2003). Early recognition and treatment of malnourished hospital patients has been proven to be cost-effective (Kruizenga, Van Tulder et al. 2005). A cross-sectional data obtained from 469 patients attending two large public hospitals has shown that the mean hospitalization costs for nutritionally-at-risk patients (€8435.2± 4890.6) was more than double of that for those not at risk (€2453.4± 2203.6). It also suggested that the cost of treating a nutritionally-at-risk patient could account for an increase in about 20% of hospitalization costs (Amaral, Matos et al. 2007).

The average cost of hospitalization for malnourished was 24% higher than the well-nourished in a recent study of 818 adults, where malnourished patients stayed in the hospital significantly longer, were more likely to be readmitted within 15 days, and had higher mortality up to three years post discharge (Lim, Ong et al. 2012).

In addition to this, malnourished people are more at risk of developing osteoporosis. Osteoporotic fractures also are now considered a major public health problem. Cashman has recently highlighted this health issue in his article while mentioning the careful estimates that the US alone has over 10 million of population with osteoporosis with a further 4 million more with osteopenia attributing them at increased risk towards osteoporosis. The estimated remaining lifetime risk of fractures in Caucasian women at age 50y, based on incidence rates in North America, is 17.5%, 15.6% and 16% for hip, spine and forearm respectively, the remaining lifetime risk for any fragility fracture approaches 40% in women and 13% in men (Cashman 2007).

Thus management of a malnourished osteoporotic population could collectively consume huge economic resources enhancing the need for timely detection and treatment of these coexisting disorders.

2.7 Nutritional status of Irish population

In Irish Population, the third national Survey of Lifestyle, Attitudes and Nutrition (SLÁN) was conducted in 2007, following previous surveys in 1998 and 2002. In addition to the young population it also included 1,207 older adults aged 45 years and over. It reports that older Irish people tend to have unhealthy diets, which contributes to increased prevalence of overweight and obesity among this age cohort (Morgan Karen, Hannah et al. 2008).

The current nutritional status of healthy older Irish has been determined in another cross-sectional study (Corish and Kennedy 2003). A sample of 874 people was recruited through interest groups for the active retired. It was obtained that, in keeping with the high prevalence of overweight and obesity in the adult Irish population, 69 per cent of older men and 61 per cent of older women were either overweight or obese. Using a BMI of less than 20kg/m² to define undernutrition, the same group of investigators found that 16 per cent of all older people (in a sample of 235) compared to 14 per cent overall (a sample of 569) fell into this group on admission to hospital.

Another study in an Irish cohort of older people has concluded that Irish recipients of Meals-on Wheels are not receiving adequate micronutrients.(O'Dwyer, Corish et al. 2009) Among this cohort over one-third of recipients were malnourished or at-risk of malnutrition.

2.8 Malnutrition and Bones in the elderly

Both micronutrient and macronutrient deficiency appears to be strongly implicated in the pathogenesis and consequences of fractures in elderly. This nutritional deficiency could eventually increase up the age-related bone loss and also attribute to frailty. Nutrients including certain minerals have been positively linked to bone density at total body, spine and hip in a cross-sectional study of 670 postmenopausal Chinese women aged 48-63 years ($p < 0.0$) (Chen, Ho et al. 2006).

These nutrients include inorganic minerals (e.g., calcium, magnesium, phosphorus, sodium, potassium, and trace elements) and vitamins (A, D, E, K, C, and certain B vitamins) and also macronutrients such as protein and fatty acids.

A detailed understanding of nutritional influences on bones is however, lacking for many nutrients. However, a review of all these dietary factors is beyond the scope of this chapter.

Before proceeding further it's prudent to briefly run through The Dietary Reference Intakes (DRIs). It is a system of nutritional guidelines developed by the Institute of Medicine (IoM) of the US National Academy of Sciences. It comprises of a comprehensive set of nutrient reference values for healthy populations that can be used for assessing and planning diets (Government of Canada 2011). The current Dietary Reference Intake recommendation is composed of:

- **Estimated Average Requirements (EAR)**, estimated to meet the requirement of 50% of the people of each particular age group and gender based on a review of the scientific literature.
- **Recommended Dietary Allowances (RDA)**, the daily dietary intake level of a nutrient considered sufficient by the Food and Nutrition Board to meet the requirements of 97.5% of healthy individuals in each life-stage and gender group. It is calculated based on the EAR and is usually approximately 20% higher than the EAR
- **Adequate Intake (AI)**, where there is insufficient evidence to determine an RDA, an AI is set, but the amount established is somewhat less firmly believed to be adequate for everyone in the demographic group.
- **Tolerable upper intake levels (UL)**, to caution that excessive intake of nutrients can be harmful in large amounts. This is the highest level of daily consumption that current data have shown to cause no side effects in humans when used indefinitely without medical supervision.

Following is a table briefly outlining the anabolic nutrients essential for bone with their required daily intake:

Table 2.1 Anabolic nutrients essential for bone with their required daily intake (Courtesy of Dr. Susan E. Brown, PhD)

Table of 20 essential bone-building nutrients		
Nutrient	Adult <u>RDA</u> or <u>AI</u> *	Common therapeutic range for bone health (daily intake)
<i>Key minerals</i>		
<u>Calcium</u> (Ca)	1000–1200 mg	800–1200 mg
<u>Phosphorus</u> (P)	1250 mg 9–18 yrs 700 mg adults	800–1200 mg
<u>Magnesium</u> (Mg)	420 mg adult males 320 mg adult females	400–800 mg
<u>Chromium</u> (Cr)	30–35 mcg, adult males 20–25 mcg, adult females	200–1000 mcg
<u>Silica</u> (Silicon — Si)	No values set to date	5–20 mg
<u>Zinc</u> (Zn)	11 mg adult males 8 mg adult females	12–30 mg
<u>Manganese</u> (Mn)	2.3 mg (AI) adult males 1.8 mg (AI) adult females	2–10 mg
<u>Copper</u> (Cu)	900 mcg adults (0.90 mg)	1–3 mg
<u>Boron</u>	No RDA established	3–5 mg

(B)

<u>Potassium</u>	4700 mg adults	4000–6000 mg
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(K)

<u>Strontium</u>	No RDA established	3–30 mg (supplements) up to 680 mg (in medications)
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<u>Vitamin D</u>	600 IU 1-70 yr 800 IU >70 yr	800–2000 IU and up, as needed
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<u>Vitamin C</u>	90 mg adult males 75 mg adult females	Oral 500–3000 mg (and upward to bowel tolerance), as needed.
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<u>Vitamin A</u>	2997 IU adult males 2331 IU adult females	5000 IU or less
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<u>Vitamin B6</u>	1.3–1.7 mg adult males 1.3–1.5 mg adult females	25–50 mg
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<u>Folic acid/folate (vitamin B9)</u>	400 mcg adults (0.4 mg)	400–1000 mcg (0.4–1 mg)
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<u>Vitamin B12</u>	2.4 mcg adults	150–1000 mcg
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<u>Vitamins K1 and K2</u>	K1: 120 mcg adult males 90 mcg adult females K2: No recommended intake	K1: 250–1000 mcg K2: 45–180 mcg MK-7 (menaquinone-7)
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Other nutrients

<u>Fats</u>	Should comprise minimum of 7% total calories. General recommendation is not to exceed 30% of caloric intake.	20–30% of total calories is perhaps more ideal
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<u>Protein</u>	0.8 g/kg per day adult males and females 125-lb person = 45 g 175-lb person = 63 g 56 g adult males 46 g adult females	1.0–1.5 g/kg
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2.9 Calcium and Vitamin D for nutrition

So far, calcium and vitamin D are the most intensively researched nutrients. Research has shown that adequate amounts of both (500 mg of calcium plus 700 IU of vitamin D3 cholecalciferol) not only attenuate the rate of bone loss with aging but also reduce the fracture risk in both community-dwelling men $n=176$ and women $n=213$ with age 65 years or more (Dawson-Hughes, Harris et al. 1997).

The recommended minimum 25(OH) D level by Holick et al is 50 nmol/L, but for maximum bone health and prevention of many chronic diseases he recommends a concentration between 78 and 100 nmol/L (Holick 2004). In 2011, the Institute of Medicine (IOM) released a report on dietary intake requirements for calcium and vitamin D for normal healthy persons (Ross, Manson et al. 2011). The Recommended Dietary Allowance (RDA) of vitamin D for adults under 70 years age is 600 IU with the RDA increasing to 800 IU after 71 years of age.

Due to Ireland's geographical position and climate Vitamin D insufficiency affects all age groups and is common throughout the entire Irish population as established by Hill et al ,who demonstrates a vitamin D inadequacy of 11% during the summer and 45% in winter in all age groups (Hill, Flynn et al. 2006). DeLappe et al identify vitamin D insufficiency (25(OH)D levels ≤ 50 nmol/l) in 75% of elderly Irish females (DeLappe, McGreevy et al. 2006). While another group of researchers at St.James's hospital, Dublin, report that 95% of non supplemented elderly hospitalised Irish population have serum 25(OH) D levels below 75nmol/l. It is also identified that standard vitamin D supplementation (typically cholecalciferol 800 IU/day) increases serum 25(OH)D by 23.8 nmol/l(Romero-Ortuno, Cogan et al. 2011).

Another analysis of 143 healthy Irish women receiving no supplementation or bone modifying treatments, it is shown that 47% are vitamin D insufficient (<50 nmol/l) and 10% of subjects have 25(OH)D concentrations less than 25 nmol/l (Lardner, Fitzgibbon et al. 2011). The prevalence of vitamin D insufficiency in Irish population and its adverse affects on musculoskeletal and non skeletal systemic health has been recently reviewed

by O'Malley et al thus emphasizing the timely management of this preventable and treatable condition(O'Malley and Mulkerrin 2011).

The adjunctive use of calcium and vitamin D supplementation for reducing fracture risk at all sites in the elderly osteoporotics has been emphasized by researchers (Kamel 2006).

Similarly, a recent qualitative systematic review of 167 appraisals has established significant beneficial bone effects of 25(OH) D at all skeletal sites in elderly men and women (Cranney, Horsley et al. 2007). Cranney also reports that intake of vitamin D-fortified foods consistently increases serum 25(OH) D in both young and older adults.

However, this has been suggested that the imprecision of different 25(OH) D assays makes it difficult to define specific thresholds of circulating 25(OH) D for optimal bone health. Standard reference preparations are needed so that serum 25(OH) D can be accurately and reliably measured, and validated. (Cranney, Weiler et al. 2008). Despite this, vitamin D and calcium play a major role in the control of bone remodelling and bone integrity.

While calcium and vitamin D are crucial nutrients for bone health, protein and other vitamins and minerals have a role to play in this area also.

2.10 Protein requirement in the elderly

Research studies (Fukagawa and Young 1987),(Campbell, Crim et al. 1994, Campbell and Evans 1996, Kurpad and Vaz 2000) have demonstrated that older adults require more protein than the RDA of 0.8 grams in order to maintain nitrogen equilibrium. However, approximately half of all women over 60 consume a diet containing less than this with studies suggesting even higher RDA for protein ranging from 1.0 to 1.5 g/kg/day).

The report of a joint WHO/FAO/UNU expert consultation mentions that there is no convincing evidence that the protein requirement of elderly people (per kg body weight) is different from the protein requirement of younger adults (World Health Organization 2007) .

This is supported by data on nitrogen balance (Campbell, Johnson et al. 2008) which showed that the mean protein requirement did not differ between younger (21–46 years) and older (63–81 years) healthy adults: 0.61 (SD 0.14) compared with 0.58 (SD 0.12) g protein/kg body weight per day. However, the low energy requirement of sedentary elderly people means that the protein to energy ratio of their requirement is higher than for younger age groups. Thus, unless the elderly people are physically active they may need a relatively protein rich diet.

2.11 Protein Energy Malnutrition (PEM)

‘PEM is also referred to as protein-calorie malnutrition caused by an imbalance between consumption of protein and energy (measured by calories) rendering it insufficient to satisfy the body's nutritional needs. This imbalance causes tissue loss, in particular of muscle tissue, with harmful functional consequences’.

PEM is highly prevalent with increasing age and has been reported worldwide.

Dietary interventions for the prevention and treatment of osteoporosis have long been focused on calcium and vitamin D and extensive evidence is available for this.

Nevertheless other nutrients, including dietary protein which represents a key nutrient for bone health have been relatively less well studied and controversial.

It has been estimated that protein deficiency malnutrition could be between 30%–60% in long-term care facilities (Rudman and Feller 1989). This was reiterated by other researchers who identified that 54% of admissions to long term care settings had PEM (Thomas, Verdery et al. 1991). Subsequently another group of researchers reported 33% of a Swedish population suffering PEM on admission to a community resident home (L., M. et al. 1999).

Constans T, has identified PEM in 4% community-dwelling elderly, 50% in hospitalized in acute care units or geriatric rehabilitation units and 30-40% in long-term care facilities.(Constans, Alix et al. 2000)

Similar results are reported by the French Health High Authority who identified 4 to 10 % in elderly persons living at home had PEM, while 15 to 38 % in those in institutional care, and 30 to 70 % in hospitalized elderly patients suffered PEM (Raynaud-Simon, Revel-Delhom et al. 2011).

The metabolic stress of insufficient protein intake, as well as the other co morbidities, further impairs an older patient's overall nutritional state.

2.12 Presence of PEM in obese population

Obesity is a growing problem globally. Considerable research has focused on this developing worldwide pandemic of obesity, including obesity among the elderly (Jenkins 2004, Popkin 2006, Jenkins, Kabeto et al. 2007)

It is associated with micronutrient deficiencies in the elderly. Despite getting sufficient calories, the obese may have vitamin and mineral deficiencies. Katherine Tucker, director of the Dietary Assessment and Epidemiology Research Center at Tufts University, advocates a very high quality diet in obese elderly to maintain good nutrient status without weight gain.

Of note, it has been established that elderly patients with concurrent obesity can often have protein malnutrition that may be overlooked. Given the positive benefits of higher protein intake on satiety and other physiologic functions, efforts should be undertaken to help obese elderly patients consume the recommended amounts of protein.

2.13 Dietary Protein and Bone health in terms of fracture risk and Bone mineral density

Dietary proteins deliver the body with the essential components for proper functioning of living cells. They cannot be synthesized in the body and are, therefore, attainable only through the dietary sources.

Protein accounts for about 30% of the mass of bone tissue, making bone one of the most protein-dense tissues in the body (Heaney 1998). Subsequently it has been demonstrated that bone turnover is dependent on continuous dietary intake of protein (Heaney 2002) hence, it is surprising that the role of protein intake in bone health has largely been viewed in terms of how it may negatively influence calcium balance.

To date, many studies have assessed the relationship between bone density and protein intake and this has been established that protein undernutrition is associated with osteoporosis. Low protein intake possibly reflecting total dietary insufficiency, has been associated with frailty and fractures in the elderly (Patterson, Cornell et al. 1992, Patterson, Cornell et al. 1992).

Several studies have documented a state of energy-protein malnutrition in elderly hip fracture patients as reviewed by Bonjour et al (Bonjour, Schurch et al. 1996). Hospitalized patients with higher protein consumption have displayed better BMD at both hip and spine along with enhanced bicipital and quadricep muscle strength in a study on 74 patients (Geinoz, Rapin et al. 1993)

Low protein and low albumin are strongly and independently associated with functional outcome after hip fracture. In hip fracture patients in particular, protein-energy malnutrition has been studied not only as a causative factor for fracture but also as an outcome determinant (Bonjour, Schurch et al. 1996).

In addition, short-term studies have suggested that acute intakes of low protein can cause a reduction of intestinal calcium absorption resulting in secondary hyperparathyroidism.

In the NHANES I survey, white women with reduced serum albumin had a higher risk of hip fracture. Using a multiple-variable analysis, in the group with serum albumin above the 75th percentile as reference (44 to 47g/l), it was shown that reduced serum albumin increased the risk for subsequent hip fracture. When serum albumin was decreased to the first percentile (<33 <40g/l) or less, the risk of hip fracture was dramatically increased by threefold ($p < 0.05$, suggesting a possible threshold effect at the extremely low values (Huang, Himes et al. 1996).

NHANES III database utilization in a study has demonstrated that in 1882 non-Hispanic white women 50 y old and older, after adjusting for age and body weight, a low protein intake was associated with a significantly lower hip bone mineral density. Mean protein intakes (g/day $\pm$ SD) in each quartile were 31 ± 8 , 50 ± 4 , 65 ± 5 , and 96 ± 22 , respectively. A dietary protein intake in the lowest quartile (0–43 g/day $n=480$) was associated with a significantly reduced BMD in the femur as compared with the quartile with the highest intake (>75 g/day $n=425$); $P=0.003$. Women with intakes in the second quartile (44–58 g/day $n=471$) also had significantly reduced BMD values compared with the highest quartile ($P=0.028$) (Kerstetter, Looker et al. 2000)

Wide-ranging epidemiological evidence suggests that when other known dietary factors are controlled, those individuals who consume low protein diets have lower BMD (Hannan., Tucker et al. , Geinoz, Rapin et al. 1993, Chiu, Lan et al. 1997, Kerstetter, Looker et al. 2000), (Promislow, Goodman-Gruen et al. 2002)

Additionally, higher protein status has been allied to better health outcomes in terms of shorter hospital stays, reduced mortality, and reduced rate of complications after a hip fracture, and general decrease of femoral bone loss in the elderly.

Several studies have documented the benefits of using supplemental protein (20 g/day) for hip fracture patients (Kerstetter, Looker et al. 2000). The significance of protein intake in minimizing age-related bone loss has previously been addressed in The Framingham Osteoporosis Study (Hannan., Tucker et al.).

Considering all, adequate protein is essential for bone growth, maintenance and renewal.

Notwithstanding, there is limited data on the positive skeletal affects of protein intake in very elderly population. For instance, higher total protein intake was associated with a reduced risk of hip fracture in men and women 50–69 years of age ($p < 0.001$), but not in men and women 70–89 years of age. These are the findings from a state wide case-control study in 18 hospitals in Utah with 1167 cases and 1334 controls (Wengreen, Munger et al. 2004) .

Nonetheless, protein is a modifiable factor in osteoporosis prevention, with dietary protein having a role in improving bone health. It has been recognized by the researchers that proteins from various dietary sources contribute to maintain bone integrity, from early childhood to old age. Along with calcium and Vitamin D, an adequate intake of proteins should be recommended in the prevention and treatment of postmenopausal and age- dependent osteoporosis (Bonjour 2005).

Sixty one studies were included in a meta-analysis carried out by Darling et al. It was used to review the effects of protein from various dietary sources such as meat, vegetable, soy etc, on Bone health. A positive association between protein intake and BMD, BMC, and a reduction in bone resorption markers was identified. However, no significant effect was found for all protein on the fracture risk reduction.(Darling, Millward et al. 2009). This may be due to the study durations not being long enough to see effects on fracture risk reduction which may need several months to years.

Bone mass is an important factor in the aetiology of fractures. Due to conflicting studies of dietary protein affects on bone mass and interplay with other factors including frailty, muscle strength and other nutrients, the exact benefits of dietary protein in reducing fracture risk remains unclear. There are contradictory results in studies about the increase or decrease in fracture risk on high protein diets.

Role of dietary protein on BMC and BMD was recently tested by Jesudason and Clifton who performed a systematic review of both cross-sectional and longitudinal studies. The overall results of this review suggested a modest beneficial association between dietary protein and bone density (Jesudason and Clifton 2011)

2.14 Dietary supplementation with protein

Improved prevalence and outcome of PEM with nutritional supplementation has long been substantiated in long-term care services with a weight gain in 50% and improvement in PEM diagnostic criteria in 63% of malnourished residents (Thomas, Verdery et al. 1991).

It has been reported by other researchers also that nursing homes with aggressive evaluation and treatment policies for malnutrition had lower prevalence and fewer complications of PEM compared to nursing homes without such policies (Abbasi AA 1993)

It has been recognized that when compared with placebo, the community-dwelling seniors supplemented with vitamins and minerals exhibit less nutritional deficiencies and improved immune cell function (High 2001);(Chandra 2002) resulting in fewer sick days, and less antibiotic use. Similarly, nutritionally supplemented subjects have exhibited improved post-vaccination immune responses rather than placebo.(Chandra 2002).

Yen has suggested that nutritional supplements have additional value in the elderly with cost-benefit analyses identifying a potential reduction in healthcare expenditures with multivitamin supplementation (Yen 2003). Reduction in medical care consumption, length of hospital stay, nurse visits and medication use is also identified with multivitamin supplementation (Arnaud-Battandier, Malvy et al. 2004).

A meta-analysis evaluating 55 randomized trials of oral protein and energy supplementation compared with placebo or control treatment in older, high-risk for people to prevent malnutrition (Milne, Avenell et al. 2006).

It reported most studies to be of poor quality, due to lack of blinding and intent to treat analysis. The trials evaluated supplements providing between 175 and 1000 additional kcal/day and between 10 and 36 g protein/day. 45% subjects were hospitalized for stroke; 16 % were community-based and 10 % in long-term care facilities. Nutritional supplementation resulted in modest improvement in percentage weight change (weighted mean difference 1.75 %, 95% CI 1.2 to 2.3), with slightly greater weight increase in patients at home or in long-term care.

Overall mortality was reduced in the groups receiving nutritional supplement, compared to control, but there was no mortality impact for patients living at home and no improvement in functional status. The greatest mortality impact was noticed in hospitalized undernourished patients aged above 75 years, and who received higher

calorie content supplements. Complication rates were lower for hospitalized patients who received supplementation, but there was no change in hospital length of stay.

International evidence for oral nutritional supplements (ONS) in community-dwelling and institutionalized elderly had been investigated in a Cochrane review. (AC, J et al. 2007). It indicated a small but consistent benefit from protein and energy oral supplementation. A subgroup analysis showed a nearly statistically significant decrease in mortality in undernourished groups who received supplementation.

Thus, extensive evidence has been offered that nutritional support is cost-effective, particularly in terms of hospital and nursing care.

2.15 Disagreement about anabolic bone effects of Dietary Protein

While several studies demonstrate that adequate protein supplementation is required to attenuate bone loss or maintain BMD, Some data also contradicts this (Anderson, Rondano et al. 1996, Sellmeyer, Stone et al. 2001, Campbell and Tang 2010) .

Therefore, the relations of dietary protein intake with BMD and bone loss in postmenopausal women and the elderly, who have the highest risk of developing osteoporosis, still remain very unclear.

2.16 Relationship between dietary protein, Calcium, and bone

It has been considered that dietary protein leaches calcium from the skeleton leading towards osteopenia. Studies also suggest that protein intake influences urinary calcium excretion to such an extent that for each 50-g increment of protein consumed, an extra 60 mg of urinary calcium is excreted.

Arguments therefore exist that if uncompensated, excessive protein, particularly animal protein, is deleterious to bone. Many researchers concur with this as increase dietary intake of protein is associated with hypercalciuria. One of these researchers reports that

the rate of calcium excretion exceeds calcium absorption in individuals with hypercalciuria, reflecting a net loss of total body calcium (Bushinsky 2002).

Theoretically, this could lead to negative calcium balance, increasing parathyroid hormone (PTH) secretion and further loss of calcium (Ca) through skeletal source. On the other hand there is evidence that dietary protein has more favourable outcomes in the presence of calcium adequacy ($>1000\text{mg/day}$) in terms of total body bone mineral content, net gain in total body bone mineral content and total body bone mineral density ($p < 0.05$) (Vatanparast, Bailey et al. 2007). This suggests that dietary protein works more effectively in presence of calcium sufficiency.

