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DEPRESSION AND BONE MINERAL DENSITY

By

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The Creator… for the wonderment of everyday life and the gift of being able to experience it.

ABSTRACT

Title: Depression and bone mineral density
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The aim of the study was to investigate the association between depression and low bone mineral density (BMD) in premenopausal females. The rationale for the study was that depression is often characterized by cortisol hypersecretion. The role of cortisol includes effects on bone metabolism and the immune system: cortisol is a bone resorption agonist through its support of osteoclastogenesis. The release of pro-inflammatory cytokines, (especially IL-1, IL-6 and TNF-α) which induce cortisol secretion, also pushes the balance of bone remodelling in favour of resorption, consequently causing loss of bone mineral density. Significant results have been reported in studies of various groups across the USA, Europe and Asia, indicating a causal role for depression in osteoporosis. However, some studies could not support this association. With both osteoporosis and depression representing growing public health concerns in South Africa, the aim of this study was to examine the association between depression and loss of BMD in a South African sample with varying levels of depression.

The study was approached from two starting points: the first used low BMD as the departure point and the second was undertaken from the diagnosis of depression. This was achieved by first investigating women where the primary concern was possible low BMD (referred to as Study 1) and secondly by assessing women whose primary diagnosis was clinically confirmed major depression (Study 2).

Study 1 involved investigation of BMD in a volunteer-based sample of 40 premenopausal women drawn from three different sources. All volunteers underwent a DEXA scan, were assessed for depression and supplied saliva for cortisol analysis. Study 2 examined the BMD of five psychiatric patients diagnosed with severe, recurrent major depression and four healthy controls.

These volunteers were required to undergo the same testing as subjects in Study 1. In addition, blood and urine samples were taken to examine bone turnover markers (bone specific alkaline phosphate, osteocalcin, urine pyridinoline cross-linked C-telopeptide and deoxypyridinoline). The pro-inflammatory status of the psychiatric patients was compared to reference ranges. The latter served as a small exploratory study and an introduction to further avenues of research.

Study 1 revealed no clear general association between depression and bone density on DEXA scores. However, a correlation was found between left femoral neck BMD and depression in those women with low BMD only. Significant differences were found though between subjects with normal and low BMD in terms of body mass index (BMI) and contraception use. Study 2 on the other hand, indicated a trend of association between depression and low BMD: subjects suffering with severe major depression were noted to have lower bone density (on DEXA) and higher bone turnover (as measured by markers of bone turnover) as well as higher cortisol levels than healthy controls. In addition, depressed subjects exhibited elevated IL-1β levels but normal TNFα levels when compared to normative data.

In conclusion, the study indicated that the effect of depression on bone density is dependent on the intensity and duration of depression. IL-1β and cortisol may be instrumental in this loss of BMD.

Key terms: bone mineral density, depression, cortisol, bone turnover, pro-inflammatory cytokines

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Chapter 1: Literature background to the study

The purpose of this study was to investigate the possible association between depression and low bone mineral density (BMD) in premenopausal females. A potential link exists between depression and low BMD via the hypothalamic-pituitary axis (HPA axis) and the hypersecretion of cortisol. Cortisol represents a link between stress and depression on the one hand and, possibly, between depression and low BMD on the other.

1.1 Rationale for the study

This study takes a psychoneuroendocrinology (PNE) approach to the analysis of physical illness. Although existing research reflects great methodological heterogeneity, associations are increasingly being reported between depression and osteoporosis (1). However, the results of several studies do not support these associations. This indicates the need for further exploration of the mechanisms of the potential link between depression and osteoimmunology. PNE has not previously been applied to the field of osteoimmunology in South Africa.

This research proposes that depression is a potential risk factor for osteoporosis. Investigating potential risk factors plays an important part in designing preventative strategies in the fight against osteoporosis and can assist in the treatment of patients (2). The study extends beyond merely listing risk factors, however, and attempts to account for the mechanism that may tie depression to the aetiology of osteoporosis. For this reason, the mediating role of cortisol in depression and osteoporosis is also explored.

High cortisol levels are commonly noted in people suffering from depression: a consistent finding in neurobiological research into depression is that 40-60% of drug-free depressed patients exhibit hypercortisolism (3). Another link has been proposed between hypercortisolism and low bone density (4). The analysis of the association between depression and BMD in this study is then a further step in defining this relationship.

Depression-induced hypersecretion of corticotrophic releasing hormone (CRH) and cortisol contributes to bone loss in patients with major depression (1). Pro-inflammatory mediators have also been connected to both depression and the loss of BMD (5, 6). These pro-inflammatory cytokines are known to be affected by cortisol secretion. Therefore, this study begins with the assumption that depression is predominantly characterized by cortisol hypersecretion, which directly or indirectly pushes the balance of bone remodelling in favour of resorption, thereby causing the depletion of BMD.

1.1.1 Overview of chapters

Chapter one offers the literature background to the study along with the aim and research questions examined in this study. The second part of this chapter *(Chapter one)* is entitled *Bone: Formation, resorption and risk factors.* Information regarding bone formation and resorption is presented. The concept of osteoporosis and its risk factors are then discussed. The literature then connects to depression and its relation to the stress response under the title *Depression and the stress response.* The final section of this chapter is entitled *Cortisol and BMD: From depression to osteoporosis.* Emphasis is placed on evidence implicating depression as causative in osteoporosis.

Chapter two is entitled *Materials and Methods.* This chapter outlines the research process for the entire study. The measurement of BMD via DEXA and bone turnover via selected markers, the assessment of depression via self-report questionnaires and the determination of salivary cortisol levels via ELISA are discussed. In addition, the assessment of the pro-inflammatory response in five psychiatric patients is also described. The statistical analyses employed are briefly described. The method used in a meta-analysis of recent literature dealing with depression and bone density is also described.

The results of the two parts of this study are presented separately. The results of the first part of the research are presented in *Chapter three,* which is entitled, *Study 1: depression in pre-menopausal females*. Descriptive and inferential statistics are used to make sense of the data. A brief discussion of the results is put forward.

Chapter four presents the results for the second part of the study. Owing to the size of the sample, emphasis is placed on descriptive statistics. A brief discussion of the results follows. The title of the chapter is *Study 2: BMD in premenopausal females with and without major depression.*

Chapter five: Integrated discussion introduces a more in-depth discussion of the overall results of the research, integrating results form Study 1 and Study 2. The results of the meta-analysis of other similar BMD and depression studies in premenopausal women are examined. The broader implications of the research are considered. The limitations of the research are described and avenues are suggested for further research.

1.1.2 Abbreviations

ACTH	Adenocorticotrophin hormone
ADAMTS	Disintegrin and metalloprotease with thrombospondin motifs
BDI	Beck Depression Inventory
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BSU	Basic structural unit
CRH	Corticotrophin-releasing hormone
DEXA	Dual-X-Ray Absorptiometry
DPD	Deoxypyridinoline
EGF	Epidermal growth factor
FACIT	Fibril associated collagens with interactive triple helix
GH	Growth hormone
GH-ILF-1	Growth hormone-insulin like growth factor I
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HPA	Hypothalamic pituitary axis
Ig	Immunoglobin
IL	Interleukin
ILGF-1	Insulin like growth factor I
LOX	Lysine oxidase
NFκB	Nuclear factor κB
OPG	Osteoprotegerin
PG	Prostaglandin
PDGF	Platelet-derived growth factor
PNE	Psycho-neuro-endocrinology
PTH	Parathyroid hormone
PVN	Paraventricular nucleus
PGW	Psychological General Well-being Schedule
Pyd	Pyridinoline
RANKL	Receptor activator of nuclear factor κ-B-ligand
SAM	Sympatho-adreno-medullary
TGF	Transforming growth factor
Wnts	Wingless-Int

1.2 Bone: formation, resorption and risk factors

Bone is a term that denotes a family of materials that have mineralised collagen fibril as the basic building block (7). Along with cartilage, bone forms the skeletal structures of most vertebrates (8). Tate (9) describes bone as "a living ecosystem… [that] fulfils scaffolding, armoring, and damping functions necessary for survival of mobile, terrestrial organisms." Aside from their easily-recognized mechanical role in acting as a support structure for the body, flat bones protect vital organs and serve as a surface for muscle attachment. Irregular bones are also involved in muscle attachment and articulation (10). Long bones are those found mostly in the appendages. These bones are longer than they are wide, and mainly function as levers. Short bones, on the other hand, are cubicle. They are found in confined spaces, where they transfer forces (10).

Within its mineral homeostasis function, bone serves as a reservoir for calcium and phosphate. Bone also houses the haemopoietic cells necessary for the formation of red and white blood cells (11).

1.2.1 The composition of bone

With such a wide range of functions, the structure of bone is rather complex. Mature bone occurs in two forms: trabecular or cancellous bone and compact or cortical bone. Cortical bone makes up the outer casing of long bones, small bones and the flat bones. The structural units of cortical bone are the Haversian systems or osteons, cylindrical structures that occur parallel to one another. These canals contain the vascular tissue that supplies the bone (12). Trabecular bone is less dense than cortical bone and comprises plates and rods. Trabecular bone is located near the ends of long bones, between the surfaces of flat bones and as the interior of small bones (11). Trabecular bone consists of networks of interconnected trabeculae with marrow-filled spaces. The marrow provides the vascularization for the bone. This marrow may be haematopoietic or fatty (8).

Cortical bone makes up 80% of the bone mass in a human and trabecular bone only 20% (12). Most bones have a dense cortical cover and trabecular core (8).

Bone consists of abundant extracellular matrix that has been mineralised. Its composition is influenced by age, skeletal site, health and nutrition (8). The main constituents of bone are minerals, collagen, water, non-collagenous proteins, lipids, vascular elements and cells. Figure 1.1 provides approximate values for the composition of axial skeletal bone. These values vary greatly according to skeletal site (13). The organisation and roles of these constituents are discussed under subsequent headings.

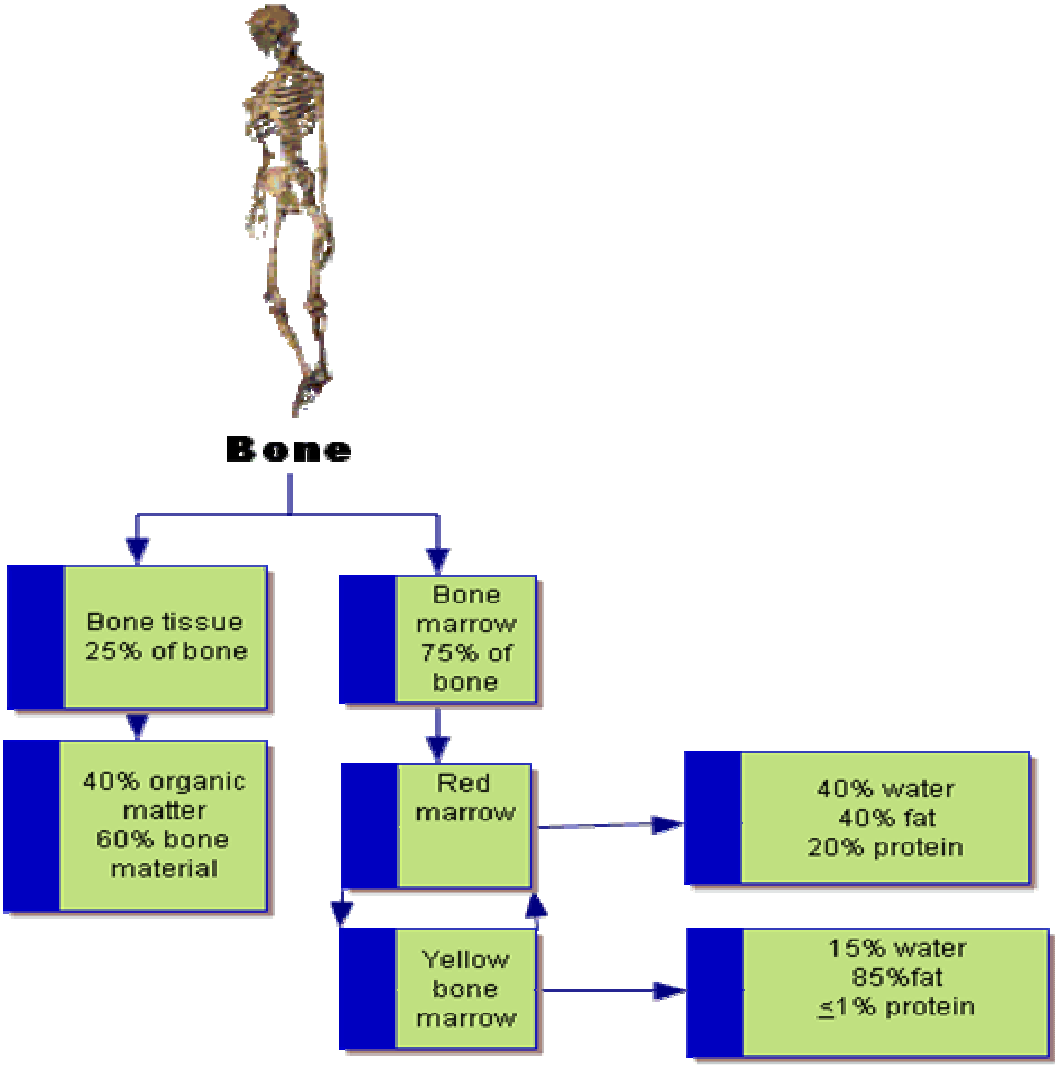

Figure 1.1 The composition of axial skeletal bone (adapted from 13)

1.2.1.1 Collagen

There are two dominant types of bone collagen namely type I (95% of bone) and type V (making up the remaining 5%), both of which are fibril-forming. The bone matrix consists of 90% type I collagen. It is the collagenous component of bone which creates the capacity for resisting pressure (14). Synthesized by osteoblasts, bone collagens are then deposited in layers known as lamellae. In healthy, mature bone the parallel deposition of collagen forms a dense configuration. In immature and some unhealthy bone, the deposits are disorganized and are known as woven bone (8).

Collagen biosynthesis occurs via the following steps (14, 15, 16):

- Translation: pro-collagen molecules are synthesised in the endoplasmic reticulum and transported to the Golgi apparatus. Modification of pro-collagen occurs via hydroxylation and glycosylation.
- Alignment of the triple helix: the pro-collagen chains are assembled into a triple helical molecule. The C and N-propeptide terminals are stabilized through the formation of intra-chain disulphide bonds. One of the functions of these propeptide terminals is to prevent inappropriate fibril formation.
- Post-translational modification: This involves the folding and final processing of the pro-collagen molecule. HSP47 is one type of chaperone involved in this process. The C-propeptides and N-propeptides of type I collagen are cleaved by proteases, such as disintegrin and metalloprotease with thrombospondin motifs (ADAMTS-2), bone morphogenetic protein 1 (BMP-1), and C-proteinase.
- Once these terminals have been cleaved, fibril assembly begins: the intermediate fibril is bound with collagen monomers in a regular pattern known as a quarter staggered array. A longer fibril is formed when intermediates fuse both laterally and longitudinally. Fibrillogenesis is affected by the kinetics of propeptide removal, the existence of the non-helical telopeptide domain, other fibrillar collagens, specific matrix constituents such as decorin, fibromodulin and biglycan and fibril associated collagens with interactive triple helix (FACIT).
- Cross-linking: The most important post-translational modification is the hydroxylation of lysine residues by enzymes such as lysyl hydroxylase to form

cross-links. Paucity of cross-linking results in, for example, osteoporosis, rupture of arteries and other connective tissue dysfunctions. The overproduction of cross-links is seen in some forms of osteogenesis imperfecta and compromises bone strength (17).

- Cross-linking of collagen fibres conveys tensile properties to bone. The cross-linking sites occur at the end of each collagen molecule. The site is only 15 to 20 amino acids in length and is a non-helical domain. Cross-linking is facilitated by the action of lysyl oxidase (LOX). LOX acts by oxidising the ε-amino groups of lysine and hydroxylysine residues to aldehyde groups. These aldehyde groups form Schiff-base-type bonds with ε-amino groups of other lysine or hydroxylysine residues in adjacent chains within the same or neighbouring molecules. Cross-linking is tissue-specific and can follow one of two major pathways, depending on whether lysine or hydroxylysine is involved. These pathways are depicted in Figure 1.2. Hydroxylysine and its subsequent oxidation increase the stability of cross-links. Hydroxylysine is therefore more abundant in cartilage and bone than it is in skin and tendons for example. Some hydroxylysine residues are further modified through the addition of galactosyl or galactosyl and glucosyl residues. Such hydroxylysine glycosides may still participate in cross-linking.
- The intermediate cross-links that are initially formed give rise to mature cross-links as the tissue ages. Pyridinium and pyrrolic cross-links are formed in skeletal tissue.

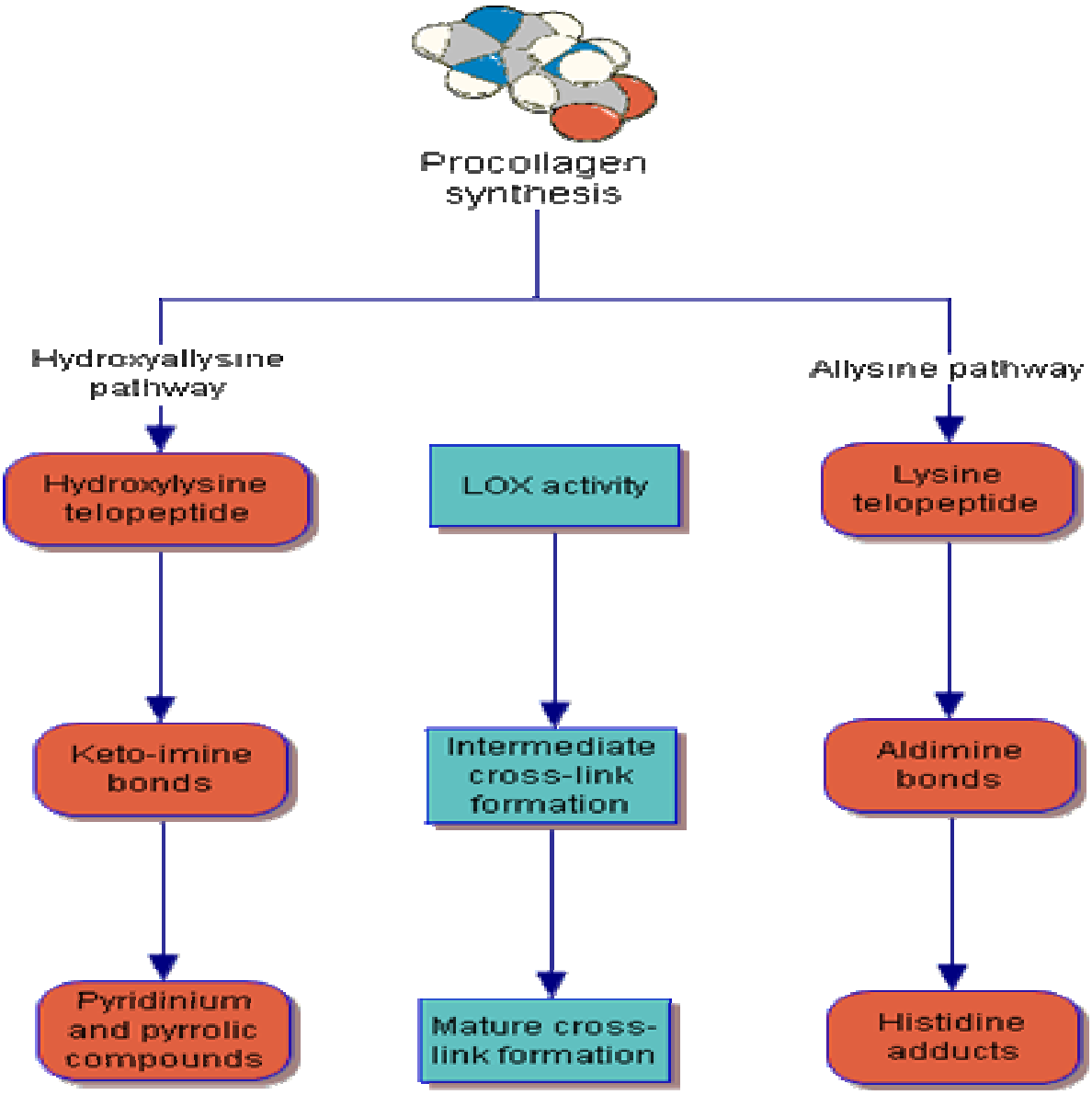

Figure 1.2 Cross-link formation via the hydroxyallysine and allysine pathways (adapted from 16; 18)

The regulation of collagen processing occurs via a number of cytokines, growth factors, oncogenes and transcription factors. The most important of those involved in the regulation of type I and type V collagens processing are depicted in Table 1.1.

Table 1-1 Regulators of collagens I and V synthesis (adapted from 14)

Growth factors	Factor	Cellular targets	Bone collagen target and type of response
	$TGF\beta_1$	Fibroblasts, osteoblasts, hepatoblasts, chondrocytes	I: enhances translation, increases mRNA stability
	$TGF\beta_3$	Fibroblasts	I: Stimulates mRNA levels
	BMP	Osteoblasts	I: Induces osteoblast differentiation, stimulates collagen I synthesis
	PDGF	Gingival fibroblasts	V: Stimulation of collagen synthesis
	EGF	Osteoblasts	I: Inhibits collagen synthesis
Cytokines	Interleukin I (IL-1)	Chondrocytes, fibroblasts	I: Enhances mRNA levels
	IFNγ	Fibroblasts	I: Suppresses collagen synthesis
	TNFα	Fibroblasts	I: Suppresses mRNA
Hormones and other factors	Vitamin D3	Osteoblasts	I: Decreases mRNA and protein levels
	Glucocorticoid	Fibroblasts	I: Suppresses collagen production and transcription in long-term cultures
	PTH	Foetal rat fibroblasts	I: Suppresses collagen production and transcription in long-term cultures
	Retinoic acid	Chondrocytes	I: Induces switches to α-1 collagen mRNA
	Ascorbate	Fibroblasts, chondrocytes, etc…	All collagens: Enhances hydroxylation and mRNA stability
Transcription factors and oncogenes	c-fos	Osteoblasts	I: Over- expression inhibits α1 mRNA levels
	Cbfa1/Runx2	Osteoblasts	I: Induces transcription

1.2.1.2 Non-collagenous proteins

The non-collagenous proteins in bone are numerous (see Table 1.2) and perform a wide variety of functions. These proteins are both endogenously and exogenously derived. The exogenous proteins include albumin and α-2-HS-glycoprotein, which are mainly serum-derived and assist in mineralization. Endogenously-derived proteins include a variety of growth factors and ground substance proteins, mainly glycoproteins and proteoglycans (19, 8).

The glycoproteins and proteoglycans that make up the ground substance of the matrix are highly anionic. This contributes to their binding capacity and is thought to play a role in calcification and aspects of bone remodelling (8). For example, osteocalcin, a molecule that is unique to bone, promotes the binding of mineral deposits (19). The phosphoprotein, osteopontin, promotes the cell adhesion of osteoclasts, which forms part of the first phase of bone resorption. It is hypothesised that cells lay down osteopontin on the mineralised matrix. Once the osteopontin becomes exposed, osteoclasts are activated and recruited. Non-collagenous proteins are also involved in the adhesion of osteoblasts to sites where bone has been eroded during resorption. Such molecules include thrombospondin, osteoadherin and bone sialoprotein. Osteonectin, another of the phosphoproteins, is involved in modulating cell division and cell migration (19).

Table 1-2 Proteins of the extracellular matrix of bone (adapted from 19)

Protein classification	Types of proteins
Collagens	I, III, V, XI, XII
Phosphoproteins	BAG-75, Bone sialoprotein, Dentin matrix protein-I, Dentin sialoprotein, matrix extracellular phosphoglycoprotein, osteopontin, osteonectin
Proteoglycans	Versican, syndecan, CS-Decorin, CS-biglycan, lumican, osteoadherin
Serum proteins	Albumin, Fetuin, IgG, IgE
γcarboxyglutamate-containing proteins	Osteocalcin, matrix Gla protein
Other	Thrombospondin, fibromodulin, proteolipids

1.2.1.3 Water and lipids

Water is a minor constituent of the bone matrix. However, the role of water should not be underestimated: water augments the strength of bone by acting as a type of plasticizer and also helps to stabilise the collagen fibril (20, 19).

Lipids are important to the formation of cell membranes. They are also involved in the initial mineralization of bone (19). Lipids are important signalling molecules in bone. In fact, the up-regulation of certain lipid mediators has been linked to pathological bone resorption and new roles of lipids are continually being discovered (21).

1.2.1.4 Inorganic contents

The mineral found in bone is an analogue of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Hydroxyapatite in bone is known as bone apatite. This mineral occurs in crystal form and contributes to the rigidity of the bone. The small size of the crystals allows them to be dissolved easily in the acidic environment created by the osteoclasts during bone remodelling (19).

The hydroxyapatite contains magnesium, carbonate, fluoride, potassium, sodium and citrate impurities, which facilitate the release of ions for homeostasis (19). Other impurities that may incorporate themselves into bone include transition metals, radioactive particles and heavy metals (8).

Apatite is deposited on type I collagen in such a manner that the crystals lie parallel to the collagen fibril (19). The process of mineralization may induce changes in the cross-links. For example, calcification of bone decreases the ratio of pyridinoline to deoxypyridinoline (16). Elevated pyridinoline cross-links are associated with a generalised fibrotic state (18).

1.2.2 Bone cells

The ecosystem that bone represents is comprised of specialised groups of cells, with each group performing its own distinctive functions. These cells are the osteoblasts, osteoclasts, osteocytes and bone lining cells. Bone cells form an interconnected network, which allows for communication and transport between the surface and deep tissues (9). The formation of bone lining cells, osteoblasts and osteocytes from a multipotent stem cell is depicted in Figure 1.3.

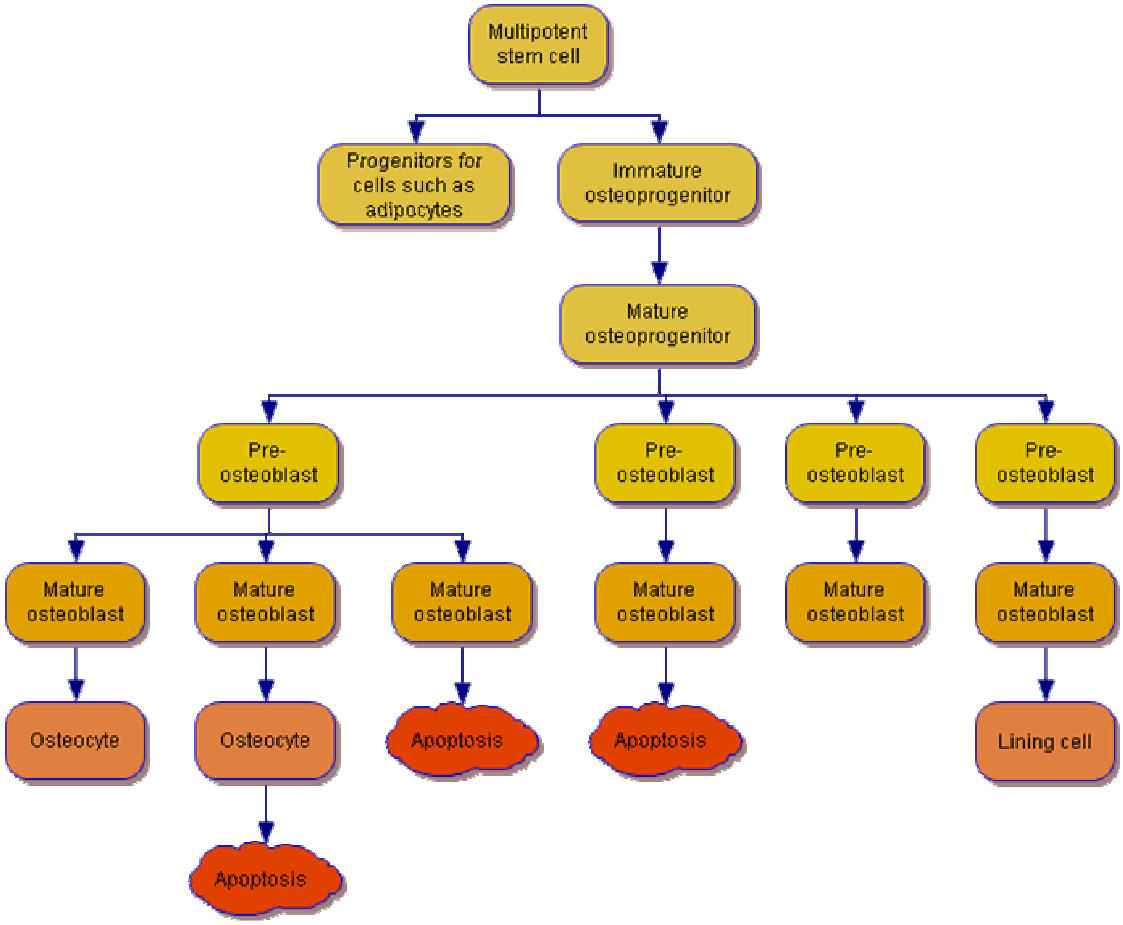

Figure 1.3 Formation of bone cells (adapted from 22, 19)

1.2.2.1 Osteoblasts

Aubin (22) describes osteoblasts as "post-proliferative, cuboidal … cells lining the bone matrix at sites of active matrix production". Osteoblasts are heterogeneous cells that arise from a multi-potent stem cell. Their main utility is to synthesize bone matrix, which is also known as the osteoid in the unmineralized state. The tasks of these osteoblasts are regulated by endocrine mediators, including parathyroid hormone (PTH), oestrogen and prostaglandins (8, 22).

Osteoblasts variably produce collagenous and non-collagenous bone matrix proteins, cytokines and growth factors. Osteoblasts secrete colony stimulating factor (CSF), receptor activator of nuclear factor κ-B-ligand (RANKL) and express osteoprotegerin (OPG), which regulate osteoclasts and bone resorption (8).

1.2.2.2 Osteoclasts

Osteoclasts are formed from the precursors of monocyte/macrophage cell lines. The osteoclasts are involved in bone resorption, removing approximately 500mg of calcium a day. These multi-nucleated cells have up to 25 nuclei and may be found in Howship's lacunae (tiny notches in the bone surface created using the acids and enzymes the cells produce) (23). One of the features that allow the osteoclasts to perform resorption efficiently is a ruffled border that contains numerous enzymes and acids, which facilitate decomposition of the matrix and bone minerals (8, 24). Once this degradation is completed, the osteoclast becomes inactivated or is apoptosed. The formation of osteoclasts is regulated by factors including RANKL, PTH, vitamin D_3, and macrophage colony stimulating factor (M-CSF).

1.2.2.3 Osteocytes

Osteocytes are located within the lacunae and represent terminally differentiated osteoblasts that have been trapped in the newly formed extracellular matrix. These cells are smaller than the osteoblasts and have lost most of their organelles. The osteocytes have a stellate shape. Their cell processes extend through the bone canaliculi (small channels in the bone) and in this way the osteocytes may connect with bone lining cells, osteoblasts, or other osteocytes. Minerals can be transported through these contiguous spaces, implying that osteocytes play an important role in mineral homeostasis. In addition, osteocytes are suspected of playing a role as sensors of damage and mechanical stimuli, which initiate bone remodelling or repair. The approximated lifespan of an osteocyte is 25 years and the remains of osteocytes are removed during remodelling (22).