A research group at Yale University and the University of Connecticut has realized that dietary protein actually causes a significant improvement in body's efficiency to absorb calcium from the diet. In this kinetic study using dual stable calcium isotopes, the high protein diet (2.1 g/kg/day) in comparison with the moderate protein diet (1.0g/kg/day) increased the body's ability to absorb calcium from the diet by more than 35%. It also caused a reduction in urinary calcium of bone origin in high protein consumers. Also no protein-induced detrimental effects were noticed on the skeleton in cases where no net difference in bone mineral mass were observed between levels of protein intake, however there was a trend toward reduced bone turnover (Kerstetter, O'Brien et al. 2005).

These researchers concluded that the hypercalciuria with high protein consumption that had troubled prior investigators was primarily due to the increased calcium absorption from the intestinal tract, and was not due to increased bone turnover. Instead, the body was simply eliminating, through the urine, some extra calcium it had absorbed.

Similar findings have been recently reported by other researchers concluding that increased dietary protein improves calcium absorption particularly when the dietary calcium level has been low (Hunt, Johnson et al. 2009); and it also consequently induces hypercalciuria as a compensatory mechanism.

Subsequently, a 15 week controlled study has attained that a high protein diet (118g/day) in a group of postmenopausal women $n = 16$ had increased their calcium absorption

without altering their bone formation and resorption markers. In addition to it, an increase in IGF-I ($P < 0.0001$) combined with a reduction in serum PTH concentrations ($P < 0.001$) in the same group uncovered the beneficial effects of protein on bone health (Cao, Johnson et al. 2011).

2.17 Relationship between dietary protein, acid, and bone

Since, it has been hypothesized that the high endogenous acid load of a high-protein diet is partially buffered by bone, leading to increased skeletal resorption; therefore, this extra calcium in the urine comes from the bones. This theory has traditionally been the base for the well quoted term ‘acid ash’ (Wachman and Bernstein 1968). Acid ash promotes that the increased prevalence of osteoporosis with aging in the contemporary cultures consuming acidic meat diets is due to ‘the life-long utilization of the buffering capacity of the basic salts of bone for the constant assault against PH homeostasis’. This presumes that a protein diet is detrimental to skeletal health by creating a long term low-grade metabolic acidosis.

As mentioned earlier, various studies have highlighted the enhanced effect of dietary protein on total body BMC and BMD particularly with adequate calcium intake (Vatanparast, Bailey et al. 2007). Interestingly, concerns have been raised previously that the favourable bone effects of calcium supplements are not just through the additional mineral that they supply but also (and possibly more so) through their provision of additional alkali salts such as citrate (New and Millward 2003)

The different concepts behind the negative bone effects of protein and evidence supporting the same has been discussed in detail and finally rejected in a recent review by Jean-Philippe Bonjour, MD who has revealed that the difference in renal acid excretion observed in response to variations in protein intake represents a normal homeostatic response. The renal tubule is extraordinarily well equipped in terms of both bicarbonate reclamation and proton secretion machinery to deal adequately with diets supplying various amounts of alkali and acid (Bonjour 2005).

In an analysis of 161 postmenopausal women, the dietary intakes of sulphur containing amino acids, protein and minerals were assessed (Thorpe, Mojtahedi et al. 2008). Areal BMD (aBMD) at the lumbar spine and total hip as measured. Increasing dietary protein was positively associated to aBMD of the lumbar spine ($p=0.04$) and total hip ($p=0.04$) of postmenopausal women but this benefit was suppressed at the lumbar spine by a negative association of sulphur from amino acids at the lumbar spine.

While suggesting that non sulphur containing amino acids are beneficial to bone, this analysis also concluded that any study evaluating the association between protein intake and bone mineral status without controlling for actual sulphur content of protein may observe no significant correlation despite real positive and negative protein effects.

Therefore, it recommends that future studies of associations between protein intake and bone density must account for the dietary acid load to produce unbiased results. This observation may reconcile reports of positive impacts of dietary protein on bone health with reports of a negative impact of the acid load from sulphur containing amino acids (Thorpe, Mojtahedi et al. 2008).

2.18 Dietary Sources of Protein

Considering the variety of proteins that are available much less is known concerning the benefits of consuming one protein versus another. Research to date is still ongoing towards finding the best protein source with anabolic bone effects.

Soy Protein

Soy Protein (SP) is low in fat content. It is shown to be beneficial to bone health with its low sulphur amino acid composition and phytochemicals such as isoflavones, saponins and phytic acid.

There are studies showing potential beneficial effects of soy isoflavones on BMD and bone turnover markers per/ postmenopausal women (Huang, Yang et al. 2006, Ma, Qin et al. 2008).

One of the largest population-based, prospective, cohort investigations examining the relationship between soy food intake and fractures involved 24,403 postmenopausal women living in urban communities in Shanghai. After classifying participants according to quintiles of soy protein (SP) and isoflavone intake and following adjustments for age and total energy intake as well as other multivariate confounders, inverse associations were identified for SP intake and fracture risk and for SI intake and fracture risk ($p < 0.001$) (Zhang, Shu et al. 2005).

However, in this study the self-reported fracture events were not verified via medical records and the women in the lowest quintile of soy intake actually had a relative risk of 0.63 for hip fracture compared to the highest quintile, which is contradictory to different other studies suggesting positive or negative effect of SP.

Further to this, there is either limited data or data showing no significant differences in BMD at 1 year post soy protein supplementation in postmenopausal osteoporosis (Anne M Kenny and Stephen J Walsh 2009). These mixed conclusions have made it hard to interpret whether soy protein is superior to other proteins to improve BMD or reduce fractures.

Milk Protein

Milk is not only a source of calcium, phosphorus, energy and vitamins; in fact, one litre of milk provides 32 to 35 g of protein. Of the six major protein types in cow's milk, four are casein proteins and the other two are whey proteins. The caseins usually make up about 80% of the protein in cow's milk however, whey protein is also responsible for numerous growth promoting elements.

Casein proteins are PH sensitive and they tend to set or gel in the acidic stomach environment. Consequently it takes twice or long for Casein to be broken down into its amino acid subcomponents than whey or other protein. These unique, time released' qualities make casein a muscle-building protein with a long-lasting and steady supply of amino acids.

Whey proteins are fast acting and hence, easily digested by the body and broken down in approximately one hour. This makes it particularly useful after resistance training or exercise.

The correlation between dairy product intake and bone health has been extensively investigated. However, research is still lacking on milk based protein effects in established osteoporosis in the elderly.

The Copenhagen Cohort Study was cross-sectional study which investigated associations of milk whey protein and meat protein intake with bone mineral content (BMC) in adolescents. A 7 day diet analysis was performed 109 participants. The mean total protein intake was 1.2 g/kg. Total and milk (0.3 g/kg) protein intake, but not meat protein intake (0.4 g/kg), was positively associated with size-adjusted BMC ($p \leq 0.05$). This positive association was not altered after correction for energy, calcium, and physical activity ($p \leq 0.01$) and did not seem to be mediated via serum insulin like growth factor-1 (A. Z. Budek 2007).

Another study of 192 healthy adolescents had shown significantly lower BMD, BMC, and insulin like growth factor-1 (IGF-1) and higher PTH with milk intakes below 55 ml/day when compared to individuals consuming over 260 ml/day (L. Esterle 2009).

Although, studies have been performed for comparisons between casein and whey protein supplementation with respect to their effects on muscle strength, lean body mass, power performances etc, however no large study has yet compared them two in accordance with their anti fracture efficacy or gain in Bone mineral content/density.

Animal Vs Vegetable Protein

Conventionally, all dietary animal protein sources are considered to be complete proteins containing all of the essential amino acids. Proteins from vegetable sources are rendered incomplete meaning they are generally lacking one or two essential amino acids. Thus a

vegetarian diet ensuring consumption of all essential amino acids need to be comprised of a variety of vegetables, fruits, grains and legumes etc.

As discussed earlier the relationship between meat intake and acid load has been examined in several studies, presumably animal proteins have a higher content of sulphur-containing amino acids per g of protein. However, this could be similar for protein from certain vegetable sources as well. If protein came from wheat sources it would have a mEq of sulphuric acid of 0.69 per g of protein, while protein from milk contains 0.55 mEq per g of protein. Thus, some plant proteins may have a greater potential to produce more mEq of sulphuric acid per g of protein than some animal proteins (Massey 2003).

Modern cultures consuming a high animal protein diet also tend to under-consume vegetables, fruits, nuts and seeds, which are sources of potassium citrate (foods high in base-forming precursors).

2.19 Conclusion

Although inconsistency between dietary protein intake, BMD, and risk of fracture is not well understood, protein intake seems to play an important and complex role in bone growth, development, and maintenance.

The clinical trials on protein intake and bone health report contradictory results and remind us that there are many variables which need to be considered when discussing the bone–protein relationship.

The authors have cautioned that the anabolic effect of dietary protein only occurs with an adequate intake of alkali equivalents, such as potassium and magnesium found in fruits and vegetables. It has been speculated that the potential anabolic effect of a high protein diet and the catabolic effects of a high acid diet interact to determine the overall net effect of any given diet (Sebastian 2005) .

Dietary protein is best considered in the context of the entire diet of each individual and the effect of dietary protein must be considered in the context of the effect on acid-base

balance and interaction with dietary calcium and phosphorus. Contemporary Diet-induced low-grade metabolic acidity could well be the most significant, and least well-recognized, of all modern bone-depleting risk factors.

Thus for preserving the bone mass, the recommended diet would be a combination of high dietary protein diet with a high alkaline load to neutralize net endogenous acid excretion. Base-forming food such as fruits, leafy green vegetables, roots, stalks etc. being a good source of dietary potassium with an alkalinizing effect could be beneficial in this regard (Heaney and Layman 2008). Thus, maintenance of acid-base homeostasis with increased protein from all sources is crucial to preserving skeletal health.

Presently, at Yale University, the results on a 18mth multi-centre trial (titled SPOON-Supplemental Protein to Outsmart Osteoporosis Now) are anticipated, which seeks to determine the effect of a natural protein supplement on bone health in elderly men and women with low levels of dietary protein intake.

Future research in this area should evaluate the role of dietary protein in preserving bone mass. Only intervention studies could reliably yield an answer. To date, no large randomized controlled trial has tested the effects of non dairy dietary protein supplementation on bone mass accumulation.

Chapter two

Malnutrition and identification and assessment of malnutrition in elderly: Section II

Identification and assessment for malnutrition

2.1 Overview

Malnutrition has a major impact on the person and on the healthcare system yet it is under recognised and undertreated. Nutritional assessment and screening should be an integral part of the overall care of the elderly.

Screening for malnutrition is based on:

- A search for risk factors of malnutrition
- An estimation of appetite and/or food intake
- Measurement of body weight
- Evaluation of weight loss compared to a previous record
- Calculation of body mass index [$BMI = \text{Weight}/\text{Height}^2$, where the weight is in kg and the height in m].

The aims of identification and assessment for malnutrition according to the European Society for Parenteral and Enteral Nutrition are as follows:

- Improvement or at least prevention of deterioration in mental and physical function
- Reduced number and severity of complications of disease and its treatment
- Accelerated recovery from disease and shortened convalescence
- Reduced consumption of resources.

There are many important aspects to nutritional assessment beyond simple measurement of body weight. It has been stated that Nutritional status assessment is beyond relying on a single marker or tool. It is predominantly an issue for older people with multiple chronic co-morbidities (Chernoff 2004). Given these complex contributing factors, a careful nutritional assessment is necessary for both the successful diagnosis of malnutrition in the elderly and the development of appropriate and comprehensive treatment plans.

A comprehensive nutritional assessment includes the following:

2.2 Anthropometric measurements

Anthropometric indicators are measurements of the human body which, when compared with standards that are typical of a reference population, can indicate abnormal nutritional status (Barasi 2003)

The objective of anthropometric measurements is to determine and monitor changes in body weight, height, composition and fat distribution and also to establish protein and energy reserves and assess risk for acute and chronic diseases.

These measure encompass

- weight, height and stature
- BMI
- Circumference
- Skin Folds
- Body composition

Other anthropometric methods include measurements of arm circumference, mid-arm muscle area, calf circumference, triceps skin-fold, and sub scapular skin-fold thickness.

2.3 Biochemical measurements

The objective of biochemical measurements is to determine stored and functional levels of nutrients and also to determine risk and monitor changes for nutrition-related diseases.

These include

- serum protein (Albumin)
- cholesterol levels
- vitamins and mineral status/micronutrients, and metabolic parameters

2.4 Clinical assessment

The objective of clinical assessment is to identify altered nutritional requirements and social or psychological issues that may preclude adequate intake.

It encompasses

- Medical History
- Signs and symptoms
- Cognitive and physical function
- Oral health
- Medication use

2.5 Dietary intake measurement

The objective of dietary intake measurement is to determine quality of the diet and to identify and evaluate dietary patterns.

It encompasses

- Food and Beverage intake
- Food security
- Supplement use
- Food preference, cultural practice, beliefs, rituals
- Dietary preference

2.6 Screening tools

Nutritional screening is a first step in identifying people who are malnourished and at risk of malnutrition. This allows early intervention or timely referral to a dietician.

A recent report by the Royal College of Physicians (2002) highlighted the need for nutritional screening and care as a regular and integral part of clinical practice. The importance of early detection and aggressive intervention is of utmost importance to arrest the downward spiral. Prompt diagnosis relies on a high index of suspicion and available screening tools.

Since no single physical finding, historical fact, or biochemical test in itself is a sufficient predictor or determinant of malnutrition, several screening tools have been developed to better document and monitor malnutrition. Selection of a suitable nutritional assessment system is however, critical towards an effective use of time and resources. Therefore, the selected tool should be validated and it should help establish reliable pathways of care for patients with malnutrition. The most common and best-evaluated are discussed below.

Subjective global assessment (SGA) (Detsky, McLaughlin et al. 1987)

SGA is a simple, routinely used and proven method for gaining a subjective judgment of nutritional status. It uses a questionnaire which records the patient's history (such as weight loss, dietary intake, functional status) and the findings of a physical examination (such as muscle and fat distribution, edema). It then specifically combines this information with the clinician's judgment. The most important parameters are percentage weight loss, oedema and a clinical measurement of muscle mass. Professionals prefer SGA because it is simple, practicable and sensitive to virtually the same degree as objective tests. Dynamometry is very useful but more difficult to perform. Biochemical parameters such as albumin level are more useful for their predictive value.

Although, SGA is an appropriate assessment tool in the different settings but it relies heavily on functional capacity and physical signs of malnutrition. As per se, it is highly dependent on the screening clinician for accuracy.

For instance, its ability to predict infection as a complication of malnutrition on initial validation was compared to six other independent methods. The SGA surpassed all six methods and exhibited 82% sensitivity and 72% specificity (Detsky, Baker et al. 1984). Nevertheless, the replication of this study with less practiced clinicians brought up much less promising outcomes (Pettigrew, Charlesworth et al. 1984).

Other researchers have also established that the reliability of SGA is only dependant on skilled clinicians

Malnutrition Universal Screening Tool (MUST) (Stratton, Hackston et al. 2004, Stratton, King et al. 2006)

It is also a valid and effective method. The MUST combines percentage unplanned weight loss, BMI and the acute disease effect.

MUST is a five-step screening tool, designed by the Malnutrition Advisory Group (MAG), a standing committee of the British Association of Parenteral and Enteral Nutrition (BAPEN). It can be used by all healthcare professionals and has been validated across various settings, like hospital wards, outpatient clinics, general practice, community settings and care homes. It too has been validated against a number of already accepted tools and was found to have good to excellent agreement with the Birmingham Nutrition Risk assessment score (BNR) in the under 65 age group (Stratton, Hackston et al. 2004)

The MUST is easier and straightforward to use and its questions dovetail more readily with the structure of recordkeeping by medical staff.

Although this Screening Tool is applicable in the acute hospital surroundings but it has not been validated yet in residential aged care facilities. Having said that it has been widely used in the clinical settings (Isenring, Bauer et al. 2009).

Nutritional Risk Screening-2002 (NRS-2002) (Kondrup, Rasmussen et al. 2003)

This tool is comprised of two steps including a first screening stage (4 simple questions) followed by a second stage which assesses nutritional status or rather malnutrition risk (Kondrup, Rasmussen et al. 2003).

This method has been validated and recognised by *The European Society for Clinical Nutrition and Metabolism- ESPEN* (Kondrup, Allison et al. 2003). It is a good selection for use in hospitalized patients. Its scores take account of the patient's nutritional status and the severity of illness.

However, NRS-2002 is not validated for elderly patients. Therefore, its discretion between adult patients and elderly patients in a geriatric unit is recommended.

Birmingham Nutrition Risk Score (BNR) (Reilly, Martineau et al. 1995).

BNR was developed in 1995 at Birmingham Heartlands Hospital (Reilly, Martineau et al. 1995).

Comparatively little supporting evidence is available to substantiate the BNR score; the original depicting study validated it by comparison with another nutrition assessment tool, but data on the ability of the BNR to predict outcomes in the elderly is lacking.

In one study comparing the BNR to anthropometric measurements of undernutrition, the BNR fell short in identifying a fifth of cases of undernutrition (Corish, Flood et al. 2004) which implies that misclassification may reduce the predictive power of the BNR tool.

Mini-Nutritional Assessment (MNA) (Guigoz, Lauque et al. 2002, Guigoz 2006)

MNA is an effortless and a validated nutritional assessment tool in elderly population. Moreover, it detects risk of malnutrition at a time when albumin levels and BMI are still normal (Guigoz, Lauque et al. 2002, Guigoz 2006).

The Mini-Nutritional Assessment (MNA) was published in 1994 for the first time (Guigo Y 1994). Since then its use has been widespread, worldwide for a rapid and economical nutrition assessment of elderly patients. Its reliability and validity has been reviewed in subsequent studies as well (Guigoz 2006).

The full MNA® has 18 items and classifies one as normally nourished, at risk for malnutrition, or malnourished. To save time in screening, Rubenstein et al developed a shortened version, the Mini Nutritional Assessment®-Short Form or MNA®-SF in 2001, and created a 2-step screening process. Six questions with the strongest correlation on the original MNA® comprised the MNA®-SF.

The complete MNA® Mini Nutritional Assessment now consists of a screening phase (used as a short form or MNA-SF) and assessment phase.

The screening phase consists of six items: a food intake item, two anthropometric parameters (recent weight loss and body mass index, or BMI), and three general parameters (mobility, physical and emotional stress, and neuropsychological).

The assessment phase has twelve items: anthropometric (calf and upper arm circumference), general (six questions on lifestyle, medication and mobility), dietary (eight questions related to number of meals, food and fluid intake, and mode of feeding), and subjective assessment (personal view of health and nutritional status).

The sum of the MNA scores of both phases distinguishes between elderly patients with adequate nutrition, MNA score ≥ 24 at risk of malnutrition, MNA score 17.5-23.5; and protein-calorie malnutrition, MNA < 17 . (Guigoz, Lauque et al. 2002).

Researchers have revised and revalidated the MNA®-SF using pooled data on the MNA® from 28 previously published studies. The new MNA®-SF incorporates the 3 cut-off points for nutritional status from the full MNA®, thus allowing the identification of those who are malnourished with just 6 questions. The new form also includes an option to substitute calf circumference when BMI is not available (Kaiser, Bauer et al. 2009).

Part 1 is designed to detect "psychological stress or acute disease" or a decline in eating or weight in the past three months, as well as current mobility or neuropathy.

Part 2 determines the presence of polypharmacy or pressure ulcers, the number of full meals eaten daily, the mode of feeding, whether the person lives independently, and the amount and frequency of specific foods and fluids psychological problems and a decrease in body mass index (BMI). MNA was designed to assess nutrient intake under normal conditions.

MNA has been considered as the gold standard nutritional assessment test for ambulatory older adults living both in the community setting as well as in long-term care facilities (Morley 1998). In addition to identifying the malnourished it also detects the persons at risk of malnutrition, which allows timely nutritional intervention if required.

For the above and other reasons it can be concluded that MNA is the first choice for its use specifically on elderly patients. The MUST and MNA are both equally accurate in identifying malnutrition risk in geriatric units so the MUST is another good choice (Guigo Y 1994, Vellas, Villars et al. 2006) followed by NRS-2002.

Evidence supporting use of MNA

The MNA has demonstrated moderate reliability and validity in the screening of malnutrition and risk of malnutrition in older adults, including both institutionalized and community-dwelling, living with or without memory impairment. Some evidence supporting its use is shown below:

Reliability of MNA

A Cronbach's [alpha] coefficient of 0.65 for MNA has been reported in a study of 59 Swedish adults with memory impairment.(Holm and Soderhamn 2003)

The MNA's equivalence as ranging from 0.51 to 0.89 has been determined in other literature also (Guigoz 2006).

Validity of MNA

In Principles of Nutritional Assessment (RS Gibson 2005) Rosalind S. Gibson states that 'Validity describes the *adequacy* with which any measurement, index or indicator reflects what it is intended to measure 'The validity of the MNA i.e., the degree to which a tool measures what it's designed to measure (nutritional status here), has been discussed extensively.

An extensive literature search has revealed that the MNA is a moderate-to-good predictor of malnutrition and the risk of developing malnutrition. Further to this, its predictive value increases with biochemical markers being added or assessment performed by a physician in order to substantiate the findings (Guigo Y 1994) .

However, another study prior to this, there was no added benefit in adding biochemical measurements, as the test was highly accurate with or without these tests (Katz, Ford et al. 1963) .

Sensitivity of MNA

The sensitivity of the MNA i.e., its ability to identify people who are malnourished-has been reported as 70% or higher in several studies (Christensson, Unosson et al. 2002, Delacorte, Moriguti et al. 2004, Thorsdottir, Jonsson et al. 2005, Wikby, Ek et al. 2006). The MNA-SF's sensitivity ranges from 86% to 100% (Cohendy, Rubenstein et al. 2001, Rubenstein, Harker et al. 2001, Visvanathan, Penhall et al. 2004).

Guigoz has also concluded in his review that the MNA "demonstrates good sensitivity compared to a variety of nutritional parameters" such as anthropometry, biochemistry, or dietary intake (Guigoz 2006).

Specificity of MNA

The specificity of the MNA i.e., its accuracy in identifying those who are not malnourished has been examined in three studies and is 70% or higher (Thomas, Zdrowski et al. 2002, Delacorte, Moriguti et al. 2004, Visvanathan, Penhall et al. 2004) The specificity of the MNA-SF ranges from 30% to 100%. (Murphy, Brooks et al. 2000, Thorsdottir, Jonsson et al. 2005)

Thus, the MNA is a non invasive, validated and sensitive tool to screen for malnutrition in elderly population. The original validation study on the full MNA[®] demonstrated the MNA[®] had a sensitivity of 96%, specificity of 98% and positive predictive value of 97% compared to clinical status (Vellas, Guigoz et al. 1999). Other studies have demonstrated MNA to be 98% accurate when compared with a comprehensive nutritional assessment which included food records and laboratory tests (Katz, Ford et al. 1963).

MNA can identify those at risk for malnutrition *before* biochemical or weight changes appear. A timely intervention could prevent adverse outcomes in patients. This is important because, as Guigoz states, "progressive undernutrition often goes undiagnosed," and malnutrition has been linked with adverse conditions including diminished cognitive function, bad teeth, and poor eyesight. Among the hospitalized elderly, low MNA scores have been associated with longer hospitalizations and higher rates of discharge to nursing homes and death. And in general, MNA scores of 27 or higher have been associated with "successful aging" and lower rates of osteoporosis and death within three years (Guigoz 2006).

The American Society for Parenteral and Enteral Nutrition (ASPEN) recently surveyed nutrition specialists, including physicians, nurses, dieticians, and pharmacists, about the MNA. It was exclaimed by the clinical staff that MNA was quick, easy to use, accurate, and objective.