1.2.2.4 Bone lining cells

Bone lining cells consist of inactivated osteoblasts or other mesenchymal type cells. These cells are considered a resting surface, comprised of flat nuclei and few organelles. However, some evidence shows that lining cells can be re-activated to osteoblasts in response to the release of PTH (22). This lining divides the bone from interstitial fluid. It also plays a role in calcium homeostasis, metabolic support of osteocytes, and the initiation of resorption by osteoclasts (8).

1.2.3 Bone development

While the formation of bone appears to have been greatly elucidated, much of the process remains enigmatic and is continually under scrutiny (20). The development of bone tissue occurs either as endochondral histogenesis or intra-membranous histogenesis. The main difference between the two processes is the existence of cartilage ruminants as intermediates in endochondral ossification (8).

1.2.3.1 Endochondral bone formation

This type of growth occurs during the formation of all bone, excluding the craniofacial skeleton and the clavicle. A cartilage anlage is formed as mesenchymal cells differentiate, becoming chondroblasts. The perichondrium is then formed and part of it becomes the periosteum. Finally, osteoblasts develop from the periosteum. Part of the cartilaginous primordium is retained for the formation of joints and growth plates, with the rest being replaced by bone. The matrix is mineralised and there is vascular invasion of the tissue (19).

Endochondral growth is accelerated during and after puberty and is responsible for the ultimate stature of the individual. Closure of the bone plates occurs when the cartilage ceases growing to be replaced by bone. Bone retains its capacity for growth and repair in the adult. The process of growth and repair is closely linked to remodelling (8, 25).

1.2.3.2 Intra-membranous histogenesis

Much of the craniofacial skeleton and clavicle is formed through intra-membranous ossification. Unlike in endochondral histogenesis, there is no cartilage intermediate. Mesenchymal cells congregate and differentiate into osteoblasts. The tissue is vascularised and osteoid is deposited. Finally, mineralization takes place (19, 25).

1.2.4 REGULATORS OF BONE DEVELOPMENT

The processes of bone development are complex and require the interplay of factors that contribute to the sound regulation of bone turnover. The regulatory molecules include (but are not confined to) vitamin D, PTH, growth factors, cytokines, prostaglandins, sex hormones and calcium and phosphate balance. A short description is offered of the roles of these regulators in bone homeostasis.

1.2.4.1 Vitamin D

Vitamin D is a steroid vitamin. It is essential for calcium metabolism and plays a vital role in bone. The conversion of cholecalciferol to vitamin D is outlined in Figure 1.4. Vitamin D acts as a transcription factor in osteoclasts and osteoblasts, thereby regulating cell growth and differentiation. $1,25(OH)_2D_3$ has a biphasic effect on osteoblasts, stimulating or inhibiting normal development, depending on whether it is introduced during the proliferation or differentiation stage. $1,25(OH)_2D_3$ stimulates expression of osteocalcin in humans and modulates the production of growth factors, such as insulin-like growth factor I (IGF-I). In addition, $1,25(OH)_2D_3$ is a potent stimulus for osteoclast formation and activity (26). $1,25(OH)_2D_3$ affects the active transport of calcium in the intestine by binding the Vitamin D receptor. This induces the synthesis of calcium-binding protein which facilitates calcium uptake (27).

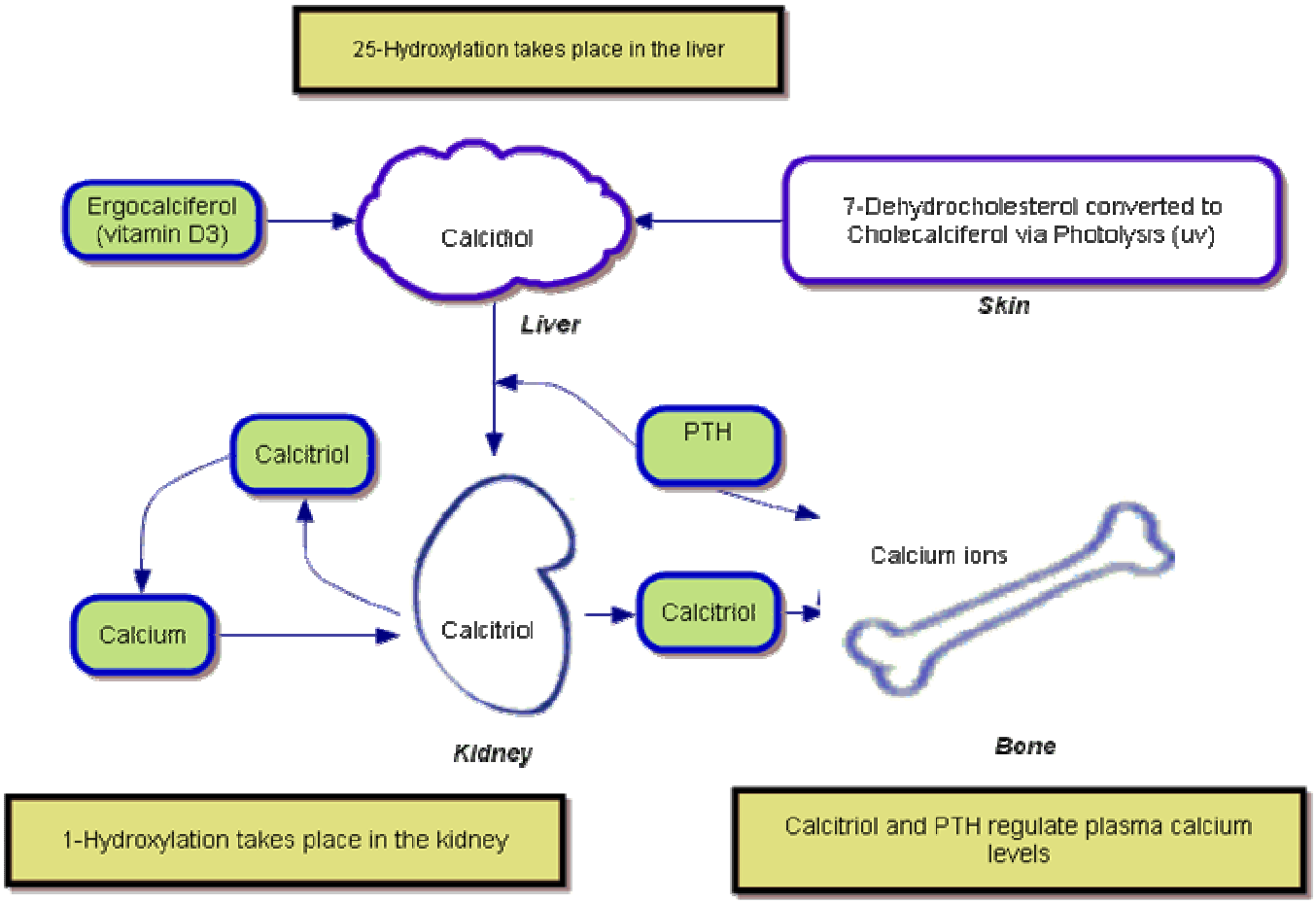

Figure 1.4 Metabolism of Vitamin D (adapted from 28)

1.2.4.2 Growth factors

Growth factors trigger mitosis, enhance differentiation and protect against apoptosis (29). The major growth factors in skeletal bone are the IGFs, the transforming growth factor-beta (TGF-β) superfamily, the fibroblast growth factors (FGF), platelet derived growth factors (PDGF), the epidermal growth factor family (EGF) and the family of morphogens called Wingless-Int (Wnts). The various effects of these growth factors are summarised in Table 1.3.

Table 1-3 Growth factors regulating bone metabolism (adapted from 21, 29, 30, 31)

Growth factor	Functions
IGF	Increases osteoblast proliferation and differentiation; decreases osteoclast activity; promotes resorption via its effects on osteoblasts; promotes matrix synthesis; up-regulates type I collagen expression
TGF-β	Role is unclear and dose-dependent. Functions may include: increasing or inhibiting osteoclast-like cell formation; decreasing or promoting bone formation via its effects on osteoblasts
FGF	Promotes osteoblast proliferation, osteogenesis and osteoclast formation
PDGF	Promotes collagen synthesis and cell proliferation; prevents bone loss
EGF	Increases resorption; increases cell proliferation
Wnts	Promotes release of glycoproteins, which increase bone growth by enhancing osteoblast proliferation

1.2.4.3 Pro-inflammatory cytokines

In Study 2 a preliminary investigation is performed on the potential link between pro-inflammatory cytokines and low BMD. There are a number of parallels and developmental links between the immune system and bone. These overlaps have been extensively investigated in the field of osteoimmunology, which aims to delineate the mechanisms involved in the relationship between immune mediators and phenomena such as pathological bone loss (6).

The most extensively studied cytokines in relation to bone metabolism are IL-1, IL-6 and TNF. These cytokines are all potent stimulators of bone resorption. IL-1 has in fact been identified as the most potent peptide simulator of this process. IL-6 has

specifically been implicated in the increased bone resorption seen in Paget's disease and cancers of the bone (32).

The discovery of a specific TNF superfamily cytokine, tumour necrosis factor-related activation-induced cytokine (TRANCE) and its receptors has enhanced understanding of how osteoclasts function and are regulated (33). This cytokine is alternatively known as receptor activator of nuclear factor κB-ligand (RANKL), osteoclast differentiation factor (ODF), or osteoprotegerin ligand (OPGL). The acronym RANKL will be used in this study.

In bone, RANKL binds to RANK, a receptor on osteoclast precursor cells. This binding aids resorption and osteoclast survival, as well as osteoclast formation from precursors. The binding of RANKL to a decoy receptor, osteoprotegerin (OPG), which is primarily expressed by bone marrow stromal cells, inhibits resorption. Figure 1.5 illustrates the relationship between CSF, RANKL, RANK and OPG.

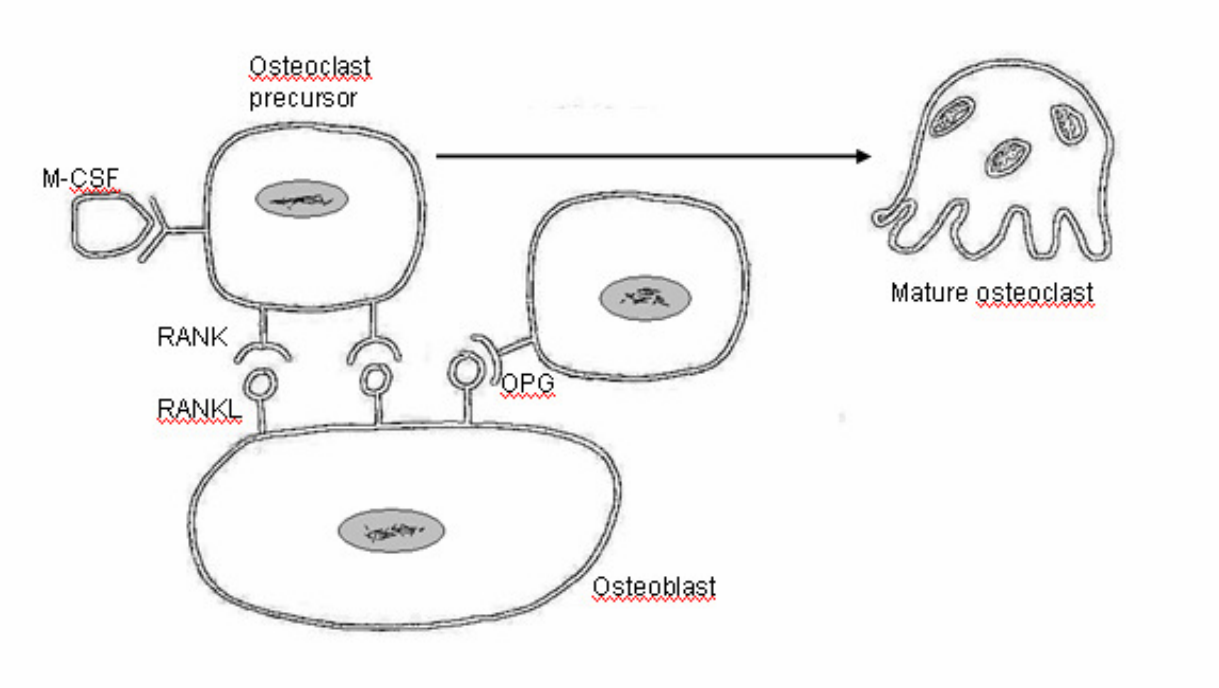

Figure 1.5 The relationship of osteoclast precursors to M-CSF, RANK, RANKL and OPG (adapted from 33, 34)

PTH and vitamin D increase the production of RANKL, while inhibiting the production of OPG. The net effect is an increase in resorption (6, 32, 35). Meanwhile, IL-1, IL-6, IL-11, IL-17 and TNF increase resorption of bone (36, 37) perhaps by increasing osteoclastogenesis and RANKL expression, while acting synergistically to increase the presentation of RANKL to osteoclasts, which leads to osteoclast maturation (33).

The prostanoids (prostaglandins and thromboxanes), leukotrienes and other lipid molecules play important roles as signal molecules (21). Prostaglandins have an effect on both the osteoblasts and osteoclast cell lines. For example, PGE_2 stimulates the production of osteoclasts and their differentiation, while extending their lifespan. PGE_2 stimulates RANK production, while inhibiting OPG expression in osteoblasts (32).

1.2.4.4 Sex hormones

Oestrogens, progesterone and androgens also assist in the control of bone metabolism. Oestrogens have effects on mature osteoblasts and mature osteoclasts, as well as on the osteoclast precursors. These effects are summarised in Figure 1.6 (dashed lines indicate inhibition, solid lines indicate stimulation). Oestrogen insufficiency is a major cause of postmenopausal osteoporosis. It is still unclear whether oestrogens inhibit osteoclastogenesis or simply hamper the functionality of the mature osteoblasts, though (38).

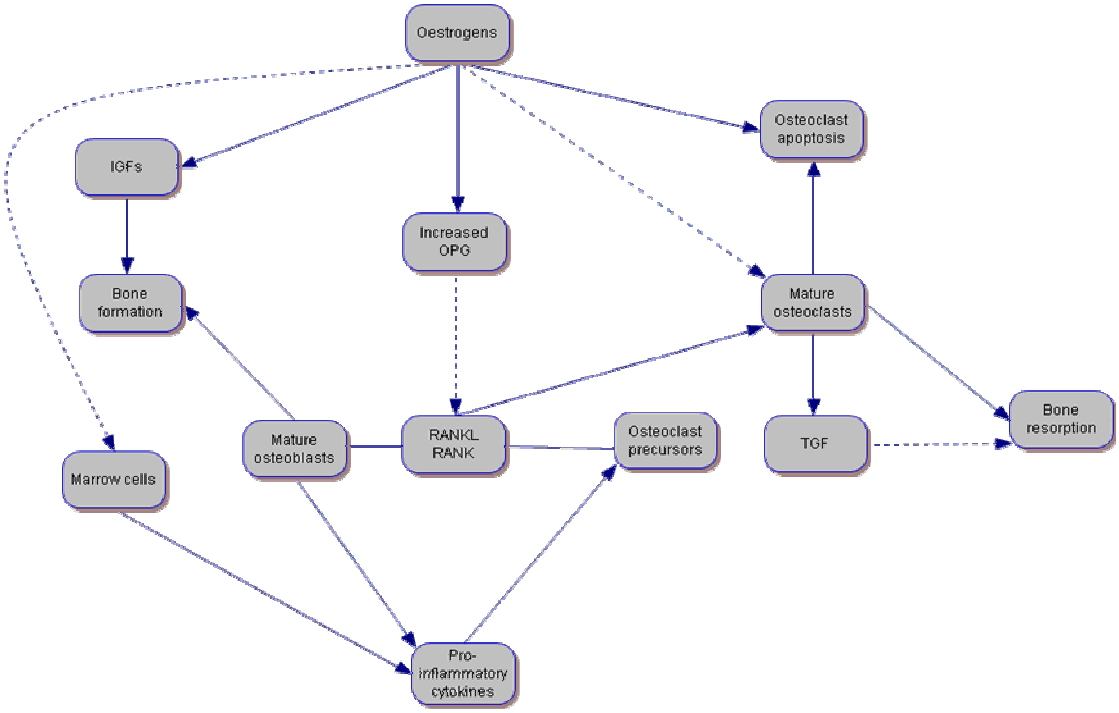

Figure 1.6 Oestrogens' effects on bone (adapted from 39)

Progesterone increases proliferation and differentiation of human osteoblasts. Its major role is to modulate oestrogens' actions on bone. Androgens inhibit bone resorption, and their effects are thought to be generally anabolic. It is now recognized that both oestrogens and androgens have overlapping roles in bone metabolism in men and women. Previously, it was believed that oestrogens were the major determinants in bone density for women, whereas testosterone fulfilled this role in men (39).

1.2.4.5 Parathyroid hormone

PTH regulates mineral homeostasis by reducing calcium excretion and enhancing tubular reabsorption of phosphate. Its catabolic effects on bone are accomplished through the bone lining cells which rapidly release calcium in response to PTH stimulation. PTH also inhibits type I collagen synthesis. Its anabolic actions include the enhancement of the early phase of osteogenesis (40).

1.2.4.6 Calcium and phosphate homoeostasis

Calcium is the fifth most abundant inorganic element in the body. It is the structural constituent of hydroxyapatite. 1% of the body's calcium is found intracellularly, while

0.1% is found in the extracellular compartments. The remaining bulk of the calcium is found in bone and teeth. Extracellular calcium concentration is maintained through dynamic equilibrium fluxes that occur in the intestine, bone and kidney (41).

Calcium is an important intracellular signal transductor and also plays a role in cell adhesion, cell proliferation and differentiation, membrane permeability, cell motility and coagulation. Within bone, calcium - as hydroxyapatite - helps ensure structural rigidity (41).

Poor calcium intake has been linked to a greater occurrence of a number of diseases, including osteoporosis (42). When the body cannot absorb enough calcium from the gut to maintain its needs, skeletal calcium is mobilised, resulting in a decreases in bone mineral mass. The body's response to this situation is depicted in Figure 1.7.

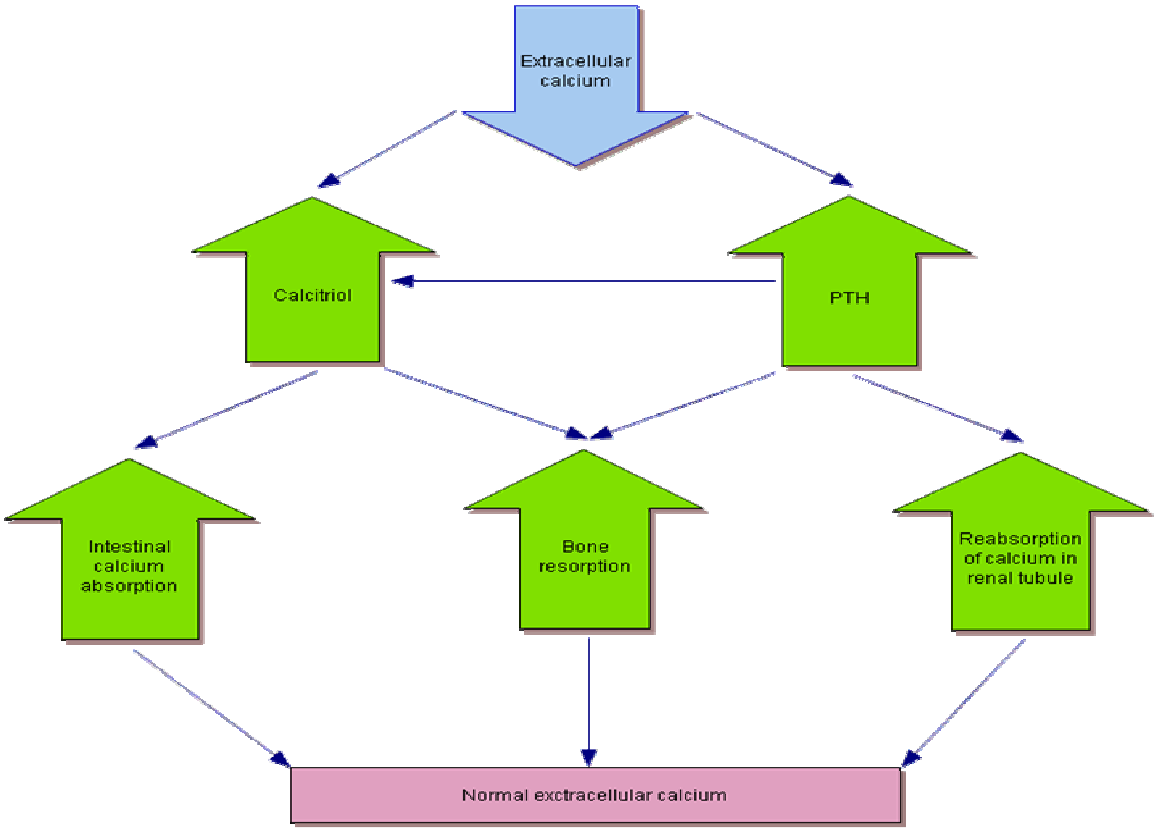

Figure 1.7 The maintenance of extracellular calcium concentration (adapted from 41)

Phosphorous is the sixth most abundant element in the body and occurs mainly in the form of phosphate, 80% of which is found in bone. Phosphate is vital for energy storage and delivery and for regulating various enzyme activities. It is an important intracellular signal transduction regulator and ensures membrane stability. Phosphate ultimately contributes to the integrity of bone (43). As seen with calcium, phosphate balance is maintained through fluxes, which occur in the intestine, bone, soft tissues and kidneys (41).

Phosphate deficiency stimulates calcitriol production. Calcitriol increases the movement of phosphate from the intestine and mobilises phosphate from bone mineral. PTH and the active form of Vitamin D both cause phosphate reabsorption. Vitamin D also causes increased mobilisation of phosphate from bone (43).

1.2.5 Remodelling

A vast array of components of the bone ecosystem (some of which have already been described) are involved in bone remodelling - the continual destruction and reconstruction of bone that occurs throughout life (42). This process is essential to the health of bone as it continually adapts to mechanical and chemical stimuli. The goal of remodelling is to remove less functional bone and replace it with a more mechanically sound support configuration, formed by fresh bone (44).

The turnover rate in cortical bone is 2-3% per year, while the turnover rate in cancellous bone is much higher. The rate of remodelling is associated with the mechanical strength of the bone (45), and perhaps also with mineral metabolism. Continual remodelling activity ensures the supply of osteoclasts, quiescent surfaces for ionic exchange, bicarbonate (for acid base balance), growth factors and cytokines, which are required for various functions in the body (46).

The remodelling cycle can be divided into two phases: during the first phase, osteoclast precursor cells are activated and penetrate the bone lining layer. These osteoclasts then fuse to form multi-nucleated osteoclasts, which adhere to the bone surface and secrete an acidic medium, consisting of protons and proteolytic enzymes that dissolve hydroxyapatite. The subsequent mechanism of apoptosis of these cells

is still unclear, but the process is regulated by local cytokines, such as RANKL, IL-1, IL-6, CSFs, PTH, calcitonin as well as 1, 25-dihydroxyvitamin D_3 (46, 44).

The second phase of remodelling involves the recruitment of osteoblasts and their precursors. Coupling signals from the resorbed matrix and osteoblasts, recruit osteoblast progenitors to the resorbed bone. Possible signallers in this process include TGF-β, IGF- I and II, BMP and PDGF. Osteoblasts fill the lacunae and lay down osteoid, which then becomes mineralised. The osteoid's main constituent is type I collagen. The collagen fibres have numerous surface-bound molecules such as the non-collagenous proteins, including fibromodulin and decorin. The removal of these molecules from the fibres may trigger mineralization (46).

As the formation of the new bone progresses, the osteoblasts become part of the bone and are known as osteocytes. These osteocytes maintain close contact with one another, via gap junctions and with lining cells and the inactive bone surface, via the canaliculi. From this central position, the osteocytes can sense changes in the chemical properties of the bone and therefore serve as important initiators of future bone remodelling. The death of osteocytes is followed by their resorption (46, 44).

When the formation of the new bone is complete, the interactive osteoblasts flatten and elongate. They are then referred to as bone lining cells. The newly formed bone is known as a basic structural unit (BSU). In cancellous bone, the BSU is called a packet or trabecular osteon, whereas in cortical bone it is known as a Haversian system or a cortical osteon (46, 44).

In young adults, resorption and formation are tightly coupled and the net change in mass is zero. However, in age-dependent bone loss (e.g. postmenopausal osteoporosis), resorption cavities are not filled completely and bone formation is therefore abnormal and thinning of the trabeculae occurs (13). In postmenopausal osteoporosis, there is also an increase in osteoclast activity without a countervailing increase in osteoblast activity. Elevated resorption has been implicated in the high incidence of fractures in postmenopausal women and in children (12). By contrast, when bone is damaged, the healing process stimulates osteoblast activity, which

moves remodelling in favour of bone formation (47). The clearance of the products of bone metabolism is beyond the scope of this study and is therefore not discussed.

1.2.5.1 Neuro-endocrine control of bone remodelling

The discovery of neural involvement in bone remodelling stemmed from the observation that some patients with nervous system disorders (the result of strokes or spinal cord injury) exhibited increased osteogenic activity (48). Recent findings delineate the role of neuropeptides and neuropeptide receptors in bone. These receptors and neuropeptides are shown in Table 1.4.

A final role-player in the control of bone remodelling is the hypothalamic-pituitary (HPA) axis. HPA axis involvement in controlling this process has long been recognized (49). The HPA axis controls the secretion of corticotrophin-releasing hormone (CRH) and, subsequently, glucocorticoids and sex hormones. Both glucocorticoids and sex hormones have an integral role to play in the regulation of bone turnover (1, 39). Furthermore, CRH has an inhibitory effect on the release of the growth hormones that encourage bone formation. It is this important sequence of events that presents a possible link between depression and low BMD and the association of the HPA axis, depression and BMD is discussed in greater detail under headings 1.3 and 1.4.

Table 1-4 Neuropeptides, the location of their receptors and their actions in bone (adapted from 48)

Neuropeptide	Location of receptors	Function of neuropeptide
α-calcitonin gene-related peptide (CGRP)	Primary osteoblasts and osteoclasts	Stimulates osteoblast proliferation, stimulates synthesis of growth factors, cytokines, collagen synthesis
Substance P	Primary osteoblasts and osteoclasts	Maintains trabecular bone integrity
Vasointestinal peptide (VIP)	Primary osteoblasts and osteoclasts	Prevents osteoclast formation
Serotonin	Primary osteoblasts and osteoclasts, bone osteocytes	Involved in regulating osteoblast differentiation and osteoclastogenesis
Glutamate	Bone and primary osteoblasts, bone osteocytes, primary bone osteoclasts	Speculative

1.2.6 Diseases of bone

Diseases of bone include osteoporosis, osteopenia, osteomalacia, rickets, Paget's disease, and a host of rare diseases such as osteogenesis imperfecta. The focus of this study is on the loss of bone density, specifically that seen in osteoporosis.

1.2.6.1 Osteoporosis

Osteoporosis is a disorder of bone that is characterized by bone fragility and an increased risk for fractures (1). This fragility results from decreases in the bone mass and BMD. Such alterations in bone mass are accompanied by changes in the microarchitecture of the bone and a reduction in the capacity to resist fracture (50). Osteopenia is also a disease of BMD loss, but the loss is not in the same order of magnitude seen in osteoporosis.

Patients with osteoporosis present with recurring episodes of acute or intermittent pain in the affected limbs following normal activity. Loss of weight, fractures and spinal deformity occur as the disease advances (13). Postmenopausal osteoporosis most frequently results in wrist, spine and femoral neck fractures (8). Further

development of the disease can take on various forms, such as cervical lordosis, thoracic kyphosis and loss of height (13). Osteoporosis fractures, which are often the endpoint of osteoporosis, are associated with increased morbidity and mortality and represent a considerable socioeconomic burden (51, 52). Osteopenic women are at greater risk for fracture than women with normal BMD and postmenopausal women suffering from osteopenia present with increased risk of developing osteoporosis compared to women with normal BMD (53).

Three main types of as osteoporosis are defined, namely primary osteoporosis type I, primary osteoporosis type II and secondary osteoporosis. These categories are described in Table 1.5.

Table 1-5 Categories of osteoporosis (adapted from 13)

Category	Type	Group affected	Diagnosis
Primary osteoporosis	Involutional type I	Menopausal/post-menopausal women	High bone turnover (>30%); vertebral crush fractures; high bone loss
	Type II	Males or females (>70 years of age)	Vertebral crush, hip and other fractures
Rare forms of primary osteoporosis	Idiopathic, juvenile	8-14 years (boys and girls)	Low bone mass for age; fractures
	Idiopathic, young adult	Young adults	Low bone mass; fractures; high and low bone turnover forms
Secondary osteoporosis	Endocrine, gastrointestinal, bone marrow disorder, connective tissue disorder, nutritional or drug-related.	People suffering from underlying diseases of the endocrine, gastrointestinal, bone marrow, connective tissue, nutritional or drug-related nature.	Underlying diseases; low bone density; fractures/pseudo-fractures

1.2.6.2 Diagnosis of osteoporosis

Despite its strong association with pathological fractures and its high morbidity, osteoporosis is often regarded as a silent disease that has the potential to remain undetected for a long time (2). The most frequently used method of diagnosing osteoporosis is dual x-ray absorptiometry (DEXA). Markers of bone turnover have also been used to determine the rate of bone formation and resorption in suspected disorders of bone density. Both DEXA and bone turnover markers are used in this study and therefore their principles are dealt with in this chapter. Their practical measurements are described in Chapter two.

1.2.6.3 DEXA

Absorptiometry techniques provide a two-dimensional reading of aerial bone density. Measures such as ultrasound and DEXA are important in live estimates of fracture risk for the diagnosis of osteoporosis (54). DEXA has its origins in x-ray spectrophotometry, which was developed in the 1970s (13). DEXA determines bone mineral content per projected area of bone.

Results of DEXA scans are expressed in g/cm^2, T-score and Z-score. Osteoporosis and osteopenia are defined using the World Health Organization criteria, which articulate BMD in standard deviations below the average in the young adults (52). These measurements are based on spinal bone density measurements and are shown in Table 1.6.

Table 1-6 diagnostic criteria for osteoporosis (adapted from 13)

Classification	Criteria
Normal	Within 1 SD for the mean BMD of a young adult
Osteopenia	1 to 2,5 SD below the mean value for a young adult's BMD
Osteoporosis	2,5 (or more) SD below the mean
Severe/established osteoporosis	2,5 (or more) SD below the mean in the presence of one or more fragility fractures

The reduction of BMD with age makes it possible to compare an individual's BMD with that of an age-adjusted reference group, using Z-scores and percentage decrease. Comparisons can also be made with healthy, young individuals (the mean

is then used to calculate the T-score or the percentage decrease) or with the fracture threshold, which represents the upper limit of BMD values found in osteoporotic patients with fractures (55).

However, the use of the WHO definitions has been questioned over recent years (55, 52). In a position paper on the use of the WHO criteria, Kanis and Glüer (56) propose that DEXA readings at the hip should be represented as T-scores, while other measurements could be expressed in relative risk or standard deviation units. One reason for this is the biological variability of BMD at different skeletal sites and the errors related to measurements at different sites.