With the revisions, the new MNA[®]-SF is claimed as a stand-alone screening tool, eliminating the need to complete the longer full MNA[®], and reducing time to screen to less than 5 minutes (Kaiser, Bauer et al. 2009). Philips MB has also identified MNA-SF

to be the most appropriate nutrition screening tool for use in community-dwelling older adults (Phillips, Foley et al. 2010).

However the full MNA® is useful in research settings and it is also used as a more in-depth screen. It is sensitive enough to detect malnutrition before changes in weight or serum protein levels are evident. It is commonly used for the free living elderly population in the community, but it can also be used in hospitals and long-term care facilities for screening, assessment and also for monitoring changes in nutritional status over time. Changes in nutritional status alter the MNA score, therefore, upon identification of malnourishment or a risk of malnourishment, MNA can be successfully used as a guide for nutritional intervention.

2.7 Conclusion: Ultimate tool for malnutrition assessment

Due to the heterogeneity in anthropometric and nutritional characteristics among different populations, assessments from a particular tool in a country population may not be readily applicable to other ones. For instance, MNA had shown sensitivity of 100% and specificity of 74.3% in a free living elderly population in Brazil (Delacorte, Moriguti et al. 2004), while on the contrary, it failed to identify persons at risk for under nutrition in another study in a Chilean population (Urteaga C.R. 2001).

A recent appraisal suggests that no one tool satisfies a set of criteria regarding scientific merit and tools are published with insufficient details regarding their intended use and method of derivation, thus with an inadequate assessment of their effectiveness. (Jones 2002)

However, a very recent study performed in Australia has shown that most validated screening tools perform well in identifying malnourishment in inpatients with the MNA identifying more at-risk patients (Young, Kidston et al. 2012) .

A wide range of estimates for PEM reflects the plasticity and multitude of diagnostic criteria used to screen for and detect malnutrition, as well as the lack of a “gold standard” to establish the presence and degree of malnutrition. Over the past decade, efforts have been made to standardize and cross validate screening and diagnostic tools based on clinical outcomes and controlled prospective studies.

Despite the usefulness of above discussed nutritional screening tools, several studies have suggested lack of awareness and knowledge regarding the availability and usefulness of the tools and insufficient time in the clinical staff’s schedule as barriers to conducting a thorough nutritional assessment on elderly patients. Obviously, a method that is quick and easy will have a better chance of being used. It has been stated by Gaithersburg, MD in Geriatric Nutrition (The Health Professional's Handbook. 2d ed) that standard references for nutritional status are validated on younger populations and the validation studies rarely include older adults.

The need of a gold-standard assessment for the nutritional state in older persons therefore still exists. Development and adoption of national recommendations for a baseline nutritional assessment protocol that is practical for routine clinical settings would greatly facilitate the introduction of nutritional assessment into clinical practice (YN. 2003).

Chapter Three

Determining fat and muscle mass in an older osteoporotic population by the use of total body DXA scan

3.1 Overview:

Dual-energy X-ray absorptiometry (DXA) is a means of measurement of the density of bone (Bone mineral density or BMD). DXA determines BMD in two dimensions, including both trabecular and cortical bone, with the results expressed as areal density (grams per square centimetre). During a DXA scan two focused (X-ray) beams with different energy levels are aimed at the patient's bones. Upon subtracting out measured soft tissue absorption the BMD can be determined from each beam absorbed by bone.

3.2 Use of DXA in Osteoporosis

DXA is the most widely used and most thoroughly studied bone density measurement technology due to its stable calibration, high precision and low radiation dose. Currently, DXA is being considered as a gold standard for the diagnosis of osteoporosis (Watts, Bilezikian et al. 2010, 2012). Densitometry performed by DXA shows the current state of bone mineral content. DXA can measure BMD at the spine, hip, forearm, heel, and in the total body.

DXA has been used for the operational classification of osteoporosis since 1994 as produced by World Health Organization (WHO) which made its basis on BMD measurements taken by DXA (1994). According to this classification osteoporosis and osteopenia has been defined as follows:

WHO classification for diagnosis of osteoporosis using BMD measurements

Classification	T Score
Normal	≥ -1.0
Osteopenia	between -1.0 and -2.5
Osteoporosis	≤ -2.5
Severe or established fractures	≤ -2.5 in the presence of one or more fragility fractures
Osteoporosis	

WHO: World Health Organisation, BMD: Bone Mineral Density.

Here the T score is the number of standard deviations the measured BMD is above or below the young adult reference BMD.

DXA is recommended for all women aged 65 years or over and for younger women with risk factors by the US Preventive Services Task Force also (Nelson, Helfand et al. 2002).

DXA uses X-rays to assess bone mineral density. The radiation dose from DXA scans varies depending on the scanner type and the site measured. The combined effective dose from AP spine, lateral spine, and hip scans on average is typically less than 3 milliSieverts (μSv) ranging from 0.08-4.6 μSv compared to that given by many other investigations involving ionizing radiation. This mean radiation dose is no more than the approximately 3 μSv radiation dose people normally get each year from background environmental radiation including radon and cosmic rays. This background radiation dose could be as higher as 10 μSv at higher altitude areas. Fan beam technology in DXA with increased resolution has resulted in increased patient radiation dose (6.7-31 μSv) but this is still relatively small.

3.3 Limitations of DXA

It is imperative to get repeat BMD measurements for the same patient being performed on the same DXA apparatus or one from the same manufacturer each time. Validation or even cross-validation in between indirect methods cannot guarantee both accuracy and reality precision. Errors between devices or endeavouring to convert measurements from

one standard criterion to another can introduce errors which may obliterate the sensitivity and accuracy of the measurements. It is thus of pivotal importance to determine the precision of all DXA measurements for quality control, result interpretation and patient monitoring.

The diagnosis of osteoporosis varies greatly depending on the measurement site, and on the number of sites measured. The spine is the preferred site for monitoring the response to treatment. Careful interpretation of the spine image and comparison of the T-scores of individual vertebrae is required as the measured BMD may be affected by factors such as vertebral fracture or degenerative changes. Spinal degenerative disease is prevalent among the elderly and may result in an artefactual increase in spine BMD measured in the AP view. Lateral spine DXA selectively measures the trabecular rich vertebral bodies and is less affected by spinal degenerative disease. Lateral spine DXA identifies more osteoporotic patients and is more sensitive to age related bone loss than AP spine DXA. The lateral spine view however, is not available on many DXA systems.

3.4 Total Body DXA

There is an increasing need for accurate and automated tools in the field of body composition measurement. Increased understanding of body composition and its association with disease risk or prevalence (particularly osteopenia and osteoporosis), makes it imperative to accurately assess the body composition. Besides muscle mass, the awareness of the importance of fat content, its distribution and keeping a note of how it changes after an intervention has also necessitated the use of an appropriate tool which could precisely evaluate these parameters in the body.

Total body DXA makes it possible to analyse the total skeleton with its regional parts, as well as it can also measure the soft-tissue composition. It has gained its significance in clinical practice due to its ability to comprehensively view the body composition. Over the past decade, it has been increasingly used not only in research and clinical practice but also in relation to direct treatment.

Total body DXA is based on a three-compartment model. It could therefore assess the three major components of the body which are: fat mass, lean mass, and bone mineral mass, with high precision and low scanning time.

Technically, bone, fat and lean tissues differentially attenuate the photons at two energy levels. The fat and lean mass are measured by the transmission at two energy levels in regions without bone. In regions with bone, the bone and soft tissue are measured, whereas the composition of the soft tissue needs to be estimated with respect to the adjacent tissue values.

Total body DXA measures bone mineral content (BMC), bone mineral density (BMD), fat-free mass (FFM), and provides estimates of percent body fat. The measurement of whole-body adiposity is expressed in grams (g) or percentage (%). The non adipose mass such as lean tissue and bone mineral content (BMC) of the regional (arms, legs and trunk) and total body is expressed in g, whereas regional and total body density is expressed in g/cm².

While there are other rapidly acquisitive imaging modalities available for analyzing the body composition e.g. quantitative computed tomography (QCT) and magnetic resonant imaging (MRI), the total radiation exposure from total body DXA is far less than that from these modalities. This extra scan equates to 0.37 μ Sv. The total radiation exposure therefore from a whole body scan is less than a Chest X-ray which is 0.50 μ Sv and it is also less than standard lateral radiographs i.e., QCT which has radiation dose (25-360 μ Sv) and is widely used in the clinical settings.

Strong correlations between body composition analysis results in whole-body regions from CT, MRI and DEXA have been shown (Kullberg, Brandberg et al. 2009). As the scanner does not have a gantry, the patient compliance level is also higher than CT or MRI, when claustrophobia is considered.

Although CT and MRI is an accurate means of measuring this, DXA is a widely available, very practical and cost effective alternative with lesser radiation for quantifying total body skeletal mass and adiposity.

Scan times for a total body scan using the DXA scanner are usually less than 10 minutes. Reduced scanning times are more convenient for patients and technical staff and have contributed to increased acceptance of DXA measurements in body composition research and clinical practice.

The use of Total body DXA nowadays range from a variety of clinical applications such as identification and prevention of bone, cardiovascular and metabolic diseases and clinical management of different chronic diseases to the monitoring of the impact of treatment regimens on body tissues and bones. Total body DXA is superior to standard DXA due to its ability of providing a better overview of bones both in the form of total body BMC and BMD. It also identifies atypical fractures which are not assessed by ordinary or standard DXA of lumbar spine and hip only.

Following is an overview of the Main Field of Clinical Applications of Whole Body DXA in Adults by Albanese et al 2003(Albanese, Diessel et al. 2003):

1. Nutritional disorders
 - Obesity
 - Overweight
 - Anorexia nervosa
2. Gastrointestinal disorders
 - Chrohn's disease
 - Celiac disease
 - Gastrectomy
3. Hepatobiliary disorders
 - Cirrhosis
 - Gallstones
4. Renal disorders
 - Chronic renal failure
 - Hemodialysis
 - Transplantation
5. Endocrinological disorders
 - Hypopituitarism

- Acromegaly
- Cushing's syndrome
- 6. Bone disorders
 - Osteoporosis
 - Paget's disease
 - Osteopetrosis
- 7. Pulmonary diseases
 - COPD
 - Fibrosis cystic
- 8. Drugs and substances
 - Corticosteroids
 - Hormones
 - Parenteral nutrition
- 9. Other disorders
 - Diabetes
 - AIDS
 - Sympathetic dystrophy syndrome
 - Amiotrophic lateral sclerosis
 - Tetraplegy
 - Duchenne muscular dystrophy

3.5 GE Lunar DXA used in our study

GE Lunar Prodigy is a fan beam system. Fan-beam systems employ multiple detectors that allow for quicker scan acquisition and clearer image resolution with a higher though still minimal radiation dose. The Prodigy employs a narrow fan beam at an angle of 4.5° , orientated parallel to the long axis of the body using peak x-ray energy of 80 kVp, a current of 3 mA and a K-edge filter produce energies at 38 and 70 keV. The Prodigy system employs 16 detectors that are energy sensitive cadmium zinc telluride, 5 cm long, allowing for rapid photon counting. Scan times for a total body scan using the Prodigy are less than 10 minutes. Reduced scanning times are more convenient for patients and technical staff and have contributed to increased acceptance of DXA measurements in body composition research and clinical practice. The instrument automatically alters scan

depth depending on the thickness of the subject, as estimated from age, height, and weight.

GE Lunar Prodigy DXA equipments utilise the enCore graphical interface software to control all aspects of scanning and analysis. This is a Microsoft Windows based application. The version we are running at St.James's hospital is version 10.51 which works under the Windows XP Professional platform. The precision error by default is $\leq 1\%$.

3.6 Analysis of Total body DXA and its parameters in SJH cohort

3.6.1 Introduction:

The nutritional state in the elderly particularly malnutrition and its effects on bone has been discussed at length in Chapter 2 of this thesis. There is substantial evidence that malnutrition of both micro and macro nutrients are directly detrimental to the human skeleton. However, the standard definition of malnutrition doesn't only comprise of a state of under nutrition but it also includes a state of over nutrition (refer to Chapter 2). Over nutrition results in Obesity which is a state of excess fat storage and exhibits a chronic imbalance between energy intake and expenditure.

Similarly under nutrition leads to reduction in skeletal muscle also known as sarcopenia. It is well recognized that human body composition changes with increasing age, however researchers are still seeking for the exact mechanism, causes and outcomes of this change. There is also substantial evidence while body fat mass or adiposity increases with age (Hughes, Roubenoff et al. 2004), there is a significant age related decline in bone and muscle mass (Doherty 2001, Kyle, Genton et al. 2001).

Scientific work in this field has exhibited conflicting results with some work exhibiting fat mass as a major contributor towards BMD (Holecki and Wiecek 2010), while some other work in favour of lean mass to be a determinant of BMD (Sahin, Guler et al. 2006). Therefore, it is not yet fully known whether it is fat or lean body mass that determines the development of bone mass.

These conflicting results suggest a complex effect of body weight with its components, fat mass and lean mass on bone.

Body fat and muscle mass could be calculated by total body DXA scan. GE Lunar Prodigy DXA scanner used in the following study can provide both total and regional analysis of body composition through high resolution, good image quality and enhanced edge detection. Therefore, this study used Total body DXA measurement to assess significance of the different body composition parameters for identifying osteoporosis.

3.6.2 Aims:

The aim of this study was to explore body composition in elderly osteoporotic population to determine the relationship of BMI, body fat parameters and body muscle parameters to bone structure in terms of bone mineral density and bone mineral content.

3.6.3 Materials and Methods:

Study Population

The study comprised of community dwelling and ambulatory elderly population attending the osteoporosis services of St. James's Hospital (SJH). Bone Health and Osteoporosis clinic in St. James's Hospital maintains electronic data base on all the patients attending for osteoporosis management. Bone Health Clinic in SJH is a specialised service for treating osteoporosis and 300 new patients per year attend. It also accepts patients locally and from tertiary centres.

A total of 157 patients were selected for study, out of these 128 female patients had their total body DXA scan and therefore their body composition outcomes were included in the analysis. All subject had a prior diagnosis of established osteoporosis with a T score of ≤ -2.5 on DXA. The exclusion criteria were primary hyperparathyroidism, hypocalcaemia, previous history of neoplasm and poor vital prognosis.

DXA measurements

Total Body dexta scan including whole body composition i.e., total body BMD, Bone Mineral Content (BMC), body fat composition and muscle mass was performed by dual-energy x-ray absorptiometry using Lunar Prodigy (GE Healthcare) which is a fan beam densitometer used in the currently available systems. Phantom calibration was performed on a daily basis. Quality assurance tests were conducted on a daily basis.

All scans were performed while the subjects were wearing light indoor clothing and no removable metal objects. Participants were placed in the supine position on the scanning table with the body aligned with the central horizontal axis. Arms were positioned parallel to the body. Forearms were pronated with hands flat on the bed. Legs were fully extended during the scan acquisition. Height and weight were measured using standardized equipment.

The system software provided the mass of lean soft tissue, fat, and bone mineral for both the whole body and specific regions. DXA was also used to provide BMD at spine, hip and total body. A brief introduction of the parameters involving fat, muscle and bone mineral used in the study is given below:

Body Mass Index (kg/m²)

Body mass index (BMI) is a measure for human body shape based on an individual's mass and height. Body mass or weight was measured by a standard calibrated weighing scale and body height was measured by a standardized stadiometer. BMI was calculated according to the formula:

$$\text{BMI} = \text{weight (kg)} / [\text{height (m)}]^2$$

Android fat percentage (%)

Android fat is the fat distribution of the adipose tissue in the midsection of the body. More particularly it is area between the ribs and the pelvis, and is totally enclosed by the

trunk region. The upper demarcation is 20% of the distance between the iliac crest and the neck. The lower demarcation is at the top of the pelvis. This pattern of fat distribution generally leads to central obesity.

Gynoid fat percentage (%)

Gynoid fat is the fat distribution of the adipose tissue primarily around the hips and upper thighs and overlaps both the leg and trunk regions. The upper demarcation is below the top of the iliac crest at a distance of 1.5 times the android height. The total height of the gynoid region is two times the height of the android region.

Total Body Fat Percentage (%)

Body fat includes essential body fat and storage body fat. Percent body fat is calculated by the following formula: $\text{fat \%} = \left(\frac{\text{fat mass}}{\text{fat mass} + \text{lean soft mass} + \text{bone mineral content}} \right) \times 100$.

Regional Body Fat Percentage (%)

It is the estimate of fat deposition across all five body regions calculated by DXA including android and gynoid, arm (arms and shoulders), trunk (neck, trunk and pelvis) and leg (legs and lateral hip area) regions.

Total body Tissue mass (gm)

Total body mass is the sum of the body's lean mass and fat mass.

Total body Fat mass (gm)

Total body fat mass consists of essential body fat and storage fat. Essential body fat is present in the nerve tissues, bone marrow, and organs (all membranes) and it is essential for normal physiological functions. Storage fat, on the other hand, represents energy

reserve that accumulates when excess energy is ingested and decreases when more energy is expended than consumed.

Lean Body Mass (gm)

It represents the weight of an individual's muscles, bones, ligaments, tendons and internal organs. DXA is a three compartment method to measure lean body mass and fat free mass. Lean body mass differs from fat-free mass. Since there is some essential fat in the bone marrow and internal organs, the lean body mass includes a small percentage of essential fat.

Fat Free Mass (gm)

The sources of essential fat are estimated with body composition measurement devices and these are then subtracted from total body weight to obtain the fat-free mass.

BMC (gm)

Bone mineral content is the amount of mineral in the specific site scanned and, when divided by the area measured, can be used to derive a value for BMD.

Statistical Analysis

All statistical procedures were performed by using IBM SPSS statistics version 19.0 software for Windows.

After testing for normality, parametric or non-parametric tests were used accordingly. The parameters which passed the tests for normality were expressed as the mean and SD and evaluated using the paired Student t-test. Otherwise, the results were expressed by the median and interquartile range (IQR) and evaluated using the Wilcoxon signed rank test.

Correlations between the bone biochemical markers and QUS parameters were assessed using the Spearman rank correlation test. A p value of ≤ 0.05 was considered statistically significant.

3.6.4 Results:

Table 3.1 Mean characteristics and body composition of the whole study population

	Mean $\pm$ SD*
Age (yr)	71.1 $\pm$ 11.5
BMI (kg/m ²)	24.8 $\pm$ 4.7
BMD Spine (g/cm ²)	0.79 $\pm$ 0.13
BMD Hip (g/cm ²)	0.74 $\pm$ 0.12
BMD Total Body (g/cm ²)	0.97 $\pm$ 0.17
BMC (gm)	1855.0 $\pm$ 363.2
Android Fat Percentage	37.0 $\pm$ 11.5
Gynoid Fat Percentage	40.5 $\pm$ 9.1
Total body Fat Percentage	34.6 $\pm$ 8.9
Regional Fat percentage	33.6 $\pm$ 8.7
Total body Tissue mass (gm)	57123.9 $\pm$ 12741.5
Fat mass(gm)	20661.7 $\pm$ 8247.2
Lean Body Mass(gm)	37074.3 $\pm$ 6476.0
Fat Free Mass(gm)	38826.5 $\pm$ 7440.8

*T-Test

Age Results:

Age was significantly negatively associated with Bone mineral content (BMC) independent of lean mass and fat free mass (p 0.003). Age was also significantly negatively associated with Bone mineral density (BMD) at hip (p0.001) and with total body BMD (0.001). There was also a negative association of age with BMD at spine; however, it did not show statistical significance (p0.86).

Age was significantly positively related to fat measures on DXA i.e., Android fat (p0.00), Gynoid fat (p0.04), Total body fat percentage (p0.009), Regional fat percentage (p0.012).

Age was significantly negatively associated with muscle measures on DXA i.e., lean mass (p0.01) fat free mass (p0.007).

A further analysis was performed by dividing the population into different age groups. The age groups and frequency of the patient population according to each particular group has been shown in the graph below.

Considering the different age groups a trend of increase in BMI was observed with increasing age. BMI was significantly higher in the oldest population i.e. 80+ years (mean 27.23 ± 6.23 , (ANOVA p 0.001). This has been illustrated in Fig 3.1 below.

Although another trend of a decrease in lean mass and fat free mass was observed with increasing age particularly in the 80+ age group, it didn't attain a significant value. Similarly a trend of increase in body fat measures was observed with increasing age though not reaching a significance level.

BMI Results:

The relationship of fat mass and muscle mass to bone mass was observed in this study in subjects matched by BMI. According to the proposed cut-off points by a World Health Organization (WHO) expert committee the cohort was divided into underweight (≤ 18.4 kg/m²), normal weight (BMI 18.5-24.9 kg/m²), overweight (BMI 25.0-29.9 kg/m²), and obese (BMI ≥ 30.0 kg/m²) groups.

Table 3.2 shows frequency of patient population falling into each BMI category.

Table 3.3 shows BMI relationship with fat, muscle and bone measurements on DXA.

Looking at a range from low BMI to high BMI, increase in BMI significantly positively correlated with BMD and BMC. The significant relationship of BMI with BMC and different BMD sites has been shown in figures 3.2 and 3.3 below.

Increased BMI was significantly positively correlated with body fat measures indicating parameters on Total Body DXA. This has been shown in fig 3.4 below.

Since there is no single or standard estimate of body fat for all individuals; the mean fat measurements of the 'normal weight' (BMI18-5-24.9) study participants were taken as reference values and the percentage difference of the mean fat measures in ' very obese'(BMI 30+) and 'underweight'(BMI up to 18.5) were then calculated.

This calculation showed:

In comparison with the normal weight population in the cohort there was 39.1% more android fat in the obese population. In contrast, underweight individuals had 71.2% lesser android fat than normal weight individuals.

In comparison with the normal weight population in the cohort there was 24.5% more gynoid fat in the obese population. In contrast, underweight individuals had 39.3% lesser gynoid fat than normal weight individuals.

In comparison with the normal weight population in the cohort there was 32.9% more total body %Fat tissue in the obese population. In contrast, underweight individuals had 52.5% lesser %Fat tissue than normal weight individuals.

In comparison with the normal weight population in the cohort there was 52.3% more regional %fat in the obese population. In contrast the underweight individuals had 33.6% lesser regional fat than the normal weight individuals.

In comparison with the normal weight population in the cohort there was 63.7% more fat mass in the obese population. In contrast, underweight individuals had 79.5% lesser fat than normal weight individuals.

Increased BMI was also significantly positively correlated with Lean mass and fat free mass on total body DXA which indicate muscle mass. This has been shown in detail in the table below:

The mean muscle measurements of the 'normal weight' (BMI18-5-24.9) study participants were taken as reference values and the percentage difference of the mean muscle indicating measures in ' very obese'(BMI 30+) and 'underweight'(BMI up to 18.5) were then calculated.

In comparison with the normal weight population in the cohort there was 12.9% more lean mass in the obese population. In contrast the underweight individuals had 13.0%% lesser lean mass than the normal weight individuals.

In comparison with the normal weight population in the cohort there was 12.5% more fat free mass in the obese population. In contrast the underweight individuals had 18.5% lesser fat free mass than the normal weight individuals.

BMD Results:

BMD spine was positively correlated with BMI (p0.00). BMD was also positively correlated with fat parameters on DXA such as Android fat (p0.004), Total body fat percentage (p0.00), fat mass (p0.004), and also with muscle parameters on DXA such as lean mass (p0.003) and fat free mass (p0.002). BMD was also significantly correlated with BMC (p0.00) and with BMD hip and TB.

BMD Hip was positively correlated with BMI (p0.00). BMI was also positively correlated with fat parameters on DXA such as Android fat (p0.008), Total body fat percentage (p0.002), fat mass (p0.02) and also with muscle parameters on DXA such as lean mass (p0.016) and fat free mass (p0.007). It was also significantly related to BMC (p0.00).