Moreover, BMD measurement at one site cannot accurately be used to predict BMD at another site (55). Arlot, Sornay-Rendu, Garnero, Vey-Marty and Delmas (57) studied the prevalence of osteoporosis by measuring BMD at various sites for the same group of premenopausal women. The research showed that the proportion of women diagnosed with osteoporosis increased when the distal radius was used as the measurement site instead of the spine or the femoral neck. The vertebrae and upper femur are the most frequently measured BMD sites because of the high frequency of pathological fractures that occur there. Vertebral measures may be confounded by the occurrence of aortic calcifications and osteophytic lesions, especially in older populations. Vertebral collapse also increases spinal mean BMD. In the presence of vertebral degeneration, DEXA measurements of the femoral neck or total hip area are frequently used (58).

Body fat content is another confounding variable in DEXA readings and while DEXA readings are corrected for body fat content, this is based on a flawed assumption that fat deposition is equal throughout the body. Accordingly, the errors of accuracy of measurements range from 2% to 10% depending on the site of measurement (56).

Kanis and Glüer (56) point out that different manufacturing types of machines also provide vastly disparate readings for the same patients and showed this when the use of Hologic and Lunar's machines on the same patients provided measurements that differed by 1 standard deviation in BMD readings for the lumbar spine.

Nevertheless, DEXA remains the gold standard in BMD measurement and osteoporosis diagnosis.

Researchers and clinicians recognize the value of using both DEXA and markers of bone turnover to predict bone status. The value of these markers is that - unlike DEXA readings - they may be used to predict bone turnover in individuals over a short period of time (54).

1.2.7 Bone turnover and its markers

There are numerous markers of bone turnover. Only those markers that are to be investigated in this study will be discussed in this section. The measurement of bone markers is based on the premise that there is dynamic equilibrium in the osteoblast and osteoclast activities and that this can be applied to predict future bone loss in individuals with a validity as high as 75%, depending on the skeletal site (54).

1.2.8 Bone formation markers

Markers of bone formation include bone specific alkaline phosphatase (BSAP), osteocalcin and propeptides of type I pro-collagen. These markers of bone formation are all proteins secreted by the osteoblasts, but reflect different stages of osteoblast differentiation. Only BSAP and osteocalcin are used in this study.

1.2.8.1 BSAP

Phosphatases are released by cells on death or during rapid turnover (4). This alkaline phosphatase is expressed during the maturation of bone matrix. This marker was chosen as it is more specific than the total alkaline phosphatase measurement. BSAP exhibits a circadian rhythm, the details of which are still speculative. Phase of the menstrual cycle affects concentration, with BSAP being elevated during the luteal phase, although not significantly. Age, gender, the existence of fractures and hormonal status also influence BSAP concentration (59).

1.2.8.2 Osteocalcin

Osteocalcin is a vitamin K dependent gamma-carboxyglutamate-containing protein consisting of 49 amino acids. It is synthesised by mature osteoblasts in bone. It is one of the most abundant non-collagenous proteins in bone, but its function is still

unclear. Osteocalcin may be a marker of late osteoblast differentiation and may play a role in bone remodelling (59, 60). Osteocalcin measures are considered reliable markers of bone formation. Serial osteocalcin correlated positively with osteoid volume, osteoid surfaces, mineralizing surfaces and bone formation rate in Brixen and Eriksen's (17) study of the correlation between osteocalcin levels and bone remodelling in osteoporosis.

The secretion of osteocalcin follows a circadian rhythm, with peaks noted at night and in the afternoon. As is seen in BSAP, osteocalcin levels rise during the luteal phase. The velocity of growth, gender, the existence of fractures, hormonal status and kidney function all influence osteocalcin secretion (59).

1.2.9 Bone resorption markers

Bone resorption forms part of the normal cycle of bone homeostasis. Markers of bone resorption include collagen degradation products (such as hydroxyproline, pyridinoline (Pyd) and deoxypyridinoline (DPD)), bone sialoprotein and osteoclast enzymes.

1.2.9.1 Collagen degradation products

With 90% of the bone matrix being made up of collagen, the structure and functional integrity of the bone is greatly reliant on this protein. Cross-linking in bone can be used as a measure of metabolism in bone collagen. Cross-linking occurs extracellularly, but the nature of the cross-link depends on the intracellular post-translational modifications that occur prior to the formation of the cross-links (17, 61).

The C-telopeptides that form the non-helical residue of the cross-links are linked to neighbouring helices via pyridinium or pyrrolic compounds (62). The release of pyridinoline and deoxypyridinoline into the bloodstream has been found to correlate significantly with bone resorption (17, 63). The measurement of DPD and Pyd were undertaken in this research.

1.2.10 Risk factors for osteoporosis

Although the causes of osteoporosis fractures are multifaceted, low BMD remains its primary predictor (64). The identification of risk factors for osteoporosis is vital in the prevention and clinical treatment of the disease in individual patients. In the study of osteoporosis, researchers often differentiate between those factors that influence the risk of falls and those that affect bone density. This section will deal only with the latter.

1.2.10.1 Age

BMD decreases with advancing age (35). Age and BMD are the strongest risk factors for hip fracture (65). A single standard deviation decrease in BMD represents a considerable increase in fracture risk (51). The risk of osteoporosis fracture doubles every seven to eight years after the age of 50 years (66).

1.2.10.2 Gender

Primary osteoporosis involutional type 1 has its onset during menopause and occurs in women. Primary osteoporosis type II occurs in men and women over the age of 70 years. The idiopathic types and secondary osteoporosis also occur in both genders (13). One reason for this is that men generally have higher BMD than women (67). Looker, *et al.* (67) add however, that body size also has an influence on the magnitude of BMD differences between men and women under the age of 50 years. Sex differences in BMD for people older than 50 years is more complicated and adjustment for body size failed to eliminate gender differences in BMD.

1.2.10.3 Menopause

The drop in ovarian oestrogen production that accompanies menopause causes accelerated bone resorption via increased osteoclastogenesis (38). Further evidence for the involvement of oestrogens in postmenopausal osteoporosis is found in studies of the effect of hormone replacement therapy on bone mass. Hadji, *et al.* (68) investigated the impact of hormone replacement therapy on BMD of the heel. Of the 2006 healthy perimenopausal women recruited from the study, 600 had received hormone replacement therapy and 1395 had not. The women using hormone replacement therapy had significantly higher ultrasonographic quantitative results

than non-users. The results remained the same even after adjustment was made for age and body mass index (BMI).

1.2.10.4 Peak bone mineral density (BMD)

Peak BMD is a major determinant of BMD in later life and has been proposed as a target for osteoporosis prevention strategies (69). Bone mass changes throughout life. Increases are well-documented in childhood and adolescence, with roughly 35% of total adult bone mass being acquired during adolescence. Bone growth peaks during this phase. Maintenance of bone mass is displayed in mid-life, with decline exhibited after approximately 50 years of age (70, 45).

Bone mineral content is greatly reliant on bone area and bone area is correlated with height. The individual's total lean tissue mass also affects bone mineral content (70) as the lean tissue adds to the dynamic load on the skeleton. The change in BMD over a lifetime partially explains age-related changes in bone turnover levels (71). Peak BMD is influenced by many of the factors mentioned in this chapter, including nutrition, genetics and lifestyle characteristics.

1.2.10.5 Body mass and body mass index (BMI)

Body mass and BMI are positive predictors of bone mass: an increase in body weight increases the load on the bone and consequently the BMD. However, the variable effect of fat mass versus lean body mass has caused some debate. Lim, *et al.* (72) found that both fat mass and lean body mass were independent contributors in an investigation into the relative contribution of these two factors to BMD in 402 age and weight-matched subjects over the age of 45 years. However, osteoporosis and obesity share a common progenitor cell and genetic predisposition (73). As a person grows older, the activity of osteoclasts increases, while osteoblast function declines. Bone marrow composition is modified to advance adipocyte presence. Despite this, obesity does not enjoy a strong association with the increased risk of osteoporosis, (74). Wang, *et al.* (74) examined 921 women aged 20 to 25 years on bone mass, bone mineral apparent density, bone mineral content, lean tissue mass, fat mass and total mass. Both lean tissue mass and fat mass were positively correlated with BMD at all skeletal sites examined, but the effect of lean tissue mass on BMD was greater

than that of fat mass. On the other hand, very low BMI is detrimental to bone health and has been associated with increased risk for osteoporotic fractures (75).

1.2.10.6 Heredity

Peak bone mass is mostly affected by heredity, with the risk of hip fracture ranging from 50% to 127% in children whose parents have suffered an osteoporotic fracture (66). The heritability of ultrasound values in the premenopausal daughters of postmenopausal women with past fractures was investigated by Drozdzowska and Pluskiewicz (76). In this study, 48 postmenopausal women and their premenopausal daughters were evaluated for bone status. In this sample, 27 of the mothers did not have a history of osteoporotic fractures. These women exhibited significantly higher BMD T-scores than their counterparts who had a history of fractures. Heritability for ultrasound values varied between 52% and 76% amongst the daughters of women with past osteoporotic fractures. This differs significantly from the whole group's value of 14% to 40%.

In an analysis of the risk factors for premenopausal osteoporosis in a group of Spanish women (N=52) by Peris, *et al.* (77), 56% of the sample was found to suffer from idiopathic osteoporosis, while the remainder suffered from secondary osteoporosis (the result of Cushing's syndrome, pregnancy osteoporosis, osteogenesis imperfecta, malabsorption renal osteodystrophy or kidney transplant osteoporosis, anorexia nervosa, hyperparathyroidism, Sheehan's syndrome, gonadal dysgenesis, epiphyseal dysplasia, ethanol or corticosteroid osteoporosis). Those with the idiopathic form of osteoporosis reported a family history of osteoporosis (48%) and exhibited associated hypercalciuria (38%) and renal lithiasis (45%).

1.2.10.7 Lifestyle factors

Lifestyle risk factors include smoking, alcohol consumption and lack of exercise (42). The mechanism through which smoking may affect BMD is still unclear, but the prevention of calcium absorption has been implicated. Heavy alcohol consumption ($\geq$ 200ml/week as defined by the Framingham Study in The North American Menopause Society, (66)) not only increases the risk for falls, but has a negative effect on BMD (66).

Load-bearing activity causes mechanical stress on the skeleton. This stress increases osteoblast activity, which generally augments BMD. Some studies indicate that the type of weight-bearing activity will determine whether or not bone mass is increased. Korpelainen, *et al.* (75) studied 407 women that fell into the lowest BMI tertile of an earlier population-based sample of 1 222 females, aged 70-73 years. The study revealed that low lifetime physical activity was a significant risk factor for low trauma postmenopausal fractures in these women. The research also revealed that high coffee consumption, which is related to sleep disturbances and diuresis, was related to an increased risk of falling. Owing to the high attrition rate (467 women from the original study did not participate, probably those who were too frail to attend the assessment), the results cannot be generalised to the frail sufferers of osteoporosis.

1.2.10.8 Drugs

Several categories of drugs that act on the central nervous system have been implicated in increased risk of hip fracture. These categories include benzodiazepines, anticonvulsants, antidepressants and opioids (78). It is unclear though, whether these drugs are associated with fracture because they increase the risk of falling or because they decrease bone density.

In a study to determine the relationship between central nervous system drugs and total femoral BMD, Kinjo, *et al.* (78) found that BMD was significantly lower in the users of anticonvulsants and opioids compared to non-users. However, the comparison between users and non-users of benzodiazepines and antidepressants was not significant. Although the study was cross-sectional, adjustments were made for age, sex, race, chronic medical conditions, smoking, alcohol intake, self-reported health, a history of wrist fracture, early menopause, cognitive impairment, activity level, metabolic activity, body mass index, vitamin D level, calcium intake and exposure to other drugs. The resulting regression model was therefore comprehensive. The representative sample consisted of 20 050 participants, aged 17 years older and, while causality cannot be inferred, the large sample size lends credibility to the data.

Central to the theory of depression-induced BMD loss is the glucorticoid cortisol. The effects of glucocorticoids on bone therefore require some exploration. Glucocorticoids are used to treat inflammation in asthma, arthritis and inflammatory bowel disease. Chronic use of these drugs leads to osteopenia and even glucocorticoid-induced osteoporosis (79). A more detailed description of the effects of glucocorticoids is presented under section 1.4.2.2 .

1.2.10.9 Medical conditions

Medical conditions such as inflammatory bowel disease, ulcerative colitis and Crohn's disease are associated with lower BMD when compared with healthy controls. Steroid therapy, low nutrient absorption and high inflammatory activity may contribute to this phenomenon (80).

Furthermore, liver disease has also been associated with osteoporosis. For example, up to 67% of primary biliary cirrhosis sufferers also have osteoporosis. Guañabens, *et al.* (60) investigated the prevalence and risk factors for osteoporosis in women with primary biliary cirrhosis. The subjects were 142 women with primary biliary cirrhosis, and 142 age-matched controls. The authors found that osteoporosis prevalence was higher in female patients with primary biliary cirrhosis than in healthy females. Regression analysis revealed that age, low BMI and the stage of the primary biliary cirrhosis were independent risk factors for osteoporosis.

1.2.10.10 Nutrition

The importance of a balanced diet has been well documented in the maintenance of bone health. The vital roles of, for example, calcium and vitamin D in bone metabolism have been discussed earlier in this chapter. The recommended daily elemental calcium intake for women ranges from 1000 mg (in premenopausal women aged 25-50 years) to 1500 mg (in women aged 65 years and older); the recommended daily intake of vitamin D is 800 IU for women at risk of deficiency (66). Substances that increase excretion of calcium, such as caffeine may represent important risk factors for low BMD (81). High sodium diets may also contribute to BMD loss as sodium causes obligate urinary calcium loss. (51).

1.2.11 Summary of risk factors for osteoporosis

Figure 1.8 provides a summary of the risk factors for osteoporosis. Age and (for women) menopause remain the most widely known risks for osteoporosis with the disease generally being described as a female disease or a scourge of advanced age. Genetic predisposition, lifestyle, low peak bone mass, poor BMI and sex hormone imbalances can all contribute to the loss of BMD and, if untreated, osteoporosis.

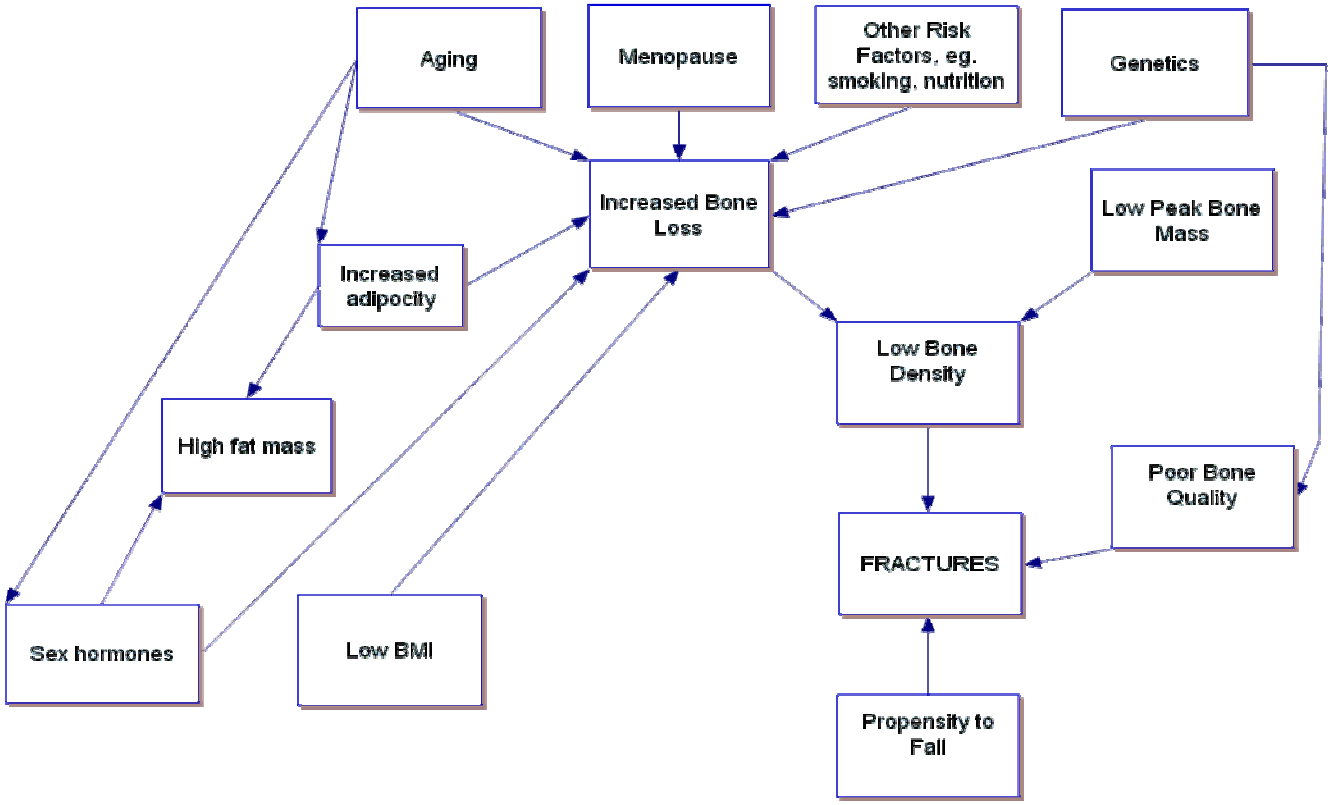

Figure 1.8 Some risk factors for osteoporosis

1.3 Depression and the stress response

Stress may be viewed as a stimulus (also called a stressor) - be it intrinsic, extrinsic, physical or psychological in nature - the reaction to stimuli or the transaction between stimulus and reaction (82, 83, 84). In biological terms, though, stress is generally defined as a state of disturbed homeostasis (82).

Albeit stress is generally regarded as effecting only negative outcomes for the body (distress), some stimuli can yield positive outcomes or eustress (84). Stress is therefore inherently paradoxical in nature: it is concomitantly adaptive and maladaptive. Marius Tausk introduced the useful metaphor of stress as the water used by firemen to douse flames. Using excess water may result in more damage than the flames alone would have produced. This could ultimately also lead to a loss of water pressure, which would hinder any further attempts at extinguishing the fire (85). The concept of stress has been transformed into the concepts of allostasis and allostatic load. Allostasis represents the means of maintaining homeostasis through change and allostatic load is the strain placed on the organism, also seen as the wear and tear of everyday life (86, 87).

1.3.1 The stress response

It was Cannon who observed that certain forces acting on an organism can cause a shift in homeostasis and result in strain on the organism. Cannon also showed that both psychological and physical stimuli can cause similar physiological changes (88). Despite Cannon's contribution to stress research, it is Hans Selye whom is considered to be the father of the field (108).

Selye was the first to describe a series of adaptive responses which the body undertakes to regain homeostatic balance after exposure to stress. These responses are known as the General Adaptation Syndrome (GAS). The GAS is divided into three main phases that occur after exposure to a stressful event (85, 89), namely:

- Alarm: rapid alterations take place in the body's internal milieu, e.g. heart rate, circulating blood glucose levels and blood pressure all increase. The body attempts to overcome shock by releasing corticosteroids from the adrenal cortex and adrenalin from the adrenal medulla.
- Resistance: this follows prolonged exposure to stress and allows the body to adapt further through building resistance to the stressor. At the same time, though, the body becomes more susceptible to the deleterious effects of other stressors.
- Exhaustion: even further exposure to the stressor causes pathological changes in the body – most notably in the immune system. Death may result.

A number of neural and endocrine structures are involved in the stress response. The structures are grouped into systems and axes, namely the hypothalamic-pituitary-adrenal axis (HPA axis), the sympatho-adrenal medullary axis (SAM axis), the dopaminergic system and the serotonergic system. These systems and axes are represented in Figure 1.9. The endocrine mediators are noted in parentheses. Both acute and chronic stresses result in activation of these axes. Even minor stressors can lead to increased cortisol levels, for example (90). Acute stress is defined as a normal physiological change in response to perceived threat. Chronic stress, on the other hand, is an abnormal ongoing physiological reaction to apparently continuing unresolved strain (91) and leads to over activation or exhaustion of the stress axes.

The two chief systems involved in the GAS are the SAM axis and the HPA axis (92, 93). The activation of the stress axes has numerous consequences for the body, which help the body to adapt to the stressful situation. Notwithstanding the inter-relatedness of the two axes, emphasis of this research falls on the HPA axis and so, only a brief overview will be offered of the SAM axis, while more information will be conveyed on the HPA axis.

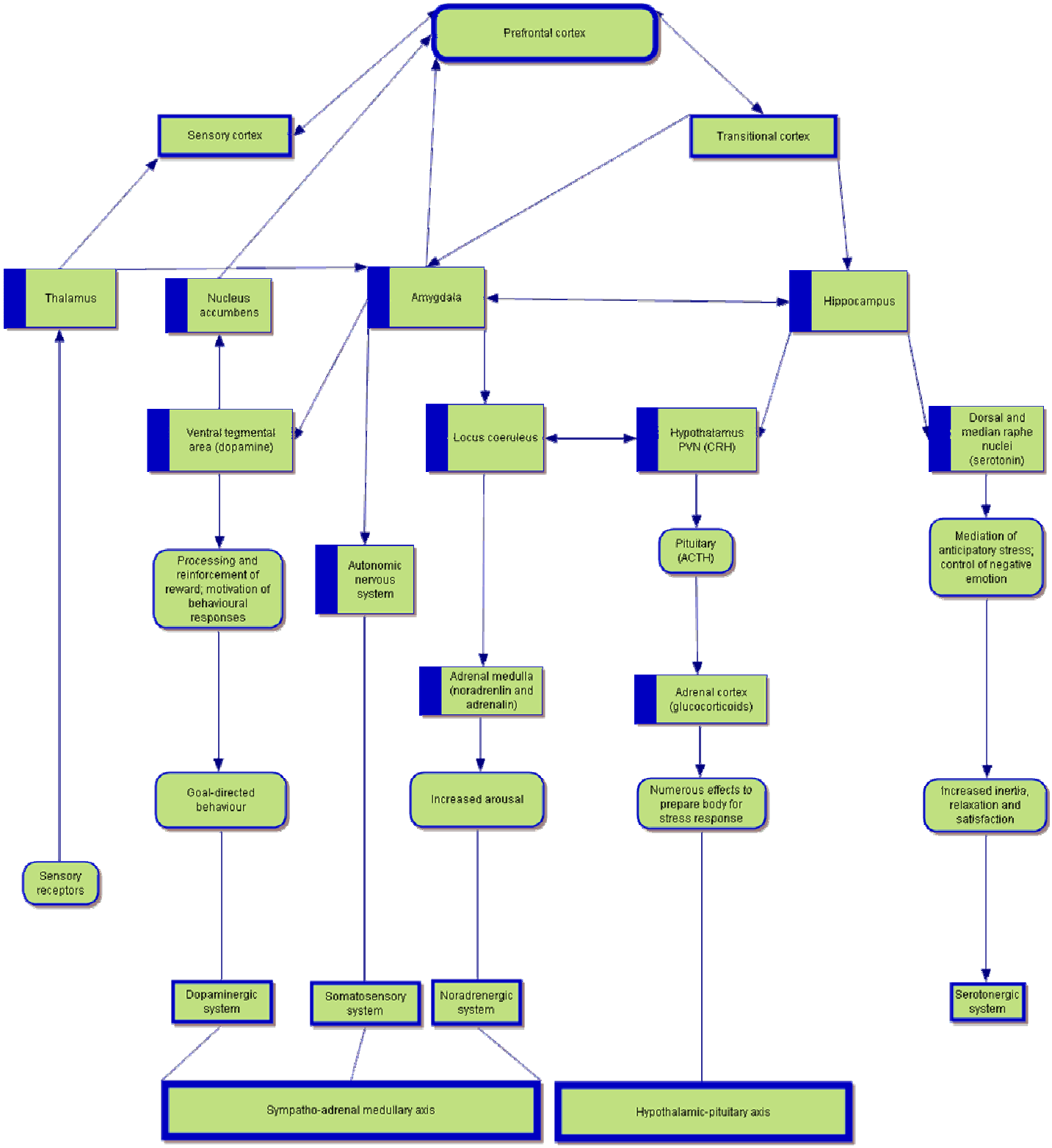

Figure 1.9 The neural and neuroendocrine structures involved in the stress response (adapted from 83, 84, 88, 94)

1.3.2 The SAM axis

Activation of the SAM axis leads to the release of the catecholamines, adrenalin and noradrenalin. Release of these catecholamines is mediated by the preganglionic sympathetic fibres emanating from the spinal cord (95). The SAM axis is responsible for the immediate “fight or flight” reaction to stress (96). Adrenalin and noradrenalin have the following effects (97, 84, 95):

- Central nervous system: both hormones cause increased irritability, anxiety, emotional disturbance and aggression. The activation of noradrenergic neurons leads to increased arousal of the reticular system.
- Bone metabolism: the release of noradrenalin is a stimulant for the release of pro-inflammatory cytokines such as interleukin 1β, which in turn stimulates bone resorption (98).
- Cardiovascular system: adrenalin and noradrenalin cause increased cardiac activity.
- Non-vascular smooth muscle: there is constriction of muscles where α-receptors predominate (e.g. sphincters) and the relaxation of muscles where β-receptors are common (e.g. digestive tract).
- Carbohydrate metabolism: increased glycogenolysis and gluconeogenesis occurs.
- Fat metabolism: lipolysis is stimulated.

Noradrenergic neurons located within the locus coeruleus and the paraventricular nucleus (PVN) of the hypothalamus have a reciprocal stimulatory effect on one another. The PVN is a vital centre for stress responses via the HPA axis (99).

1.3.3 The HPA axis

The HPA axis integrates stimuli that relate to stress. It is activated when the person experiences exhaustion, loss of control or a perceived loss of control (100). Stress-induced activation of the HPA axis elicits responses from the neuroendocrine, peripheral nervous and immunological systems. The central effector of these responses in humans is the glucocorticoid, cortisol.

The hypothalamus is the epicentre of various endocrine and autonomic responses that govern behaviour and rhythmicity of body functions. The organ contains diverse specialized cells that help regulate these functions. The hypothalamus performs its endocrine regulation via three mechanisms (101):

- The secretion of neurohormones from the paraventricular nucleus (PVN). These hormones have a direct effect on the body.
- The secretion of regulatory hormones that moderate the manufacture and systemic release of hormones from the anterior pituitary.
- By exerting influence on the autonomic nervous system that innervates endocrine organs (this includes an interactional relationship with the SAM axis).

Corticotrophin-releasing hormone (CRH) neurons are scatter throughout the central nervous system. In the hypothalamus, these neurons are found in the PVN. CRH is a hypophysiotropic peptide that is released into the anterior pituitary via the hypophyseal portal blood (97). In the anterior pituitary, CRH stimulates the release of pro-opiomelanocortin (POMC), which is cleaved to form adrenocorticotropic hormone (ACTH) and related peptides. ACTH, in turn, stimulates the release of glucocorticoids, particularly cortisol from the adrenal cortex. Vasopressin augments the actions of ACTH, but CRF release is inhibited via negative feedback actions of glucocorticoids (97, 100).

1.3.4 The glucocorticoids

Glucocorticoids are potent anti-inflammatory molecules, which also evince mineralocorticoid activity and are secreted by the adrenal cortex (102). Glucocorticoid receptors are widely distributed in the cytoplasm of human cells. They are bound to a protein complex, including heat shock proteins HSP90 and HSP70, when not activated. The classical mechanism of glucocorticoids involves ligand-receptor binding. After ligand binding, the receptor translocates to the nucleus, binds to the glucocorticoid response element (GRE) in a promoter of the target gene and there it regulates transcription. The non-genomic mechanism of glucocorticoids involves a signal transduction pathway that stimulates transcription factors (103).

Repression of transcription can occur through cis-repression or trans-repression. In cis-repression, the glucocorticoid receptor binds to a negative GRE and physically obstructs the transcription machinery or the regulating transcription factor. Trans-repression is achieved through the receptor interacting with the transcription factor and preventing it from activating transcription (79).

By far the most influential of these glucocorticoid molecules in the stress reaction is cortisol. Some mammals, such as rats, secrete the glucocorticoid, corticosterone, while humans secrete seven times as much cortisol as corticosterone (102). Cortisol is found in the blood as free cortisol and bound (predominantly to corticosteroid-binding globulin and albumin). Cortisol has effects on metabolism, behaviour, immunity, the CRH neurons and diurnal rhythms (94). The release of cortisol follows a diurnal pattern, stimulated by a pulsative ACTH and CRH release. Cortisol levels peak prior to waking and decrease throughout the day, reaching their lowest levels in the evening (104, 102). Table 1.7 provides the normative values for salivary cortisol in adult females aged 21 to 30 years and 31 to 50 years.

Table 1-7 Normative values of adult salivary cortisol (105)

Time	Concentration (ng/ml) range for adult females aged 21 to 30 years	Concentration (ng/ml) range for adult females aged 31 to 50 years
Morning	2.72 to 13.48	0.94 to 15.15
Evening	Difficult to detect to 3.59	Difficult to detect to 1.81

1.3.5 Metabolic effects of cortisol

Cortisol is responsible for, amongst other functions, the mobilization of amino acids and fats for the provision of energy and the synthesis of new compounds required for responding to stressors (106). Cortisol also inhibits the effects of growth hormone and the sex hormones on fat, muscle and bone. Combined with its insulin resistance effect, cortisol can stimulate increased adiposity (95). The direct effects of cortisol on bone are described under section 1.4.

1.3.6 Effects of cortisol on immunity

A reciprocal regulatory role exists for the HPA axis and the immune system (84). The central nervous system's modulation of the immune system is mediated by intricate bidirectional relationships between the nervous, endocrine and immune systems (93). Inadequate secretion of cortisol can lead to septic shock and, while excessive secretion of cortisol can lead to deficient immunity (87). Both scenarios can endanger life.

Lymphoid and myeloid tissues respond to neuroendocrine secretions mediated by the HPA axis and their activities are consequently up or downregulated. Inflammatory mediators such as cytokines and lipid mediators of inflammation activate the HPA axis via the CRH neurons in the PVN, pituitary corticotroph or adrenal cortex. Thus, peripheral inflammatory events transform non-cognitive stimuli into stimuli that are recognizable to the central nervous system (87).

1.3.7 Effects of cortisol on mood, affect and behaviour

Acute stress results in a transient increase in plasma cortisol. Augmenting the stress response is a resulting partial resistance to feedback inhibition of cortisol release. This involves rapid desensitization of glucocorticoid receptors in the brain (83). Acute stress has also been shown to decrease the number of glucocorticoid receptors in the hippocampus, which results in further inhibition of negative feedback, and an increase in circulating cortisol levels. Acute stresses are accompanied by changes in affect and cortisol secretion is associated with momentary mood states: negative affect has been consistently linked with increased cortisol secretion, while positive affect has been associated with both increased and decreased cortisol levels (90, 107).

Chronic stress propagates a longer term increase in cortisol secretion. The effects of chronic stress vary according to the coping mechanisms that the individual has developed. Inadequately controlled high levels of arousal of the stress axes pose a potential risk to the organism's health - both physical and mental (83, 108). This health risk is linked to continual inflammatory response or repression of immunity as a consequence of HPA axis overstimulation or exhaustion.

Exhaustion of the HPA axis is seen in the hyposecretion of cortisol. This has been associated with post-traumatic stress disorder, fibromyalgia and chronic fatigue syndrome (95, 109, 110, 111). Thomsen, Kvist, Andersen and Kessing (112) compared 989 patients with adrenocortical insufficiency to a cohort of 124 854 patients with osteoarthritis and found that patients with adrenocortical insufficiency are more likely to suffer from affective disorders than their counterparts without this shortfall.

On the other hand, hypersecretion of cortisol has been linked to the desensitisation of central glucocorticoid receptors and a resistance to negative feedback. Both receptor desensitisation and feedback resistance act as triggers for the onset of depression, anorexia nervosa, obsessive-compulsive disorder and anxiety (83, 113, 95). Accompanying these changes is the enhanced release of arginine-vasopressin, IL (interleukin) -1, IL- 6, tumour necrosis factor alpha (TNFα) and interferon (IFN), all of which further increase the release of cortisol.

Bao, Meynen and Swaab (114) argue that there are observations that point to a causal role for hypercortisolism in major depression. Hypersecretion of cortisol has been shown to precede the onset of major depression in adolescents that are at risk for the disorder (as a result, for example, of bereavement) and is linked to the chronicity of the disorder (115). Eggers (91) however, points out that there is no clear indication that the initial stage or mild cases of depression are caused by cortisol hypersecretion. However, high cortisol is linked to the severity of major depressive symptoms. The fact that cortisol hyper and hyposecretion have been described in depression may be a manifestation of the subtype of the disorder (116). The questions of how much cortisol is enough to induce depression (if cortisol can in fact be declared as causative), what level of depression is caused by cortisol and the chronicity of cortisol-induced major depression are still therefore under scrutiny.

Nevertheless, one of the most abundantly documented behavioural effects of cortisol dysregulation relates to depression. The clinical model for cortisol hypersecretion is Cushing's syndrome in which cortisol production is typically three times higher than normal and the circadian rhythm is repressed. Two-thirds of Cushing's patients exhibit psychopathology – most notably, major depression (91, 117).

Furthermore, long-term corticosteroid therapy has been shown to alter mood. In a study by Naber, Sand and Heigl (118) both depressive and manic symptoms of organic origin were reported in a significant proportion of 50 ophthalmic patients being treated with corticosteroids after only eight days of treatment. Brown, Vera, Frol, Woolston and Johnson (119) assessed 13 prednisone-treated patients and controls in a long-term follow-up study of mood and memory. Initial results indicated higher levels of depression in patients on prednisone versus controls. Follow-up assessment indicated a worsening of depressive symptoms over time in the chronic prednisone treatment group.

Aside from the direct effects of cortisol on the immune system that may lead to depression, activation of the stress axes lead to behaviours that put the individual at greater risk for ill health, such as alcohol consumption, sleep dysregulation, hyper- or hypophagia, etc… (120). There is specific evidence to suggest a primary role for cortisol hypersecretion in insomnia (121), decreased restraint in eating snack foods in times of stress and increased emotional eating (122). These behaviours enhance the negative effects of stress and further perpetuate depression.

There is an overwhelming amount of evidence to suggest a central role for cortisol in depression. Further discussion of such research is presented later in the chapter. A brief overview of depression is first offered before the stress response's role in this disorder is discussed.

1.3.8 Depression

Stress can give rise to depression and plays a role in modulating the latter disorder's severity and long-term outcomes (109). Within the realm of psychopathology, depression is classified under the Mood Disorders. Mood Disorders are characterised by pathological mood and associated complaints (123).

Depression is a heterogeneous disorder. It is typified by depressed mood, anhedonia and a host of vegetative symptoms. Nosologically, depression is divided into various depressive disorders that are classified according to severity, duration of symptoms, presence of psychotic symptoms and the chronicity of the disorder, e.g. major depressive episode and dysthymia (124). This study focuses on those patients currently exhibiting sufficient depressive symptoms to be diagnosed with a depressive disorder or that have a confirmed history of depression. As the disorder has a wide presentation amongst sufferers, a brief description of the diagnostic criteria is required for context. The diagnostic criteria of the DSM-IV-TR are listed for Major Depressive Disorder, Major Depressive Disorder, Single Episode and Major Depressive Disorder, Recurrent (123).

Depression is a widely occurring disorder with a lifetime prevalence of 20% (125). Women are generally more prone to the disorder, with twice as many women diagnosed with the disorder as men (123). The common occurrence of the illness does not decrease its potency and its debilitating outcomes rival those of diseases such as coronary artery disease, chronic lung disease and arthritis (126). Major depression tends to be chronic. Approximately 75% of people hospitalized for a depressive episode will face a recurrence of the disorder within five years of their treatment (123).

Table 1-8 DSM-IV-TR criteria for Major Depressive Episode (123)

A	Five or more of the following have been present during the same two-week period and represent a change from the person's previous functioning. At least one of the symptoms is either depressed mood or loss of interest or pleasure (i.e. 1 or 2).
1	There is depressed mood most of the day, nearly every day as indicated by either subjective report or observations made by others. In children and adolescents this may present as irritable mood.
2	There is markedly diminished interest or pleasure in all, or almost all activities most of the day nearly every day as indicated by either subjective report or observations made by others.
3	The person has displayed significant weight loss when not dieting or weight gain (such as a change of more than 5% of body weight in a month), or there has been decrease of increase in appetite nearly everyday. In children this may present as the failure to make expected weight gains.
4	Insomnia or hypersomnia is noted nearly everyday.
5	Psychomotor retardation is observed by others nearly everyday.
6	The person is fatigued or has lost energy nearly everyday.
7	The person experiences feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly everyday. These feelings may not simply be in the form of self-reproach or guilt about being sick.
8	The person experiences a diminished ability to think or concentrate or is indecisive nearly everyday (as indicated by either subjective report or observations made by others).
9	There are recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan or a suicidal attempt or a specific plan for committing suicide.
B	The symptoms do not meet the criteria doe a mixed episode.
C	The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning.
D	The symptoms are not due to the direct physiological effects of a substance or a general medical condition.
E	The symptoms are not better accounted for by bereavement (i.e. the symptoms have lasted for more than two months or are characterised by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms or psychomotor retardation).

Table 1-9 DSM-IV-TR criteria for Major Depressive Disorder, single episode (123)

A	a single major depressive episode is present.
B	The major depressive episode is not better accounted for by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder or psychotic disorder not otherwise specified.
C	There has never been a manic episode, a mixed episode, or a hypomanic episode. This exclusion does not apply, though, if the manic like, mixed like or hypomanic like episodes are substance or treatment induced or are due to the direct physiological effects of a general medical condition.
	If the full criteria are currently met for a major depressive episode, specify:
	mild, moderate, severe with or without psychotic features.
	With catatonic features
	With melancholic features
	With atypical features
	With postpartum onset
	If the full criteria are not currently met for a major depressive episode, specify:
	In partial remission, in full remission,
	Chronic
	With catatonic features
	With melancholic features
	With atypical features
	With postpartum onset

Table 1-10 DSM-IV-TR criteria for Major Depressive Disorder, recurrent (123)

A	Two or more major depressive episodes have presented. To be considered discrete episodes, there must be an interval of at least two consecutive months in which criteria are not met for a major depressive episode.
B	The major depressive episodes are not better accounted for by schizoaffective disorder and are not superimposed on schizophrenia, schizophreniform disorder, delusional disorder or psychotic disorder not otherwise specified.
C	There has never been a manic episode, a mixed episode, or a hypomanic episode. This exclusion does not apply, though, if the manic like, mixed like or hypomanic like episodes are substance or treatment induced or are due to the direct physiological effects of a general medical condition.
	If the full criteria are currently met for a major depressive episode, specify:
	Mild, moderate, severe with or without psychotic features.
	With catatonic features
	With melancholic features
	With atypical features
	With postpartum onset
	If the full criteria are not currently met for a major depressive episode, specify:
	In partial remission, in full remission,
	Chronic
	With catatonic features
	With melancholic features
	With atypical features
	With postpartum onset
	Specify
	Longitudinal course (with and without inter-episode recovery)
	With seasonal pattern

1.3.9 How depression is connected to the stress response

Depression has been linked to HPA axis abnormality. Both states are associated with alterations in affect, arousal, cognitive capacity, neuroendocrine and autonomic functioning (109). Although patients with major depressive disorder rarely have physical signs of hypercortisolaemia as in Cushing's disease, they do present increased levels of serum cortisol and major depression patients also have a decreased number of glucocorticoid receptors (2), which indicates a blunted response to negative feedback.

Gold and Chrousos (109) suggest that research should examine the subtypes of depression separately, given that melancholic depression is more regularly associated with hypercortisolaemia and atypical depression with insufficient cortisol secretion. While subtypes of depressed patients do present with varying symptoms and neuroendocrine findings, hyper-reactivity of the HPA axis generally remains a central feature of depression (101, 127), with hypercortisolism being a common finding in 40-60% of depressed people for more than 40 years (1, 3). Depressed patients specifically exhibit higher ACTH and baseline cortisol levels than healthy subjects (4). The higher resting plasma cortisol in depressed patients means that any further stressor results in substantial cortisol response, which then propagates a vicious cycle.

In their process-oriented model for HPA axis dysregulation in depression, Parker, *et al.,* (3) describe a pattern of hypersecretion of cortisol that is driven by hypersecretion of ACTH and CRF in the acutely depressed. In chronic depression there is an increased sensitivity of the adrenals to ACTH. Enlarged adrenals and hyper-responsivity to ACTH is state and not trait-dependent. The enlarged adrenals also produce more cortisol per molecule of ACTH than normal. But, the adrenals return to normal size and production volume once the state has passed. There is a potentiation effect, though, and consequently the hyper-responsivity to ACTH can continue a little longer than the state. Therefore ACTH levels can be near normal in a chronically depressed patient, while cortisol levels rise significantly.

Tafet, Toister-Achituv and Shinitzky (128) posit that elevated cortisol levels enhance serotonin uptake in vivo and in vitro. Attenuation of serotonin uptake through the use of treatments such as selective serotonin reuptake inhibitors has long illustrated that increased serotonin uptake causes depressive symptoms.

1.3.10 Depression: a PNE approach

With the fruition of theories of the biopsychosocial nature of diseases, the aspects of mental illness that may influence physical sickness are increasingly being investigated in an expansion of the mind-body research terrain. These post-modern perspectives on the mind-body question - such as those represented in psychoneuroendocrinology - seek to survey multi-directional interactions that may exist between mind and body (94). This section explores the possible bidirectional nature of the relationship between depression and medical illness.

1.3.10.1 Depression: the result of medical illness

The organic basis of mental disorders has been fecund terrain for questions since even before Philippe Pinel's contribution to mental health (129). It is known that chronic or high intensity stress may bring about psychopathology. In addition, researchers have shown that life-threatening medical illnesses serve as the most frequent stressful life events associated with the onset of major depression. Patients with myocardial infarction, diabetes mellitus, human immunodeficiency virus (HIV), cancer, stroke and Parkinson's disease all exhibit higher incidence of major depression than patients without these disorders (130).

Through a meta-analysis of the comorbidity of diabetes and depression, Andersen, Freedland, Clouse and Lustman (131) conclude that there is an increased prevalence of depression in diabetes patients. It has subsequently been suggested that a metabolic corollary of diabetes is structural alteration of the brain that leads to depression. Kessing, Nilsson, Siersma and Andersen (132), however, could not find a correlation between diabetes and increased risk of developing depression. Their analysis of two cohorts derived from the Danish National Hospital Register (91 507 patients with diabetes and 108 487 with osteoarthritis) yielded no significant differences between the two groups in terms of the risk of being readmitted to a hospital with a diagnosis of depression following a previous admission for diabetes or osteoarthritis. The outcome measure (readmission to hospital) in this study must be questioned, though, given that patients with mild depression that did not require hospitalisation would not have been included in the analysis.

Other patient populations where depression's occurrence is above the general population include patients with peripheral vascular disease (PVD) (133) and chronic heart failure (CHF) (134). Both CHF and PVD may result in a loss of independence for the patient, pain, decreased mobility and a lowered capacity to perform daily tasks, all of which may lead to depression (133).

Of course, a number of features may contribute to these depressive outcomes. Genetic predispositions, lack of coping skills and poor interpersonal relations must be considered risk factors in patient populations. Also, the increased prevalence of depression in medically ill samples does not point to the direction of causality. What is known is that depression amplifies physical symptoms, can affect social and occupational functioning, diminishes compliance to treatment and is related to the onset of diseases that decrease longevity.

This evidence supports the classic contention that depression may be an outcome of medical illness. There is growing evidence to support the corollary - that depression may cause medical illnesses. This is not only in respect of the functional impairment resulting from depression or merely because of the susceptibility of depression patients to pain syndromes and poor health habits, though: depression has direct adverse physiological consequences that lead to increased morbidity and mortality (130).

1.3.10.2 Depression: the cause of physical illness

The negative affect that accompanies depression has been linked to cancer (through immune down-regulation), auto-immunity (through increased immune sensitivity), impaired wound healing (through impeding key cytokine distribution) and susceptibility to infectious diseases (through the overall dysregulation of the immune system) (120). Chronic infection gives rise to chronic inflammation, which has been associated with a four-fold increase in risk for atherosclerosis and a three-foldincrease in mortality in individuals with cardiovascular disease. Inflammation is also linked to ageing disorders such as arthritis, osteoporosis, type 2 diabetes, Alzheimer's disease and periodontal disease (135).

Ketterer, Mahr & Goldberg (136) make a distinction between behavioural (or psychobehavioural) mediators that influence a medical condition (such as in the case of non-compliance with a treatment regime) and psychophysiological mediators that act via the overstimulation of the stress axes to cause illness. There is growing perception that medical conditions may be psychophysiological in origin and this attests to the centrality of stress-disease relationships in modern medicine (136).

There is growing evidence that even minor depression can cause significant immune changes (137). The increased HPA axis activation and hypersecretion of cortisol that accompany depression lead to immunosuppression and inflammation. Immunosuppression is characterised by decreased lymphocyte proliferation and weakened T cell virus-specific responses as well as destabilized T cell memory. Immune activation in depression is seen through the increased production of pro-inflammatory cytokines, acute phase proteins, chemokines and adhesion molecules (137). As the immune system is disrupted, illness behaviours such as insomnia and anxiety may arise, which further activate the stress axes (135, 93). This places depressed patients in greater jeopardy of morbidity and mortality (138, 5).

Frequently observed cytokine changes in depressed subjects are the increases in IL-6 and C-reactive protein, with increased IL-1β and TNFα also being reported (139). The release of inflammatory mediators is triggered by the activation of the sensory nervous system and the HPA axis. There is then enhanced release of TNF, IL-1 and IL-6 from T cells results (5). These alterations in the immune system are possibly caused by the hyper-activation of the HPA axis. Alternatively, they may be precipitous in this interaction. However, the research is not without debate: in an examination of the pro-inflammatory cytokine levels in patients with major depressive disorder, Simon *et al.* (140) discerned a general inflammatory state. The 49 depressed subjects exhibited significantly higher levels of nine pro-inflammatory cytokines (MCP-1, MIP-1α, IL-1α, IL-1β, IL-6, IL-8, GM-CSF, IFNγ and eotaxin) compared to healthy age and gender matched controls (140). Similarly, significantly increased levels of TNFα and IL-6 were found in SSRI treatment resistant depressed patients compared to healthy cohorts (141). In contrast, though, Marques-Deak,

Neto, Dominguez, Solis, Kurcgant, Sato, Ross and Pardo (142) found no significant differences in pro-inflammatory cytokine levels between 46 subjects suffering from depression and 41 healthy controls.

1.4 Cortisol and bone mineral density (BMD): from depression to osteoporosis

Over the past few years, the link between depression and medical illnesses has enjoyed much attention from researchers. One of the many physical conditions to which depression has been linked is decreased bone density (BMD). In fact, depression is increasingly being reported as a risk factor for decreased BMD. However, the effect of depression on BMD remains controversial. Some of the results of research into the association between BMD and depression are presented in this section. The possible mediating role of cortisol is also discussed and a model for depression-induced osteoporosis is offered.

1.4.1 Low bone mineral density (BMD) and depression: current views

The link between depression and lowered BMD has been investigated using varying methods of BMD quantification (mainly DEXA and CT scans) and disparate tests of depression (e.g. the Beck's Depression Inventory, the Hamilton Depression Scale and the General Health Questionnaire). The anatomical regions chosen for assessment of BMD also differ across studies. These distinct research methods have contributed to inconsistent results.

Evidence to contradict findings of a link between depression and low BMD is found in the work of Reginster, Deroisy, Paul, Hansenne and Ansseau (143) who aimed to determine whether or not women most at risk for developing depression also had the highest risk of developing osteoporotic fractures. DEXA was used to analyze the bone densities of 121 postmenopausal women (aged 48-77 years). No significant correlation was found between BMD of the spine, femoral neck or non-dominant hip and vulnerability for the development of depression.

Schweiger, Weber, Deuschle and Heuser (144) also investigated whether an increase in bone density loss occurs in concomitance with major depression. Twenty-

one healthy people and 18 cohorts hospitalized for treatment of depression were assessed in a longitudinal study. Follow-up readings were taken approximately two years after the first measurements. The researchers concluded that bone density had decreased in both groups in the interval between the first and second measurements. This was evident even when adjustments were made for age, initial bone density and gender. However, the loss of bone was more prominent in persons suffering from depression. Bone loss for men with depression was 6,9mg/cm^3, SD=5,5 and in women with depression it was 3,8mg/cm^3, SD=4,3. This contrasted clearly with the rate of bone density loss in men and women without depression (males 3,9mg/cm^3, SD=4,2 and females 0,9mg/cm^3, SD=8). Initial bone density and age did not correlate with this annual bone loss. Owing to the small sample size, though, that the statistical power of the results is limited.

Robbins, Hirsch, Whitmer, Cauley and Harris (145) assessed an older population, consisting of 1 566 black and white men and women older than 65 years of age. The researchers concluded that the mean total hip BMD was lower in subjects with higher depression scores, even after adjusting for risks for developing osteoporosis ($p<0.001$).

Yazici, Akinci, Sütçü, Özçazar (146) point out that low BMD has been reported in patients suffering from various mental disorders, including depression. Their study of 25 premenopausal females suffering from major depression and 15 healthy controls included the analysis of BMD (using DEXA as well as serum calcium, phosphate, alkaline phosphatase, PTH, vitamin D, osteocalcin, cortisol, sodium, calcium, creatinine and DPD). The results show a link between major depressive disorder and BMD. However, no correlations were found between the severity or duration of the depression and the degree of bone loss.

Mussolino, Jonas, and Looker (147) highlight the role played by gender in the association between depression and low BMD. These researchers utilized data from 5 171 participants in the Third National Health and Nutrition Examination Survey (NHANES III) to examine the hypothesized link between depression and bone density. The participants were aged between 20 to 39 years. BMD was measured using DEXA. Using the Diagnostic Interview Schedule (DIS), the presence of Major

Depressive Episode and dysthymia was determined for every individual. After adjusting for factors such as smoking, physical activity and calcium consumption, it was concluded that Major Depressive Episode and dysthymia are correlated with lower BMD in the proximal femoral region in men, but not in women.

Kahl, Greggersen, Rudolf, Stockelhuber, Bergmann-Koester, Dibbelt and Shweiger's (2) research into markers of bone turnover in young females exhibiting Borderline Personality Disorder and comorbid depression shows comparable results. The values of BMD of these 22 patients were significantly lower than those of patients with Borderline Personality alone. A control group consisting of 20 healthy females was also included in the study and further analysis revealed significant differences between the three groups. In contrast with Yazici, *et al.*'s (146) results, differences were noted in the bone turnover marker levels for the three groups: the group with comorbid depression displayed the highest levels of fasting cortisol, PTH and 1, 25 – Hydroxy-vitamin D of the three groups.

A cohort study of Asian men also supports the findings that depression and low BMD are linked (148). The subjects were 2 000 men from Hong Kong, aged between 65 and 92 years. Their BMD was measured using DEXA and the measures for the lumbar spine, total hip and total body were evaluated alongside scores on the Chinese version of Geriatric Depression Scale (GDR). It was found that 40,83% of the depressed subjects recorded a T-score lower than –1, but greater than –2,5 (the definition of low BMD), while only 29,13% of the non-depressed subjects fell into this category. Interestingly, though, depressed subjects with a BMD T-score less than –2,5 amounted to 1,78% of the overall depressed number, while the percentage of non-depressed subjects totalled 1,91% of their group. Relative risk calculation yielded valuable information: at a 95% level of confidence, depression was associated with a 1,4-fold relative risk of having a BMD T-score lower than or equal to –1.

Further research by Yazici, *et al.* (126) into the relationship between major depression and BMD, however, refutes these findings. Thirty-five premenopausal women meeting the DSM-IV-TR criteria for major depressive episode were compared (on BMD and markers of bone turnover (osteocalcin and C-telopeptide) with thirty

premenopausal women that did not have major depression. The results suggested that mild to moderate depression did not cause osteoporosis. Yazici and colleagues do suggest that factors such as duration of depression, number of episodes and cortisol levels may be confounding variables in such studies.

Coelho, *et al.*'s (149) results also challenge the association of BMD and depression. These researchers evaluated 102 Portuguese women aged between 40 and 80 years on their depressive symptoms and their general psychological well-being. 47,1% of the women in the study suffered from osteoporosis and these subjects presented significantly higher scores on the Beck's Depression Inventory, (a test that gauges the level of clinical depression) than those without osteoporosis ($p=0.045$). The confounding variables of age, body mass, daily energy, vitamin D and zinc intake, as well as caffeine ingestion and physical activity were adjusted for in this sample and the mean depression values remained higher for the women with osteoporosis. Logistic regression evinced that BMD was significantly associated with low BMD. Interestingly, no significant difference was noted between the groups in the mean scores for the Psychological General Well Being Schedule, which is an indicator of subjective feelings of well-being or distress. As it is a cross-sectional analysis, the study obviously does not clarify whether depression initiates osteoporosis or vice-versa or if the two factors share a pathophysiological pathway.

The argument therefore continues over whether depression is a potential cause of osteoporosis, or whether the two are discrete clinical entities (126). Although depression may be more frequently diagnosed in subjects with low BMD than in the general population (1), studies into this field are mainly cross-sectional and therefore do not confirm causality. The exception to this is the follow-up study conducted by Schweiger, *et al.* (144).

It is proposed that the relationship between low BMD and depression is bi-directional and that both factors are active participants in the proverbial vicious cycle (1). Issues such as gender, severity and chronicity of depression continue to confound research results and necessitate further exploration.
The mechanism underlying the possible link between depression and low BMD also has need of elucidation. Brown, Varghese and McEwen (4) have proposed cortisol as

a mediator in the relationship between depression and osteoporosis. This research takes up this proposal and supplements it with the notion that the effectors of this relationship may also include pro-inflammatory cytokines.

1.4.2 Depression-induced bone mineral density (BMD) loss: a proposed trajectory

It remains unclear whether the increased cortisol release is pathogenic for depression (involved in its pathophysiology) or simply epiphenomenal (secondary to depression) and the result of anxiety and tension that accompany depression (150). What is clear though is that the dysregulation of the HPA axis and the resulting hypercortisolism are strongly associated with depression. But how is this related to low BMD?

Bone is built through the process of remodelling, which is described earlier in this chapter. This process is mediated by a number of factors, including glucocorticoids and cytokines. Glucocorticoids and pro-inflammatory cytokines are linked to the activation of osteoclast activity and, therefore, resorption. The release of these substances is influenced by the HPA axis (2). Although the dysregulation of the HPA axis is likely to be a cause for depression, the depressive disorder further perpetuates the disruption of the HPA axis (109, 137, 3). The dysregulation in the HPA axis that precedes or that is induced by depression is largely suspected of influencing BMD. In fact, Yirima, *et al.* (151) are of the opinion that major depression is one of the chief medical conditions that contributes to decreased BMD. The endocrine factors that are potentially linked to this bone loss in depressed people include cortisol, cytokines, gonadal hormones and sympathetic agonists (151). Figure 1.10 illustrates this proposed relationship. A description of the figure follows along with the existing evidence regarding the relationships of the factors to BMD loss.

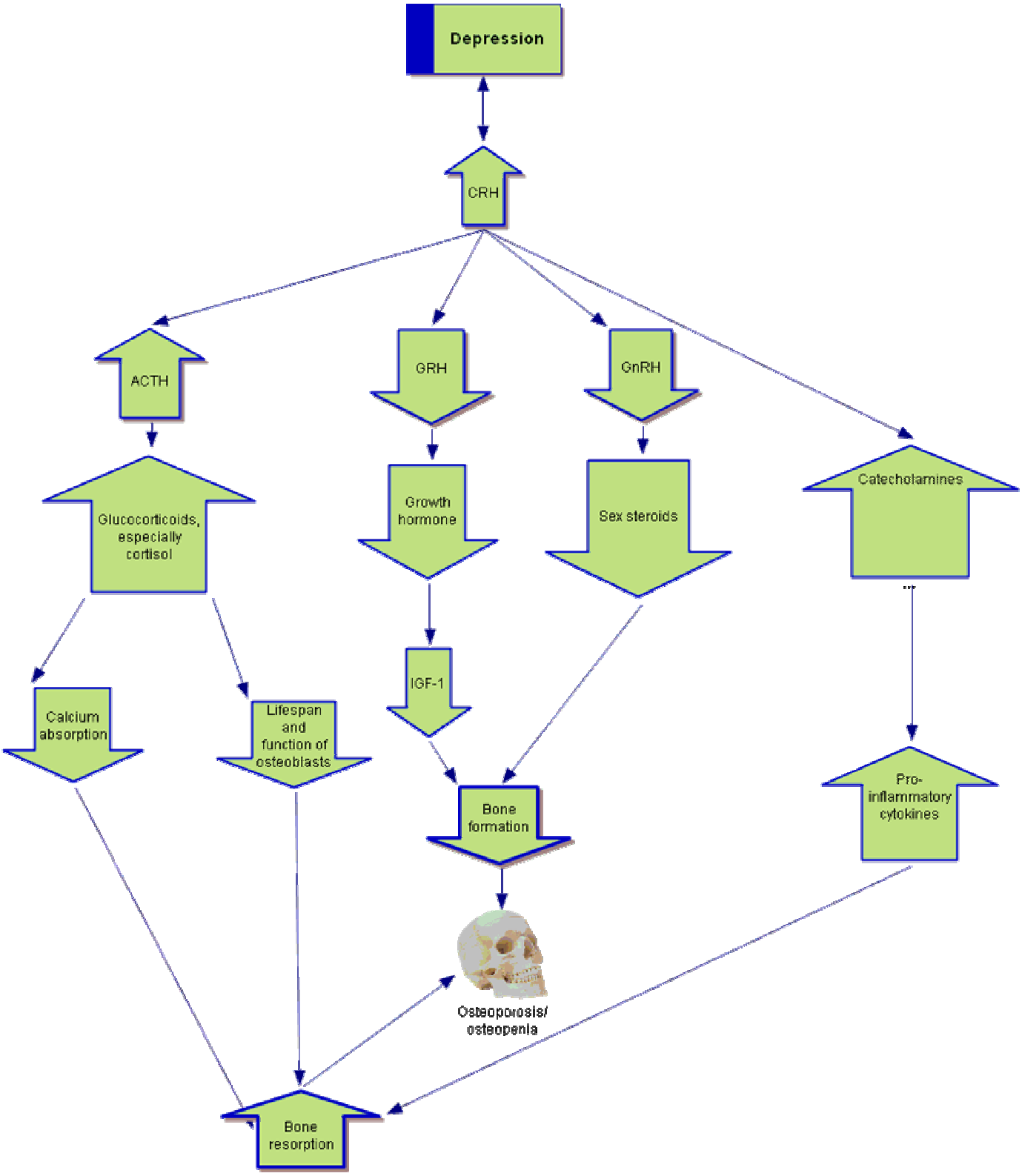

Figure 1.10 The relationship between depression, low bone mineral density (BMD) and cortisol (4, 152)

1.4.2.1 IGF-1 and sex hormones

CRH secretion diminishes the activity of the GH-insulin-like growth factor 1 (GH-ILF-1) axis and lowers the secretion of sex hormones. This in turn leads to decreased bone formation and the increased release of inflammatory mediators such as IL-6 - which act to increase bone resorption (1). Additionally, TRANCE has been found to have a direct effect on immune cell functioning. Activated T lymphocytes support osteoclastogenesis by also increasing the release of pro-inflammatory cytokines, specifically, IL-1, IL-6, IL-17 and TNFα (33).

As mentioned, IL-6 is responsible for the inhibition of osteoclast differentiation and activity, as it stimulates OPG expression. Glucocorticoids and oestrogens have an inhibitory effect on IL-6, which promotes loss of BMD. Dovio, Sartori, Masera, Racca and Angeli (153) examined the inhibitory effects of both oestrogens and physiological levels of cortisol on IL-6 production by human osteoblast-like cell lines. Their findings suggest that it is cortisol, rather than oestrogens, inhibit IL-6 and IL-1β production in osteoblast-like cells. An inhibitory effect of oestrogens could not be demonstrated in the same cell lines despite cells having receptors for both glucocorticoids and oestrogens. The potential inhibitory effect of cortisol on IL-6 during times of stress is therefore significant in BMD loss.

1.4.2.2 Glucocorticoids and catecholamines

Cortisol hypersecretion perturbs the renal handling of sodium and calcium, leading to hypertension and the loss of BMD. The evidence for and against the involvement of cortisol in osteoporosis is not uncontroversial. Qvist, Christgau, Pederen, Schlemmer & Christiansen (154) found no difference in the diurnal variation of C-Terminal telopeptide of type I collagen (CTx) for subjects with and without normal diurnal variation in serum cortisol. Interestingly, though, the circadian rhythm of serum CTx was also unaltered by menopausal stage or antiresorptive drug therapy. Meanwhile, Vergély, Lafage-Proust, Caillot-Augusseau, Millot, Lang and Estour (155) analysed the relationships between serum levels and circadian variations of cortisol and osteocalcin in nutritionally deplete (anorexia nervosa) and nutritionally replete (Cushing's syndrome) patients. Their results showed a significant decrease in osteocalcin levels accompanied hypercortisolism in both groups.

Clues to the mechanism of cortisol-induced BMD loss that may occur in depression are found in research on glucorticoid-induced osteoporosis. Glucocorticoids are used in the treatment of a number of conditions, including auto-immune diseases, respiratory conditions and gastrointestinal disorders. They are also utilized for immunosuppression in organ transplant patients. The side-effects of the drugs include weight gain, diabetes, adrenal insufficiency, depression and loss of BMD (79). Long-term exposure to glucocorticoids, even in modest doses, results in a loss of BMD, with significant reduction in BMD generally exhibited within the initial few weeks of therapy and will continue at a slower pace years after glucocorticoid use (156). Furthermore, significant bone loss leads to increased fracture risk. The process of trans-activation that is associated with the side-effects of chronic glucocorticoids use, including the loss of bone density. Humphrey*, et al.,* (79) assessed the effects of glucocorticoid receptor ligands on OPG production and on the expression of OPG and RANKL in osteoblast cultures. Findings suggested that glucocorticoids enhance RANK signalling, inhibit IFN-β production (IFN-β is an inhibitor of osteoclast differentiation), stimulate RANKL formation and inhibit OPG production by osteoblasts. This pushes remodelling in favour of resorption.