BMD Total body was positively correlated with BMI (p0.00). BMI was also positively correlated with fat parameters on DXA such as Android fat (0.001), Gynoid fat (p0.016),

Total body fat percentage (p0.004), Regional %fat (p0.005), fat mass (p0.001) and also with muscle parameters on DXA such as lean mass (p0.0001) and fat free mass (p0.0001). It was also significantly related to BMC (p0.001) and total body tissue mass (p0.001).

BMC Results:

BMD Spine and Hip were significantly positively associated with BMC (P 0.001 for each).

Fat parameters such as android and gynoid fat (p 0.001 and 0.04), Total body fat percentage (p 0.009), Regional fat percentage (p 0.012) were all positively correlated with BMC.

BMC was significantly positively correlated with BMI (p0.001), Android and Gynoid fat (p 0.001 and 0.02), Total body fat percentage (0.003), Regional fat percentage (0.004), Fat mass (0.001).

Total body fat percentage was associated with bone mass independent of weight. This study also reflects that fat mass represents a positive influence on bone mass in our osteoporotic population independent of lean mass. BMC was significantly positively correlated with Lean mass in gm (0.001) and Fat Free mass in gm (0.001).

In comparison with the normal weight population in the cohort there was 9.2% more BMC in the obese population. In contrast the underweight individuals had 26.1% lesser BMC than the normal weight individuals.

Lean mass and Fat free mass (Muscle parameters) Results:

Lean mass in gm was positively associated with total body tissue mass (0.00) and fat parameters such as with Fat mass (0.00), BMI (0.00), while it was not significantly related with regionally distributed fat such as Regional fat (0.92), Android fat(0.30),

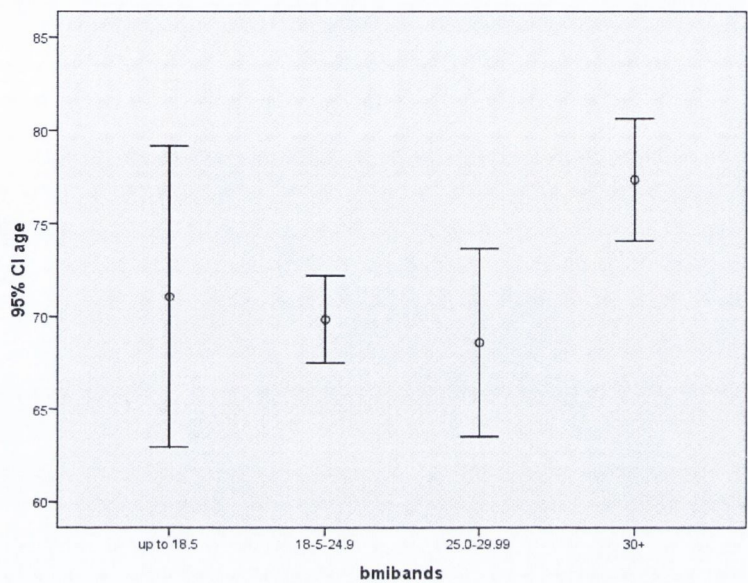
Gynoid fat (0.86), total body fat percentage (0.89) . *Lean mass* was also significantly positively associated with Fat free mass (0.00)

Fat Free mass was positively associated with total body Fat mass (0.00), BMC (0.00) and BMI (0.00). However, it was not significantly related with regionally distributed fat such as Android fat (0.13), Gynoid fat (0.84), Regional %Fat (0.773).

Fracture History Results:

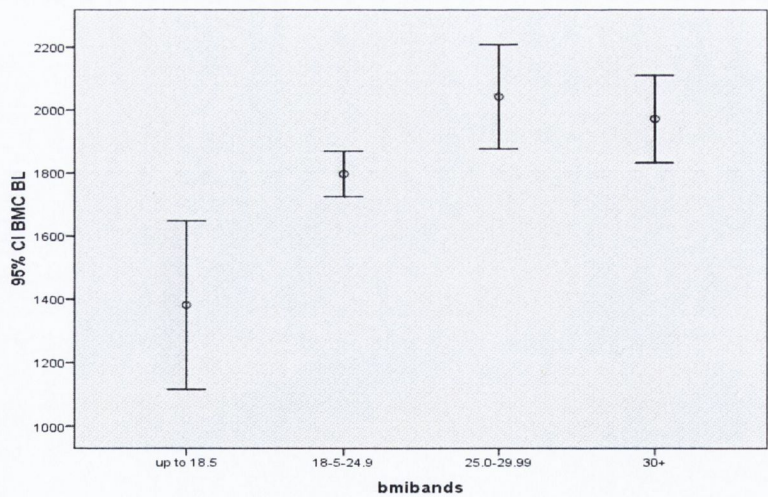
No significant association of the history of hip, vertebral, non vertebral fracture was seen with the fat parameters or lean and fat free mass on the Total body DXA scan, thus expressing fat and muscle being non predictive of fracture risk in the elderly independent of body weight. Past medical and drug history of the patients also had no significant effect of the T scores at hip, spine and total body when calculated.

Figure 3.1 Means with 95% Confidence Interval of Age Vs BMI Bands *



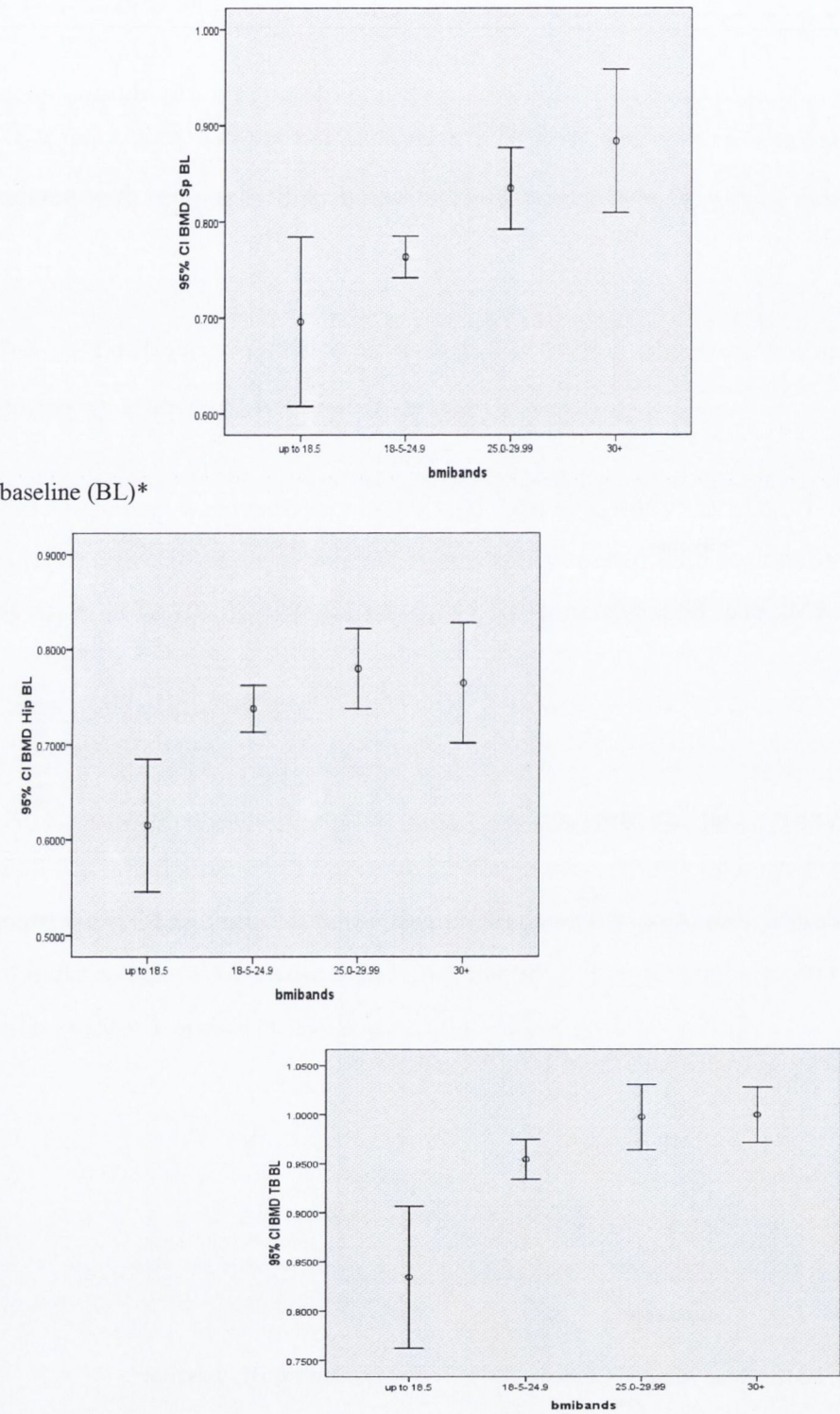
* ANOVA ($p \leq 0.001$), BMI: Body Mass Index

Figure 3.2 Means 95% Confidence Interval of Bone mineral content versus BMI bands at baseline*



* ANOVA ($p \leq 0.001$), BMC: Bone Mineral Content, BMI: Body Mass Index, BL: baseline

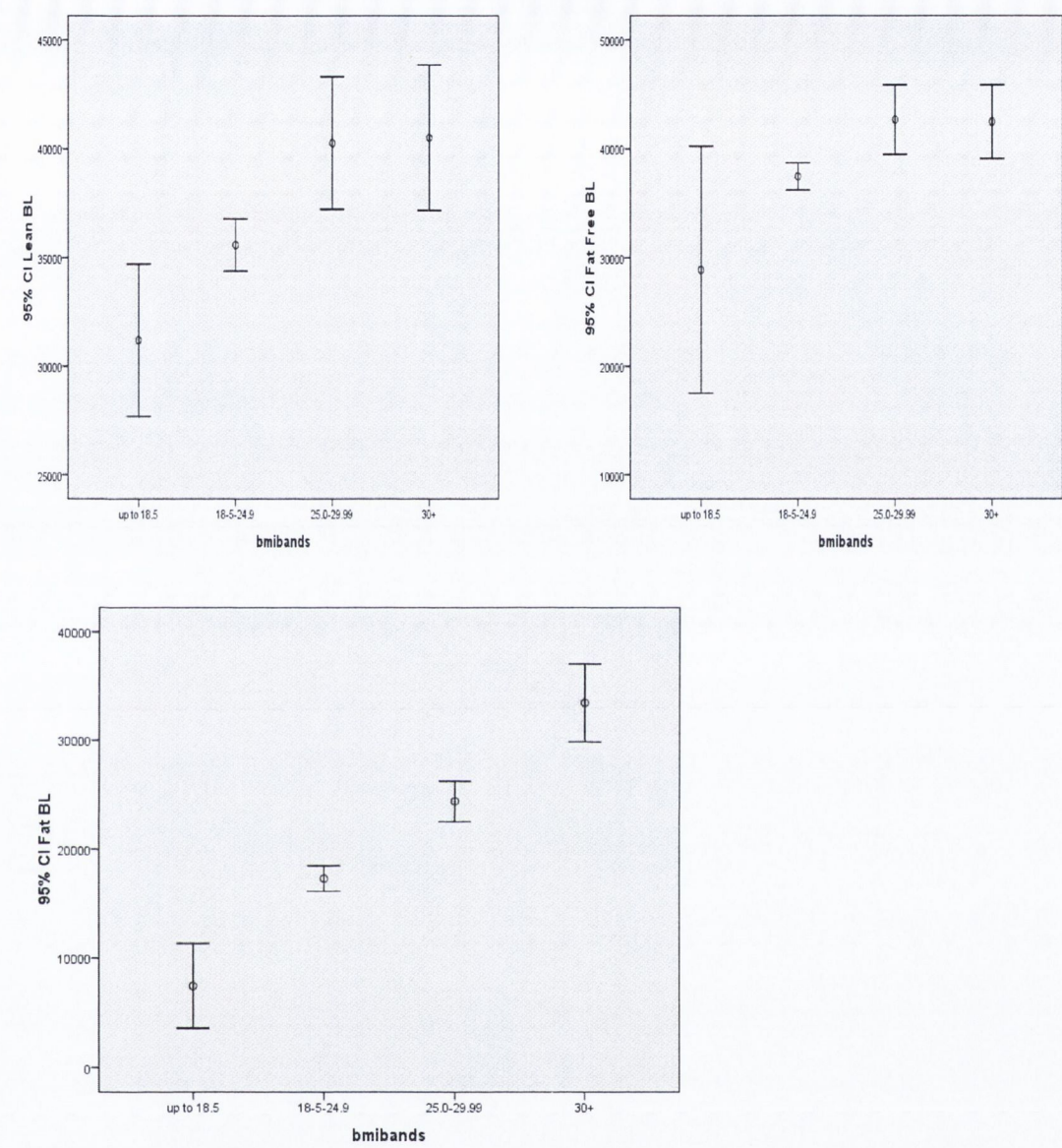
Figure 3.3 Means with 95% Confidence Interval of Bone mineral density (BMD) versus BMI bands at



*ANOVA $p \leq 0.001$

BMD Sp: BMD at spine, BMD TB: BMD at total body, BL: baseline

Figure 3.4 Means with 95% Confidence Intervals of Lean mass, fat free mass, fat mass versus BMI bands at baseline*



*ANOVA $p \leq 0.001$, BL: baseline, BMI: Body Mass Index

Table 3.2 Frequency of study population in each BMI category

BMI (kg/m ²)	%Freq. in cohort
≤18.4	5.7
18.5-24.9	55.4
25.0-29.9	23.5
≥30	15.3

Table 3.3 BMI relationship with fat, muscle and bone measurements on DXA

	Cohort divided into categories according to BMI				P value*
	up to 18.5	18-5-24.9	25.0-29.99	30+	
	Expressed in means $\pm$ SD (95% Confidence Interval)				
Android Fat Percentage	15.8 $\pm$ 9.6 (6.8,24.7)	33.3 $\pm$ 9.0 (31.2,35.4)	42.2 $\pm$ 8.6 (38.9,45.4)	49.5 $\pm$ 6.3 (46.5,52.5)	$\leq$ 0.001
Gynoid Fat Percentage	25.7 $\pm$ 9.5 (16.9,34.5)	38.3 $\pm$ 7.9 (36.4,40.1)	43.5 $\pm$ 7.08 (40.8,46.1)	49.0 $\pm$ 6.67 (45.7,52.2)	$\leq$ 0.001
Total body Fat Percentage	18.8 $\pm$ 9.49 (10.0,27.6)	32.2 $\pm$ 6.97 (30.5,33.8)	37.9 $\pm$ 6.55 (35.4,40.4)	44.9 $\pm$ 6.14 (41.8,47.9)	$\leq$ 0.001
Regional Fat Percentage	18.2 $\pm$ 9.1 (9.7,26.6)	31.1 $\pm$ 6.7 (29.5,32.7)	36.7 $\pm$ 6.3 (34.3,39.2)	43.7 $\pm$ 5.9 (40.7,46.7)	$\leq$ 0.001
Total body tissue mass(gm)	38666.1 $\pm$ 4264.4 (34722.1,42610.1)	52915.5 $\pm$ 7015.3 (51242.7,54588.2)	64654.4 $\pm$ 8722 (61336.7,67972.1)	69308 $\pm$ 0465.5 (58785.6,79830.4)	$\leq$ 0.001
Fat mass (gm)	7448.4 $\pm$ 4185.6 (3577.3,11319.5)	17288.1 $\pm$ 4903 (16119,18457.1)	24386.9 $\pm$ 4904.9 (22521.1,26252.7)	33465.8 $\pm$ 6998.3 (29867.6,37064)	$\leq$ 0.001
Lean mass	31209.7 $\pm$ 3794.4 (27700.4,34719.0)	35568.8 $\pm$ 5018.8 (34390.1,36783.5)	40267.8 $\pm$ 7993.7 (37227.1,43308.4)	40513.2 $\pm$ 6500.8 (37170.8,49855.6)	$\leq$ 0.001
Fat Free mass	2877.4 $\pm$ 12294.9 17506.5,40248.3)	37451.3 $\pm$ 5201.0 (36201.7,38700.5)	42689.9 $\pm$ 8223.3 (39501.2,45878.6)	42485.4 $\pm$ 6593.1 (39095.5,45875.3)	$\leq$ 0.001
BMD Spine	0.695 $\pm$ 0.115 (0.607,0.784)	0.763 $\pm$ 0.1 (0.74,0.78)	0.834 $\pm$ 0.1 (0.79,0.87)	0.883 $\pm$ 0.1 (0.80,0.95)	$\leq$ 0.001
BMD Hip	0.615 $\pm$ 0.9 (0.54,0.68)	0.737 $\pm$ 0.1 (0.71,0.76)	0.779 $\pm$ 0.1 (0.73,0.82)	0.764 $\pm$ 0.1 (0.70,0.82)	$\leq$ 0.001

*ANOVA

3.6.5 Discussion:

The role of nutrition in skeletal mineralization has been discussed at length in Chapter 2 of this thesis. The nutrients known to be significant are not only calcium and vitamin D but also protein and calories. Therefore, any form of malnutrition may also affect total skeleton mineralization.

Age has been associated with decrease in muscle mass and increase in adiposity (Evans and Campbell 1993, Song, Ruts et al. 2004). The exact mechanism behind this observation remains obscure. This age related change in the body composition has been considered as a contributory factor towards osteoporosis in the elderly.

The results of our analysis also show that increasing age is significantly associated with an increase in fat deposition in the body and a decrease in muscle mass.

However, this analysis also exhibits that age related decline in BMC and BMD has been independent of the body muscle and fat composition. This outcome very well exhibits that age is an independent predictor of osteoporosis and any age related gain or loss in adiposity or muscle mass is not the only accountable factor towards osteoporosis in the elderly. Nonetheless fat and muscle remain important determinants of bone mass in the osteoporotic population.

Both Obesity and Osteoporosis are believed to be connected to each other at molecular and cellular levels (Rosen and Bouxsein 2006). This concept is based on the exploration of an overlap between the genetic and environmental factors influencing both diseases and the association of physiological aging with both a high incidence of osteoporosis and bone marrow adiposity (Rosen and Bouxsein 2006).

Hence the relationship between bone and fat metabolism is becoming very topical. It has been substantially exhibited that total body fat mass has positive impact on bone density and it negatively relates to fracture risk. Therefore, any variation in fat mass is positively related with variations in bone density (Reid 2008). Extensive literature has explored that

increased BMI is correlated with high BMD or BMC and that a decrease in body weight leads to bone loss.

Our study exhibited similar results in support of this, where BMI generally had significantly positive influence on BMD hip, spine and total body and also on total body BMC. Moreover, while considering a range from low BMI to high BMI, increase in BMI was significantly positively correlated with BMD at both hip and spine and total body BMC ($P \leq 0.00$ for each).

In contrast to this, patients with high BMI or greater fat deposition in their bodies get osteoporosis too. Our study has shown, while BMI largely increases as adiposity increases, due to differences in body composition, it is not an accurate indicator of body fat; for example, individuals with greater muscle mass also had higher BMIs. The thresholds between "normal" and "overweight" and between "overweight" and "obese" are therefore disputed for this reason.

Accordingly, it must be taken into account that BMI is a general term reflecting a correlation between groups depending on general mass and it can only serve as a non-accurate means of estimating adiposity or fat deposits. Further explaining this, BMI when divided into categories such as low BMI or high BMI doesn't take hold of several other factors such as body size and muscle mass. BMI also doesn't reflect varying proportions of fat, bone, cartilage, water weight etc. therefore it is an inaccurate measure for the lean mass and fat tissue.

For the past several decades obesity has been primarily diagnosed by using the body mass index (BMI). It is well understood now that BMI could well reflect excess weight but it doesn't measure excess fat therefore overestimating adiposity or obesity in individuals with increased muscularity, and it could also well underestimate adiposity in individuals with less lean body mass.

The results of our study replicate the facts mentioned in above paragraph. We calculated the percentage difference between fat and muscle parameters of normal BMI group from

the lowest or highest BMI groups. There were significantly higher and lower percentages of fat in the highest and lowest BMI groups respectively.

On contrast, considering the percentage differences of muscle parameters in these groups had shown that the 'obese' population (according to BMI category) also had considerably higher percentage of muscle than the normal weight population and the underweight population had the lowest percentage of muscle in reference to the normal weight population.

These results also support the outcomes of a US based large study published in 2008 (Romero-Corral, Somers et al. 2008). In this cross-sectional study 13,601 subjects from the Third National Health and Nutrition Examination Survey (NHANES 111) were examined. The results from this study revealed while BMI-defined obesity achieved high specificity (95% for men and 99% for women), BMI showed poor sensitivity (36% for men and 49% for women). Despite this undercounting of obesity by BMI, BMI values in the intermediate BMI range of 20-30 were found to be associated with a wide range of body fat percentages.

This study thus explored that the diagnostic accuracy of BMI to diagnose obesity is limited, particularly for individuals in the intermediate BMI ranges. It was therefore suggested to use direct but simple measures of body fat and measures of body fat distribution to further stratify the individuals in different BMI groups according to their level of adiposity.

Our study reflects that the fat or adiposity reflecting parameters have significant positive effects on bone mineral density and BMC. This effect has been independent of BMI, lean and fat free mass. In our study the regional fat percentage contribution was almost similar as total body fat percentage and also contributory towards better bone mineralization.

Current scientific evidence on direct measurements of body fat parameters has established fat mass as a major contributor towards BMD and this evidence has also linked this contribution with the local mechanisms of osteoblast and adipocyte differentiation, and the hormonal activity of adipose tissue (Holecki and Wiecek 2010).

Contrarily, some studies upon the effect of fat mass on bone have also yielded conflicting results. These studies have negatively linked higher proportions of fat and higher adiposity markers such as serum leptin concentrations with the bone mass (Blum, Harris et al. 2003).

Negative or no correlations of adipose tissue to bone mass have also been observed in independent groups; suggesting that excessive fat mass may not be protective against low bone mass (Janicka, Wren et al. 2007).

Similarly, in a large-scale cross-sectional study of 7137 men, 4585 premenopausal women, and 2248 postmenopausal women, Significant trends were determined for higher risks of osteoporosis, osteopenia, and non spinal fractures with higher percentage body fat (Hsu, Venners et al. 2006). The results of these studies have supported contrasting conclusions when compared to our study and the studies mentioned above (Reid 2008).

Multiple potential mechanisms could also be involved in explaining the interaction between adiposity and bone. One straightforward explanation is that greater fat mass imposes a greater mechanical stress on bone, and in response, bone mass increases to accommodate the greater load.

Thus, fat mass may exert its beneficial effects on the skeleton not only due to its mechanical load but besides this there is likelihood of fat itself acting as an endocrine marker of growth factors responsible for skeletal augmentation. This has been speculated by Garnett whose work has exhibited fat mass relationship with IGF-I and estrogen levels in pre pubertal children (Garnett, Hogler et al. 2004). IGF-I and estrogen are undoubtedly recognized as bone augmenting factors.

Hence, the greater mechanical load exerted on the skeleton by additional weight probably partially explains the correlation between adipose tissue and the skeleton, in addition to this a number of other physiological mechanisms may also uphold this correlation. Likewise, complex genetic backgrounds and other interacting metabolic and regulatory pathways could also sustain the bone and fat mass relationship. Having said that, it is a

complex issue, and this complexity is reflected in divergent results concerning the effect of adiposity on bone tissue.

In addition to above discussion upon relationship of fat or adiposity with osteoporosis, our study has also shown positive influence of lean mass and fat free mass on bones. Although both fat and muscle reflecting parameters in this study were positively correlated with each other, this influence of muscle mass on bones has been independent of adiposity and BMI. It reflects that our study also collaborates well with the previous studies in support of lean mass as an independent contributor towards bone strength.