Glucocorticoids have both direct and indirect effects on BMD. The direct effects include inhibition of osteoblast replication and bone matrix synthesis, as well as the induction of osteoblast apoptosis (157). Reduction in bone matrix synthesis is achieved by decreasing the synthesis of type I collagen and modulating the expression of mRNA encoding Furthermore, glucorticoids directly stimulate osteoclast synthesis (117).

The indirect effects of glucocorticoids include the inhibition of growth hormone secretion and renal Ca^{2+} re-absorption. There is also a decrease in interstitial Ca^{2+} absorption and stimulation of parathyroid hormone secretion. Approximately 60% of patients with Cushing's syndrome suffer from low BMD (117).

In a year long multicentre, randomized, double-blind control clinical trial conducted by Cohen and colleagues in 1999, 17% of patients exhibited vertebral fractures within a year of initiating oral glucocorticoid treatment (156). Even orally-administered

treatment periods as short as 12 weeks have been known to induce significant bone loss (158). Intriguingly enough, fractures in people with glucocorticoid-induced osteoporosis occur at a higher BMD level than fractures in people with involutional osteoporosis (156).The use of inhaled glucocorticoids does ameliorate some side-effects, but dose-dependent BMD loss at the hip has been observed in such treatments (156).

Glucocorticoids can also regulate the RANKL:OPG ratio. Increasing RANKL and decreasing OPG shifts the ratio in favour of osteoclastogenesis, thereby inducing osteoporosis (33). And, as Black (82) has pointed out, one of the early events in the stress response is the activation of nuclear factor κB (NFκB). Glucocorticoids also decrease osteoblast numbers *in vivo* and *in vitro* via either increasing apoptosis or decreasing differentiation to the osteoblast lineage in favour of adipocyte differentiation (103).

The release of CRH has a stimulatory effect on the locus coeruleus, which in turn stimulates further release of CRH from the PVN of the hypothalamus. The release of catecholamines from the locus coeruleus causes increased arousal. In addition to the general metabolic up-regulation that occurs in the presence of catecholamines, there is also an increased release of pro-inflammatory cytokines in response to elevated catecholamine levels (98). These cytokines have been implicated in increased bone resorption, as described in section 1.2. In addition, IL-1 can stimulate the increased production of CRH, which further perpetuates the immune dysregulation (93).

1.5 The scope of the problem

While it is largely accepted that stress plays a defining role in physical health, debates continue around the extent to which such information can be accepted as axiomatic. The mind-body debate is taken up in this research, with depression being examined as a contributor to and not only a consequence of physical illness – specifically diseases of low bone density.

The extrapolated prevalence of depression in South Africa is 2 355 768 (159) and is understood to be on the rise (160). Depression is frequently cited as a trigger, cause

and comorbid condition in medical illnesses (4). As such, the medical consequences and cohorts of depression are wide-ranged and have strong implications for both treatment and prevention (161, 162). Although the causal relationship between osteoporosis and BMD has not yet been confirmed with absolute certainty, significant results have arisen from studies on both men and women of varying ages (for example 149, 145, 146) and these results have been used as guidelines for this research.

Any association between depression and BMD loss would have a major impact on how depression is treated and on how its impact on life is viewed. This is especially significant in the light of the fact that current statistics reflect that South African is also affected by the high worldwide prevalence of disorders of bone density: osteoporosis is on the rise amongst young adults and an increasing percentage of black women is being affected (161). From an economic point of view, the expenses related to osteoporosis treatment are tremendous. Furthermore, work-place productivity is influenced by the fact that osteoporosis accounts for more hospitalization time amongst women over the age of 45 years, than the more public diabetes mellitus, myocardial infarction and breast cancer (161). Should depression prove to be a significant risk factor for osteoporosis, this may bring about a shift in paradigms regarding the screening of, prevention and treatment of depression.

1.5.1 Osteoporosis in South Africa

Osteoporosis claims a place in the World Health Organization's list of the world's ten most serious diseases. In spite of this, the disease is not afforded high priority in South Africa (161). According to Bateman (161) most South African medical aids have removed osteoporosis from their list of prescribed minimum benefits, resulting in fewer people being able to access preventative medicine for this silent disease. The worldwide epidemiological profile of osteoporosis is cause for alarm and Bateman (161) contends that South Africa is not immune to the high prevalence of this disorder.

In South Africa, BMD statistics are comparable for White, Black, Asian and Coloured races (163). This differs somewhat from evidence that African Americans have a higher BMD, and also a lower incidence of osteoporosis than white Americans (164).

Solomon (165) reported that black South Africans exhibited a lower rate of post-menopausal femoral neck fractures than Western Europeans. Such fractures are indices of osteoporosis. However, this pattern has been questioned in light of processes such as urbanization and western acculturation. Vorster (166) studied the effects of urbanization on the markers of bone turnover of postmenopausal black females in the North West Province of South Africa and found that women living in urban areas may lose bone more quickly than their rural counterparts. Black women form urban areas showed a pattern of bone loss with age that resembled white urban females and differed from those of rural black women. The most likely contributor to this pattern is a higher fat mass to lean mass ratio in urbanized versus rural black females. Smoking, alcohol consumption and stress levels were not found to be significantly different in urban and rural black women in that study. Vorster conceded that Thiazide use may have played a role in this observed effect, though.

1.5.2 Depression in South Africa

Estimates of depression cases in South Africa vary. The US Census Bureau's extrapolated estimate is almost 2,5 million (159). Bhagwanjee, Parekh, Paruk, Petersen and Subedar (167) illustrated that the weighted prevalence of depressive disorders and generalized anxiety in South Africa is 23,9% (the depressive disorders contributed 20,2% to this total). This was concluded to be higher than findings in other community-based studies conducted in industrialized Western regions. Pillay and Kriel (168) determined that 21% of the 422 women over the age of 21 years attending district-level clinical psychology services in the Msunduzi municipality in 2004 suffered from depression. This is marginally higher than the world estimate for lifetime prevalence (125).

The link between stress and depression has been strongly represented in the literature (84, 88). A basic premise of this field is that stressful events can lead to depression (151). Resource-poor rural communities in South Africa are at particular risk from stressors such as climate change, HIV/AIDS, limited land access and overall low quality of life (169). It is not surprising then that the South African Depression and Anxiety Group (160) proposes that depression's prevalence is growing in rural communities (160).

It is, however, not only rural communities where stressors may give rise to increased depression statistics. For example, the stressors facing specific urban communities, such as correctional services and police officers, have been well-documented. Consistent high levels of stress in correctional services officials have serious outcomes such as heart disease, hypertension, alcoholism, anxiety, as well as depression and suicide (170). Furthermore, high levels of crime and violence have contributed to a dramatic increase in burnout amongst South African police officers (171). Burnout has a moderate correlation with depression, with evidence for co-development of the two (172).

The normal neural and neuroendocrine reactions involved in stress are known to be deranged in depression (83, 3). It is the dysregulation of the HPA axis that is suspected of playing a role in diminishing BMD. Low BMD is more frequently reported in depressed people than in the general population (1). The role that lifestyle and environment can play in the loss of BMD in South Africans warrants further scrutiny. It has, for instance, not been considered whether stress levels and depression could contribute to the occurrence of osteoporosis in South Africa. With a lifetime prevalence estimated at 20%, depression is another major health concern for most countries (125). In South Africa, the disorder has reached alarming proportions (168). With depression (and concomitant alterations in the HPA axis) emerging as a potential risk factor for osteoporosis in Europe, America and Asia (149, 2) it is time to investigate the phenomenon in South Africa.

1.6 Aim and hypotheses

This research explored whether depression is related to low BMD in females. In addition, the role of cortisol in lowering BMD and depression was investigated. A limited exploration of pro-inflammatory cytokine levels was employed to ascertain if there are any trends in the BMD, cortisol and bone turnover profiles of depressed patients. This research is a limited exploration of whether or not depression is a risk factor for low BMD in women that have not yet experienced menopause.

The aim of the study was to investigate the possible association between depression and low bone density in premenopausal females. This aim was to be achieved

through answering the following research questions:

- What is the strength of association between depression and BMD in premenopausal females?
- In what manner are cortisol levels and BMD associated?
- What is the relationship between BMD and cortisol levels in depressed and non-depressed persons?

The study was approached from two starting points: the first uses low BMD as the departure point and the second uses depression as the preliminary point. This was achieved by first investigating women where the primary concern was possible low BMD (referred to as Study 1 or the first part of the research) and then by assessing women whose primary diagnosis is depression (Study 2 or the second part of the research). Therefore:

- Study 1 investigated the depression levels of pre-menopausal female volunteers from DEXA units, while
- Study 2 investigated the bone density of premenopausal female volunteers, from a psychiatric clinic, diagnosed with major depression.

The objectives of Study 1 involved the determination of BMD via DEXA, saliva cortisol levels via ELISA and depression by self-report on the Beck Depression Inventory and the Psychological General Well-being Schedule. Women visitng DEXA units in Pretoria were asked to volunteer for this research. Any significant correlation found between BMD on DEXA and depression was to be further investigated through the quantification of urinary and plasma markers of bone turnover. The hypotheses put forward in the first part of the study are:

1. $H_0$1: there is no difference between the depression levels of females with low BMD and those with normal BMD.
2. H1: women with low BMD exhibit higher levels of depression than women with normal BMD.
3. $H_0$2: the females that present with low BMD and those with normal BMD exhibit similar salivary cortisol levels

4. H2: females with low BMD exhibit higher salivary cortisol levels than those with normal BMD.
5. $H_0$3: cortisol and depression levels do not predict bone density.
6. H3: BMD can be predicted through cortisol and depression levels.

The objectives of Study 2 involved the assessment of BMD (as reflected by DEXA), saliva cortisol levels (determined by ELISA) and depression as measured on the Beck Depression Index and the Psychological General Well-being Schedule in women diagnosed with depression and attending outpatient treatment at a psychiatric unit. Any trend of correlation found between BMD on DEXA and depression was to be further investigated through the quantification of urinary and plasma markers of bone turnover. The hypotheses put forward in the second part of the study are:

7. $H_0$4: there is no difference in the BMD levels of females with and without depression.
8. H4: women with depression exhibit lower BMD than women without depression.
9. $H_0$5: the salivary cortisol levels in females do not differ between those that present with depression and those without depression.
10. H5: the salivary cortisol levels are higher in females with depression than in those without depression.
11. $H_0$6: the levels of pro-inflammatory cytokines are within normative range in women with depression
12. H6: the levels of pro-inflammatory cytokines are above the normative range in women with depression

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Chapter 2: Materials and methods

The aim of the study was to investigate the possible association between depression and low bone density in premenopausal females. In order to accomplish this aim, two avenues of research were followed. The first part, Study 1, involved the comparison of depression levels in premenopausal females with normal and low BMD. Study 2 involved the assessment of BMD in premenopausal females with and without depression.

The research design employed is quantitative. It involved the once-off completion of questionnaires along with a quantification of bone density via DEXA and the collection of saliva for the measurement of cortisol via ELISA. Markers of bone turnover were measured in urine and plasma and pro-inflammaotry cytokines were quantified in serum. No intervention was employed. The nature of the sampling and the lack of differential treatment mean that the study is not experimental. The main limiting factors for this research were funding, time and the lack of volunteers that met the inclusion criteria. A number of variables needed to be investigated, but finances diminished the capacity to select a large subject group for enhanced statistical power.

2.1 Sample

DEXA units of five hospitals in the Pretoria region were approached to assist with the recruitment of subjects. Ultimately, only three hospitals provided subjects. Permission to conduct the research at the hospitals was obtained from the relevant parties. The participating health professionals scanned their databases for subjects that met the criteria for the study.

One psychiatric clinic was approached for volunteers for Study 2. The admission records of the females fitting the age criteria were scrutinized by the psychiatric registrar and women with a diagnosis of severe chronic major depressive disorder, recurrent without psychotic features or postpartum onset

were approached to volunteer for the study. The diagnosis could include the specification for remission (i.e. in partial or full remission).

The Ethics Committee of the University of Pretoria also sanctioned to use of an advertisement to recruit volunteers. Interested persons were invited to speak to the investigator regarding the study. Every volunteer was briefed on the study and supplied with the informed consent leaflet (Appendix A: Informed Consent). The opportunity was then afforded for the volunteer to ask questions and to decide on participation. Those subjects agreeing to participate in the study were asked to sign the informed consent form.

Subjects were required to complete the questionnaire (Appendix B: Questionnaire). Every subject was provided with four vials for saliva collections and the method of collection was explained. Subjects were also given a pamphlet explaining the process in detail (Appendix C). Individuals were then asked to collect saliva as directed at the indicated times. Subjects were requested to return the saliva samples within a week from the date of commencement of the study.

The correlation between depression and DEXA BMD results was to be investigated further through the quantification of markers of bone turnover. Cytokines were to be measured for a preliminary investigation into the pro-inflammatory status of subjects. Urine and blood samples were to be collected at Du Buisson, Bruinette and Kramer Pathologists or at the Clinical Research Unit at the University of Pretoria.

The intentional exclusion criteria for the study were set prior to recruitment and are noted as:

- Age (under 20 years or over 40 years)
- Current pregnancy
- Known infectious or auto-immune disease

- Known inflammatory disease
- Metabolic disorders related to bone density loss
- Psychiatric illnesses other than major depression or dysthymia
- The chronic ongoing use of medication with effect on bone mineral density
- The current use of hormone replacements
- The existence of known metabolic or endocrine disorders other than those directly related to bone density loss (e.g. diabetes, COPD, rheumatic diseases, liver and kidney diseases, Cushing's disease, hyperthyroidism)
- Use of medication to prevent bone loss (e.g. oestrogens or fluorides)

Unintentional exclusions include:

- Illiteracy: the questionnaires were presented in written format and therefore people that could not read or write could not participate.
- Language: those that could not understand English adequately were not capable of answering the questionnaires and therefore were not included in this study.

2.2 Ethical considerations

The Ethics Committee of the University of Pretoria approved the study (Protocol number 90/2006). Written informed consent was obtained from all participating sites and subjects. Once subjects had signed the informed consent leaflet, they were randomly assigned a unique code which was to be kept confidential and used on all questionnaires and labels. The investigator was not privy to the code and it was therefore stressed that the code had to be recorded accurately for all investigations. Subjects were required to nominate a doctor and to inform that doctor of participation in the research. Anomalous DEXA readings were forwarded to the nominated doctor using the subject's code.

2.3 Measuring bone mineral density (BMD)

Bone density was measured using Dual-emission X-ray Absorptiometry (DEXA). DEXA is a non-invasive method that is utilised for the evaluation of skeletal integrity (1). On of the method's advantages is that it does not require subjective assessment of results. Furthermore, the exposure of the patient to radiation is low. The effective dose of radiation for DEXA ranges from 0,07µSv for a six minute DEXA of the forearm to 4,6µSv for a DEXA of the entire body lasting 16 minutes (1).

Readings were obtained from the databases of sites 1 and 2 after permission for access was granted by subjects. Volunteers from site 3 and the psychiatric clinic underwent free DEXA scanning for purposes of the study. Lumbar (L1-L4), total hip and femoral neck BMD's were measured using the following machines:

Table 2-1 The DEXA machines used in the study

Site	Machine
1	Hologic Discovery
2	Lunar Prodigy
3 (including psychiatric patients)	Hologic QDR 1000

The Hologic QDR 1000 was the first commercially available DEXA machine. This was most recently upgraded to the Hologic Discovery series bone densitometer. Prodigy is GE Lunar's latest densitometer (1, 2, 3).

DEXA delivers readings in terms of the amount of bone at the measured site (bone mineral content), area of measurement and BMD adjusted for age and BMI. T-scores offer the BMD referenced against a young adult range and the Z-scores are referenced against an age-matched reference group (4). Figures 2.1 and 2.2 show DEXA results for an individual. Figure 2.1 is an example of the image produced by the DEXA scan and Figure 2.2 shows the referenced result.

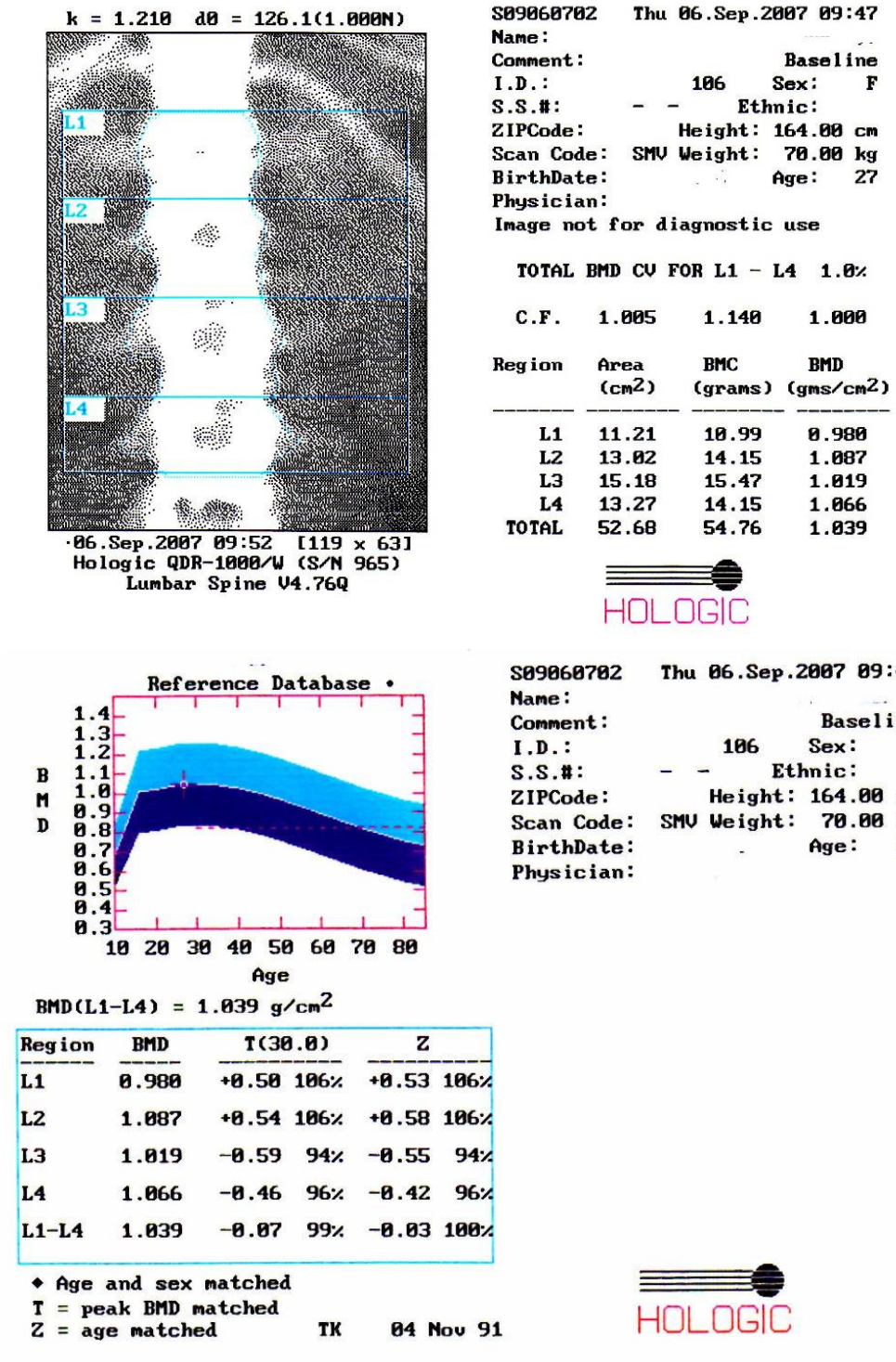

Figure 2.1 Hologic QDR 1000W image of lumbar region (top) and graph of referenced lumbar results (bottom). Permission obtained for use of the image from Professor D Van Papendorp and the subject.

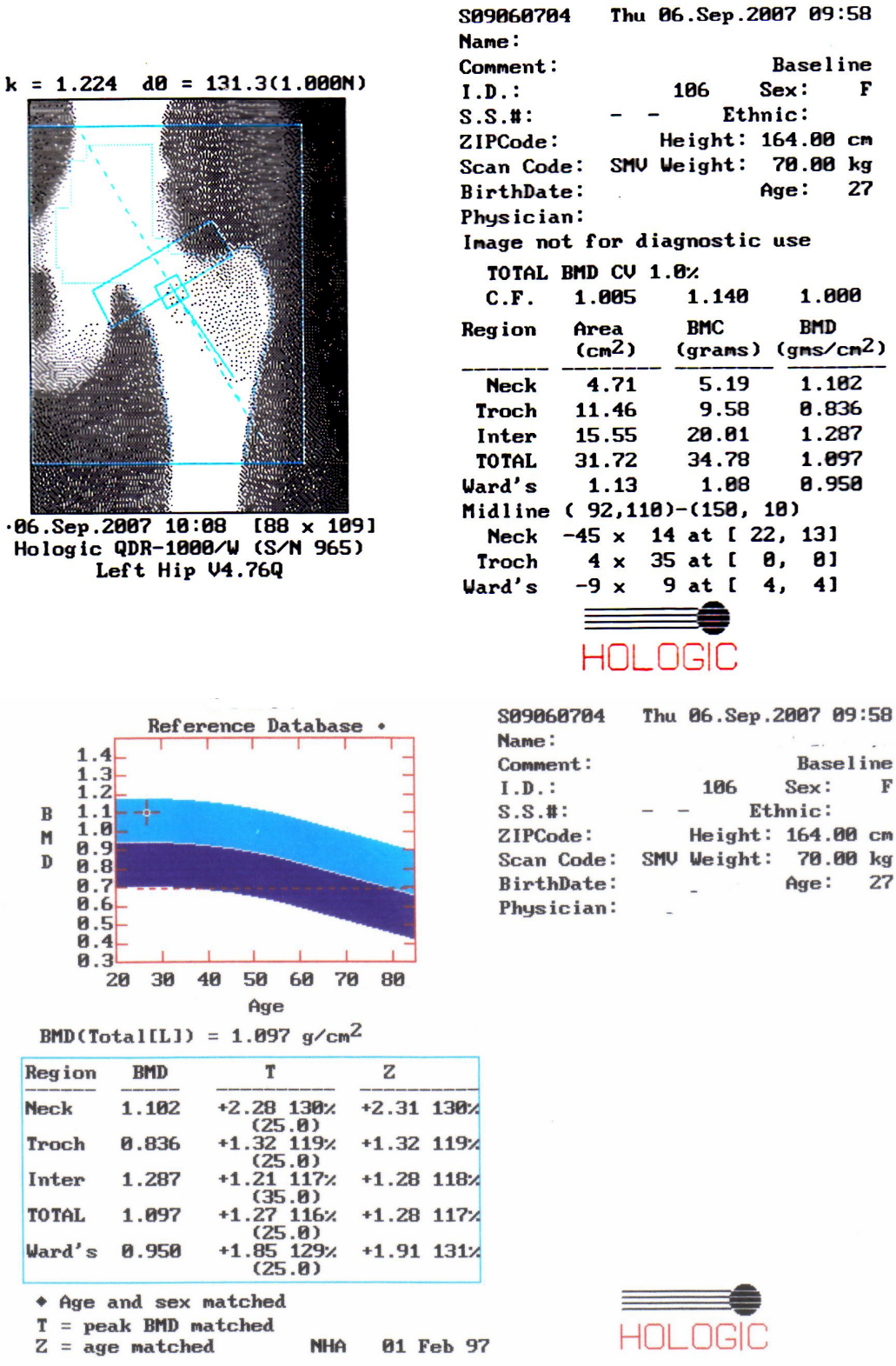

Figure 2.2 Hologic QDR 1000W image of left hip (top) and graph of referenced left hip results (bottom) (permission obtained for use of the image from Professor D Van Papendorp and the subject)

2.4 Measuring salivary cortisol

Cortisol is an essential measurable product of the HPA-axis and is often used as a marker of stress, anxiety and depression (5). Salivary cortisol measures are considered a reliable reflection of total values and have been found to correlate highly with plasma levels (5*).* Nonetheless, there exists considerable within-individual variation in cortisol levels (5, 6). One method of limiting the effects of such variations is to establish person-specific averages, but this prevents cross-level interaction analysis. A multi-level approach allows more accurate comparisons. For this reason, four collections were done over a day. Collections occurred in the subject's natural settings. The first collection was done in the morning (approximately 30 minutes post-waking), early afternoon (six and a half hours post-waking), late afternoon (11 and a half hours post-waking) and bedtime (approximately fifteen hours post-waking). Subjects rinsed their mouths with water and five minutes later they spat into tubes. Subjects were instructed to masticate (on an empty mouth) or to sit in a room where they could smell food in order to stimulate salivation.

Solid phase enzyme immuno-assays (DRG Instruments) were employed for this study. The procedures supplied by the kit manufacturers were followed without deviation. The DRG cortisol ELISA kit is a solid phase ELISA that is based on the principle of competitive binding. The 96 microtitre wells are coated with a monoclonal antibody. This antibody is directed at an antigenic site on the cortisol molecule. The cortisol molecule competes with cortisol horseradish peroxidase conjugate for binding with the antibody. After incubation, the wells are washed to remove unbound conjugate. The bound conjugate is inversely proportionate to the cortisol concentration. Substrate solution added to the sample produces a colour, the intensity of which is inversely proportionate to the cortisol concentration.

The assay procedure is as follows:

1. 50µl of standard is dispensed into the wells.
2. 50µl of sample is dispensed into the selected wells.
3. 250µl of conjugate is dispensed into the sample and standard wells. The plates are mixed thoroughly for ten seconds.
4. The wells are incubated for 60 minutes at room temperature.
5. The wells are shaken out and rinsed three times with wash solution (diluted).
6. 200µl of substrate are added to the wells.
7. The plates are incubated for 15 minutes at room temperature.
8. The reaction is stopped by adding 100µl of stop solution to every well.
9. The absorbance is determined at 450 $\pm$ 10nm within ten minutes of the addition of the stop solution.

2.5 Questionnaires

All subjects were required to complete a biographical data sheet and two psychological questionnaires, namely the Psychological General Well-Being Schedule (PGW) and the Beck Depression Index (BDI). The PGW is a concise self-administered questionnaire that assesses subjective psychological health or quality of life within the past month of the subject's life (7). The index was developed by Harold Dupuy in 1977. According to McDowell and Newell (8) the test was developed for the American Health and Nutrition Examination Survey (HANES I). The PGW contains 22 items rated according to frequency or intensity.

The results cover six domains: anxiety, depressed mood, positive well-being, self-control, vitality and general health. Overall psychological well-being is derived from the total score. Normative data is derived from the HANES I data and the reference values appear in Table 2.2. In addition, the six domains are classified as in Table 2.3.

Table 2-2 Reference standards for the Psychological General Well-being Schedule (PGW) (8)

Scores	Classification
73-110	Positive Well-Being
61-72	Moderate distress
0-60	Severe distress

According to Rasmussen and Nørholm (9) and Bowling (10), the PGW has been utilized in several key studies in the United States and the test was developed using adult subjects from the United States. Its generalizability to other countries is being investigated. For instance, Rasmussen and Nørholm (9) have translated the test into Dutch for use on that population. Existing studies suggest that the test exhibits high validity and reliability (9). The correlation validity of the PGW has been established in Fazio's study of 195 students and Fazio is quoted (in 8) as stating that the PGW "emerged as the single most useful instrument in measuring depression."

A potential weakness of the test is that it has not been adapted for the South African population and no normative data exists for this country. A second query regarding the statistical properties of the test involves the validity of the six separate domains. Taylor *et al.* (7) suggest that the PGW is one-dimensional. Although further research is required to fortify this criticism, The Beck Depression Inventory- second edition (BDI) was utilised as a means of controlling for any anomalies in the depressed mood domain.

Table 2-3 Domains: Topics and classification of scores (8, 11)

Domain	Range of scores	Low score	High score
Anxiety	0-25	Extremely bothered by nervousness; very tense, anxious; worried, upset; felt under heavy pressure	Not bothered by anxiety or nervousness. Low tension; not anxious; little or no stress and strain
Depressed mood	0-15	Intensely or often felt depressed, downhearted and blue, hopeless	Never or rarely depressed, downhearted, blue or hopeless
Positive well-being	0-20	Low spirits, unhappy, never or seldom felt life was interesting or cheerful	In excellent spirits; happy with life; daily life is interesting; felt cheerful
Self-control	0-15	Very concerned or disturbed about losing self-control, seldom felt emotionally stable	In definite control of behaviour, thoughts, emotions and feelings; emotionally stable
General health	0-15	Often bothered by illness, bodily disorders, need help in caring for self, worried or fearful about health	Rarely if ever bothered by illness; healthy enough to do things; not fearful or worried about health
Vitality	0-20	Low in energy, seldom waking fresh and rested, dull, sluggish, tired, worn-out	Full of energy, pep, waking fresh and rested, felt active, vigorous, never felt tired or worn-out

The BDI was developed by Beck, Steer and Brown (12). This inventory allows the researcher to determine to what extent the patient's symptoms match those of the diagnostic criteria of depression according to the DSM-IV-TR. There are 21 items, which are rated on a Thurstone scale. The scores are then added and the respondent's experience of depressive symptoms is classified as minimal, mild, moderate or severe. The split-half reliability coefficient is 0.86 for this test, indicating a high internal consistency, while the test-retest reliability is in the 0.70's range. This indicates a high reliability. The validity of the test is also

consistently high (13). One of the main limitations of the test is its high face validity. This means that the respondent may easily be able to manipulate the outcome of the test.

2.6 Biochemical markers of bone turnover

The measurement of bone turnover markers was planned in the event that an association was found between depression and BMD on DEXA. The measures were only carried out for Study 2. Bloods and urine were taken from individuals during one morning session after an overnight fast. The specimens were immediately taken to the laboratory at Du Buisson, Bruinette and Kramer via courier. All determinations for bone turnover were done by Du Buisson, Bruinette and Kramer.

The biomarkers of bone turnover measured were BSAP, osteocalcin, urine pyridinoline (Pyd) cross-linked C-telopeptide and deoxypyridinoline (DPD). Osteocalcin and BSAP served as markers of bone formation, while bone resorption was calculated using urine Pyd cross-linked C-telopeptide and DPD. Creatinine was measured in urine for the correction values of DPD.

2.6.1.1 Bone formation markers

The Metra Osteocalcin ELISA kit (Quidel) was employed in the determination of osteocalcin. The kit consists of two monoclonal antibodies that act against osteocalcin. The first antibody is the capture antibody, while the second recognizes the N-terminal of the protein, allowing for quantitative analysis.

The BSAP was determined using the Access Ostase assay (Beckman Coulter, Inc.). The assay consists of a monoclonal mouse antibody specific to BSAP. The reaction vessel contains paramagnetic particles that are coated with anti-mouse polyclonal antibody. The serum is added to the reaction vessel and BSAP binds to the anti-BSAP mouse monoclonal antibody. The bound materials remain suspended in a magnetic field and the unbound material is washed

away. A chemiluminescent substrate (Lumi-Phos*530) is introduced into the vessel and the reaction generates light, the intensity of which is measured via luminometer. The light is directly proportional to the concentration of BSAP in the sample.