Researchers have associated lean mass with BMD via biomechanical forces through muscle insertions as a larger cross-sectional area in muscle generally produces more powerful contractions. A recent publication has reinforced this by observing the role of muscle strength and body composition in osteoporosis (Rikkonen, Sirola et al. 2012).

This recent study involved the measurement of muscle strength (grip and isometric knee extension strength) and examination of the lean tissue distribution along with whole body composition in 979 postmenopausal women. The outcomes revealed that both muscle strength and lean mass were independently associated with postmenopausal osteoporosis.

Reduced muscle mass or sarcopenia is with low BMD and osteoporosis in middle aged and elderly males also. It is established in a recent European study of 679 men with age ranging from 40-79 years. In this study sarcopenia was associated with a 3-fold higher risk of osteoporosis (OR=3.0; 95%CI= 1.6, 5.8) compared with those with normal muscle parameters. Interestingly, likewise our analysis higher fat mass was also positively associated with BMD in this study (β coefficient 0.020, 95%CI= 0.008, 0.032).

Despite all the independent mechanisms behind positive influence of fat and muscle on bones, these parameters could well be interrelated to each other. Researchers have highlighted the need for further larger studies in future to alleviate the ambiguity of fat and lean mass effects on skeletal health (Zhao, Jiang et al. 2008).

3.6.6 Conclusion:

To preserve BMD, maintaining or increasing lean mass would appear to be an appropriate strategy for avoiding osteoporosis and its complications. Further studies on the mechanisms leading to sarcopenia could provide the basis for prevention and establishment of therapeutic methods that would contribute to an increase in the standard of living among elderly people.

Our study collaborates with the literature available in support of positive influential effect of lean mass and adiposity in osteoporosis however the inconsistent findings reflected in other studies intricate this relationship and therefore necessitate a new approach to alleviate this obscurity.

Longitudinal studies with large sample size, powerful design, and careful data analysis will be needed to conclusively show the true effect of fat mass and lean mass on bone. Continued work in this area will generate substantial research interest in the upcoming years, and it will help identify the factors leading to the development of therapeutic interventions that can be used to treat osteoporosis and malnourishment simultaneously. It may also become a non invasive method for determining appropriate nutritional support.

Malnourishment by strict definition hereby ranges from under nutrition to over nutrition as previously discussed. The age related changes in body composition involving excessive fat accumulation and concomitant muscle and bone loss could be prevented by attaining a healthy life style and maintenance of calorie intake and energy intake balance. The role of weight maintenance at a healthy level in the prevention of osteoporosis therefore remains a significant proposal and it needs to be widely escalated.

Finally, our study reflects that total body DXA provides a reasonable estimate of body composition in people of different weights ranging from low body weight to obesity. It may also become a non invasive and quick method for determining appropriate nutritional status. Total body DXA gives a significant amount of information in a short time which would otherwise be obtained by a trained clinical nutritionist requiring more time for detailed assessments and cost for different anthropometric measures.

Chapter Four

Treatment of osteoporosis with recombinant Parathyroid Hormone, its effects on total body composition, and factors determining response to this therapy.

Analysis of Total body DXA and change in its parameters on rPTH in SJH cohort

4.1 Introduction

The therapeutic use of recombinant parathyroid hormone in osteoporosis has been discussed at length in chapter one of this thesis. Intermittent administration of recombinant parathyroid hormone (rPTH) has exhibited significant anabolic effects on human skeleton in terms of bone mineral density (BMD) gain at both spine and hip regions of patients as shown on their DXA scans (Compston 2007, Black, Bouxsein et al. 2008, Boonen, Marin et al. 2008). Besides the BMD effect of rPTH, there are certain other potential effects of rPTH which remain to be studied. These would include total body muscle and fat composition and total body BMD. In addition, other factors which might affect treatment response need to be considered such as previous drug or fracture history or other medical co morbidities.

It is well recognized that excess endogenous parathyroid hormone has been associated with a risk of sarcopenia (Visser, Deeg et al. 2003). However, no information is available on the effects of exogenous recombinant PTH and its effects on total body muscle and fat composition measured by total body DXA scan. There is also a lack of evidence in relation to total BMD gain on DXA scan after use of rPTH in osteoporosis.

Likewise, it is also recognized that a certain number of patients fail to respond to rPTH treatment. It's our own personal experience at St.James's hospital that certain number of patients doesn't respond to rPTH treatment. Currently a bone mineral density (BMD) gain shown on the DXA scan at the completion of treatment is used to identify a response to rPTH treatment. As bone markers correlate well with BMD they could be used to identify an inadequate response to treatment at an early stage in the treatment.

The focus of this research chapter is to identify potential factors that might contribute to a suboptimal response to rPTH treatment.

4.2 Aims

The objectives of this investigation were:

- To identify any biochemical parameters potentially associated with inadequate response to rPTH.
- To identify what percentage of patients will inadequately respond to rPTH treatment?
- To identify whether total body fat and muscle composition at baseline could affect the response to rPTH treatment.
- To identify whether body composition in particular changes during rPTH treatment.

4.3 Materials and Methods:

4.3.1 Study Population:

Ethics permission was sought from AMNCH/SJH ethics committee prior to treatment of the patients with rPTH. All the patients included in the study were attending the Osteoporosis Services of St. James's Hospital (SJH). The Bone Health and Osteoporosis clinic in St. James's Hospital maintains a data base on all the patients attending for osteoporosis management. Bone Health Clinic in SJH is a specialised service for treating osteoporosis and 300 new patients per year attend. It also accepts patients locally and from tertiary centres.

The referral sources of the patients included in the study were as follows:

- General practitioners and hospital based consultants
- Respiratory clinic for patients with steroid induced osteoporosis
- Gastroenterology clinics including coeliac and inflammatory bowel clinics
- Orthogeriatric team referrals for inpatient elderly population with fractures
- The clinical nurse specialist led osteoporosis pre assessment clinic.
- Patients attending the Bone Health Service who had failed to response to previous therapy for osteoporosis.

Inclusion criteria

Patients with established osteoporosis and those with osteoporosis induced fragility and vertebral fractures were considered suitable unless they had one or more of the exclusion criteria.

Exclusion criteria

1. Hypersensitivity to PTH or any of its excipients
2. Pre-existing hypercalcemia
3. Severe renal impairment: Glomerular filtration rate less than 30mls/min

4. Metabolic bone diseases other than primary osteoporosis (including hyperparathyroidism and Paget's disease of the bone)
5. Unexplained elevations of alkaline phosphatase
6. Prior external beam or implant radiation therapy to the skeleton
7. Patients with skeletal malignancies or bone metastases
8. Renal stones in the last 5 years
9. Alcohol abuse
10. Unfused epiphyses
11. Pregnancy

Additional exclusion criteria for the purpose of this study included

1. Elevated endogenous PTH levels
2. Significant hepatic impairment
3. Severe cognitive impairment
4. History of significant non-vertebral fracture (hip fracture) within the last 6 months.

Patient training and education

All patients were given instructions on the injection pen use by trained nursing staff. They were advised on potential side effects and details for contacting clinical nursing staff were given to each patient. Their GPs were informed about their treatment. Any other osteoporosis treatment was discontinued before commencing rPTH. All the individuals were given the same dose of calcium and vitamin D supplements i.e., 1000 mg of calcium and 800IU of cholecalciferol on commencement of rPTH treatment. The patients were regularly followed up for assessment and monitoring of treatment compliance.

4.3.2 Total body DXA assessment

Total Body DXA scan was performed using a Lunar Prodigy (GE Healthcare) and this included total body BMD, Bone Mineral Content (BMC), body fat and muscle composition. The specifications and methodology involved in use of this DXA scanner are explained in chapter 3 of this thesis. A total body DXA scan was performed at baseline and at 1 yr. on rPTH.

In addition to total body composition, parameters specific at regions of interest were also examined such as lean tissue mass, body fat, and bone mineral content .DXA was also used to provide BMD at spine, hip and total body. A brief introduction of the parameters involving fat, muscle and bone mineral used in the study has been given in chapter 3 of this thesis.

The parameters examined in this analysis with the relevant values are expressed as follows:

Android fat percentage (%), Gynoid fat percentage (%), Total Body Fat Percentage (%), Total body Tissue mass (Gm), Total body Fat mass (Gm), Lean Body Mass (Gm), Fat Free Mass (Gm), Bone mineral content-BMC (Gm), Bone mineral density- BMD (gm/cm²).

Radiological definition of a non responder:

Definition of a non responder was determined by 1 year BMD response at the spine on DXA. A non responder was defined by a failure in achieving BMD gain of 3% at the spine by the end of the 1year treatment. A 3% change in BMD corresponds to the least significant change (LSC) necessary in order to provide 95% confidence interval that the measured change in BMD for an individual patient is a true biologic change, and that it does not simply reflect the inherent imprecision of the DXA scanner (Baim, Wilson et al. 2005). The value above thus ignores BMD gain as opposed to variability secondary to a

precision error. This 3%BMD gain at spine is accepted internationally and it is used in osteoporosis research (Sebba, Bonnick et al. 2004).

4.3.3 Study design

128 patients were selected to prospectively participate in this study. The patients in this study were divided into two main groups at 1 year of the treatment. Patients with a BMD gain of 3% or more, 1 year after the treatment were considered to be responders.

Inversely, patients who failed to show a gain of 3% or more in their BMD at 1 year of treatment were placed in the non-responder group.

The changes in BMD were expressed as the percentage change from baseline and after 1 year of the treatment.

4.3.4 Biochemical Measurements

Blood sample measurements taken from these patients at baseline, 6mth and 1 year of the treatment were used in the analysis. Samples were taken during attendance at a scheduled clinic.

The biochemical variables assessed in the study were: serum markers of bone turnover (osteocalcin- OC, procollagen type I N-terminal propeptides- PINP, serum type I collagen C- terminal telopeptide breakdown products- CTX) and other biochemical variables (serum parathyroid hormone- PTH, Vitamin D, serum corrected calcium- Ca, serum phosphate- PO₄, 24 hour urinary calcium- 24hrUrCa). In this study Hypercalciuria was defined according to the international standards which suggest a value of 24 hr Urinary calcium >7.5 mmol/24hr for male and >6.25mmol/24hr to establish hypercalciuria.

The blood samples for the biochemical bone markers were spun and separated immediately, with the serum being frozen within 1 hour at -70 °C. Routine biochemistry was performed by standard techniques. Biochemistry and immunoassays were performed

on Roche Modular Cobasc system which is a biochemical and immunoassay automated analyser into a single integrated system. Vitamin D was analysed using a chemiluminescence immunoassay by Applied bio system API 4000. Bone markers including CTx, P1NP, OC and PTH were analysed by Roche Modular Cobasc system by using chemiluminescence enzyme immunoassay utilizing the commercial kits.

4.3.5 Statistical analysis

Baseline characteristics of the study population were expressed as the mean and standard deviation (SD). The difference between the total body, hip and spine BMD, fat composition and muscle mass at baseline and, at 12mth on PTH treatment was calculated for the patients. The changes in BMD were expressed as the percentage change from baseline and at 1yr of the treatment.

1 sample KS was applied to assess normality of the different parameters. After testing for normality, parametric or non-parametric tests were used accordingly. Changes that passed the tests for normality were expressed as the mean and SD and evaluated using the paired Student t-test. Man Whitney u test was used for non-parametric measures. For correlations, spearman was used for non-normal and Pearson was used for normal distribution. A p value of ≤ 0.05 was considered statistically significant.

The effects of past medical history, drug history and fracture history on the two groups; responders and non-responders were separately analysed by using descriptive cross tabulations; Chi square test was used for this method. Two-sample Student's t-test was performed to determine statistical differences between means. Logistic regression and step- wise multiple regression analyses were used to evaluate the relationships between the variables.

4.4 Results:

Regardless of excellent team-work and reassuring approach towards our patients, the dropout rate was 16% at 1 year on treatment. However, despite some side effects experienced by the patients, in general, the majority of patients tolerated this drug very well. Overall the patients in the study were satisfied and contented with the level of care provided and the drug itself.

The most common reasons for drop out were as follows in a descending order of frequency:

- Flu like symptoms including headache and dizziness- 4%
- Nausea and dyspepsia- 4%
- Compliance issues to treatment- 3%
- Pre-existing diagnosis of malignancy not disclosed by the subjects before recruitment- 2%
- Hypercalcemia- <1%
- Hot flushes/ skin rash- <1%
- Treatment refusal for nonspecific reasons- <1%

Out of the total of 128 patients only those individuals were selected for analysis that had been compliant with rPTH at 1 year and besides they also had complete sets of their biochemical bone markers and total body DXA scans. The total number of individuals included in the analysis was therefore reduced to 51 female patients. Out of these 51 patients *16 were non responders and 35 were responders to rPTH* according to the definition mentioned in methodology.

4.4.1 Results on Past Medical and drug history:

The coexisting medical conditions and previous medication use including bone medications were not statistically different among the responder and non responder groups. The frequencies of different co morbidities and previous medication use are expressed in table 4.1

Table 4.1 Past Medical History and medication use in responders and non responders at baseline

	Responders	Non - Responders
<i>Past Medical History</i>		
COPD	11.4%	6.3%
Hypothyroid	11.4%	6.3%
Hyperthyroid	5.7%	0%
Diabetes Mellitus	8.6%	6.3%
Arthritis	2.9%	0%
CRF	0%	0%
Malabsorption	11.4%	6.3%
<i>Smoking Status</i>		
Current Smoker	8.6%	0%
Ex Smoker	11.4%	31.3%
<i>Relevant med use</i>		
Steroid inhalers	8.6%	12.5%
Steroid Oral	2.9%	0%
Eltroxin	8.6%	6.3%
Diabetic meds	5.7%	6.3%
Loop Diuretics	2.9%	12.5%
Thiazide Diuretics	2.9%	6.3%
Statins	14.3%	12.5%
Antidepressants	2.9%	31.3%
Neuroleptics	2.9%	26.7%
<i>Bone Medications</i>		
rPTH	0%	0%
Denosumab	0%	0%
Vit D or analogues	2.9%	0%
HRT or related meds	0%	0%
Strontium Ranelate	8.6%	12.5%
Ibandronate	2.9%	12.5%
IV Bisphosphonates	2.9%	0%
Risedronate	31.4%	31.3%
Alendronate	22.9%	31.3%
Ca/Vit D supplements	74.3%	93.8%

* Chi_squared comparison of proportions P value: ≥ 0.05

4.4.2 Biochemistry and Biochemical Bone marker results:

Table 4.2 shows means and standard deviation of the serum biochemistry and serum biochemical bone markers of the whole cohort at baseline, 6months and 1 year of rPTH treatment. The difference overtime among all serum biochemical measurements including bone formation markers between the two groups is shown in table 4.3

The difference in biochemistry of the responder and non responder groups was measured at Baseline, 6 months and 1 yr on rPTH treatment. Due to increased variability of bone formation markers (P1NP and Osteocalcin) among individuals, percent change was considered to observe the difference between these values.

Bone turnover markers P1NP, OC and CTx all were positively correlated to one another at baseline ($p < 0.00$ for all). There was a significant increase with time in Percentage change in OC and percentange change in P1NP among the whole cohort and this increase was at its highest at 6months of treatment ($p \leq 0.01$). However these measures did not show a statistically significant difference between responder and non responder groups at 6months and 1Year (Table).

Serum corrected calcium was raised over time, particularly at 6months in the whole cohort ($p \leq 0.01$) as shown in Fig 4.3. Although, there was a trend of increased serum calcium over time in both groups, it was not significantly different between the two groups (Fig 4.4)

Other biochemical measures including serum magnesium, phosphate, vitamin D and PTH did not show any significant difference over time between the two groups.

24hr Urinary calcium was significantly increased in the whole cohort over duration of study ($p \leq 0.01$) as shown in fig 4.1. There was no significant difference at baseline in 24hr Urinary calcium between the two groups. The mean scores for 24 Hr Urinary calcium was significantly increased at 6 months in the responder group ($p \leq 0.0005$) as shown in fig 4.2.

24 hr urinary calcium was also positively correlated with serum magnesium ($p \leq 0.01$) and it was negatively correlated with OC ($p \leq 0.01$) and P1NP ($p \leq 0.01$).

Table 4.2 Biochemical changes overtime in the whole cohort

Units		Baseline	6 months	1 year
Mean ± SD*				
24hr Urinary Calcium	(mmol/l)	3.4±2.4	5.4±2.5	4.9±2.3
Serum calcium	(mmol/l)	2.26±0.1	2.46±0.2	2.44±0.2
Serum Vitamin D	(nmol/l)	75±28.8	71±17.1	70±17.1
Serum PTH	(pg/ml)	36.75±19.4	74.7±120.0	43.07±68.7
Serum Phophate	(mmol/l)	1.73±4.4	1.01±0.1	1.07±0.1
Serum Magnesium	(mmol/l)	0.8±0.08	0.74±0.1	0.74±0.08
Serum Osteocalcin percentage change	%		321.39±314.9	278.24±221.3
Serum PINP percentage change	%		656.23±863.0	470.07±613.8

*T-Test

Table 4.3 Biochemical changes overtime between Responders Vs Non Responders

	Reference Values	N	Baseline	6 months	1Yr	P Value*
24hr Urinary Calcium	2.5-7.5mmol/l					
Non Responders		16	4.6±2.7	5.3±1.5	5.2±1.9	0.03
Responders		35	2.87±2.07	5.5±2.8	4.8±2.4	
Serum calcium	2.2-2.7mmol/l					
Non Responders		16	2.26±0.1	2.5±0.2	2.5±0.3	0.24
Responders		35	2.26±0.1	2.4±0.1	2.4±.1	
Serum Vitamin D	50-80nmol/l					
Non Responders		16	71.5±21.0	72.4±18.8	70.8±14.7	0.54
Responders		35	77.8±31.8	71.2±16.6	69.9±18.3	
Serum PTH	10-65pg/ml					
Non Responders		16	32.3±9.2	50.4±63.3	60.0±110.3	0.20
Responders		35	38.7±22.4	85.8±137.9	35.3±37.08	
Serum Phosphate	0.8-1.4mmol/l					
Non Responders		16	0.97±0.2	1.02±0.4	0.98±0.2	0.43
Responders		35	2.08±5.3	1.01±0.1	1.05±0.1	
Serum Magnesium	0.7-1.0mmol/l					0.20
Non Responders		16	0.80±0.08	0.79±0.08	0.75±0.1	
Responders		35	0.81±0.08	0.71±0.09	0.74±0.06	
Serum Osteocalcin percent change	%					
Non Responders		16		P value 0.65**	263.3±192.3	P value 0.74**
Responders		35			285.0±235.7	
Serum P1NP percent change	%					
Non Responders		16		P value 0.87**	303.1±275.9	Pvalue 0.19**
Responders		35			546.3±708.1	

* ANOVA with repeated measures with a Greenhouse-Geisser correction

Fig 4.1 24 hr Urinary calcium at baseline, 6months and 12 months of rPTH*

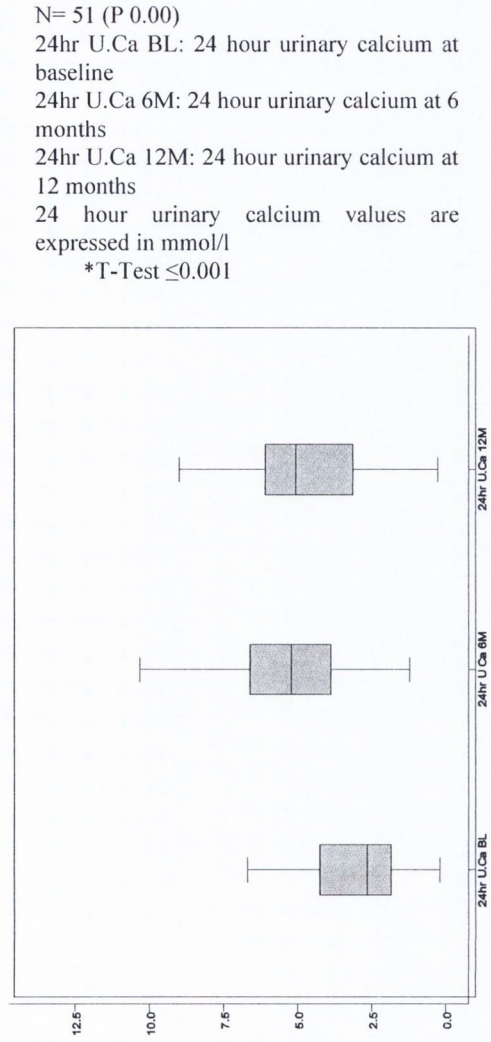
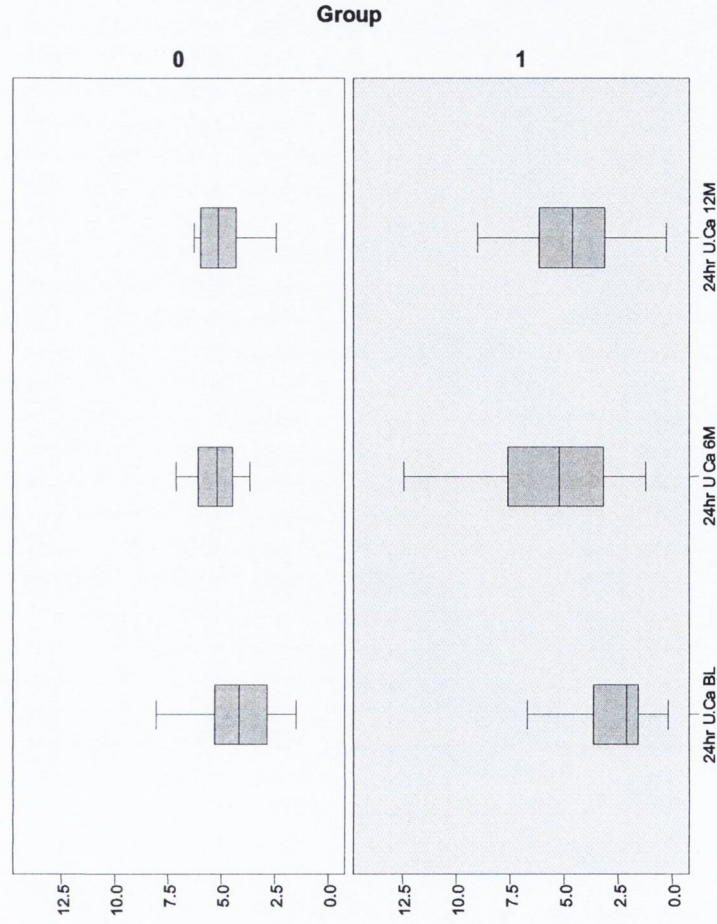


Fig 4.2 24 hr Urinary calcium in responders (1) and non responders (0) at baseline, 6months and 12 months of rPTH*



* ANOVA with repeated measures with a Greenhouse-Geisser correction $p \leq 0.03$

Fig 4.3 Serum Corrected Calcium changes in the whole cohort overtime*.