2.6.2 Bone Resorption Markers

The β-CrossLaps/serum kit (cobas®) immunoassay kit was selected for the measurement of the degradation of type I collagen (Pyd cross-linked C-telopeptide). The type I collagen fragments measured are the C-terminal telopeptides. The kit is based on the sandwich binding of the anti-β-CrossLaps monoclonal antibody to the C-terminal telopeptides released into serum. Streptavidin-coated microparticles and a monoclonal anti-β-CrossLaps-specific antibody labelled with ruthenium complex are added to the sample and sandwich complex becomes bound to the solid phase via the interaction of biotin and streptavidin. The mixture is aspirated into a measuring cell in which the microparticles are magnetically bound to the surface of an electrode. Unbound particles are removed. A voltage is applied to the electrodes in the measuring cell. This causes a chemiluminescent emission which is measured by photomultiplier.

For the determination of DPD, the Immulite®/Immulite® 1000 Pyrilinks-D Analyzer (DPC) was utilised. Deoxypyridinoline is stable in stored urine and is unaffected by diet (14). The kit is based on the principles of solid phase chemiluminescence enzyme-labelled immunoassays. A bead coated with monoclonal murine anti-DPD antibody is placed in the Immulite®/Immulite® 1000 Test Unit. Samples are diluted where necessary and added to the Test Unit as per the operator's manual. The urinary DPD results are normalized to the urinary creatinine concentration. Urinary creatinine was determined using kinetic colorimetry using the Hitachi P-modular analyzer.

2.7 Cytokines

The cytokines chosen for this study were IL-1β and TNFα. Originally, the levels of IL-1α were also to be determined, but lack of funding precluded this. These cytokines were chosen because of they are frequently cited in osteoimmunology studies involving the pathological loss of BMD (15, 16, 17, 18). The testing of IL-1β and TNFα was made possible through the generosity of Professor M Viljoen and Mr Priyesh Bipath (both from the Department of Physiology, University of Pretoria). The cytokine determinations were conducted by Mr Bipath.

2.7.1 IL-1β

Human IL-1β ELISA kit (Diaclone) was used in this determination. The wells of the microtitre strips are coated with a monoclonal antibody specific for IL-1β. Serum was centrifuged at 1000 x g for 10 minutes. The washing, standard preparation, control preparation and the preparation of biotinylated anti- IL-1 β and the Streptavidin-HRP were carried out as per the instructions in the kit. The concentration of IL-1β was determined via absorbance, which is measured at 450nm.

2.7.2 TNF-α

The Human TNFα ELISA kit (Diaclone) was utilised to determine TNFα levels in subjects. Serum was collected in pyrogen/endotoxin free tubes. Serum was centrifuged at 1000 x g for 10 minutes. The wells of the microtitre strips are coated with a monoclonal antibody specific fro TNFα. The washing, standard preparation, control preparation and the preparation of biotinylated anti-TNFα and the Streptavidin-HRP were carried out as per the instructions in the kit. The concentration of TNFα was determined via absorbance, which is measured at 450nm.

2.8 Outline of the data analysis for Studies 1 and 2

Statistical examination for Studies 1 and 2 began with descriptive analysis of the data. Owing to the small sample size for Study 2, only descriptive statistics were employed. The means and standard deviations for relevant variables were calculated.

In Study 1, the first step of inferential analysis involved determining the strength of association between BMD and depression. The DEXA readings and results from the questionnaires were analyzed using the Pearson correlation coefficient for

- DEXA and BDI score
- DEXA and PGW depressed mood subscale score (referred to as PGW(depression) subscale)

The second step involved the determination of average cortisol levels. The statistical model suggested by Hruschka, *et al.* (6) was utilized. This model did not require that all subjects provide the same number of readings. The variables in the model are:

- CORT*ij*, representing cortisol on the *j*th measure for the *i*th person.
- β_0 is the intercept and represents the population mean for cortisol six hours post waking
- β_1 is the population's average diurnal slope
- ε_{ij} is the *i*th person's *j*th reading deviation from her own mean. Its variance represents within-individual variance not accounted for by time.
- b_i is a random variable that represents any deviation of the *i*th person's readings from the overall mean. Its variance represents between-individual variance not accounted for by time.
- TIME is defined as the hours since waking.

The following model describes the data model for cortisol readings collected at four points during a day:

$$CORT_{ij} = \beta_0 + \beta_1 \times \text{TIME} + b_i + \varepsilon_{ij}$$

The mean for the *i*th individual is represented by $(\beta_0 + b_i)$

The intra-individual correlation coefficient (ICC) is calculated as

$$ICC = \frac{\tau^2}{\tau^2 + \sigma^2} = \frac{Var(b_i)}{Var(b_i) + Var(\varepsilon_{ij})}$$

τ^2 = between-individual variance; σ^2 = within-individual variance

The ICC provides an expected correlation for any pair of readings on the same subject. The ICC also indicates the proportion of total variance that can be attributed to between-individual differences. It is also a measure of reliability for a single measure of cortisol. The higher the ICC, the more reliably a single measure of cortisol reflects between-individual differences (if the ICC $\geq$ 0.80, then a single readings is considered reliable). The following steps were performed:

- A population model for cortisol diurnal cycle was created.
- Between- and within-individual variances were calculated using the $CORT_{ij} = \beta_0 + \beta_1 \times TIME + b_i + \varepsilon_{ij}$ model and ICC equation.

The third stage of analysis involved determining the differences between women with normal and women with low BMD. Fisher's Exact Probability and the Mann-Whitney tests were used to compare the two groups on categorical and continuous variables. Correlation analysis was performed using the Pearson and Spearman correlation coefficients to determine any within-group correlations.

At the fourth stage, a regression model for low bone density was to be created in the event of significant associations between depression and BMD. The aim

of this analysis was to determine what proportion of variance in BMD can be accounted for by depression and cortisol levels.

2.9 Outline of process

This research is divided into two studies, Studies 1 and 2. Study 1 explored depression levels in premenopausal female volunteers recruited from three DEXA units. These volunteers underwent DEXA evaluation, completed the BDI and the PGW and provided saliva samples for cortisol analysis via ELISA. The determination of bone turnover marker values and cytokines was to be carried out only if significant associations were found between DEXA and depression scores. This decision was taken in order to prevent the unnecessary testing of subjects and because of financial constraints.

Study 2 investigated the BMD status of premenopausal women diagnosed with major depression. These women were volunteers from a psychiatric clinic. The volunteers underwent DEXA scanning, completed the BDI and PGW and provided saliva samples for cortisol quantification. Again, the determination of bone turnover marker values and cytokines was to be carried out only if significant associations were found between DEXA and depression scores. The pro-inflammatory status of the psychiatric patients was determined through quantification of IL-1β andTNFα. This was to be compared to referenced normative data.

2.10 Meta-analysis

Owing to the limitations imposed by the small sample in Study 2, it was considered valuable to conduct a meta-analysis of similar research. The aim of this review was to describe and quantify the statistical link between depression and the loss of bone mineral density as described in journal articles published between January 1997 and May 2007. A comprehensive search was undertaken to identify all quantitative studies undertaken between January 1997 and May 2007 and relevant to the loss of bone density in premenopausal

women as a result of depression. The databases searched were: MEDLINE, SCOPUS and SCIENCE DIRECT.

Quantitative studies involving depression and bone mineral density were selected. Drug trials were excluded. Where trials included males and females, only the females' scores were analysed. Initially, ten studies were identified, but only six met the criteria for inclusion in the analysis. Articles were excluded on the basis of insufficient information about results, the use of all-male samples and the use of methods other than dual x-ray absorptiometry to ascertain femoral and lumbar bone density. No negative studies were identified. The list of studies found and decision on their inclusion or exclusion are indicated in Table 5.1. No additional hand searching was undertaken. Where information was missing or incomplete, the authors were contacted. Only one author replied and therefore the remaining incomplete studies were excluded from analysis. Data were collected by a single researcher and recorded in an Excel spreadsheet.

Information was collected regarding subject biographical data (age, sex, size of group), depression score (on whichever instrument used), BMD DEXA or CT scores, BMD resorption and development. From the articles examined, deoxypyridinoline (DPD), osteocalcin, bone specific alkaline phosphatase and cross-laps (CTx) were variably used to measure bone turnover and cortisol was measured as a mediator in the depression and BMD association. Only variables that appeared in three or more studies were included in the analysis. The outcomes measured were:

- Depression (measured with HAMD)
- BMD (Lumbar and femoral neck measured by DEXA)
- Cortisol (measured in serum)
- Osteocalcin (measured in serum)

Table 2-4 Studies identified and decisions on inclusion/exclusion

Reference	Number	Inclusion	Reason for exclusion, (if applicable)
Schweiger U, Weber B, Deuschle M, Heuser I. Lumbar bone mineral density in patients with depression: evidence of increased bone loss at follow-up. American Journal of Psychiatry 2000; 157: 118-120. (19)	1	No	BMD measured via CT scan. Results therefore not compatible with other studies.
Yazici KM, Akinci A, Sütçü A, Ozçakar L. Bone mineral density in premenopausal women with major depressive disorder. Psychiatry Research 2003; 117: 271-275. (20)	2	Yes	
Altindag O, Altindag A, Asoglu M, Gunes M, Soran N, Deveci Z. Relation of cortisol levels and bone mineral density among premenopausal women with major depression. Journal of Clinical Practice 2007; 61: 416-420. (21)	3	Yes	
Amsterdam JD, Hooper MB. Bone density measurement in major depression. Progress in Neuro-Psychopharmacology and Biological 1998; 22: 267-277. (22)	4	No	Insufficient data presented in article.
Herran A, Amado JA, Garcia-Unzueta MT, Vazquez-Barquero JL, Perera L, Gonzalez-Macias J. Increased Bone Remodeling in first-episode major depressive disorder. Psychosomatic Medicine 2000; 62: 779–782. (23)	5	Yes	
Michelson D, Stratakis C, Hill L, Reynolds J, Galliven E, Chrousos G, Gold P. Bone mineral density in women with depression. The New England Journal of Medicine 1996; 335: 1176-1181. (24)	6	No	Results contained four postmenopausal women.
Kavuncu V, Kuloglu M, Kaya A, Sahin S, Atmaca M, Firidin B. Bone metabolism and bone mineral density in women with mild depression. Yonsei Medical Journal 2002; 43: 101-108. (25)	7	Yes	
Yazici AE, Bagis S, Tot S, Sahin G, Yazici K, Erdogan C. Bone mineral density in premenopausal women with major depression. Joint Bone Spine 2005; 72: 540-543. (26)	8	Yes	
Leo R, Tesauro M, Rizza S, Fortuna E, Bianchi F, Bertoli A, Pecchioli C, Troisi A, Di Lorenzo G, Siracusano A, Lauro R. Low bone mineral density in premenopausal women with major depression. 2003; 14: S1-S159. (27)	9	No	Reference only presented as an abstract. No author information available.
Vrkljan M, Vizner B, Bekiæ M, Thaller V, Sonicki Z. Can long-term major depression cause osteoporosis? Acta Clin Croat 2001; 40:179-184. (28)	10	No	Insufficient data presented in article.

The outcomes of the included studies appear in Table 5.2. The data was analysed using SAS. A general variance-based method was used to calculate effect with 95% confidence levels. The goal of the analysis was to estimate difference measures. Therefore a general variance-based method based was chosen (29). The estimated variance was calculated for each study. This was used to calculate the rate difference of each study. This allowed the data to be read as a normative curve. The weight for each study was determined and then the products of the weights and the rate differences were summed and divided by the sum of weights. For every set of variables, the summary estimate of rate difference (∓) was calculated using the formula

$$\mp = \sum \frac{w_i t_i}{w_i}$$

where, $w_i = \frac{1}{\text{var}iance_i}$

where, $\text{var}iance_i = \sqrt{\frac{esd^2}{n_1} + \frac{esd^2}{n_2}}$

and, $t = \frac{em - cm}{\text{var}iance_i}$

The 95% confidence interval (CI) for the estimate of effect (derived with the abovementioned formulae) was then estimated using the formula

$$95\%\ \text{CI} = \mp \pm 1.96 \times \sqrt{\text{var}iance_s}$$

where, $\text{var}iance_s = \frac{1}{w_i}$

Table 2-5 Outcomes of included studies

Variable name	Study reference no.	Experimental mean	Experimental SD	Experimental N	Control mean	Control SD	Control N
Depression measured on the Hamilton Depression Scale (HAMD)	2	22.600	3.900	25.000	Not administered	Not administered	15.000
	3	28.100	6.700	36.000	11.600	5.200	41.000
	5	20.600	4.800	19.000	Not administered	Not administered	19.000
	7	17.600	4.300	42.000	5.900	4.000	42.000
	8	22.400	4.800	35.000	6.800	3.900	30.000
Total lumbar bone density measured by DEXA (g/cm^2)	2	0.978	0.143	25.000	1.108	0.085	15.000
	7	1.16317	0.12357	42.000	1.1609	0.12848	42.000
	8	1.000	0.110	35.000	0.937	0.400	30.000
	3	0.768	0.112	25.000	0.859	0.118	15.000
Femoral neck bone density measured by DEXA (g/cm^2)	7	0.9840	0.11269	42.000	0.997	0.12134	42.000
	8	0.863	0.130	35.000	0.745	0.511	30.000
	2	14.500	8.900	25.000	14.100	5.500	15.000
	3	21.200	20.900	36.000	9.300	4.200	41.000
Serum cortisol (µg/dl)	7	14.380	5.800	42.000	14.840	5.100	42.000
	2	5.900	3.200	25.000	6.200	3.500	15.000
	3	24.900	15.700	36.000	32.700	17.900	41.000
Osteocalcin (ng/ml)	5	16.500	6.500	19.000	11.000	3.600	19.000
	7	8.350	5.800	42.000	6.080	6.100	42.000
	8	24.100	10.700	35.000	23.200	5.700	30.000

2.11 References

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Chapter 3: Study 1 - depression in premenopausal females

This chapter presents the results of the main study (Study 1), the aim of which was to investigate the association between depression and BMD, using BMD as a starting point. The chapter begins with the statistical description of the sample and continues with the inferential data that is directed at testing the hypotheses put forward in Chapter 1. These are:

- $H_0$1: there is no difference between the depression levels of females with low BMD and those with normal BMD.
- H1: women with low BMD exhibit higher levels of depression than women with normal BMD.
- $H_0$2: the females that present with low BMD and those with normal BMD exhibit similar salivary cortisol levels
- H2: females with low BMD exhibit higher salivary cortisol levels than those with normal BMD.
- $H_0$3: cortisol and depression levels do not predict bone density.
- H3: BMD can be predicted through cortisol and depression levels.

The discussion at the end of the chapter focuses on the outcomes with regard to the hypotheses of Study 1. A more comprehensive, integrated discussion of Study 1 and Study 2 is presented in Chapter 5.

3.1 Demographic characteristics of the sample

Forty-four subjects volunteered for the study. Four of the subjects were retrospectively excluded from the study because they had previously been diagnosed with psychological disorders other than depression (two with bipolar disorder, one with generalized anxiety, one with anorexia nervosa). Twenty-five of the subjects were derived from site 1, two from site 2 and the remaining 13 from site 3.

The characteristics of the sample (N = 40) are summarised in Table 3.1. The mean age of the sample was 25.8 (SD = 5.2; range = 20 to 37 years). The

mean body mass index (BMI) was in the normal range (21.7; SD = 3.1; range = 15.6 to 30.1).

Table 3-1 Demographics of the sample

Variable	Mean	SD	Minimum	Maximum
Age (years)	25.8	5.2	20	37
Height (cm)	167	6.6	155	178
Mass (kg)	60.9	9.0	40	80
BMI	21.7	3.1	15.6	30.1
Alcohol Intake (units)	1.3	1.9	0	7

Only four (10%) of the sample listed their highest level of education as high school, while the rest (36; 90%) attended a tertiary educational institution. Most of the subjects listed Afrikaans as their vernacular (62.5%), twelve (30%) stated that English is their home language, one (2.5%) Sotho and the remaining two (5%) spoke other languages at home. The majority of the sample was white (36; 90%), with two black and two Asian subjects also included. Thirty-seven (92.5%) of the subjects were employed and the rest (3; 7.5%) were unemployed. Twenty-five (62.5%) were in a relationship and 15 (37.5%) were single. Four (10%) of the sample admitted to smoking. The mean alcohol intake of the sample was 1.3 units per week (SD = 1.9; range = 0 to 7 units).

3.2 Psychological and medical history

In order to exclude prior diagnosis of depression as a variable in later analysis, explorations of the relationships between a prior diagnosis of depression and BDI, then PGW and then BMD were planned. However, only five (12.5%) of the sample had been diagnosed with depression prior to the study. The rest (35; 87.5%) had never been diagnosed with any psychological disorder. The two groups were too uneven and the smaller group much too small to conduct the analysis.

The presence of medical illness was assessed through the biographical questionnaire. All subjects denied suffering from any diagnosed thyroid, parathyroid or adrenal gland diseases.

3.3 Medication

Table 3.2 shows the proportion (by number of subjects and then percentage) of subjects using specific medications. No subjects reported using anti-convulsants, calcitonin, thyroid hormones, alendronate, cyclosporin, anabolic steroids or bisphosphonates. However, 25 (62.5%) were using contraception at the time of testing, one (2.5%) used inhaled steroids, four (10%) were using anti-depressants and two (5%) were receiving corticosteroid treatment.

Table 3-2 Current medication use

Substance	Proportion using (N)	Proportion using (%)
Contraception	25	62.5
Inhaled steroids	1	2.5
Antidepressants	4	10
Corticosteroids	2	5

3.4 Physical exercise and calcium intake

The participation in weekly physical activity and the number of kilometres walked daily are represented in Tables 3.3 and 3.4, respectively. The sample was not a particularly active one, with 15 (37.5%) participating in less than an hour of exercise per week and 14 (35%) walking less than a kilometre a day.

Table 3-3 Time spent in physical activity per week

Hours of exercise per week	0-1 hour	1-2 hours	2-3 hours	3-4 hours	> 4
Proportion of sample (N)	15	6	6	7	6
Proportion of sample (%)	37.5	15	15	17.5	15

Table 3-4 Distance walked per day

Distance of walking per day	0 to 1 km	1 to 2 km	2 to 3 km	3 to 4 km	> 4 km
Proportion of sample (N)	14	13	8	3	2
Proportion of sample (%)	35	32.5	20	7.5	5

Furthermore, only 22 (55%) of the women reported consuming adequate amounts of calcium per day.

3.5 DEXA

The mean DEXA readings and T-scores for the total lumbar spine, left femoral neck and total left femur are recorded in Table 3.5. The group was highly skewed towards normal total lumbar BMD, with 27 (67.50%) of the sample exhibiting T-scores higher than -1. Thirteen (32.50%) of the sample exhibited osteopenic T-scores (-1>T-score>2.5). There were no osteoporotic (T-score below -2.5) subjects. The mean lumbar BMD was 1.011 (SD = 0.116) and the corresponding mean T-score was -0.382 (SD = 1.051). This falls within the normal range.

For the femoral neck the sample was also skewed towards normal BMD. T-scores were higher than -1 for thirty-seven (92.5%) of the sample. Only three (7.5%) of the sample exhibited osteopenic T-scores (-1>T-score>-2.5). There were again no osteoporotic (T-score below -2.5) subjects. The mean BMD score for the left femoral neck is 1.025 (SD = 0.191), which corresponds to a T-score of 1.511 (SD = 1.793), which also falls in the normal range.

The sample was again skewed towards normal DEXA score on total femur BMD. Here, the total left femoral BMD T-scores were higher than -1 for 33 (82.5%) of the sample. The remaining seven (17.5%) of the sample exhibited osteopenic T-scores (-1>T-score>2.5). There were once again no osteoporotic (T-score below -2.5) subjects. The mean BMD for the total femur was 0.964 (SD = 0.129), which is transformed to a T-score of 0.155 (SD = 1.079), which again falls in the normal range.

Table 3-5 Mean bone mineral density (BMD) on DEXA

Variable	Mean	SD	Minimum	Maximum
Lumbar spine (g/cm^2) N=40	1.011	0.116	0.834	1.288
Lumbar spine T-score	-0.382	1.051	-2.100	2.19
Left femoral neck (g/cm^2) N=40	1.025	0.191	0.666	1.471
Left femoral neck T-score	1.511	1.793	-1.700	5.610
Left femoral total (g/cm^2) N=40	0.964	0.129	0.710	1.239
Left femoral total T-score	0.155	1.079	-1.900	2.430

3.6 Beck Depression Index (BDI) and Psychological General Well-being Schedule (PGW) results

In order to test the validity of the two depression tests, Pearson correlations were calculated for the BDI total and the PGW subscales. Table 3.7 reflects these correlations. The BDI was negatively correlated with all PGW subscales. All these correlations were significant at the $p<0.05$ level, with three correlations (those between the BDI and anxiety, positive well-being and self-control) significant even at the $p < 0.0001$ level. However, these correlations only ranged from -0.469 to -0.688, indicating that the correlations were not very strong. This means that the PGW scores were inversely associated with the BDI scores. This is logical, as high PGW subscale scores indicate more positive well-being (i.e. low depression and anxiety, good general health, vitality and self-control and positive well-being); while a high BDI indicates depression.

Table 3-6 Correlation matrix for Beck Depression Index (BDI) and Psychological General Well-being Schedule (PGW) subscales

Variable	BDI	p-value
PGW (depression)	-0.521	0.0006
PGW (anxiety)	-0.688	<0.0001
PGW (positive well-being)	-0.621	<0.0001
PGW (self-control)	-0.616	<0.0001
PGW (vitality)	-0.560	0.0002
PGW (general health)	-0.469	0.0023

Although the PGW provides a means of assessing anxiety, positive well-being, vitality and general health as well as depression, this study focused only on the depression score. The mean scores for BDI and the PGW subscale are reflected in Table 3.7. The mean BDI score (mean = 6.9; SD = 6.5) fell within the range indicating the existence of minimal depressive symptoms (0-13). The scores ranged widely from 0 to 32. The mean score on the PGW (depression) subscale is considered to be high at 12.4 (SD = 2.0) (range for subscale is 0-15). This indicates that the subjects were never or rarely felt depressed, downhearted, blue or hopeless.

Table 3-7 Depression scores on the Beck Depression Index (BDI) and Psychological General Well-being Schedule (PGW) subscale

Variable	Mean	SD	Minimum	Maximum
BDI	6.9	6.5	0	32
PGW (depression)	12.4	2.0	7	15

The number of subjects suffering from minimal, mild, moderate and severe depression based on the BDI is illustrated in Figure 3.1. Few subjects (6; 15%) reported symptoms that warrant the categorisations mild, moderate or severe. 34 (85%) fell in the minimal depression category.

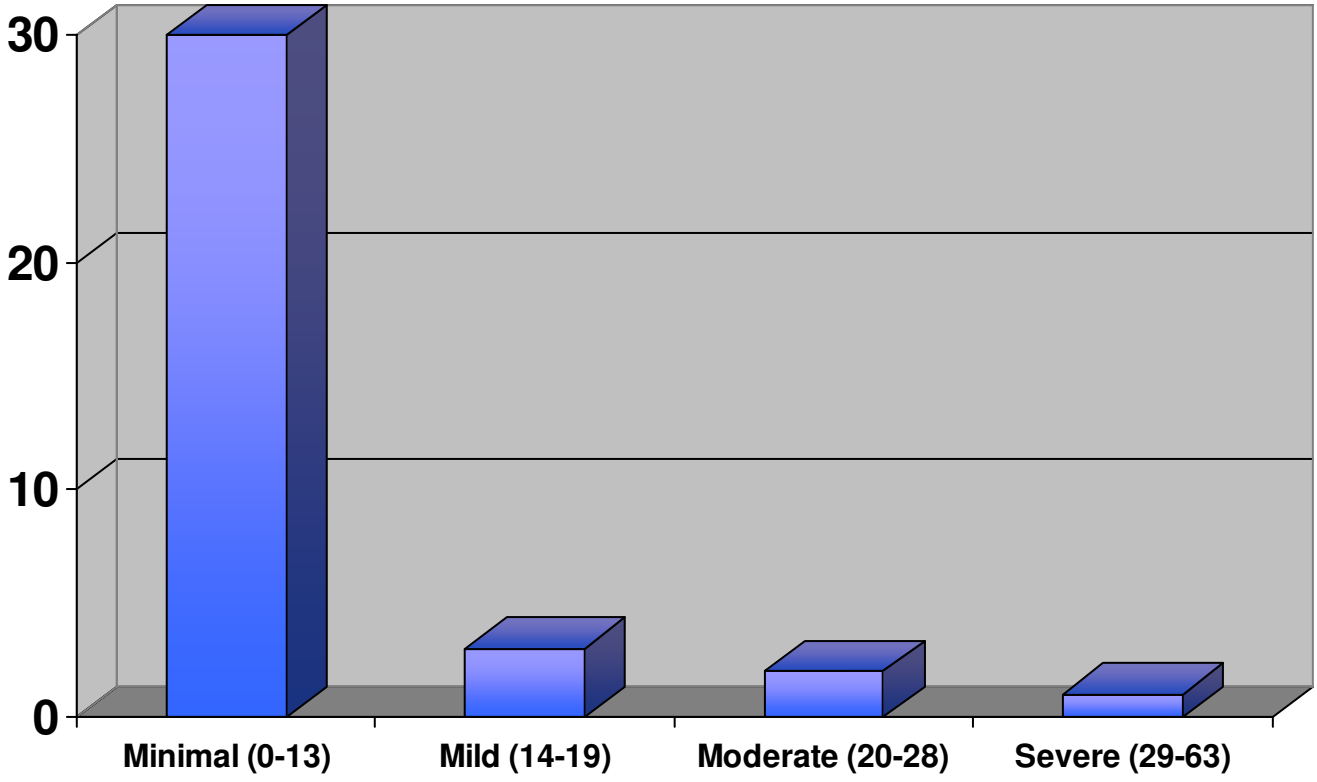

Figure 3.1 Number of subjects in Beck Depression Index (BDI)

3.7 Saliva cortisol

The mean sample values for cortisol levels are provided in Table 3. 8. The morning reading was within average range for adults aged 21 to 30 years, but the 18:00 (mean = 6.051; SD = 2.510) and bedtime readings (mean = 5.438; SD = 2.409) were higher than the expected value of 3.59ng/ml. According to the insert of the DRG kit used, the mean value for salivary cortisol in adults ranges from 1.2 to 14.7ng/ml Therefore, the mean over 24 hours (mean = 6.825; SD = 2.092) was within the normal range.

Table 3-8 Mean cortisol values for sample (N = 36)

Time of measurement	N	Mean (ng/ml)	SD
07:00	39	8.693	4.123
13:00	38	7.208	2.221
18:00	40	6.051	2.510
Bedtime	38	5.438	2.409
Mean over 24 hours	37	6.825	2.092

3.8 Correlations

The correlations between BMD and demographic features (age and BMI), lifestyle factors (walking, exercise, cigarette smoking and alcohol intake), depression and cortisol are noted in Table 3.9. The Pearson correlation coefficient was calculated for the sample. The levels of significance are noted below the correlation coefficient.

Table 3-9 Sample correlation matrix for bone mineral density (BMD), demographic and lifestyle factors, depression and cortisol readings

Variable	Left femoral neck (g/cm^2)	Left femoral neck T-score	Left femoral total (g/cm^2)	Left femoral total T-score	Lumbar spine ($g/cm^{2)}$	Lumbar spine T-score
Age	0.152 0.349	0.134 0.410	-0.072 0.657	-0.078 0.631	0.342 0.031*	0.314 0.049*
BMI	0.462 0.003*	0.450 0.004*	0.566 0.0001*	0.561 0.0002*	0.484 0.002*	0.503 0.0009*
BDI	-0.085 0.601	-0.157 0.335	0.103 0.527	0.051 0.755	0.291 0.069	0.173 0.285
PGW depression	0.127 0.434	0.152 0.349	0.029 0.859	0.044 0.789	-0.172 0.290	-0.132 0.416
Alcohol intake	-0.154 0.341	-0.155 0.339	-0.145 0.371	-0.143 0.379	-0.183 0.259	-0.186 0.252
Walking	-0.116 0.475	-0.069 0.672	-0.060 0.712	-0.033 0.839	-0.025 0.879	0.031 0.848
Exercise	-0.98 0.547	-0.097 0.551	0.165 0.309	0.161 0.321	0.029 0.858	0.032 0.847
Cortisol 07:00	-0.162 0.353	-0.149 0.390	0.103 0.555	0.103 0.555	-0.035 0.842	-0.021 0.903
Cortisol 13:00	0.024 0.895	-0.026 0.886	0.031 0.864	-0.005 0.979	-0.009 0.961	-0.0973 0.584
Cortisol 18:00	-0.131 0.446	-0.128 0.458	0.153 0.373	0.153 0.374	0.089 0.605	0.099 0.566
Cortisol Bedtime	-0.336 0.052	-0.339 0.049*	-0.017 0.923	-0.027 0.878	-0.034 0.847	-0.052 0.772
Mean cortisol	-0.239 0.179	-0.247 0.166	0.089 0.622	0.076 0.673	-0.078 0.668	-0.099 0.580

The symbol * indicates significant correlation ($p<0.05$). Significant correlations were found between all DEXA readings and BMI. These significant correlations were all positive, indicating that higher BMI readings are positively correlated to high DEXA scores. The Pearson values were low, though, ranging from 0.450 to 0.561 (p ranges from 0.0001 to 0.049), indicating that these correlations were not very strong. There was a significant correlation between the lumbar T-score and age ($r = 0.314$; $p = 0.049$). This indicates that the older subjects exhibited higher lumbar T-scores than their younger counterparts. Bedtime cortisol was significantly correlated with left femoral neck T-score ($r = -0.339$; $p = 0.049$). This indicates that high cortisol levels were seen in low left femoral neck T-scores; however, the correlation is weak.

An analysis of bone turnover markers was to be conducted in the event that BMD and depression were significantly correlated. The purpose of this was to confirm the depression and BMD link at the biochemical level. Since no such correlations were found, and in view of the high cost involved in the assessments, the markers were not measured.

After collating the descriptive data from the questionnaires and the DEXA readings, two groups of subjects were identified – those with low BMD and those with normal BMD. All three readings for BMD were considered, namely total lumbar BMD, left femoral neck BMD and total left femoral BMD. Subjects with normal BMD on all three readings were classed in Group 1 and the rest of the subjects in Group 2. This was done to differentiate those with one or more low BMD readings from those with normal BMD. A Mann-Whitney test was executed to confirm that this division of groups was sound and that the groups did in fact differ significantly on BMD. The results of the test are noted in Table 3.10. DEXA results for the left femoral neck, the left femoral total and the total lumbar spine all varied significantly from Group 1 to Group 2, as did their T-scores: the difference between the two groups on the left femoral neck BMD showed a p-value of 0.0004 and 0.0008 on the T-score. The differences on the left femoral total score, the total lumbar spine score and their

respective T-scores were all significant at $p < 0.0001$. This confirms that the division of groups was sound.