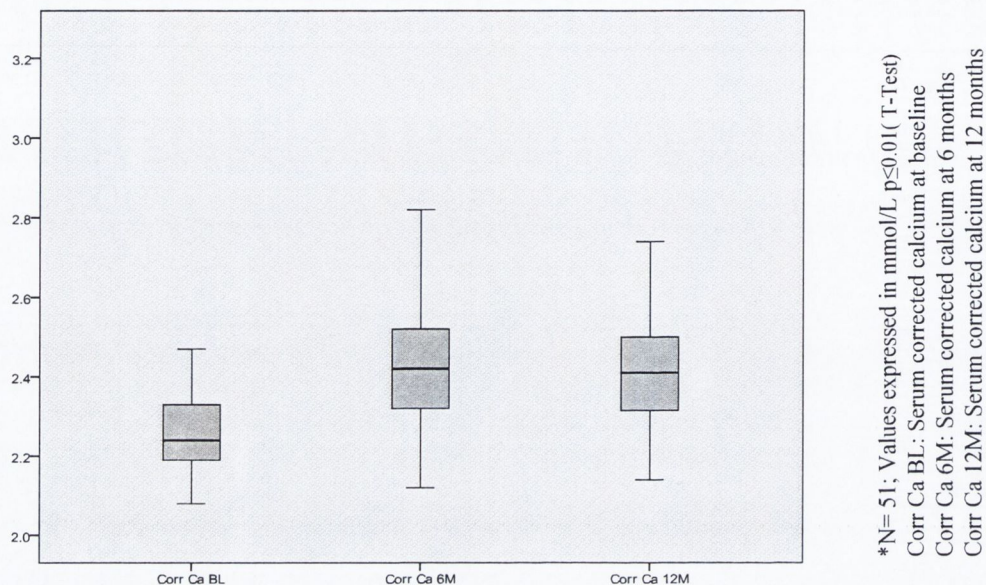
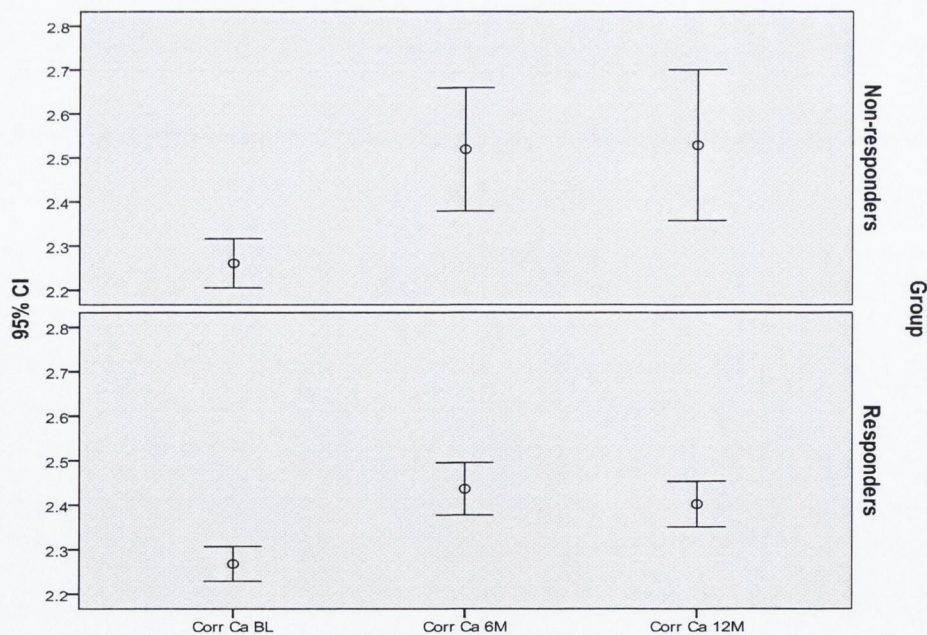


Fig 4.4 Serum Corrected Calcium changes in both groups considering means with 95% confidence interval*.



*N= 51; Values expressed in mmol/L $p \geq 0.05$ (ANOVA)
Corr Ca BL: Serum corrected calcium at baseline
Corr Ca 6M: Serum corrected calcium at 6 months
Corr Ca 12M: Serum corrected calcium at 12 months

4.4.3 Body Composition results on total body DXA:

Table 4.4 shows all parameters expressing total body composition in the whole cohort. Measurements are expressed in mean $\pm$ standard deviation (SD) and standard error of means (SEM). The difference in total body composition between two groups is shown in Table 4.5 (expressed as mean $\pm$ SD and SEM) and Table 4.6 (expressed as mean rank).

All parameters at baseline and 1 year were significantly correlated in pairs $P < 0.00$. On calculation of the mean difference in the total body DXA parameters among the whole cohort from baseline to 1 yr, rPTH made no significant difference to the fat and muscle of the population at 1 year. It showed significantly increased BMD spine at 1 year (mean $\pm$ SD from 0.78 ± 0.1 at baseline to 0.85 ± 0.1 at 1 year $P < 0.00$). BMD at hip and total body did not get the significant P value. However, at 1 year on rPTH treatment there was a trend in an increase in these parameters also in the general cohort. (Table 4.4)

BMD and Fat parameters did not show a statistically significant difference among the two groups at baseline (Table 4.5)

BMC and muscle mass parameters did not show a statistically significant difference among the two groups at baseline (Table 4.6)

The percentage change from baseline at 1 year in the total body composition is shown in Table 4.7 (expressed as mean $\pm$ SD and SEM) and Table 4.8 (expressed as mean rank).

When the cohort was divided into responder and non responder group, BMD percentage gain at hip in the responder group was significantly higher (mean 1.4 ± 4.2) as compared to the non responder group (mean -1.7 ± 3.1) ($p < 0.01$) . No significant differences in percentage change in the other parameters were observed between both groups (Table 4.7)

The Percentage change in Lean mass, Fat free mass and Bone mineral content (BMC) were significantly lower in the non responder group (Table 4.8)

Table 4.4 Total Body Composition at baseline and 1yr for the whole cohort*

	Baseline	1 Year	P Value**
	Mean± SD (SEM)		
Android fat	38.74±10.6 (1.4)	37.91±12.1 (1.7)	0.29
Gynoid fat	42.62±8.4 (1.1)	39.94±10.4 (1.4)	0.07
TotalBodyFat Percentage	36.31±8.6 (1.2)	36.13±9.1 (1.2)	0.65
RegionalFat Percentage	34.83±8.6 (1.2)	35.52±9.3 (1.3)	0.40
Tiissue mass	55644.6±12739.2(1783.8)	56366.96±13756.7(1926.3)	0.66
TotalBodyFat mass	21222.14±7684.0(1075.9)	21433.57±8646.6(1210.7)	0.52
Lean Body mass	35651.76±6274.5(878.6)	35082.54±7613.5(1066.1)	0.45
Fat Free mass	37573.94±6399.3(896.0)	37605.75±5921.3(829.1)	0.91
Bone mineral content	2243.0±2739.4(387.4)	1840.72±392.8(55.5)	0.29
BMD Hip	0.756±0.10 (0.0)	0.759±0.11 (0.0)	0.46
BMD Total body	0.967±0.07 (0.0)	0.961±0.08 (0.0)	0.33
BMD Spine	0.789±0.1 (0.0)	0.853±0.1 (0.0)	0.000

N= 51; Age: 71.1 ± 11.5

*Means± Standard Deviation and Standard Error of Mean

**Paired Samples T Test

Table 4.5 Total Body Composition of Responders Vs Non Responders at baseline*

	Group	N	Mean± SD	SEM	P Value**
BMD Spine	Non-responders	16	0.83±0.08	0.02	0.03
	Responders	35	0.76±0.11	0.01	
BMD Hip	Non-responders	16	0.78±0.12	0.03	0.23
	Responders	35	0.74±0.10	0.01	
BMD Total body	Non-responders	16	1.00±0.09	0.02	0.03
	Responders	35	0.95±0.06	0.01	
TotalBodyFat%	Non-responders	16	37.35±9.01	2.25	0.56
	Responders	35	35.83±8.51	1.43	
RegionalFat%	Non-responders	16	36.38±8.91	2.22	0.57
	Responders	35	34.12±8.51	1.43	
Tiissue mass	Non-responders	16	61342.38±13144.55	3286.14	0.02
	Responders	35	53040.00±11839.51	2001.24	
TotalBodyFat mass	Non-responders	16	23227.94±7963.05	1990.76	0.21
	Responders	35	20305.20±7489.71	1265.99	

*Mean± Standarad deviation and Standard error of mean among groups

** Independent samples T test

Table 4.6 Total Body Composition of Responders Vs Non Responders at baseline*

Group		N	Mean Rank	Sum of Ranks	P Value*
Lean Body mass	Non-responders	16	29.75	476.00	0.2
	Responders	35	24.29	850.00	
Bone Mineral Content	Non-responders	16	31.22	499.50	0.09
	Responders	35	23.61	826.50	
Fat Free mass	Non-responders	16	28.13	450.00	0.2
	Responders	33	23.48	775.00	

* Mann-Whitney U test

Table 4.7 Percentage change in body composition of two groups at 1 year*

Group		N	Mean± SD	SEM	P Value**
TotalBodyFat%	Non-responders	16	-0.21±7.2	1.8	0.86
	Responders	35	-0.72±10.8	1.8	
RegionalFat%	Non-responders	16	13.19±20.3	5.0	0.41
	Responders	35	6.98±26.9	4.5	
TotalBodyFatmass	Non-responders	16	0.08±9.2	2.3	0.47
	Responders	34	-0.03±14.2	2.4	
BMD Hip	Non-responders	16	-1.73±3.1	0.7	0.01
	Responders	35	1.40±4.2	0.7	
BMD Total body	Non-responders	16	-0.95±6.3	1.5	0.63
	Responders	35	-0.30±3.3	0.5	

* Mean± Standarad deviation and Standard error of mean among groups

** Independant sample T test

Table 4.8 Percentage change in body composition of two groups at 1 year

	Group	N	Mean Rank	Sum of Ranks	P Value*
Android fat	Non-responders	16	28.13	450.00	0.2
	Responders	35	25.03	876.00	
Gynoid fat	Non-responders	16	30.34	485.50	0.07
	Responders	35	24.01	840.50	
Tissue mass	Non-responders	16	20.94	335.00	0.05
	Responders	35	28.31	991.00	
Lean Body mass	Non-responders	16	19.25	308.00	0.01
	Responders	35	29.09	1018.00	
Bone mineral content	Non-responders	16	19.03	304.50	0.02
	Responders	33	27.89	920.50	
Fat Free mass	Non-responders	16	19.06	305.00	0.02
	Responders	33	27.88	920.00	

* Mann-Whitney U test

4.5 Discussion:

This study reflects that all the patients being treated with rPTH did not improve bone strength by exhibiting a certain level of BMD gain and therefore they did not achieve optimal skeletal benefit despite of being compliant to the treatment.

It has been established in the past that both compliance and sufficient calcium and vitamin D supplementation are the primary requirements to attain a response to the osteoporosis treatment and additionally, the other requirement is a treatment period of at least one year to allow sufficient time for the treatment to be fully effective (Diez-Perez and Gonzalez-Macias 2008). Both of these requirements were fulfilled by the patients in our study as only those participants were included in the analyses who were fully compliant with rPTH at 1 year.

The coexisting medical conditions, bisphosphonate or other osteoporotic medication use and any other drug history did not have any detrimental bone effects in our study cohort and there was a general trend in increase in BMD at 1 year of the treatment. Exogenous PTH induced positive effects on BMD and markers of bone formation in postmenopausal women with established osteoporosis have already been established regardless of previous long-term exposure to antiresorptive therapies. (Boonen, Marin et al. 2008). Conclusively, past medical and drug history also could not be marked for being responsible for a poor response to the treatment.

Monitoring the effects of therapy suggests whether the drug is having its expected skeletal response or not. However, there is very limited availability of the evidence determining the factors responsible behind a suboptimal or no response. Therefore, clinical trials should be carried to attain a pragmatic approach evaluating the contributory factors towards an inadequate treatment response. This will subsequently enable either to prevent or treat those factors or at least to make changes in the therapy in patients who have shown a deterioration or less expected change in their bone density. This study was an effort to determine any potential predictors of inadequate response to rPTH which in general is a very potent and useful anabolic drug to treat osteoporosis.

Serum biochemical markers of bone turnover were included in the analysis due to their significance both in clinical literature and in practice. Biochemical bone markers have been used by the researchers in the field of osteoporosis since a decade. These are proven as an approach to predict individuals at fracture risk with accelerated bone loss, to identify appropriate treatment options for these individuals, to assess relative efficacy of treatment and to promote treatment compliance (Szulc and Delmas 2008, Garnero 2009). Despite, studies are not particularly suggestive of any guidelines concerning the use of bone turnover markers in monitoring anti-osteoporotic therapy in population (Szulc and Delmas 2008).

Our study demonstrates that changes in biochemical bone markers are not always indicators of degree of response to rPTH treatment. Therefore accepting the great use of these biochemical bone markers in osteoporosis as mentioned above, none of these is a definite indicator of response to rPTH treatment in Osteoporosis. Some correlation

between early bone markers rises and late BMD (bone mineral density) response in collective group of patients has been mentioned in the literature available, still it fails to identify an individual patients' response to treatment.

We have been seeking for the predictors of response to osteoporosis treatment at St.James's Hospital (SJH). Work from this research on different cohorts has been presented in different national and international meetings (Najia, Martin et al. 2011, Siddique, Chan et al. 2011, Najia, Kin et al. 2012, Najia, NgK et al. 2012).

Due to the immense variability of the values of biochemical bone markers among the individuals, a percentage change from baseline in these values was considered for our analysis. As mentioned in the results of this study, no parameter significantly differed enough between the responders and non responders to predict the treatment outcomes. This implied that while the increase or decrease in these markers during the course of treatment could be suggestive of drug compliance however they may not indicate if a particular individual was responding to it. Thus attention should be given to other biochemical parameters as well for being suggestive of a treatment response or no response.

When 24 hour urinary calcium excretion is considered as a valuable tool either in identifying osteoporosis or in determining response to its treatment, no clear-cut indications for the inclusion or exclusion of urinary calcium excretion measurement as a primary or first line diagnostic tool in osteoporosis are currently available. This probably occurs because there is no comprehensive data on its prevalence and role in postmenopausal women with osteopenia or osteoporosis.

The effect of rPTH treatment on 24 hour urinary calcium excretion particularly not widely studied. Some studies do show that 24 hour urinary calcium is increased overtime on rPTH treatment (Miller, Bilezikian et al. 2007) however the beneficial or detrimental aspects of it while on rPTH are still not very well understood.

Subsequent studies are suggestive of rPTH effects on calcium metabolism in a pattern consistent with the known actions of endogenous PTH (Satterwhite, Heathman et al. 2010). Satterwhite demonstrates the pharmacologic effect of rPTH following once-daily subcutaneous administration with a transient and modest increase in serum calcium, which returns to pre dose levels prior to administration of the next dose. Since 24 hr urinary calcium has not been measured

in this study, though not confirmed but it could well be assumed that this calcium absorption also lead to hypercalciuria which is not detrimental to skeleton(Satterwhite, Heathman et al. 2010).

Hypercalciuria is generally believed to be detrimental to bone health (Ryan and Ing 2012) in non rPTH treated population . It has been suggested in the past that hypercalciuria could well be a pathological factor responsible for low bone mass in population with osteopenia or osteoporosis.

However it is crucial to determine the type of hypercalciuria in a particular individual before considering this factor being responsible for osteoporosis in that individual. The three commonly described types of hypercalciuria are renal, absorptive and fasting or resorptive hypercalciuria. It has mostly been proposed that resorptive hypercalciuria is linked with bone loss. Having said that some researchers have pointed towards the emerging evidence that hypercalciuria of intestinal origin may also be associated with bone loss or osteoporosis. An example of this is a retrospective analysis of a cohort of 319 patients with postmenopausal osteoporosis or osteopenia. The researchers in this analysis have described low spinal bone mineral density among patients with absorptive hypercalciuria with intestinal hyperabsorption of calcium and kidney stones and therefore they have linked intestinal calcium hyperabsorption to postmenopausal low bone mineral density (Odvin, Poindexter et al. 2008).

Contrarily, other researchers have proposed that in the absence of any PTH pathology, absorptive type of hypercalciuria is not a compensatory phenomenon, but probably the marker of disturbed cell calcium transport, involving both intestinal and bone tissues. In fact, patients with absorptive hypercalciuria less frequently show bone disease and a consequential reduction in their dietary calcium greatly increases the probability of bone loss in these subjects (Sella, Cattelan et al. 2008).

More to the point, practically a clear demarcation between absorptive, resorptive (renal leak) hypercalciuria can be very difficult or subtle. Considering the mechanism of absorptive hypercalciuria a study involving histomorphometric analysis of bone, has shown that the bone loss in absorptive hypercalciuria is due to reduced bone formation rather than increased bone resorption as seen in resorptive hypercalciuria (Heller, Zerwekh et al. 2007) .

Keeping this into account and referring to our study it is well established that rPTH increases bone formation and does not lower the bone turnover; which has also been reflected by the

significant BMD spine gain in our responder group, therefore the bone loss linked to absorptive hypercalciuria is not applicable in this case. In fact better calcium absorption reflected by increased urinary calcium secretion in the responder group reflects the better action of rPTH in this particular group which has subsequently shown significant positive bone effects. Hence hypercalciuria observed in individuals on rPTH treatment in the absence of any renal or PTH impairment is an indicator of a good response to the treatment and is absorptive in nature.

Osteoporosis is linked with sarcopenia. The effects of endogenous PTH on human muscle were observed in a large prospective study of 1008 males and females aged 55-85 years. Measurement of appendicular skeletal muscle mass using DXA was one of the parameters in this study. High PTH levels (≥ 4.0 pmol/litre) were associated with an increased risk of sarcopenia, compared with low PTH (< 3.0 pmol/litre): odds ratio = 2.35 (1.05–5.28) based on muscle mass. With per unit increase in PTH, the risk of sarcopenia was 3.52 (95% CI 1.43–8.67) based on appendicular skeletal muscle mass (Visser, Deeg et al. 2003) .

However, to date no study so far has searched for the fact if the frail patients given rPTH treatment for osteoporosis get further sarcopenia or in any case if a pre-existing low muscle mass affect the response to this treatment. The effects of PTH on skeletal muscle have been observed in animal studies and recombinant forms of PTH have been used for this, however, the effects of rPTH on human muscle still need to be explored.

Since decades, both PTH 1-84 and PTH 1-34 had been linked with impaired energy production, transfer, and utilization in skeletal muscle of rats (Baczynski, Massry et al. 1985). Later researchers demonstrated that perfusion of the isolated rat liver with PTH-(1-84) induced the production of bioactive IL-6 and the IL-6sR which could then increase the circulating levels of these cytokines in vivo (Mitnick, Grey et al. 2001). This soon after became applicable in the elderly, when elevated levels of IL-6 were linked with smaller muscle area, less appendicular muscle mass and a lower muscle strength in a well-functioning 70-79 year old population (Visser, Pahor et al. 2002) . Nevertheless, the effects of rPTH treatment directly on human skeletal muscle still remained unexplored.

The measurement of body composition by total body DXA in our study population, while on rPTH treatment was an innovative step to explore its affects on human body further

than monitoring the bone effects only. There was no significant difference in the muscle or fat parameters in the whole cohort after 1 year of rPTH treatment. When the percentage change in these values at 1 year was observed discretely among the two groups, the responders had shown if they had more muscle at 1 year post rPTH treatment. The percent change can quantify how much one group differs from another group relative to that other group. Higher percentage change in the responder group's muscle mass could explain one factor behind its good response to the treatment.

Conclusively, it is very reassuring to comprehend that unlike the animal models recombinant PTH does not detriment human muscle and fat and simultaneously it augments the bone radically.

4.6 Conclusion:

The biochemical bone markers have not been proven as indicators of response to rPTH treatment. Definite standard reference values should be determined to establish universally applicable cut-offs which could help monitoring an optimum treatment target. More evidence is also requisite to establish that biochemical bone turn over markers could be used to monitor treatment response like they are used in monitoring compliance.

Hypercalciuria is a frequent feature in population with reduced bone density. In contrast to previously held opinion, hypercalciuria is not necessarily detrimental to bones. Conversely, low urinary calcium at six month could indicate suboptimal response to treatment at 1 year. This thus enables to timely curtail the treatment in individuals not likely to gain benefit from rPTH. These individuals could then be commenced on alternate treatment for example a bisphosphonate.

This ultimately would improve cost effectiveness while treating Osteoporosis, because rPTH is an expensive (though highly effective) treatment in addition to the fact that osteoporosis population is growing with serious cost effects on patients themselves and society.

However, to substantiate this, a meticulous understanding is required to clearly distinguish between intestinal absorptive, bones damaging resorptive (renal leak) hypercalciuria. Studies using radioisotope calcium measurement will allow the finest understanding of the mechanism of hypercalciuria in different clinical scenarios.

rPTH remains an effective anabolic osteoporotic medication regardless of coexisting medical conditions and previous exposure to different medications including osteoporotic agents.

Our study also reflects that unlike endogenous PTH in pathological conditions, where it is continuously elevated recombinant PTH treatment during osteoporosis does not appear to negatively affect the body fat and muscle composition. In particular where the risk of sarcopenia is concerned for example in elderly population where it frequently coexists with other co morbidities.

Chapter Five

Quantitative Heel Ultrasound in Osteoporosis

There are several clinical techniques for BMD measurement. These include DXA, Quantitative computed tomography (QCT) and Quantitative heel ultrasound (QUS), each of which have their own advantages and shortcomings. Appropriate choice of technique and measurement site is important for the accurate diagnosis of osteoporosis.

5.1 Overview

In osteoporosis, bone fragility results from both bone loss and changes in trabecular micro architecture. This can be quantified by bone histomorphometric parameters. Non-invasive techniques for measuring bone mineral density (BMD) play an important role in the clinical diagnosis of osteoporosis and in monitoring its progression.

Quantitative ultrasound (QUS) is a quick, non-invasive and inexpensive method to measure bone strength. Moreover, the device is portable, making it easy to use in the field in contrast to other bone measuring techniques. It does not use any ionised radiation.

QUS for the measurement of bone has been used for more than two decades. Langton described the use of BUA measurement in the calcaneus as a potential indicator of hip fracture risk (Langton, Palmer et al. 1984). The concept finds its basis in the theory that the speed of sound and attenuation of sound wave are affected by the density, compressibility, viscosity, elasticity and structure of the material it is travelling through.

The calcaneus bone is mainly chosen for assessment as it is an easily accessible site and is comprised of about 90% trabecular bone, so has a high metabolic turnover rate and a similar pattern of bone loss to the spine (Gluer, Wu et al. 1994) . In contrast to the X-ray techniques, QUS transmits ultrasonic waves through the calcaneus. Reflected ultrasonic waves from the bones are the basis of bone density measurement i.e. the higher the bone density the faster ultrasound waves are bounced off bone.

QUS uses frequencies in the range of 0.2 to 1.0 MHz and most systems measure broadband ultrasonic attenuation (BUA in dB/ MHz It means how much sound is absorbed by the bone) and the speed of sound (SOS in m/s) of the calcaneus in the heel. Some heel QUS devices provide additional ultrasound variables derived from the mathematical combination of both BUA and SOS, defined as stiffness index (SI), to improve precision. Stiffness index is calculated by the following equation: $SI = (0.7 \times BUA + 0.28 \times SOS) - 420$.

As per DXA the QUS also produces results in terms of BMD (g cm^{-2}) and a heel T-score.

Different systems produce different values both in absolute terms and in relation to age-matched subjects. It is not possible to extrapolate the findings from one instrument or technique and apply it to another, and this limits the amount of generally applicable evidence.

5.2 Use of QUS in osteoporosis

Since its introduction, the use of QUS has increased vastly. It has been widely used for research and clinical purposes and as an alternate method free of ionizing radiation for non-invasive assessment of skeletal status in osteoporosis. While ionizing radiation attenuates at the atomic level, ultrasound is attenuated at the macroscopic structural level. It is therefore also suggested that BUA depends on the macroscopic structure of the cancellous bone in addition to the BMD assessed by using the ionizing radiation techniques.

Studies have shown QUS parameters to be significantly associated with bone structure independent of BMD, rendering QUS to be an effective tool for investigating bone tissue (Portero, Arlot et al. 2005, Wuster, de Terlizzi et al. 2005) .

Low QUS parameters are stronger predictors of low bone mass than clinical risk factors. According to National Osteoporosis Foundation (NOF) recommendations the individuals found to have low QUS parameters (as defined by machine-specific normative data) may either be referred for confirmation of the diagnosis by axial (preferably hip) BMD measurement or be advised to receive preventative therapy if other strong clinical risk factors are present.

Due to all these advantages, the measurement of peripheral bone density is growing outside secondary care centres.

5.3 Limitations for QUS use in osteoporosis

National Osteoporosis Foundation has recommended that in most cases QUS measurements alone cannot be used to monitor bone loss or assess response to therapy in an individual patient.

The precision of QUS to detect changes in bone is generally reported to be poorer than that of DXA and also the time period to follow individual subjects remains 2-3 times that for DXA, for a given rate of change. This leads to its limited use in long term monitoring of the patients on treatment (Sahota, San et al. 2000).

There is no established cut off T score value to determine osteoporosis on heel US, However data provided by the manufacturers with the devices often quote the WHO criteria indicating that a heel T-score of ≤ -2.5 is indicative of osteoporosis. However, The International Society for Clinical Densitometry Position Development Conference panellists have concluded that it is inappropriate to use WHO T score criteria for peripheral densitometry, as this could underestimate the prevalence of osteoporosis (Miller, Njeh et al. 2002).

Therefore, theoretically QUS cannot be used to diagnose osteoporosis quoting the WHO criteria for osteoporosis i.e., T-score of ≤ -2.5 . This is in contrast to portable ultrasound machines that are frequently used in community screenings.

Correlation between ultrasound-measured BMD and DXA-measured BMD is poor. National Osteoporosis Foundation has stressed this in its position statement on the use of QUS in osteoporosis. It declares that appropriate axial BMD T-score cut-offs for intervention cannot be directly translated into identical intervention cut-offs for QUS parameters. No universal cut-off for QUS parameters can be produced at present as there are no universally accepted reference phantoms allowing cross-calibration and standardisation between different manufacturers' devices.

The lack of agreement between apparently identical QUS parameters assessed in the same population using different manufacturers' devices makes it unlikely that cross-calibration equations can be derived in the short-term and hence it is not likely to provide a standardised normal range such as has been produced for hip BMD as assessed by DXA. Arbitrary cut-offs for diagnosis as used by some manufacturers, may not only be incorrect, but also possibly lead to some patients being falsely reassured or alarmed.

Several researchers have given arguments either in favour or opposition of QUS use and its validity in identification of osteoporosis.

In an interesting study, heel and axial DXA T-scores were measured for 215 women, mean age 64.6 years. Of these, 71 patients were found to be osteoporotic on axial densitometry and 144 patients were not osteoporotic (McCauley, Mackie et al. 2006). Only 15 of the 71 patients (21.1%) who were found to be osteoporotic on axial densitometry had heel T-scores of ≤ -2.5 . However, 90% of patients who had a T-score above the lower threshold of -2.2 did not have osteoporosis, whilst 90% of patients who had a T-score below the upper T-score threshold of -1.2 were osteoporotic. The approximate thresholds that had been calculated for the heel densitometer were that the patients with a heel T-score of above -1.2 were very likely to have normal bone density on axial densitometry, whilst patients with heel T-score of below -2.2 were very likely to have osteoporosis at the hip or spine. It was recommended that patients with heel T scores

that lie between these two thresholds should be referred for axial densitometry (McCauley, Mackie et al. 2006)

Another limitation to the use of QUS is the availability of different QUS scanners using different ultrasound principles and being applied to different anatomical sites. Such as devices could either use water-based or direct- contact, some are mostly applied to the heel while some are applicable to the phalanges, radius and tibia. It makes it difficult to compare the results of one device to another.

A very recent study rendered QUS as more sensitive than DXA in diagnosing osteoporosis with sensitivity being 17.1% for DXA and 46.4% for QUS, $P < 0.01$ (Li, Li et al. 2013). However, considering the sample number relatively small including 140 postmenopausal women the investigator suggested its validation in a larger population.

So, the validity of QUS in the measurement of bone health and the relationship between QUS output and body composition is still being assessed in the osteoporotic population.

5.4 QUS study at St. James's Hospital

A cross-sectional study was carried out for the purpose of examining the relationships of QUS with biochemical bone markers, fracture, past medical history, age and anthropometric status of patients, at St. James's Hospital. The T score obtained by QUS was also compared with the T score measured by central DXA scan.

The study comprised of community dwelling elderly population attending the osteoporosis services of St. James's Hospital (SJH). Bone Health and Osteoporosis clinic in St. James's Hospital maintains data on all the patients attending for osteoporosis management. Bone Health Clinic in SJH is a specialised service for treating osteoporosis and 300 new patients per year attend. It accepts patients locally and from tertiary centres.

A total of 183 patients were assessed for this study. Out of these, multiple analysis' were performed on 60 patients who had a complete set of data including their past medical,

drug and fracture history and also had a standard DXA scan around the time of their QUS.

The DXA measurements were performed by GE Lunar prodigy DXA scanner by trained technologists. BMD was measured both at hip and at L2-L4 in spine. All QUS measurements were performed by trained clinical nurse specialists. Measurements were made in accordance with the standard procedure provided by the manufacturer.

Following is the detail of this study.

5.4.1 Analysis of QUS Vs Bone markers in SJH cohort

Introduction:

QUS and its association with markers of bone turnover have been poorly assessed in osteoporosis and the literature is still lacking in both the number and magnitude of the studies performed in this area. Although, several studies have sought to use biochemical markers to select patients at risk of rapid bone loss for subsequent BMD measurement on a DXA, they have failed to demonstrate a consistent relationship between marker results and bone loss. Previous studies have demonstrated QUS can assess the parameters of bone quality other than density, such as three-dimensional structure of bone for the BUA and bone elasticity for the SOS. But relatively little is known about the ultrasound data and its relationship with the biochemical disturbances that are responsible factors for osteoporosis.

This cross-sectional analysis examined the potential relationships between the QUS parameters and biochemical markers of bone metabolism in an elderly population with an established diagnosis of osteoporosis.

Materials and Methods:

60 elderly community dwelling subjects attending Bone Clinic in St.James's Hospital were consecutively recruited for this cross-sectional study. All subject had a prior diagnosis of established osteoporosis with a T score of ≤ 2.5 on DXA and they were about to commence treatment with anabolic bone agents. The exclusion criteria were primary hyperparathyroidism, hypocalcaemia, previous history of neoplasm and poor overall prognosis.

Ultrasound of Bone

Bone mass was assessed by speed of sound (m/second) using a QUS device Achilles insight lunar by GE. For all subjects, speed of sound was measured at the left calcaneus. The measurement was taken in a temperature- controlled environment and was performed by a trained clinical nurse specialist only. Standardization and calibration with standards were performed before the measurements.

The QUS parameters measured through the heel were: Attenuation (broadband ultrasound attenuation BUA, expressed in dB/MHz) and speed (SOS, speed of sound, expressed in m/s) and T score.

Biochemical Analysis

The biochemical variables assessed in the study were: serum markers of bone turnover (osteocalcin, procollagen type I N-terminal propeptides PINP, serum type I collagen C-telopeptide breakdown products CTX) and other biochemical variables (serum parathyroid hormone PTH, Vitamin D, serum corrected calcium, serum phosphate (PO₄)).

The blood samples for the biochemical bone markers were spun and separated immediately, with the serum being frozen within 1 hour at -70 °C. Routine biochemistry was performed by standard techniques. Biochemistry and immunoassays were performed on Roche Modular Cobasc system which is a biochemical and immunoassay automated

analyser into a single integrated system. Vitamin D was analysed using a chemiluminescence immunoassay by Applied bio system API 4000. Bone markers including CTx, P1NP, OC and PTH were analyzed by Roche Modular Cobasc system using chemiluminescence enzyme immunoassay using commercial kits.

Statistical Analysis

All statistical procedures were performed by using IBM SPSS statistics version 19.0 software for Windows. After testing for normality, parametric or non parametric tests were used accordingly. The parameters which passed the tests for normality were expressed as the mean and SD and evaluated using the paired Student t-test. Otherwise, the results were expressed by the median and interquartile range (IQR) and evaluated using the Wilcoxon signed rank test. Correlations between the bone biochemical markers and QUS parameters were assessed using the Spearman rank correlation test. A p value of ≤ 0.05 was considered statistically significant.

Results:

The Anthropometric data of cohort and the mean values of the QUS measurements of the calcaneus and of the biochemical bone markers are shown in Table 5.1. Past medial history and current medication use including bone medications in the cohort have been described in Table 5. 2.

Table5.1 Anthropometric data, quantitative ultrasound of the calcaneus and biochemical markers

Measurements*		Mean ± Standard Deviation
N= 60		
T DXA Spine		-3.28± 0.87
<i>Anthropometric data</i>		
Age (years)		71.07± 15.4
Body Mass Index (Kg/m2)		25.23±5.13
<i>Quantitative ultrasound of the calcaneus</i>		
BUA (dB/MHz)		90.47±14.27
SOS(m/s)		1510.88±24.70
T score		-2.74±1.32
<i>Serum Biochemical markers (units)</i>	<i>Ref Range</i>	2.27± 0.129
Calcium (mmol/l)	2.2-2.70	
25-Hydroxyvitamin D (nmol/l)	50-80	75.28± 26.3
PTH (pg/ml)	10-65	36.03± 18.1
Magnesium (mmol/l)	0.7-1.0	0.80±0.1
Phosphate (mmol/l)	0.8-1.40	0.99±0.1
P1NP (ng/ml)	≤80	32.01± 23.3
Osteocalcin (ng/ml)	11-50	18.8± 11.2
CTx (ng/ml)	0.1-0.7	0.47± 2.06

*T-Test

PTH Parathyroid hormone, BUA Bone attenuation; SOS speed of sound

Table 5.2 Past Medical History and medication use expressed in percentage

Percentage Frequency		Percentage Frequency	
<i>Past Medical History & Relevant meds</i>		<i>Bone Medications</i>	
COPD	20	Use of Bone meds	81.7
Arthritis	8.3	rPTH	0
Malabsorption	10	Denosumab	1.7
Hypothyroid	10	Vit D or analogues	1.7
Hyperthyroid	2	HRT or related	3
Chronic Renal Failure	3.3	StrontiumRanelate	16.7
Diabetes Mellitus	10	Bonviva	3.3
		IV Bisphosphonate	3
		Residronate	8.3
		Alendronate	41.7
		Ca/Vitamin D supplement	81.7
<i>Medications</i>		<i>Smoking Status</i>	
Diabetic meds	3	Current Smoker	10
Steroid inhalers	13.3	Ex-Smoker	15
Oral Steroids	5		
Eltroxin	6.7		
Loop Diuretics	6.7		
Thiazide Diuretics	15		
Statin use	20		
Antidepressants	18.3		
Neuroleptics	8.3		

* Chi-square comparison of proportions P value: ≥ 0.05

Interpretation of results:

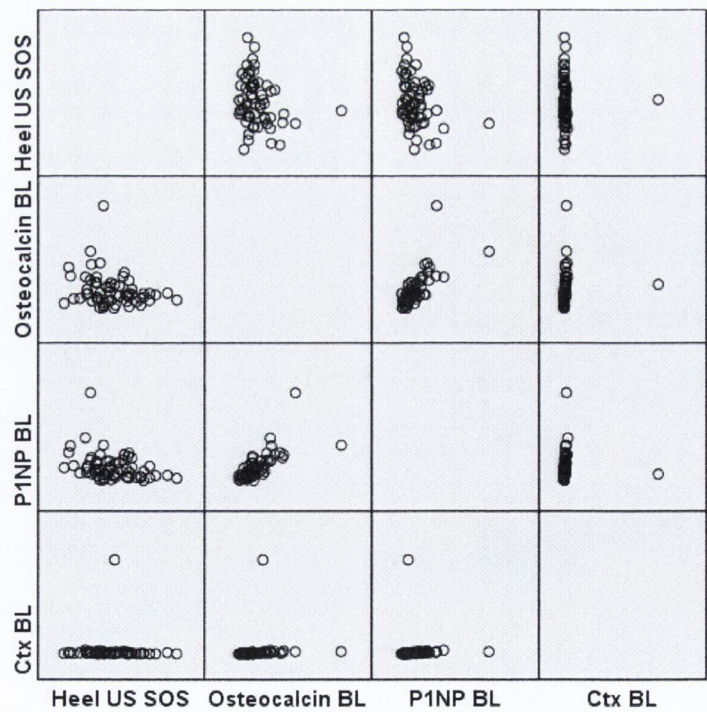
Table 1 shows the results of routine biochemistry and biochemical bone markers expressed in means and standard deviations. Figure 5.1 shows the correlations among the biochemical bone markers and QUS parameters.

The bone turnover markers (P1NP and OC) showed a significantly negative association with Heel US SOS (p 0.00, 0.025 respectively). CTx had a linear association with Heel US SOS (p 0.000). This has been shown in Figure 5.1.

The bone turnover markers (P1NP, OC showed a negative association with Heel US T score (p 0.017, 0.10, respectively). CTx had a linear association with Heel US T score (p 0.03). This has been shown in Figure 5.2.

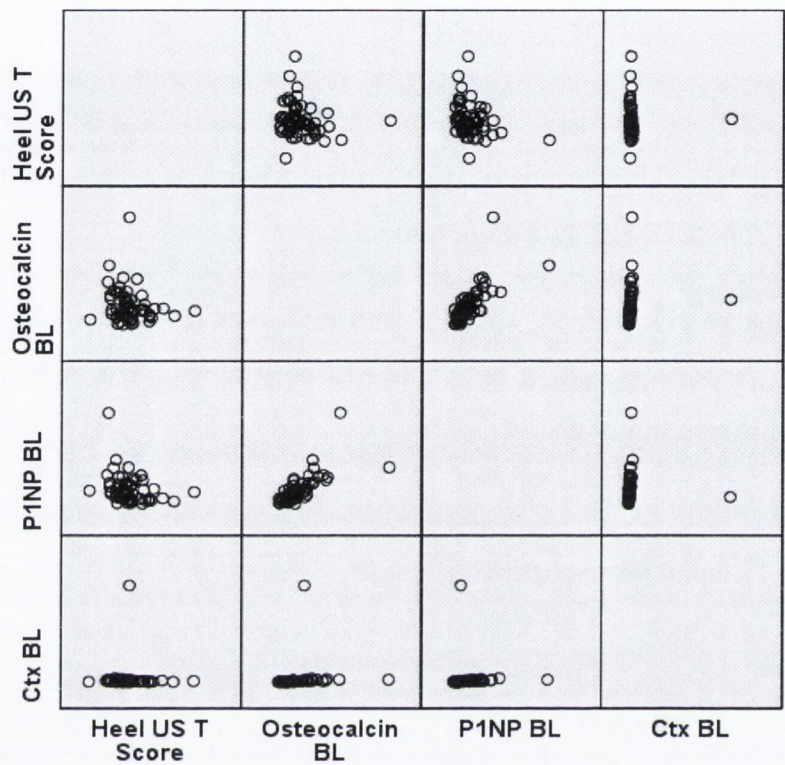
These bone markers (P1NP, OC and CTx) also relatively negatively correlated with Heel US BUA (P 0.27, 0.11 and 0.02 respectively).

Figure5.1: Matrix plot of OC, P1NP and CTX at baseline against Heel US SOS*



*Pearson Correlations
BL: baseline values.

Figure 5.2: Matrix scatter plot of OC, P1NP and CTX baseline against Heel US T score*



*Pearson Correlations

BL: baseline values.

Discussion:

Our study had shown that QUS parameters were significantly lower in the patients with higher bone turnover. These results are similar to a study in which QUS was used to assess the skeletal status in a population of uremic patients (Montagnani, Gonnelli et al. 1999). The researchers considered a group with high and a group with normal to low bone turnover on the basis of bone biochemical markers. Amplitude-dependent speed of sound (AD-SOS) and ultrasound bone profile score (UBPS) at phalanxes were significantly ($p < 0.01$) lower in the high bone turnover than in low bone turnover group.

Similarly in a study of 36 subjects with acromegaly, results suggested significantly lower QUS values (QUI, SOS and BUA) and increased bone turnover in male patients with active acromegaly (Kastelan, Dusek et al. 2007)

In a Spanish prospective, multicentre study, 368 women with 195 fracture cases and 173 controls were included in an analysis. Mean age was 76.2 ± 3.2 years and mean follow-up was 2.3 ± 1.2 years (Nanchen, Cornuz et al. 2009) . This group found that although the heel QUS discriminated between low-trauma non-vertebral fracture patients and controls, combining the QUS with two urinary markers of bone resorption, Urinary total pyridinolines and total deoxypyridinolines , did not discriminate better than a single test between those at risk and those not at risk. Therefore, the combination of bone resorption markers and QUS was not shown to be better than either test alone.

Conclusion:

The above results indicate that heel US parameters such as SOS, T score and BUA are sensitive to the rate of bone turnover in patients. This could be explained by the fact that an osteoporotic bone absorbs less ultrasonic waves compared to a denser bone with a different microstructure.

In conclusion, QUS of bone seems to be a relevant tool with a greater sensitivity for assessing osteoporosis in the severely osteoporotic population with high bone turnover.

However, the relevance of QUS cannot be fully ascertained until some longitudinal data are forthcoming.

5.4.2 Analysis of QUS Vs Fractures in SJH cohort:

Introduction:

Clinical utilisation of heel quantitative ultrasound (QUS) depends on its power to predict clinical fractures. This is particularly important in settings that have no access to

DXA-derived bone density measurements. DXA equipment is expensive, non portable, involves radiation exposure, and is usually restricted to tertiary care hospitals or specialized clinics. Therefore, in some geographical areas, there are clear limitations in the accessibility to this technique. Thus, the introduction of new alternative methods such as QUS to evaluate the status of the bone would be of interest.

Studies have shown that QUS has been able to discriminate subjects with osteoporosis from those without osteoporosis. In estimation of hip fracture risk, QUA has given a similar positive predictive value to DXA.

In recent times, a study examined the association of spine osteoarthritis and bone mineral density in 1082 community-dwelling ambulatory older women aged 50-96 years. The BMD was measured at the hip and posteroanterior (PA) and lateral lumbar spine using DXA. The investigators noted that under the WHO classification, women with spinal osteoarthritis were more likely to be given a diagnosis of osteoporosis of the femoral neck and hip than those without spinal osteoarthritis but less likely to receive such a diagnosis when BMD was based on the PA spine (14.4% vs. 24.5%). Thus they advocated that, in women aged 65 years and older who are likely to have spinal osteoarthritis, DXA of the hip should be used for identification of osteoporosis (Schneider, Bettencourt et al. 2006). However, DXA of the hip still includes cortical bone, so findings can be influenced by degenerative changes, leading to a decrease in the ability to detect osteoporosis.

QUS has been reported to predict osteoporotic fractures in large prospective studies. In 1990 a prospective study of 1414 elderly women from residential accommodation was published (Porter, Miller et al. 1990). Measurements were performed at the calcaneus of all women aged over 69, using the original Langton device. Over a follow up period of two years 73 women sustained a hip fracture. The women in the fracture group had a significantly lower BUA than those in the non-fracture group with BUA index (40.3 ± 19.3 versus 50.92 ± 2.2 db/MHZ) $p \leq 0.001$ (Porter, Miller et al. 1990).

QUS is emerging as a low-cost screening technique that is able to identify women at risk for the osteoporosis and that may be used by general practitioners in primary care and in ambulatory settings. Several cross-sectional studies have illustrated that the values of the

parameters measured with QUS, principally at the calcaneus, are lower in women with a history of osteoporotic fracture, regardless of the BMD values determined by DXA (Nguyen, Center et al. 2004). Conclusively, QUS could offer a prospective prediction of fracture risk comparable to that of axial DXA.

The aim of the present study was to examine the association of heel quantitative ultrasound QUS, with vertebral, hip and incident low energy non-spinal fractures in an elderly Caucasian osteoporotic population.

Clinical significance of the study:

This study investigated the predictive value of quantitative ultrasound (QUS) measurements and other potential predictors of osteoporotic fractures in the elderly. The aim was to assess the suitability of US measurements as a tool for detecting individuals at risk of fractures and to investigate whether baseline QUS measurements predicted non-spine fracture among men and women.

Materials and Methods:

Bone mass was assessed by speed of sound (m/second) using a QUS device Achilles Insight Lunar by GE. For all subjects, speed of sound was measured at the left calcaneus. The measurement was taken in a temperature- controlled environment and was performed by a trained clinical nurse specialist only. Standardization and calibration with standards were performed before the t measurements.

The Lunar Achilles QUS systems are designed specifically to give T-scores (using the Stiffness Index) that are closely aligned with the DXA T-scores at the spine and hip.

BMD was measured at the lumbar spine (L1–4) and left hip using dual energy X-ray absorptiometry on a GE Lunar Prodigy.

All statistical analyses were performed by using IBM SPSS statistics version 19.0 software for Windows. After testing for normality, parametric or non parametric tests were used accordingly. The parameters which passed the tests for normality were expressed as the mean and SD and evaluated using the paired Student t-test. Otherwise, the results were expressed by the median and interquartile range (IQR) and evaluated using the Wilcoxon signed rank test. Correlations between the different fracture sites and QUS parameters were assessed using a bivariate analysis (Spearman rank correlation test). To verify the assumptions, the Levenes test of equality of error variances was requested. Assumptions of linearity and normality of the residuals were also checked. A p value of ≤ 0.05 was considered statistically significant.

Results:

The anthropometric data, QUS parameters and past medical, drug history has been given in table 1 and table 2 respectively. A further categorization of QUS scores according to fracture history is shown in Table 5.3 below.