Table 3-10 Significance of group differences on DEXA bone mineral density (BMD)

	Group 1 (N=26)					Group 2 (N=14)					p-value
Characteristic	**Mean**	**SD**	**SEM**	**Minimum**	**Maximum**	**Mean**	**SD**	**SEM**	**Minimum**	**Maximum**	
Left femoral total (g/cm^2)	1.026	1.109	0.021	0.821	1.239	0.848	0.069	0.019	0.710	0.955	<0.0001
Left femoral T-score	0.673	0.923	0.181	-0.990	2.430	-0.806	0.569	0.152	-1.900	0.100	<0.0001
Left femoral neck (g/cm^2)	1.098	0.157	0.034	0.886	1.471	0.889	0.139	0.037	0.007	1.191	0.0004
Left femoral neck T-score	2.194	1.643	0.322	-0.500	5.610	0.243	1.338	0.358	-1.700	3.090	0.0008
Lumbar spine total (g/cm^2)	1.069	0.101	0.019	0.938	1.288	0.903	0.036	0.009	0.834	0.974	<0.0001
Lumbar spine T-score	0.157	0.888	0.174	-0.990	2.190	-1.383	0.373	0.099	-2.100	-0.670	<0.0001

3.9 Categorical between-group differences

Between-group differences were analysed using Fisher's exact probability for categorical items and measures. These results are described in the following sections.

3.9.1 Medication

The use of these medications by the subjects in Groups 1 and 2 are shown in Table 3.11. Only one (2.5%) subject in Group 1 used inhaled steroids, four (10%) used antidepressants and two (5%) used corticosteroids. The subjects in Group 2 did not use any of these medications. There were no significant differences in the use of these medications according to Fisher's exact probability. However, a significant difference was noted between the proportions of current users of contraception. Significantly more subjects in Group 2 were using contraception at the time of testing.

Table 3-11 Current medication use across groups

Substance	Group 1 proportion of using (N=26)	Group 1 proportion using (%)	Group 2 proportion using (N=14)	Group 2 proportion using (%)	Fisher's exact probability (p-value)
Inhaled steroids	1	2.5	0	0	1.000
Antidepressants	4	10	0	0	0.533
Corticosteroids	2	5	0	0	0.278
Contraception	10	38.5	13	92.9	0.002

3.9.2 Smoking

Two subjects each from Groups 1 and 2 admitted to smoking (see Table 3.12). Both smokers in Group 1 smoked 10 to 20 cigarettes a week. One subject from Group 2 smoked less than 10 cigarettes per day and one smoked more than two packs per week.

Table 3-12 Cigarette smoking across groups

Cigarettes smoked per week	Group 1 (N=26)	Group 1 (% of total sample)	Group 2 proportion (N=14)	Group 2 proportion (% of total sample)
0	24	60	12	30
<10	0	0	1	2.5
10-20	2	5	0	0
>2 packs	0	0	1	2.5

Fisher's exact probability for a comparison of the two groups is 0.222. There was therefore no significant difference in the number of cigarettes smoked by subjects in the two groups.

3.9.3 Calcium intake and physical exercise

Of the 22 subjects that stated they take in sufficient calcium, 15 (37.5%) came from Group 1 and seven (17.5%) from Group 2. These results are reflected in Table 3.13.

Table 3-13 Calcium intake across groups

	Group 1 proportion (N=26)	Group 1 proportion (%)	Group 2 proportion (N=14)	Group 2 proportion (%)
Intake sufficient	15	37.5	7	17.5
Intake insufficient	11	27.5	7	17.5

Fisher's exact probability for the two groups with regard to calcium intake is 0.744. This reflects that there is a relatively even distribution of sufficient and insufficient intakes across both groups.

The participation in physical activity is represented in Table 3.14. Fisher's exact probability for comparing the two groups on physical activity was 1.000. There was therefore no significant difference in the time subjects of Groups 1 and 2 spend on physical activity per week.

Table 3-14 Time spent in physical activity per week across groups

Hours of exercise per week	Group 1 proportion (N)	Group 1 proportion (%)	Group 2 proportion (N)	Group 2 proportion (%)
0-1 hour	10	25	5	12.5
1-2 hours	4	10	2	5
2-3 hours	4	10	2	5
3-4 hours	4	10	3	7.5
> 4 hours	4	10	2	5

The number of kilometres walked daily is shown in Table 3.15. Fisher's exact probability for the distance walked per day across the two groups is 0.779. This is not significant. Hence, it can be concluded that the difference in distance walked daily by subjects across the two groups was not significant.

Table 3-15 Distance walked per day across groups

Distance of walking per day	Group 1 proportion (N)	Group 1 proportion (%)	Group 2 proportion (N)	Group 2 proportion (%)
0 to 1 km	9	22.5	5	12.5
1 to 2 km	9	22.5	4	10
2 to 3 km	6	15	2	5
3 to 4 km	1	2.5	2	5
> 4 km	1	2.5	1	2.5

3.10 Continuous between-group differences

The mean, SD and SEM for DEXA readings and T-scores (total lumbar spine, left femoral neck and total left femur) and depression (on BDI and PGW) are summarised in Table 3.16. The two groups were compared across these variables using the Mann-Whitney T-test ($p \leq 0.05$).

3.10.1 Demographics

The Mann-Whitney test values indicate no significant differences between the groups on age (p = 0.954) or alcohol intake (p = 0.127). However, there was a significant difference between the groups on BMI (p = 0.032), with Group 1 exhibiting significantly higher BMI.

3.10.2 Depression

The comparison of the two groups on BDI revealed no significant difference (p = 0.249). Similarly, the PGW difference was not significant (p = 0.749)

Table 3-16 Significance of between group differences

Characteristics	Group 1 (N=26)					Group 2 (N=14)					p-value
	Mean	SD	SEM	Min	Max	Mean	SD	SEM	Min	Max	
Age	25.9	5.2	1.026	20	36	25.6	5.4	1.444	20	37	0.954
BMI	22.6	3.1	0.616	18.4	30.1	20.0	2.1	0.571	15.6	22.3	0.032
Alcohol intake	1.1	1.9	0.381	0	7	1.8	1.7	0.448	0	4	0.127
BDI	7.6	6.9	1.349	0	32	5.7	5.9	1.581	0	21	0.249
PGW depression	12.4	1.9	0.369	8	15	12.4	2.4	0.635	7	15	0.749

3.10.3 CORTISOL

The following model was used for the cortisol readings. It is described in detail in Chapter two:

$$CORT_{ij} = \beta_0 + \beta_1 \text{ x TIME} + b_i + \varepsilon_{ij}$$

The results for Group 1 and Group 2 on the model are noted in Table 3.17. The standard error is represented in parentheses.

Table 3-17 Summary of results of diurnal cortisol levels analysed using a multilevel model

	Group 1	Group 2
Fixed effects		
Intercept (β_o)	9.219 (0.659)	10.432 (0.968)
Overall slope	-1.016 (0.264)	-1.266 (0.378)
Variance components		
Var(b_i) = τ^2	0.272 (0.024)	0.184 (0.372)
Var (ε_{ij}) = σ^2	6.664 (1.318)	7.282 (3.143)
ICC	0.039	0.025

The slope is negative for both groups. The slope and intercepts of the two models are very similar, illustrating that similar readings are expected for both groups for the cortisol levels at the time of waking and the rate of change in the diurnal cortisol readings. The between-individual variance (Var(b_i) = τ^2) is very small relative to the Var (ε_{ij}) = σ^2, which represents the variance of the within-individual effects not accounted for by time. The small ICC for each group indicates that the use of a single cortisol for an individual in this study would have been unreliable.

3.11 Within-group correlations

The correlations between BMD, depression and cortisol are noted in Table 3.18. The Pearson correlation coefficient was calculated for the larger group (Group 1, N = 26) and the Spearman correlation was used for the smaller group (Group 2; N = 14). The levels of significance are noted below the correlation coefficient.

Significant correlations ($p \leq 0.05$) were found in Group 1 between:

- left femoral neck T-score and BMI (r = 0.388; p = 0.050)
- left femoral total BMD and BMI (r = 0.449; p = 0.021)

- left femoral total T-score and BMI (r = 0.455; p = 0.019)
- lumbar BMD and age (r = 0.533; p = 0.005)
- lumbar T-score and age (r = 0.479; p = 0.013)

These correlations in females with normal BMD are not very strong, however, and range from 0.388 to 0.455. What they do indicate though, is that in females with normal BMD,

- the higher the left femoral neck BMD , the higher the BMI and
- the older the female, the higher the lumbar BMD score.

Significant correlations ($p \leq 0.05$) were found in Group 2 between:

- left femoral neck BMD and the depression score on the PGW scale (r = 0.643; p = 0.013)
- left femoral neck T-score and the depression score on the PGW scale (r = 0.669; p = 0.009)

This indicates that, with regard to women that fall into the low BMD category, the left femoral neck BMD is higher in females with low depression. These are moderately strong correlations.

3.12 Regression

A regression model using BMD as the dependent variable, with depression scores and cortisol levels as the independent variables, was planned at the start of the research. However, for the following reasons, the regression was not attempted:

- Only six subjects exhibited BDI scores high enough to be categorised as anything other than minimal.
- Only one significant correlation was found between cortisol (N=38) and BMD (this was for the cortisol measure taken at 18:00 and the correlation was weak at 0.339).
- No significant correlation was found between BDI scores and BMD.

Table 3-18 Within-group correlations

Variable	Group 1 Left femoral neck (g/cm^2)	Group 2 Left femoral neck (g/cm^2)	Group 1 Left femoral neck T-score	Group 2 Left femoral neck T-score	Group 1 Left femoral total (g/cm^2)	Group 2 Left femoral total (g/cm^2)	Group 1 Left femoral total T-score	Group 2 Left femoral total T-score	Group 1 Lumbar spine (g/cm^2)	Group 2 Lumbar spine (g/cm^2)	Group 1 Lumbar spine T-score	Group 2 Lumbar spine T-score
Age	0.233 0.252	0.217 0.457	0.185 0.365	0.197 0.499	-0.099 0.631	-0.115 0.695	-0.123 0.549	-0.158 0.590	0.535 0.005*	0.074 0.801	0.479 0.013*	0.137 0.639
BMI	0.379 0.056	0.055 0.852	0.388 0.050*	0.009 0.976	0.449 0.021*	0.169 0.563	0.455 0.019*	0.152 0.603	0.308 0.125	0.416 0.139	0.369 0.064	0.222 0.445
BDI	-0.110 0.592	-0.416 0.139	-0.209 0.305	-0.450 0.106	0.023 0.909	-0.023 0.938	-0.052 0.799	-0.074 0.802	0.312 0.121	0.115 0.695	0.132 0.520	-0.012 0.968
PGW depression	-0.101 0.625	0.643 0.013*	-0.077 0.708	0.669 0.009*	-0.104 0.609	0.393 0.165	-0.893 0.665	0.438 0.117	-0.361 0.070	0.126 0.667	-0.329 0.101	0.239 0.410
Alcohol intake	-0.191 0.351	0.179 0.539	-0.164 0.422	0.105 0.719	-0.161 0.431	0.399 0.157	-0.135 0.510	0.338 0.237	-0.119 0.564	0.118 0.688	-0.095 0.644	-0.101 0.973
Walking	0.016 0.938	-0.178 0.543	0.046 0.822	-0.017 0.954	0.193 0.345	-0.399 0.158	0.204 0.315	-0.252 0.385	0.154 0.453	-0.322 0.262	0.212 0.299	-0.069 0.816
Exercise	-0.080 0.697	-0.906 0.758	-0.047 0.819	-0.139 0.634	0.279 0.167	0.125 0.671	0.291 0.149	0.105 0.722	0.155 0.449	-0.316 0.270	0.204 0.317	-0.488 0.077
Cortisol 07:00	-0.208 0.341	0.213 0.506	-0.192 0.381	0.221 0.491	0.283 0.191	0.375 0.229	0.278 0.199	0.362 0.248	0.078 0.723	0.424 0.169	0.0936 0.671	0.459 0.133
Cortisol 13:00	-0.149 0.495	0.507 0.111	0.220 0.312	0.506 0.112	-0.009 0.966	0.217 0.522	-0.064 0.772	0.215 0.526	-0.013 0.952	-0.028 0.935	-0.166 0.448	-0.017 0.961
Cortisol 18:00	-0.150 0.484	-0.091 0.779	-0.142 0.507	-0.091 0.779	0.256 0.227	0.0527 0.871	0.249 0.240	0.054 0.868	0.144 0.503	0.175 0.587	0.155 0.468	0.199 0.534
Cortisol Bedtime	-0.303 0.159	-0.376 0.255	-0.312 0.148	-0.369 0.264	0.105 0.633	0.009 0.977	0.083 0.708	0.006 0.985	0.104 0.636	-0.087 0.799	0.067 0.762	-0.054 0.875
Mean cortisol	-0.316 0.152	0.101 0.768	-0.327 0.137	0.107 0.755	0.221 0.322	0.289 0.388	0.196 0.383	0.280 0.404	-0.009 0.969	0.240 0.477	-0.056 0.804	0.277 0.409

* Indicates significant at $p \leq 0.05$

3.13 Discussion

The $H_0$1 assumes that there is no difference in the depression levels of females with low BMD and those with normal BMD, i.e. between Groups 1 and 2. The H1 therefore assumes that there is a difference. Given the debate in the literature, this study questioned whether or not women with low BMD exhibit higher levels of depression than women with normal BMD. The Mann-Whitney tests comparing BDI and PGW(depression) across the two groups reveal p-values of 0.249 for the BDI and 0.749 for the PGW. Neither of these p-values is significant. Therefore the $H_0$1 is accepted and it is concluded that there is no difference in the depression levels of females with low BMD and those with normal BMD.

The $H_0$2 put forward is that the salivary cortisol levels in females will not differ between those that present with low BMD and those with normal BMD (i.e. between Groups 1 and 2). The H2 is that the salivary cortisol levels in females are higher in those with low BMD than those with normal BMD. The population models for the low BMD and normal BMD groups revealed similar slopes and intercepts. This implies that the two groups showed similar cortisol readings. Therefore the $H_0$2 is accepted and it is concluded that there is no difference in the cortisol levels of females with low BMD and those with normal BMD.

The $H_0$3 is that cortisol and depression levels do not predict bone density. The H3 put forward is that BMD can be predicted through cortisol and depression levels. The regression analysis could not be conducted and therefore this hypothesis could not be tested in this study.

Chapter 4 : Study 2 – Bone mineral density (BMD) of premenopausal females with major depression

The results of study 1 revealed no clear general association between depression and bone mineral density (BMD) on DEXA. However, the sample in Study 1 showed low depression levels on the self-report measures employed. For this reason, it was decided that the possibility of and association between clinically significant depression and BMD should be explored.

Therefore, the aim of the second part of the study was to examine the association between depression and low bone density by using depression as a starting point. In this part of the study, patients known to be suffering from recurrent severe major depression (current or in full or partial remission) were compared with healthy controls in terms of their DEXA results, bone turnover markers and cortisol levels. A small exploratory component is added to the study and examines the pro-inflammatory status of the depressed patients. This represents a limited study of the theory that pro-inflammatory cytokines will mediate BMD loss in depressed patients.

Finding patients with no comorbid conditions (psychiatric or medical) proved exceptionally difficult. Only nine volunteers were found for the study and Study 2 therefore represents a pilot study into the association between BMD and severe major depression.

The hypotheses put forward for this part of the study are:

- $H_0$4: there is no difference in the BMD levels of females with and without depression.
- H4: women with depression exhibit lower BMD than women without depression.
- $H_0$5: the salivary cortisol levels in females do not differ between those that present with depression and those without depression.

- H5: the salivary cortisol levels are higher in females with depression than in those without depression.
- $H_0$6: the levels of pro-inflammatory cytokines are within normative range in women with depression
- H6: the levels of pro-inflammatory cytokines are above the normative range in women with depression

4.1 Demographic characteristics

Originally, Study 2 was to make use of a large sample of volunteers. However, difficulty in obtaining willing participants as well as time and financial constraints curtailed this possibility. Nine subjects were identified by the Department of Psychiatry at the University of Pretoria for the second part of the study. The severely depressed patients (Group 1) were volunteers from a psychiatric clinic. Controls (Group 2) were chosen that did not suffer from depression. Two subjects from site 1 and two from site 3 consented to be tested as controls.

The demographics of the two groups are summarised in Table 4.1. Although the means for all variables are given, with such a small sample it is more useful to discuss the medians. The age medians differed greatly, with Group 2 (N = 4) exhibiting a much younger median (23 years; range = 10 years) than Group 1 (N = 5), where the median age was 28 years (range = 6 years). The BMI medians also differed widely, with Group 1 showing a much higher median (24.7; range = 9.6) than Group 2 (BMI median = 21.4; range = 4.6). Group 1's median BMI also lies close to the maximum value for healthy BMI (i.e. 25). The subjects of both groups take in similar amounts of alcohol per week (median for Group 1 = 2 units; range = 11; median for Group 2 = 1.5 units; range = 2).

Table 4-1 Demographics of the two groups

Variable	Mean	Median	Minimum	Maximum	Range	Interquartile range
Age: Group 1 (years) N=5	26.6	28	23	29	6	3
Age: Group 2 (years) N=4	25.5	23	23	33	10	5
BMI: Group 1 N=5	25.6	24.7	21.7	31.2	9.6	1.4
BMI: Group 2 N=4	21.9	21.4	20.2	24.8	4.6	2.9
Alcohol Intake (units): Group 1 N=5	3.6	2	0	11	11	3
Alcohol Intake (units): Group 2 N=4	2.5	1.5	1	3	2	5

4.2 Psychological and medical history

By definition, all the subjects in Group 1 have been diagnosed with depression. Interestingly, though, when asked if they had ever been diagnosed with a psychological disorder, three subjects answered “No”.

Of the five subjects that suffer from depression, two were undergoing psychotherapy and four were using antidepressants. None of the subjects reported suffering from any serious medical illnesses. None of the subjects used inhaled steroids, corticosteroids, anticonvulsants, calcitonin, thyroid hormone, alendronate, cyclosporin, anabolic steroids or bisphosphonates. Three subjects were utilising contraception (one from Group 1 and the other two from Group 2).

4.3 Calcium intake and physical exercise

Group 1 was relatively evenly spread across the exercise and walking spectrum with two subjects spending an hour or less on exercise and two walking a kilometre or less a day (Tables 4.2 and 4.3). In Group 2, one subject spent up to an hour on exercise per week and one walked up to a

kilometre a day. In addition, the calcium intake for both groups differs, with three subjects from Group 2 stating that they take in enough calcium on a daily basis, but only one subject in Group 1 reporting adequate calcium intake.

Table 4-2 Time spent in physical activity per week across groups

Hours of exercise per week	0-1 hour	1-2 hours	2-3 hours	3-4 hours	> 4
Proportion of Group 1 (N = 5)	2	0	2	1	0
Proportion of Group 2 (N = 4)	1	1	0	0	2

Table 4-3 Distance walked per day across groups

Distance of walking per day	0 to 1 km	1 to 2 km	2 to 3 km	3 to 4 km	> 4 km
Proportion of Group 1 (N=5)	2	1	1	1	0
Proportion of Group 2 (N-4)	1	2	1	0	0

4.4 DEXA

The mean DEXA readings and T-scores for the total lumbar spine, left femoral neck and total left femur and are recorded in Tables 4.4 to 4.6. The median lumbar spine BMD on DEXA was within normal limits for both groups (T-score > -1). The T-scores for both groups also fell in the normal range, although Group 2 exhibits a slightly higher median than Group 1. Group 2 also exhibited greater variability amongst subjects with regard to lumbar BMD, with both the ranges and the interquartile ranges greater in Group 2.

Table 4-4 Mean lumbar bone mineral density (BMD) on DEXA across groups

Variable	Mean	Median	Minimum	Maximum	Range	Interquartile range
Lumbar spine Group 1 (g/cm^2) N=5	1.034	1.038	1.004	1.054	0.050	0.019
T-score Group 1 (N=5)	-0.120	-0.080	-0.400	0.070	0.470	0.170
Lumbar spine Group 2 (g/cm^2) N=4	1.057	1.065	0.899	1.197	0.298	0.242
Lumbar spine T-score Group 2 (N=4)	0.095	0.160	-1.300	1.360	2.660	2.170

For the femoral neck (Table 4.5), again the Group 2 medians were higher than those of Group 1, but both groups' readings still remained in the normal range. Again, Group 2 exhibits greater variability amongst its members than Group 1 does. This is seen on the ranges and interquartile ranges for the BMD score and the T-score.

Table 4-5 Mean femoral neck bone mineral density (BMD) on DEXA across groups

Variable	Mean	Median	Minimum	Maximum	Range	Interquartile range
Left femoral neck Group 1 (g/cm^2) N=5	1.046	0.973	0.903	1.283	0.380	0.141
T-score Group 1 (N=5)	1.774	1.120	0.490	3.910	3.42	1.270
Left femoral neck Group 2 (g/cm^2) N=4	1.100	1.015	0.899	1.471	0.572	0.332
T-score Group 2 (N=4)	2.280	1.505	0.5	5.61	5.11	2.960

On total femur BMD, Group 2 once again reflected higher medians for the BMD score and the T-score. Although Group 2 also showed greater variability than Group 1 in the BMD scores, it was Group 1 that had a higher T-score range.

Table 4-6 Mean total femur bone mineral density (BMD) on DEXA across groups

Variable	Mean	Median	Minimum	Maximum	Range	Interquartile range
Left femoral total Group 1 (g/cm^2) N=5	0.914	0.923	0.801	1.035	0.112	0.125
T-score Group 1 (N=5)	-0.226	-0.150	-1.150	0.770	1.9200	1.020
Left femoral total Group 2 (g/cm^2) N=4	1.046	1.043	0.955	1.144	0.189	0.143
T-score Group 2 (N=4)	0.860	0.820	0.100	1.700	1.600	1.200

4.5 Beck Depression Index (BDI) and Psychological General Well-being Schedule (PGW) results

The results of the BDI and PGW are recorded in Table 4.7. Group 1 exhibited a higher median than Group 2 on the BDI (27 vs. 5). While the median for Group 2 fell within the minimal category (range = 2.5), Group 1's median fell within the moderate category, with a range of 44. This points to a fairly heterogeneous group with regards to depression scores. The PGW (depression) score for Group 1 was borderline, while Group 2's PGW median is definitely an indication that the subjects in Group 2 are seldom depressed.

Table 4-7 Scores on Beck Depression Index (BDI) and Psychological General Well-being Schedule (PGW) for the two groups

Variable	N	Mean	Median	Minimum	Maximum	Range	Interquartile range
BDI Group 1	5	29.6	27	14	58	44	13
BDI Group 2	4	4.8	5	3	6	3	2.5
PGW (depression) Group 1	5	6.8	8	0	13	13	7
PGW (depression) Group 2	4	12.5	13	10	14	4	2

4.6 Cortisol

The sample was too small to justify the use of the model described in Chapter three. Therefore, only the mean of the cortisol level was calculated per group for the four readings and for the day. The means, medians and ranges are supplied in Table 4.8. For all the times of measurement, the median reading for cortisol is higher in Group 1. Group 2, however, shows greater variability in its readings.

Both groups reflected normal median values for the morning. The median of cortisol readings over 24 hours were also average (median for Group 1 = 8.344; median for Group 2 = 6.45). However, both groups reflected elevated medians for 18:00 (median for Group 1 = 6.600; median for Group 2 = 4.500) and bedtime (median for Group 1 = 6.500; median for Group 2 = 5.250).

Table 4-8 Cortisol levels (ng/ml) for the two groups

	Mean	Median	Minimum	Maximum	Range	Interquartile range
07:00						
Group 1 (N=4)	10.038	10.200	9.250	10.500	1.250	7.925
Group 2 (N=3)	11.133	8.400	7.500	17.500	10.000	10.000
13:00						
Group 1 (N=5)	8.520	9.000	5.600	11.250	5.650	1.75
Group 2 (N=3)	7.717	7.900	5.750	9.500	3.750	3.750
18:00						
Group 1 (N=5)	6.630	6.600	4.750	8.500	3.750	1.700
Group 2 (N=3)	5.200	4.500	3.600	7.500	3.900	3.900
Bedtime						
Group 1 (N=5)	6.790	6.500	6.000	7.750	1.750	1.300
Group 2 (N=3)	6.750	5.250	5.000	10.000	5.000	5.000
Mean cortisol over 24 hours						
Group 1 (N = 5)	8.216	8.344	7.300	8.875	1.575	1.131
Group 2 (N = 3)	7.700	6.450	5.525	11.125	5.600	5.600

4.7 Markers of bone turnover

The information pertaining to marker scores is found in Table 4.9. The median values for Group 1 were higher than those of Group 2 for all parameters. This points to a trend of higher bone remodelling (both formation and resorption) in depressed patients when compared with non-depressed subjects. Group 1 exhibited elevated median DPD:Cr (9.000) and BSAP (16.100) values. DPD:Cr, is a marker of resorption, which indicates that Group 1 has abnormally high rates of bone resorption. BSAP is an indicator of bone formation. This shows that the rate of bone formation is also elevated in Group 1.

Table 4-9 Markers of bone turnover across groups

Marker	Reference range	Mean	Median	Min	Max	Range	Interquartile range
BSAP (µg/l) Group 1 (N=3)	*≤ 14.3*	12.833	16.100	5.300	17.100	11.8	11.8
BSAP (µg/l) Group 2 (N=4)		9.625	10.300	6.800	11.100	5.700	2.850
DPD:Cr Group 1 (N=5)	*3-7.4*	8.880	9.000	5.900	12.300	6.400	1.000
DPD:Cr Group 2 (N=3)		7.933	7.100	6.700	10.00	3.300	3.300
Osteocalcin (ng/ml) Group 1 (N=5)	*3.7-10*	6.440	7.000	4.100	7.500	3.400	0.800
Osteocalcin (ng/ml) Group 2 (N=4)		5.875	4.850	2.800	11.000	8.200	4.250
β-CrossLaps (ng/ml) Group 1 (N=5)	*0.025-0.573*	0.477	0.501	0.396	0.500	0.104	0.091
β-CrossLaps (ng/ml) Group 2 (N=4)		0.425	0.397	0.251	0.655	0.404	0.212

4.8 Cytokines

The results of cytokine testing are recorded in Table 4.10. Group 1's median IL-1β reading (14.669pg/ml) is well above the normative value of 4.721pg/ml. This indicates very high levels of this pro-inflammatory cytokine. There is also a great deal of variability between subjects (range and interquartile range = 11.374 and 7.982, respectively). However, the group's median TNFα reading is within the normative range (1.333).

Table 4-10 Cytokine levels for Group 1 (N=5)

Cytokine	Reference	Mean	Median	Minimum	Maximum	Range	Interquartile range
IL-1β (pg/ml)	*Mean = 4.721pg/ml*	12.258	14.669	6.128	17.502	11.374	7.982
TNFα (pg/ml)	*Mean < 7.8pg/ml*	2.906	1.333	0.197	7.379	7.182	4.317

4.9 Meta-analysis results

The outcomes of the steps leading to and including the $w_i t_i$ values are noted in Tables 4.11 to 4.15.

Table 4-11 Depression on HAMD for meta-analysis

Study	Variance	Rate difference (t_i)	Weight (w_i)	$w_i t_i$
2	Data missing	Data missing	Data missing	Data missing
3	1.38074	11.9501	0.72425	8.6548
5	Data missing	Data missing	Data missing	Data missing
7	0.90620	12.9111	1.10351	14.2476
8	1.07948	14.4513	0.92637	13.3873

Table 4-12 Lumbar DEXA scores (g/cm^2) for meta-analysis

Study	Variance	Rate difference (t_i)	Weight (w_i)	$w_i t_i$
2	0.036050	-3.60607	27.7390	-100.029
7	0.027499	0.07273	36.3651	2.645
8	0.075359	0.83599	13.2697	11.093

Table 4-13 Femoral neck DEXA scores (g/cm^2) for meta-analysis

Study	Variance	Rate difference (t_i)	Weight (w_i)	$w_i t_i$
2	0.037819	-2.40641	26.4440	-63.6352
7	0.025546	-0.50888	39.1444	-19.9197
8	0.095848	1.23111	10.4332	12.8444

Table 4-14 Serum cortisol scores (µg/dl) for meta-analysis

Study	Variance	Rate difference (t_i)	Weight (w_i)	$w_i t_i$
2	2.27707	0.17566	0.43916	0.07714
3	3.54455	3.3572	0.28212	0.94716
7	1.19174	-0.38599	0.83911	-0.32389

Table 4-15 Osteocalcin scores (ng/ml) for meta-analysis

Study	Variance	Rate difference (t_i)	Weight (w_i)	$w_i t_i$
2	1.10737	-0.27091	0.90304	-0.24464
3	3.82903	-2.03705	0.26116	-0.53199
5	1.70464	3.22649	0.58663	1.89277
7	1.29881	1.74776	0.76994	1.34566
8	2.08666	0.43131	0.47924	0.20670

The final results of the analysis are noted in Table 4.16. Significant values ($p \leq 0.05$) are indicated with *. The HAMD is significantly different across depressed and non-depressed groups in the identified studies ($p < 0.001$). This indicates that the depressed and control groups did evince a significant difference in terms of depression scores. The lumbar BMD also differed significantly across depressed and non-depressed groups ($p < 0.0001$). However, no significant differences were found across groups for the femoral neck BMD, serum cortisol or osteocalcin.

Table 4-16 Final results of meta-analysis

Variable	N	$\sum w_i t_i$	$\sum w_i$	$\mp = \sum \frac{w_i t_i}{w_i}$	z-score	p-value
HAMD	304	36.2897	2.75413	13.1765	21.8671	<0.001*
Lumbar BMD	266	-86.2905	77.3738	-1.11524	9.80993	<0.0001*
Femoral neck BMD	266	-70.7105	76.0216	-0.93014	8.10990	5.5511
Serum cortisol	201	0.70042	1.56039	0.44887	0.56071	0.57499
Osteocalcin	304	2.66849	3.00001	0.88650	1.54065	0.12340

4.10 Discussion

The $H_0$4 is that there is no difference in the BMD levels of females with and without depression. The H4 is that women with depression exhibit lower BMD than women without depression. There appears to be a trend in the data: higher BMD scores and T-scores were reflected in Group 2, the non-depressed subjects. However, there were also differences is the groups relating to age and BMI, both of which were higher in Group 1. Therefore, age and BMI may theoretically have had an influence on the DEXA results.

The $H_0$5 is stated as: the salivary cortisol levels in females will not differ between those that present with depression and those without depression. The H5 is that the salivary cortisol levels are higher in females with depression than in those without. The trend in the data appears to be that cortisol levels across all four times and the 24 hour mean were higher in Group 1 (the depressed subjects).

The final hypothesis of the study is based on a small, exploratory section of the research. The $H_0$6 is that the levels of pro-inflammatory cytokines are within normative range in women with depression. The H6 proposed that the levels of pro-inflammatory cytokines are above the normative range in women with depression. The cytokine results varied greatly, with the IL-1β median lying higher than the normative range, while the TNFα median was within normative range. It is therefore not possible to conclude that the levels of pro-inflammatory cytokines were higher than the normative range in depressed women. However, the high degree of variability in the subjects' results suggests that further exploration is required.

The meta-analysis results indicate that existing data confirm a relationship between depression and loss of bone density in terms of DEXA lumbar BMD scores. However, no association was found between depression and femoral neck BMD values ($p = 5.5511$) or on depression and osteocalcin values ($p = 0.12340$). Moreover, the evidence did not suggest a mediating role for cortisol ($p= 0.575$). This analysis is however not sufficient to reject the possibility of an

association between BMD loss and depression via cortisol dysregulation. More studies are required to test the hypothesis further. In particular, intervention studies would contribute greatly to clarity in this arena. Future research should therefore include studies of larger samples, should include quality of life measures and should be longitudinal.