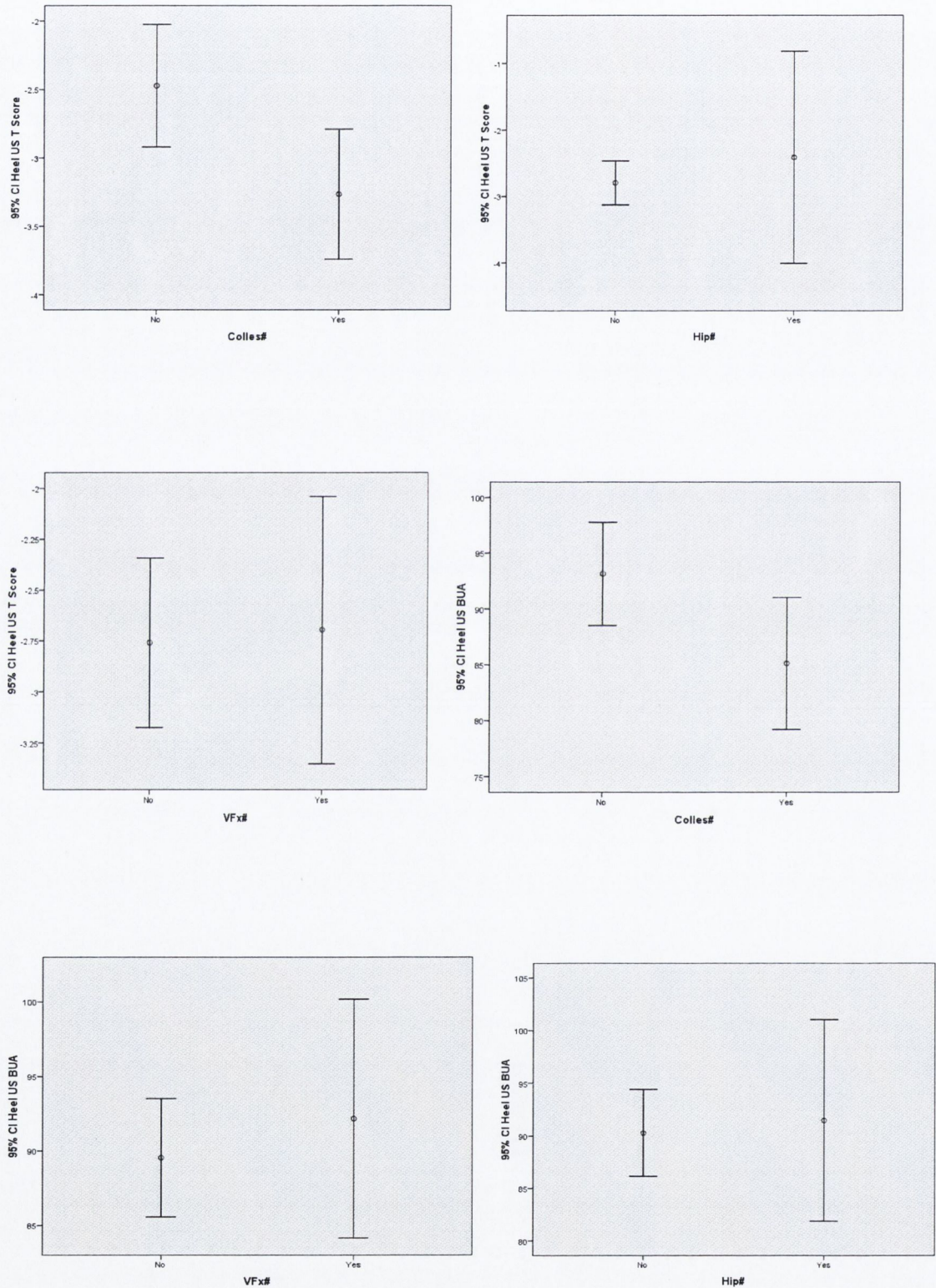
QUS T score was significantly lower in Colles fracture group (p 0.02) with a mean of 3.26 (SD: 1.01, CI: 0.15; 1.429). QUS BUA was also significantly lower with Colles fracture group (p 0.03) with a mean of 85.12 (SD: 12.63, CI: 0.69; 15.34). No association was seen between QUS T score and BUA with hip, vertebral and non vertebral fracture sites. This has been shown in Figure 5.3 below.

Table5.3: Means and SD of QUS parameters in different

Fracture site	Fracture frequency (%)	QUS BUA	QUS SOS	QUS T score
Vertebral	54	92.17±17.62	1512.28±26.82	-2.70±1.44
Hip	17	91.47±12.46	1502.98±24.4	-2.41±2.07
Colles	50	85.12±12.63	1503.45±20.87	-3.26±1.01
Other	46	93.77±13.55	1512.05±25.60	-2.63±1.06

*Independent Samples T-Test

Figure 5.3: Heel US T Scores and BUA* scores Vs Fracture sites (Means with 95% Confidence Intervals) **



* BUA: bone ultrasound attenuation, ** Independent samples T-Test

Discussion:

The results of this cross-sectional analysis showed that both BUA and T score measurements of the calcaneus were identified as relatively strong predictors for Colles fracture in elderly people.

Our results support the previous results from case– control studies that ultrasound measurements can discriminate between fracture and control groups.

In Japan, a multicentre prospective study investigated the relationship between baseline heel QUS measurements and non-spine fracture risk. There were 4,028 subjects (1,004 men and 3,024 women), with mean age of 67.5 ± 8.9 years, in the study who were followed for a mean period of 5 yrs. (Fujiwara, Sone et al. 2005) SOS, BUA, and SI obtained from heel QUS predicted non-spinal fractures in Japanese men and women.

Increased susceptibility to fracture is the most significant clinical manifestation of all metabolic bone diseases including osteoporosis. Since effective treatments exist to minimize fracture risk, it is essential that new screening strategies are developed to detect a population at risk. QUS of the calcaneus has been shown in many prospective studies to predict vertebral and non-vertebral fracture risk independent of BMD. QUS has been proven to be independent predictor of risk of non-spinal fragility fractures including wrist fractures (Thompson, Taylor et al. 1998) (Diez-Perez, Gonzalez-Macias et al. 2007). Similar to our analysis, lower values of QUS parameters were independently associated with the risk of Colles fracture.

Several prospective studies involving women of 65 years and older have shown that fracture risk assessment with QUS measurements is feasible. A meta-analysis of 14 prospective studies inclusive of nearly 43,700 individuals (Marin, Gonzalez-Macias et al. 2006), demonstrated the relationship between low QUS values and increased fracture risk independent of age and other covariates. Depending on the QUS parameter, the type of fracture, and the ultrasound measurement site, the estimated relative risk ranged between 1.23 and 1.94 for each SD decrease in QUS measurements, being statistically significant in all circumstances

As mentioned above, heel QUS discriminated between low-trauma non-vertebral fracture patients and controls in a Spanish cohort of 368 patients (Nanchen, Cornuz et al. 2009)

Recently, a meta-analysis of 21 studies considered heel QUS measurements at baseline and fracture outcomes in follow-up (Moayyeri, Adams et al. 2012). All four QUS parameters (broadband ultrasound attenuation [BUA], speed of sound [SOS], stiffness index [SI], and quantitative ultrasound index [QUI]) were associated with risk of different fracture. Meta-analysis of studies with QUS measures adjusted for hip BMD showed a significant and independent association with fracture risk (RR/SD for BUA = 1.34 [95%CI 1.22-1.49]).

Several studies have previously demonstrated BUA as a good predictor of low BMD and an independent predictor of hip fracture. It has been suggested that the QUS parameters of broadband ultrasound attenuation (BUA) and speed of sound (SOS) depend not only on bone density but also on bone structure and elasticity (Gluer, Wu et al. 1993). The International Society for Clinical Densitometry (ISCD) states that validated heel QUS devices predict fragility fracture in postmenopausal women and men over the age of 65, independently of central DXA BMD in a position paper. Although central DXA has been shown superior to QUS but the ISCD recommends that in settings where there is no availability of standard DXA facilities pharmacologic treatment can be initiated if the fracture probability, as assessed by heel QUS, using device specific thresholds and in conjunction with clinical risk factors, is sufficiently high (2013 ISCD Official Positions – Adult).

The above discussion clarifies the role of QUS in improving estimates of fracture risk as mentioned in previous studies. Similarities to our results are evident. In clinical practice clinical risk factors have been widely used to screen patients for osteoporosis yet they have proven to be poor determinants of low BMD at spine, hip and other non vertebral sites. Hence patients often present with prevalent fractures before any preventive measures have been put in place. Therefore, our study effectively shows that a relatively inexpensive, portable and easy to use QUS device can be effectively used for the screening of osteoporosis and prevention of fractures. Therefore, this could potentially reduce the socioeconomic impact of this disease.

Conclusion:

In this cross-sectional study we found that low values of BUA and T score can predict the risk for fractures in elderly people. Hence, QUS may be a valid alternative to evaluate fracture risk in situations where DXA is not accessible.

Whereas QUS is increasingly incorporated to clinical practice, no firm conclusions can yet be drawn regarding its potential use in fracture risk assessment. Ultimately though QUS may have a role in improving estimates of Colles fracture risk; this is at best a proxy for the assessment of bone density to identify osteoporosis. QUS of the heel cannot, therefore, be recommended to justify initiation of treatment but it could initiate the investigation or monitoring of patients suspected of being at risk for severe osteoporosis or fracture.

5.4.3 Analysis of QUS Vs Age in SJH Cohort:**Introduction:**

Clinical studies have shown that QUS parameters are sensitive to age- related changes. To further evaluate this, the effect of age on QUS measurements was also assessed in the same cohort of the patients.

Materials and Methods:

The QUS device (Achilles Insight Lunar by GE) and its measurement parameters (BUA, SOS and T Score) remained the same as used in the above study.

The same software (IBM SPSS statistics version19.0 software for Windows) was used for statistical analysis.

The above mentioned osteoporotic population cohort was subdivided into three bands/quartiles depending on the age of each individual patient at time of QUS i.e., 50-69 yrs, 70-79 yrs and ≥ 80 yrs.

The correlation of T score measured by QUS with patient age in this cohort was observed by one way descriptive statistical analysis.

Results:

The mean QUS T scores as per age quartiles has been shown in figure 5 below. As mentioned above the anthropometric data, QUS parameters and past medical, drug history has been given in table1 and table2 respectively.

QUS T score was significantly negatively associated with increasing age (p 0.01). It is shown in figure 5.4 below. Interestingly when the age of the patient population was separately observed in quartiles, the T score on QUS was worse in the oldest population aged ≥ 80 yrs (mean -3.09, SD 1.094, 95% CI -3.72 to -2.46) as compared to those aged 50-69 yrs (mean -2.50, SD 1.130, 95% CI -3.02 to -1.99) and those aged 70-79 yrs (mean -2.83, SD 1.549, 95% CI -3.50 to -2.16). This has been illustrated in figure 5.5 below.

Figure5.4: Heel US T score association with increasing age

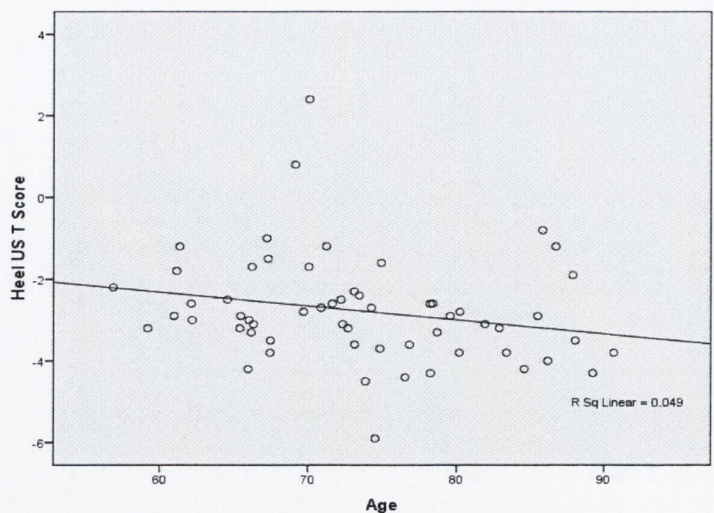
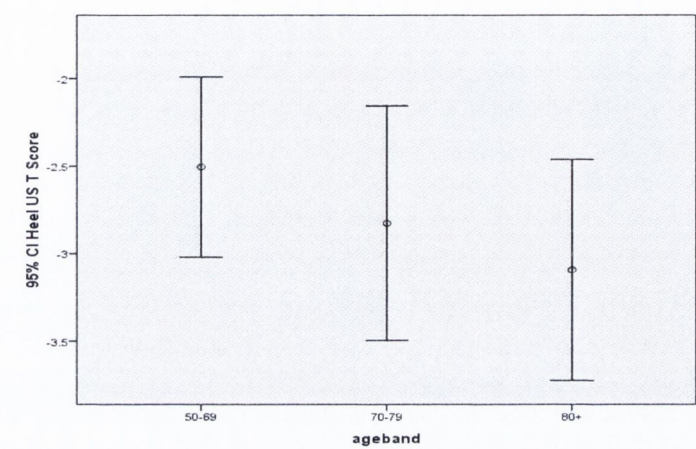


Figure 5.5: QUS T score by age quartiles (Means with 95% Confidence Intervals)*



*ANOVA

Discussion:

Looking at the prior studies it is evident that the QUS measurement parameters have previously been shown to have a significant negative relationship with age.

A large study on 1630 individuals aged between 30-85yrs also compared age-related changes in bone mass measured by QUS (Lunar, Achilles Plus) and dual-energy X-ray absorptiometry (DXA). With aging, women show a decrease in cortical area and medullary expansion in the bones of the lower limbs. It is well understood that decreased thickness of cortical bone shell results in a reduction in loading capacity and bone strength, which subsequently causes local and systemic deterioration of bone architecture and therefore, potentially becomes evident as an adverse outcome.

Age-related bone loss has been proven to be significantly larger using QUS rather than DXA at all sites in women (Gudmundsdottir, Indridason et al. 2005). The definite negative correlations between age and all of the QUS parameters, such as speed of sound (SOS), broadband ultrasound attenuation (BUA), and estimated heel bone mineral density (BMD) ($p<0.0001$) have been demonstrated in other studies as well (Rhee, Lee et al. 2009).

Conclusion:

Considering the documented evidence in the literature as discussed above, the similar results in our study also demonstrate an increased sensitivity of QUS with increasing age, which implies that QUS could effectively be used as a tool for age based screening in osteoporosis.

5.4.4 Analysis of QUS T score Vs DXA T score in SJH cohort:**Introduction:**

Researchers have carried out relatively less direct comparisons between the T scores measured by QUS and hip DXA. This analysis was carried out in order to compare the T scores measured on a routine DXA at hip and spine with the T scores measured by QUS heel ultrasound. The population was categorized as osteoporotic based on T score at spine measured by DXA.

Materials and methods:

QUS and DXA data were statistically compared and their relationship was analysed. Pearson coefficient analysis was used for this purpose with the help of IBM SPSS statistics version 19.0 software for Windows.

Results:

All 60 pt were severely osteoporotic as shown in table 4 below.

The means of T score on QUS in this analysis showed that T score was sensitive enough to identify osteoporosis and it was comparable with T spine on DXA (Figure 5.6).

T score of hip on DXA was significantly positively correlated with T score QUS ($p \leq 0.00$).

No significant correlation of T score on QUS was observed with T Spine on DXA ($p \geq 0.9$).

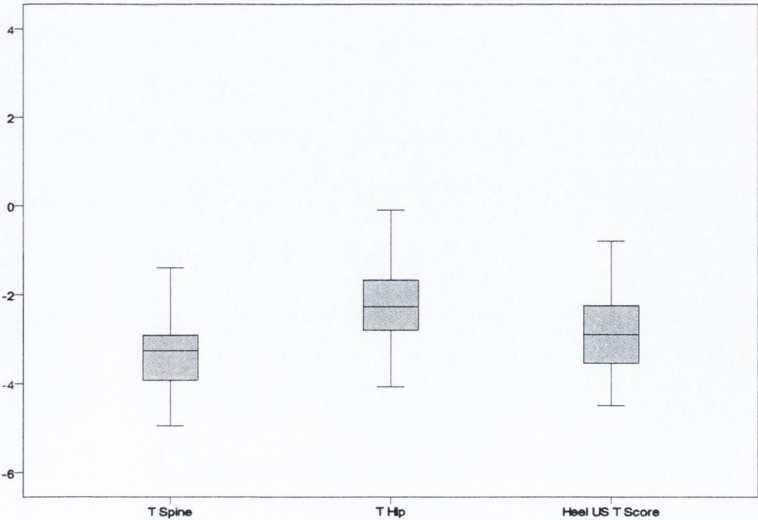
T spine DXA was positively correlated with T hip DXA ($p \leq 0.01$).

BMI in 50% of the patient cohort was between 18.5 and 25, which is considered normal. BMI was positively correlated with T spine ($p \leq 0.00$) and T hip ($p \leq 0.02$) on DXA.

Table 5.4: Mean T scores on central DXA and calcaneal QUS.

	Mean± SD	P value
T Spine	-3.28±0.87	0.0001
T QUS	-2.7±1.32	0.0001
T Hip	-2.3±1.01	0.0001

Figure5.6: Comparison of DXA T scores with Heel US T scores (Means with 95% Confidence Intervals)*



*T-Test ($p 0.001$)

Age was significantly negatively associated with T hip ($p \leq 0.02$), T spine ($p \leq 0.02$) on DXA, and T QUS ($p \leq 0.01$). Therefore one of the findings was that increasing age was identified as a negative predictor and increasing BMI was identified as a positive predictor of T spine on DXA in this analysis.

Discussion

In our analysis where the mean $\pm$ SD for T spine on DXA was -3.28 ± 0.87 the mean T score on QUS was measured as -2.7 ± 1.32 .

This result was similar to the conclusion drawn in a recent systematic analysis which explored seven studies. In this paper the prevalence of osteoporosis was the same and T score of < -3.65 for QUS was equivalent to a T-score < -2.5 for DXA (Floter, Bittar et al. 2011).

While there has been no consensus regarding the diagnosis of osteoporosis using QUS measurements of the calcaneus, several studies have demonstrated significant correlations between QUS and standard DXA parameters (Trimpou, Bosaeus et al. 2010).

A strong predictive relationship was seen between hip bone mineral density and QUS parameters. (Collinge, Lebus et al. 2010) In this retrospective analysis of 129 patients T score on QUS was compared with T-scores (hip or radius) QUS T-score cut offs of greater than -0.9 resulted in 90% sensitivity (defining low osteoporosis risk) and a threshold of -1.6 or less resulted in a specificity of 80% (defining high osteoporosis risk). Hence it provided some more evidence in support of using QUS in identifying osteoporosis in low to high risk population (Collinge, Lebus et al. 2010).

This result is in agreement with our study in which a mean QUS T score of -2.7 is significantly comparable with a low DXA T score at hip. In fact QUS T score in our study is more sensitive in identifying osteoporosis than the DXA T score at Hip. This could be explained by the fact mentioned previously that DXA of the hip still includes cortical bone, so findings can be influenced by degenerative changes with increasing age,

therefore leading to a decrease in the ability of standard DXA to detect osteoporosis as compared to heel QUS.

Nevertheless, there is, as discussed in the previous analyses above, increasing evidence that the current T score definition of osteoporosis (-2.5 SD) cannot be universally applied to different densitometry techniques or sites, mainly due to differences not only in age-related bone loss at different sites but also in the young adult reference population used, along with the technological differences and the discordance in BMD measurements at different sites. Thus, T scores for different devices are not equivalent, owing to differences in the prevalence of osteoporosis identified for identical T scores. This makes it unclear as what thresholds to use when interpreting peripheral bone measurements such as QUS.

Conclusion:

Our study reflects that standard DXA particularly at hip of elderly population is not superior to heel QUS in identifying osteoporosis. However, specific guidelines need to be established for correct interpretation of diagnostic tests and in order to treat the patients appropriately. Determining absolute fracture risk and identifying device-specific T score thresholds also needs to be established.

Despite few limitations heel QUS remains a relatively effective, economical and safe tool to highlight the high risk patients in clinical settings particularly when Standard DXA is not available.

5.5 Collective Conclusion from the QUS study analyses in SJH cohort:

In summary, all the analyses in this study reveal that QUS of bone evaluates characteristics of bone that are influenced, at least partially, by age, BMI and bone turnover markers.

QUS is also capable to detect bone related changes in population with Colles fracture. In our study QUS has identified osteoporotic patients who have already been diagnosed as osteoporotic by the so far gold standard DXA measurements. Therefore its use is comparable with Standard DXA.

However, further research should be conducted in this area, because it is possible to improve the number diagnoses by varying the cut off T-score. Furthermore, using QUS of the calcaneus is a helpful tool for assessing fragility fractures, whether or not they are associated with osteoporosis.

To aid in the diagnosis of osteoporosis, use of Achilles T-scores corresponding to the WHO classifications identifies moderate and high-risk individuals requiring additional testing with DXA. Of note, there is yet no standardisation of ultrasound devices. Therefore the development of quality standards for these but also the cross-calibration of QUS scanners is required, so that results from different devices can be compared. Nevertheless, QUS remains a useful technique which can be effectively used in areas where standard DXA is not available.

Chapter Six

Conclusion

The aim of this study was to explore the relationship of recombinant PTH (rPTH) and nutritional status (in terms of body composition) in osteoporosis and factors identifying response to this treatment. It investigated the effect of muscle and fat mass at baseline and in response to rPTH at 1 year of treatment in elderly osteoporotic population.

We reviewed the evidence to date about use of rPTH in osteoporosis and effects of nutrition in bone health. . We also identified DXA as a useful tool to assess body composition in elderly osteoporotic population. Another arm of this study investigated the role of Qualitative heel ultrasound (QUS) in identification of severe osteoporosis and fracture risk in the elderly.

It is established from a detailed review of literature in this study that rPTH is generally well tolerated and treatment compliance rates are favourable. rPTH is best reserved for the treatment of patients with osteoporosis at high risk of fracture, or for patients with osteoporosis who have unsatisfactory responses to or intolerance of other osteoporosis therapies.

The longitudinal arm of this study identifies that rPTH remains an effective anabolic osteoporotic medication regardless of premorbid conditions and previous exposure to different medications including osteoporotic agents.

The longitudinal arm of this study also demonstrates that changes in biochemical bone markers are not always indicators of degree of response to rPTH treatment. It is a novel finding of our study that the degree of hypercalciuria at six months significantly positively associate with treatment response at one year. In contrast to previously held

opinion, hypercalciuria is not necessarily detrimental to bones. Conversely, low urinary calcium at six month could indicate suboptimal response to treatment at 1 year. This thus enables to timely curtail the treatment in individuals not likely to gain benefit from rPTH. These individuals could then be commenced on alternate treatment for example a bisphosphonate.

Unlike endogenous PTH in pathological conditions, where it is continuously elevated recombinant PTH treatment during osteoporosis does not appear to negatively affect the body fat and muscle composition. In particular where the risk of sarcopenia is concerned for example in elderly population where it frequently coexists with other co morbidities.

We therefore conclude from the longitudinal arm of this study that prevention of osteoporosis and its complications is possible by preserving BMD through maintaining or increasing lean mass. Particularly in association with rPTH treatment maintaining or increasing muscle mass can enhance likelihood of good response.

The cross-sectional analysis of our study population before commencement of rPTH clearly shows that if patients have adequate fat and muscle mass they are more likely to have good bone health. This study also implies that fat and muscle are independent predictors of BMD regardless of body mass index. Our study agrees with the literature available in support of positive influential effect of lean mass and adiposity on BMD.

Although this study did not assess, it is presumed that people taking adequate calories and a nutritiously balanced diet could attain better muscle and fat mass. The role of adequate nutrition at a healthy level in the prevention of osteoporosis therefore remains a significant proposal and it needs to be widely escalated.

Our study reflects that total body DXA provides a reasonable estimate of body composition in people of different weights ranging from low body weight to obesity. It may also become a non invasive and quick method for determining appropriate nutritional status. Total body DXA gives a significant amount of information in a short time which would otherwise be obtained by a trained clinical nutritionist requiring more time for detailed assessments and cost for different anthropometric measures.

In many European countries rPTH use is limited because of its cost as it is an expensive (though highly effective) treatment. Defining an algorithm or a precise rule to perform certain measures such as body muscle and fat mass, 24 hour urinary calcium etc during rPTH treatment can distinguish the patients likely to optimally respond to the treatment at an earlier stage. These patients can therefore be continued to have the full course of treatment, while alternate treatment options should be considered for other patients.

Lastly, the cross-sectional analysis of a subgroup in our study cohort has established that QUS precisely detected bone related changes in population with Colles fracture. QUS has also correctly identified more than 95 percent of osteoporotic patients in this cohort who were previously diagnosed as severely osteoporotic by the so far gold standard DXA measurements. Changes in QUS parameters also correlate well with baseline BMI, increasing age and biochemical bone markers. QUS of bone therefore, evaluates characteristics of bone that are influenced, at least partially, by age, BMI and the bone turnover markers.

Conclusively, QUS of the calcaneus is a helpful tool for identifying severe osteoporosis in the elderly. Besides it can assess fragility fractures, whether or not they are associated with osteoporosis. It is a useful technique to be effectively used in areas where standard DXA is not available. Still, there exists more room for research in this area to standardise this equipment widely.

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