Chapter 5: Integrated discussion

Both osteoporosis and depression feature prominently in world epidemiological studies as growing threats to human health. While South Africa's contingent of osteoporosis sufferers is comparable to international prevalence, high levels of crime, violence and other stresses are contributing to rising depression in the country (1, 2, 3, 4).

This study was designed on the premise that the disruption of the HPA axis that accompanies depression has the potential to perturb bone remodelling in favour of resorption, leading to the loss of BMD that is most frequently associated with osteoporosis. The accumulation of evidence indicating that depression is associated with illnesses such as cancer, auto-immunity and a host of inflammatory disorders (including osteoporosis) points strongly to the involvement of immune changes in depression (5, 6, 7). A very likely starting point for these immune changes is the HPA axis, which is generally overactive in depressed patients, causing a hypersecretion of cortisol (8). It is now generally accepted that the nervous system can exert an influence on the immune system, but the mechanisms and consequences of this influence are complex (9). The HPA axis integrates stimuli that relate to stress. It is activated when the person experiences exhaustion, loss of control or a perceived loss of control (10). Stress-induced activation of the HPA axis elicits responses from the neuroendocrine, peripheral nervous and immunological systems. The central effector of these responses in humans is cortisol. Cortisol can act directly on bone, causing resorption by suppressing type I collagen production, up-regulating osteoclastogenesis and even decreasing the osteoblast population (11, 12, 13). Furthermore, stress causes the secretion of pro-inflammatory cytokines IL-1β, IL-6 and TNFα (and an upregulation of their receptors). These pro-inflammatory cytokines have been shown to increase osteoclast maturation and tilt the balance of remodelling towards uncontrolled inflammation and loss of BMD. In a healthy system, the high cortisol levels, in turn, cause the decreased secretion of these cytokines via negative feedback. However, a general inflammatory state and hypercortisolism have been noted in depressed patients (14).

From the 1980's, researchers have noted that depression was often an outcome of osteoporosis (15). Depression has since emerged as a potential risk factor and not only a consequence for osteoporosis in premenopausal women. A number of researchers (e.g. 17, 18, 19) have presented evidence for the occurrence of depression-induced osteoporosis in various communities, including premenopausal women. The main limitation of most of the studies is that they are cross-sectional and therefore cannot be used to infer causality. In addition, most sample sizes are small and their statistical power therefore limited. These studies have, furthermore, been challenged by results which indicate no association between depression and BMD loss (20, 21).

Unfortunately, the link between depression and BMD loss has not yet been explored in the South African context. The aim of this research was therefore to investigate the potential association between depression and low bone density in South Africa. The challenge of fulfilling this aim was approached via two avenues: the first avenue of the research (Study 1) examined the association of BMD, cortisol and depression in a random sample of premenopausal women. The second avenue of the research (Study 2) involved the exploration of these variables in a group of psychiatric patients diagnosed with severe recurrent major depression in contrast to healthy controls.

5.1 Study 1

Study 1 began with the investigation of BMD in a volunteer-based sample of 40 premenopausal women drawn from three different sources. All volunteers underwent a DEXA scan, filled in a questionnaire and were requested to supply saliva samples for analysis. Before analysis of the depression scores began, prior diagnosis of depression was to be ruled out as a confounding variable. Too few subjects reported a prior diagnosis of depression for statistical analysis to be significant.

5.1.1 What Study 1 reveals about depression and bone mineral density (BMD)

Study 1 revealed no association between depression and bone density as reflected by DEXA scores. It was, however, necessary to determine if there was any difference between women with low BMD and those with normal BMD. The sample was therefore split according to DEXA results. The women that displayed normal DEXA readings at all three anatomical sites (femoral neck, total femur and lumbar spine) fell into the first group. The women with T-scores below -1.5 (i.e. osteopenic or osteoporotic) formed the second group. The sample was highly skewed towards normal BMD and therefore the two groups were unevenly split, with a very small contingent of subjects falling into the low BMD group.

The groups differed significantly on BMI and the use of contraception, but were found to be similar with respect to the use of other medications, smoking, calcium intake, exercise, age and alcohol use. More importantly, the groups were similar with regard to depression and cortisol levels. Therefore, the results again did not illustrate any correlation between depression and BMD. However, the possible role played by BMI and contraception use in influencing the BMD in depressed patients should be investigated further.

Despite there not being enough evidence to confirm the link between depression and bone density in Study 1, some within-group correlations yielded intriguing results. It was illustrated that, in subjects with low BMD, the left femoral neck BMD was negatively correlated to the depression score (i.e., the more severe the depression, the lower the left femoral neck BMD), but only if depression was measured on the PGW (depression) subscale and not on the BDI. While it is tempting to state that severe depression is related to BMD loss at the left femoral neck, it must be emphasised that this sample evinced only borderline PGW (depression) scores. This contrasts with Coelho, *et al.*'s (16) findings, which showed that depression was significantly associated with low BMD when the BDI was used as a measure of depression, but that there was no association when the PGW score was used. The question of how self-report measures influence reporting of symptoms is

beyond the scope of this study, but is perhaps a viable reason for the disparate associations across studies. It is possible that the measure used to determine depression influences the outcomes. Accordingly, issues such as validity, reliability and even transparency of tests cannot be ignored. The diagnosis of psychopathology is not a straightforward exercise, and the diagnosis of depression in particular is complicated by the instability of depressive disorders and the occurrence of sub-clinical syndromes. Depression is commonly diagnosed in categories (such as the DSM-IV) or according to theoretical frameworks (e.g. 22). Authors such as Angst, Sellaro and Merikangas (23) describe depression along a continuum, while researchers such as Van Praag, De Kloet and Van Os (24) use a syndromal approach. Each of these modes of diagnosis has its strengths and weaknesses.

Creed (25) argues strongly for less variation in the literature on depression and physical illness. Creed presents six main problems that characterise depression research. These are listed in italics along with the strategies introduced or the limitations of the current research in contending with these challenges.

- *There is a lack of clear definition of depression in research involving physical illness. Propagators of Endicott's criteria for depression in clinical practice deem questions related to somatic symptoms in depression (fatigue, sleep disturbance, weight changes and decreased concentration) to be too closely related to symptoms of physical illness. Endicott's criteria therefore substitute questions related to these symptoms for the four criteria social withdrawal/not talking, brooding/pessimism, non-reactive mood and tearful/depressed appearance*. Potential participants that suffered from physical illness were omitted from the study. Although multivariate regression could have been used to eliminate the effect of fatigue, sleep, weight and concentration on results, the small sample discouraged such technique.

- *The validity of using certain assessments in the physically ill has not been clearly established.* Two widely-utilised and extensively researched instruments were used for the study. The instruments' validities are discussed in Chapter two.
- Different thresholds are used for depression. For example, the BDI categorizes scores according to severity while the PGW only indicates whether or not a person is considered depressed, without indicating the level of depression. *Analyses were conducted using the continuous scores only to provide more clarity.*
- *Selection bias exists for certain treatment centres.* Unfortunately, only one psychiatric unit could be accessed to assist in the study.
- *Sociodemographic factors are heterogeneous.* Given the limited response from volunteers, subjects were not screened for sociodemographic variables prior to entrance to the study.
- *Appropriate controls are omitted.* As the sampling method was random, the pre-selection of controls was not part of the method.

An added difficulty within the South African context is the lack of normative data for tests of psychopathology (26).

5.1.2 Other findings of Study 1

The lumbar DEXA score in subjects with low BMD was positively correlated to age. Given the relatively young age of the sample (range = 20 to 37 years), this is not surprising. The attainment of peak bone mass only occurs in late adolescence and early adulthood and the loss of BMD is only appreciable after the age of 50 years (27, 28) when the risk of osteoporosis increases (29).

Furthermore, the left femoral total T-score in subjects with low BMD was positively correlated with BMI. It is known that extremes of BMI can be detrimental to BMD (30, 31). On the one hand, very high BMI is linked to obesity and the deposition of fat in bone tissue. This fatty bone does not have the same integrity as healthy bone and is prone to fracture (32). On the other hand, very low BMI is linked to osteoporosis, especially in young girls

suffering from anorexia (27, 33). However, normal BMI contributes to BMD when lean mass is taken into consideration (30, 31, 32).

Other interesting findings of Study 1 related to cortisol. There was no association between cortisol and BMD. However, the cortisol levels for both groups in Study 1 were elevated at 18:00 and bedtime. A flattened cortisol curve may therefore be characteristic of this sample. In addition, no evidence could be found for an association between cortisol and depression scores in this study. This result may imply that the level of depression was not high enough to illustrate an effect. Alternatively, the type and stage of depression may have played a role.

Although Study 1 offered only an indication of an association between depression and BMD of the femur neck in depressed subjects, the depression levels of the subjects were not particularly high. It was postulated therefore that the degree of depression in this sample was not high enough for a clear relationship to be seen. The possibility of the severity of depression being important in BMD loss was investigated in Study 2.

5.2 Study 2

Study 2 examined the BMD of five psychiatric patients diagnosed with severe, recurrent major depression and four healthy controls. These volunteers were also required to undergo a DEXA scan, to complete a questionnaire and supply saliva samples. In addition, blood and urine samples were taken to examine the status of bone turnover markers in these nine subjects. The pro-inflammatory status of the depressed patients was investigated and compared to reference ranges. The study was limited by great difficulties in obtaining volunteers with a single diagnosis of severe recurrent major depression. Extensive comorbid psychiatric and medical diagnoses were noted throughout the patient population of the psychiatric clinic that agreed to assist in the study. With limited time and funding, it was considered pragmatic to complete Study 2 with the five psychiatric patients that volunteered.

5.2.1 What Study 2 reveals about depression and bone mineral density (BMD)

Study 2 specified a trend of association between depression and low BMD on DEXA. Study 2's results also denoted a tendency of association between depression, bone density (on DEXA) and bone turnover (on formation and resorption markers) and cortisol. Study 2 indicated that women diagnosed with severe major depression evince lower BMD (on DEXA) and increased overall bone turnover (as measured by markers of bone turnover) and higher cortisol levels than their healthy cohorts. However, the meta-analysis results are less clear: the meta-analysis revealed that depression is associated with low lumbar BMD, but not low femoral BMD. In addition, the meta-analysis does not show association between depression and bone turnover or depression and cortisol levels. The pattern of increased bone turnover with coincident loss of BMD seen in Study 2 has been noted in, for example, postmenopausal women with breast cancer who have been treated with aromatase inhibitors (34). However, the mechanism behind the bone loss in those cancer patients was oestrogen-dependent and factors such as cortisol and cytokines were not assessed.

5.2.2 Other findings of Study 2

Study 2 indicated variability in the cytokine profiles of depressed patients. Depression has long been associated with a pro-inflammatory immune response. Specifically, a higher level of pro-inflammatory cytokines and acute phase proteins have been identified in depressed patients (14). Bone remodelling has also been found to be linked to inflammation and is even considered to be a controlled inflammatory reaction (35). The involvement of the TNF superfamily and the discovery of their role in modulating bone remodelling has leant credence to this conceptualisation. A host of pro-inflammatory cytokines, including the two examined in this study (IL-1β and TNFα) increase the expressions of OPG and RANKL, thereby enhancing osteoclast maturation and, ultimately, advancing bone resorption (13, 36, 37). The implication is therefore that osteoporosis may be an immune mediated disorder.

Eskandari, *et al.* (38) have shown that women with major depressive disorder exhibit increased levels of 24-hour pro-inflammatory cytokines, but decreased levels of anti-inflammatory cytokines. These increased pro-inflammatory cytokines were also associated with low BMD. Financial constraints precluded comparison of the depressed patients with the controls on two measures of pro-inflammatory cytokines (IL-1β and TNFα). However, Study 2 did indicate a trend similar to that exhibited in Eskandari, *et al.*'s (38) research. The small group of patients diagnosed with severe recurrent major depressive disorder showed evinced levels of IL-1β that were higher than normative data. TNFα levels, though, were not elevated above the normative range. One possible reason for this result is propagated by Marques-Deak, *et al*. (14) who theorise that cytokine profiles in depressed subjects may differ as a result of the depression subtype (e.g. severity, chronicity, recurrence and the presence of melancholic symptoms). It is possible that the heterogeneity in the cytokine results in the current study reflects a degree of depressive subtyping, but such a debate should be entered into with great caution, given the small sample size. On the other hand, the elevated IL-1β and normal TNFα readings are consistent with the findings of Simon *et al.* (39) who reported a generalised pro-inflammatory response in depressed patients, with the exception of the TNFα levels.

5.3 Overall findings

While Study 2 indicates a trend of association between depression, BMD on DEXA and cortisol, Study 1 offers no substantial evidence for such a link. So, although depression and cortisol levels could not be used in a regression model to predict BMD, the severity of depression does emerge from this research as a potential factor for explaining the marked differences in Study 1 and Study 2's results. Once more, however, the role of confounding variables such as age, contraception use and BMI cannot be ignored.

Although it is clear that Study 1 and Study 2 differed greatly in their outcomes regarding the association between depression and BMD, in both Study 1 and Study 2, the evening and bedtime cortisol readings were elevated. The factors

responsible for the elevated cortisol was not clear from the results of this study and could not summarily be attributed to depression: even though the group suffering from major depression exhibited higher cortisol levels than their healthy counterparts, all groups showed evidence of elevated cortisol at night.

Although a negative correlation was found between bedtime cortisol levels and left femoral neck T-scores in Study 1, the correlation was weak. This is consistent with Reynolds, *et al.*'s (40) report that elevated peak cortisol levels are associated with accelerated BMD loss in the femoral neck for women.

5.4 Limitations and recommendations for further studies

- This study has limited statistical power because of the small sample size. This is unfortunately a trend of the research on this topic. The greatest barriers to a larger sample were financial and time constraints. This study has value, however, in that it can serve as the basis for a larger study or can be included in future meta-analyses on depression and bone density. This data should be supplemented with longitudinal studies and larger samples that are more representative of the population to resolve the association of depression with BMD.

- One of the greatest disadvantages of this study is that it is a cross-sectional study without time-series measurements. This essentially means that causality cannot be inferred and the results may only be used to imply an association. The use of follow-up measures, such as those employed by Schweiger, Weber, Deuschle and Heuser (19) should be considered.

- Subjects did not always return the questionnaires or saliva in a timely fashion. This means that a number of added variables could not be controlled for. Time of measurement effects need to be considered a limiting factor in this research. Hence, future studies should refine the

sequence and timing of testing and a shorter period should be allocated for evaluations to be completed.

- Although the study tried to deal with the issue of subtypes of depression by specifying the inclusion of only severe recurrent major depressive patients in Study 2, better screening of patients is recommended. Most subjects were recruited on the basis of a single psychiatrist or registrar's opinion and no indication was given of the possible existence of specifiers, such as melancholic or atypical features. Given the findings that subtypes of depression present with different cytokine and cortisol profiles (14, 39), it is logical to try and ensure that groups are as homogeneous as possible.

- Furthermore, research should concentrate on whether or not bone loss occurs only during a depressive episode or if a history of depression is enough to begin the negative effects. This study could not explore any influence of prior diagnosis of depression, since there were only four subjects with such a diagnosis. The effects of long-term low-level depression were not analysed in this research and may also offer an opportunity for depression-induced osteoporosis if the depression is chronic or recurrent.

- Although this research focuses on the detrimental effects of depression, some researchers have pointed out that the treatment of depression may be as deleterious as the disorder itself. Recent evidence suggests that the use of selective serotonin reuptake inhibitors in the treatment of depression may in fact contribute to increased bone resorption in both the elderly and growing children (41, 42). This research did not investigate the correlation of antidepressant use and BMD. It is recommended that future studies not only study the effects of depression on BMD, but also examine the effects of the treatment of depression on BMD.

- Finally, large community-based studies such as the NHANES III, Geelong and Mr Os studies (43, 44, 45) have shown an association between depression and BMD in men. Future South African studies should also include male subjects to allow for international comparison.

5.5 Conclusions

This research confirms a trend of association between depression and low BMD, but only when the depression is a severe major depression. A possible increased overall bone turnover is also noted in subjects with severe major depression and may provide clues as to the mechanism of depression-induced bone loss, which is also associated with increased cortisol and IL-1β in these patients. Low levels of depression do not appear to influence BMD. However, age, BMI and contraception use presented as confounding variables in the research and therefore need to be investigated further.

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Appendix A

DEPRESSION AND BONE MINERAL DENSITY
PATIENT INFORMATION LEAFLET AND INFORMED CONSENT

I am a Master's student in Human Physiology at the Department of Physiology, University of Pretoria. You are invited to volunteer to participate in my research project on depression and bone density. This information leaflet is to help you to decide if you would like to participate in the study. **PLEASE READ AND UNDERSTAND THIS DOCUMENT BEFORE THE START OF THE STUDY.**

Before you agree to take part in this study you should fully understand what is involved. If you have any questions, do not hesitate to ask me. You should not agree to take part unless you are completely happy about what is expected of you. In the best interests of your health, it is strongly recommended that you discuss with or inform your personal doctor of your possible participation in this study, wherever possible.

WHAT WILL THE RESEARCH EXAMINE?

The purpose of the study is to establish whether a link exists between depression and low bone density. This will involve the following:

- You may have participated in a bone density scan recently. I would like your permission to obtain your results from the hospital and analyze them. If you have not participated in a scan in the last year, you will be required to undergo a DEXA scan to assess your bone density.
- You will be required to complete a set of questionnaires. The completion of the questionnaires may take about 40 minutes. The questionnaires will be collected from you before you leave. They will be kept in a safe place to ensure confidentiality. I will be available to help you to fill in the questionnaires or to fill them in on your behalf, should it be necessary.
- I will also provide you with 4 little containers in which you will have to collect your saliva in a particular way over a day (please read the document entitled "**SALIVA COLLECTION:** DEPRESSION AND BONE MINERAL DENSITY").
- Depending on your results, you may be requested to donate 40ml of blood and 50ml of urine. These collections will be done by the staff at Du Buisson, Bruinette and Kramer Pathologists in Pretorius Street, Pretoria. Alternatively, Dr AME (Ilse) Du Plessis (PR. NO. 1578324) will be on site to draw the blood.

PLEASE NOTE THAT NO FORM OF TREATMENT WILL BE OFFERED DURING THIS RESEARCH.

WHO WILL HAVE ACCESS TO MY INFORMATION?

The implication of completing the questionnaire is that informed consent has been obtained from you. Data that may be reported in my dissertation and scientific forums (such as journals) will not include any information that identifies you as a participant in this study. All information obtained during the course of this study is strictly confidential. Your doctor will, however, have access to your results. He or she will therefore be able to assist you in making informed decisions about serious problems picked up by your test results.

Your participation in this study is voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will involve no penalty or loss of benefits, but as data is anonymous, you must understand that you will not be able to recall your consent, as your information will not be traceable.

WHAT IS THE DURATION OF THIS RESEARCH?

The study will last for up to 1 week per patient (in total). You will visit the investigator to:

- Discuss the research and to complete the informed consent form.
- Complete the questionnaires.
- Have blood drawn and to provide a urine sample.
- You will also be given saliva collection vials, which are to be returned to the investigator after the last collection. Please see the document entitled: "**SALIVA COLLECTION:** DEPRESSION AND BONE MINERAL DENSITY"

It is important that you let the investigator know of any medicines (both prescriptions and over-the-counter medicines), alcohol or other substances that you are currently taking.

HAS THE RESEARCH RECEIVED ETHICAL APPROVAL?

This protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS RESEARCH?

Your participation in this research is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other medical care. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest. If it is detected that you did not give an accurate history or did nor follow the guidelines of the research and the regulations of the research facility, you may be withdrawn from the research at any time.

CAN ANY OF THESE RESEARCH PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

Venipunctures (i.e. drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in a bruise at the puncture site, or less commonly fainting or swelling of the vein, infection and bleeding from the site. To limit the chance of this happening, the procedures are performed under sterile conditions by experienced personnel. A total of 40 ml of blood and 50ml of urine will be collected over the course of the entire study. For a day you will be required to collect your own saliva. This saliva will have to be stored in a specific manner (please see the document entitled: "**SALIVA COLLECTION:** DEPRESSION AND BONE MINERAL DENSITY") and returned to the investigator within a specific timeframe or else it cannot be used in the study.

Your blood, saliva and urine will be stored under scientific conditions until it is time for their analysis. Any materials collected from you and not used in this study will be appropriately destroyed and not used without your consent in other studies.

The DEXA procedure is a means of checking your bone density and it is NOT an invasive procedure. You will eat normally on the day of the DEXA. You will be asked to remove any metal objects you may be wearing and you may have to wear a gown. **If you are pregnant, you may NOT undergo this test.** The test will take approximately 30 minutes. It is painless and no complications are expected from such a procedure. The effective radiation dose from this procedure is about 0.01 mSv, which is about the same as the average person receives from background radiation in one day.

WHAT ARE THE RISKS INVOLVED IN THIS INVESTIGATION?

As mentioned, drawing blood does have the potential to cause a bruise at the puncture site, or less commonly, fainting or swelling of the vein, infection and bleeding from the site. This is why the procedure will be conducted by qualified technicians in a sterile environment at Du Buisson, Bruinette and Kramer Pathologists in Pretorius Street, Pretoria or by a qualified physician, Dr Ilse Du Plessis.

Exposure to radiation during the DEXA is approximately the same as the average person receives from background radiation in one day and is therefore not expected to cause harm. However, pregnant women that take this test risk harm to the foetus through the radiation exposure.

ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS STUDY?

No pregnant women may participate. Always heed the advice of your doctor and, should she/he recommend that you not participate in the study, please adhere to this. Please do not participate in this study if you have undergone a barium examination or have been injected with a contrast material for a computed tomography (CT) scan or radioisotope scan less than two weeks ago. This could affect the DEXA scan.

DISCONTINUING YOUR PARTICIPATION

Please inform the investigator of your intention to withdraw from the study as soon as you have decided to do so.

INSURANCE AND FINANCIAL ARRANGEMENTS

Neither you, nor your medical aid will be required to pay for the assessments carried out.

SOURCE OF ADDITIONAL INFORMATION

If at any time between your visits you feel that any of your symptoms are causing you any problems, or you have any questions during the research, you are advised to consult your doctor. Please inform the investigator of any difficulties you may experience.

CONFIDENTIALITY

All information obtained during the course of this research is strictly confidential. Data that may be reported in, for instance, scientific journals will not include any information which identifies you as a patient in this research. In

connection with this research, it might be important for domestic and foreign regulatory health authorities and the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, as well as your personal doctor, to be able to review your medical records pertaining to this research.

Any information uncovered regarding your test results or state of health as a result of your participation in this research will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this research but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases in which a law exists compelling us to report individuals infected with communicable diseases. In this case, you will be informed of our intent to disclose such information to the authorized state agency.

INFORMED CONSENT

Kindly fill in the following information. Please note that this information will not be used by the investigator to break confidentiality. Should the investigator need to contact you, however, these details will be necessary.

Telephone number (work):_____

Cellular phone number or after hours contact number:_____

E-mail address: _____

Please inform the investigator of any changes to your contact details.

I hereby confirm that I have been informed by the investigator, Catherine Govender about the nature, conduct, benefits and risks of the study titled "Depression and bone mineral density". I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding the clinical investigation.

I am aware that the results of the research, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a research report.

I may, at any stage, without prejudice, withdraw my consent and participation in the research. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the research.

Patient's name _____

(Please print)

Patient's signature _____ Date _____

I, Catherine Govender herewith confirm that the above patient has been informed fully about the nature, conduct and risks of the above research.

Investigator's name Catherine Govender

Investigator's signature _____ Date _____

Witness's name* _____ Witness's signature _____ Date _____
(Please print)

*Consent procedure should be witnessed whenever possible.

INFORMED CONSENT FOR PARENTS / GUARDIANS (on behalf of minors under 18 years old)

Catherine Govender has provided me with a copy of the Patient Information Leaflet and Consent Form regarding this clinical investigation and has fully explained to me the nature, risks, benefits and purpose of the research. She has given me the opportunity to ask any questions concerning both the drug and the research. It has been explained to me that I will be free to withdraw my child from the research at any time, without any disadvantage to future care. I have understood everything that has been explained to me and I consent to my child to participating in this clinical research.

Parent/Guardian(s) Name _____
(Please print)

Parent/Guardian(s) Signature _____ Date _____

Patient's Name _____
(Please print)

Patient's Signature * _____ Date _____

(*Minors competent to understand must participate as fully as possible in the entire procedure.)

Investigator's Name Catherine Govender

Investigator's Signature _____ Date _____

Witness's Name _____ Witness's Signature _____ Date _____
(Please print)

VERBAL PATIENT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned, Catherine Govender have read and have explained fully to the patient, named .....and/or is/her relative, the patient information leaflet, which has indicated the nature and purpose of the research in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the research and the alternative treatments available for his/her illness. The patient indicated that he/she understands that he/she will be free to withdraw from the research at any time for any reason and without jeopardizing his/her subsequent injury attributable to the drug(s) used in the clinical research, to which he/she agrees.

I hereby certify that the patient has agreed to participate in this research.

Patient's Name _____

(Please print)

Investigator's Name Catherine Govender

Investigator's Signature _____ Date _____

Witness's Name _____ Witness's Signature _____ Date _____

(Please print)

Appendix B

						For office use only	
Respondent number						V1	
How old are you (in years)?						V2	
What is the highest level of education you have achieved?	primary school		high school		tertiary education	V3	
	1		2		3		
What is your home language?	English	Afrikaans	Sotho	Zulu	Other	V4	
	1	2	3	4	5		
Gender	female			male		V5	
	1			2			
Race/ethnicity	Asian	Black	Coloured	White	Other	V6	
	1	2	3	4	5		
Relationship status	in a relationship			single		V7	
	1			2			
Employment status	employed		retired		unemployed	V8	
	1		2		3		
Have you ever been diagnosed with a psychological disorder?	yes			no		V9	
	1			2			
If you answered "yes" on the question above, please provide your diagnosis and the earliest date of your diagnosis.						V10	
Are you currently undergoing psychological treatment?	yes		no			V11	
	1		2				
If you answered "yes" to the question above, is the therapy for the diagnosis you wrote down?	yes		no			V12	
	1		2				
Are you *currently* using oral contraception?	yes		no			V13	
	1		2				
If you answered "yes" to question 12, please name (including dosage) the contraception.						V14	
If you answered "yes" to question 12, how long have you been using this contraception?	less than a month	2 to 6 months	7 months to 1 year	1 to 2 years	more than 2 years	V15	
	1	2	3	4	5		

***Only for those who are not using oral contraception at the moment.* Have you used oral contraception in the past?**	yes			no		V16	
	1			2			
If yes, for what period?	0-6months	7 months to 1 year	2 to 5 years	more than 5 years		V17	
	1	2	3	4			
If yes, when did you stop?	0-6 months ago	7 months to 1 year ago	1 to 2 years ago	more than 2 years ago		V18	
	1	2	3	4			
Weight (in kilograms)						V19	
Height (in centimetres)						V20	
How much do you smoke in a week?	Nothing	less than 10 cigarettes	10-20 cigarettes	1-2 packs	more than 2 packs	V21	
	1	2	3	4	5		
How much alcohol do you drink in a week? Please name the drink and provide an estimated amount, e.g. 1 glass of red wine or 6 beers or nothing						V22	
Do you use any of the following medications?	yes			no			
inhaled steroids	1			2		V23	
anticonvulsants (medication that prevents fits or seizures)	1			2		V24	
calcitonin	1			2		V25	
thyroid hormone	1			2		V26	
alendronate (Fosamax)	1			2		V27	
corticosteroids	1			2		V28	
cyclosporin	1			2		V29	
anabolic steroids	1			2		V30	
biphosphates	1			2		V31	
antidepressants (medicine for depression)	1			2		V32	
Do you have any of the following medical problems?	yes			no			
thyroid problems	1			2		V33	
parathyroid problems	1			2		V34	
adrenal gland problems	1			2		V35	

Menstrual history							
Days per month; how many days does your period last (on average)?						V36	
Times per year; how many times in a year do you menstruate (on average)?						V37	
Flow is; Is the blood flow during menstruation light, medium or heavy?	light		medium		heavy	V38	
	1		2		3		
Please name any regular organised sporting activity that you have participated in more than once a week over the past 5 years.							
How often have you participated in regular sports and leisure time physical activity, excluding walking, over the past 5 years?	0-1 hour per week	1-2 hours per week	2-3 hours per week	3-4 hours per week	more than 4 hours per week	V39	
	1	2	3	4	5		
How many kilometers do you walk per day? (20 minutes of brisk walking is roughly the equivalent of 1,6km)	0 to 1	1 to 2	2 to 3	3 to 4	over 4	V40	
	1	2	3	4	5		
Were you considered more active than others of your age and gender when you were…	yes			no			
…between the ages of 14-21 years?	1			2		V41	
…between the ages 22-35 years?	1			2		V42	
…36years and over?	1			2		V43	

Do you take in enough calcium on a typical day?	yes	no	V44	
	1	2		
Women need approximately 1000mg of calcium per day and young adults (up to the age of 24 years) approximately 1200mg. Calcium comes from various sources, for example;				
1 slice of bread OR 30g of cereal OR 1 cup rice/noodles/pasta	*20mg calcium*			
a pancake, waffle or slice of French toast	*100mg calcium*			
sardines with bone	*400mg calcium*			
30g chocolate	*50mg calcium*			

Please choose the answer that is most applicable to you. Place an "X" on the number under the answer that you have chosen.

How have you been feeling in general during the past month?	in excellent spirits	in very good spirits	in good spirits mostly	up and down in spirits a lot	in low spirits mostly	in very low spirits	**V45**
	5	4	3	2	1	0	
How often were you bothered by any illness, bodily disorder, aches or pains during the past month?	every day	almost everyday	about half of the time	less than half of the time	rarely	none of the time	**V46**
	0	1	2	3	4	5	
Did you feel depressed during the past month?	to the point that I felt like taking my life	to the point that I didn't care about anything	very depressed almost everyday	quite depressed several times	a little depressed now and then	never felt depressed at all	**V47**
	0	1	2	3	4	5	
Have you been in firm control of your behavior, thoughts, emotions or feelings during the past months?	yes, definitely so	yes, for the most part	generally so	not too well	no and I am somewhat disturbed	no and I am very disturbed	**V48**
	5	4	3	2	1	0	
Have you been bothered by nervousness during the past month? (Have you felt nervous during the past month?)	extremely so	very much so	quite a bit	some, enough to bother me	a little	not at all	**V49**
	0	1	2	3	4	5	
How much energy, pep or vitality did you have during the past month?	very full of energy - lots of pep	fairly energetic	my energy varied quite a bit	generally low in energy	very low in energy or pep most of the time	no energy or pep at all	**V50**
	5	4	3	2	1	0	

I felt downhearted and blue during the past month (I felt sad durin the month)	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V51**
	5	4	3	2	1	0	
Were you generally tense or did you feel any tension during the past month?	extremely tense most of the time	very tense most of the time	fairly tense several times	a little tense a few times	my tension level was quite low	I never felt any tension at all	**V52**
	0	1	2	3	4	5	
How happy, satisfied or pleased have you been with your personal life during the past month?	extreme-ly happy	very happy most of the time	generally satisfied	sometime s fairly happy	generally dissatisfied and unhappy	very dissatisfied and unhappy	**V53**
	5	4	3	2	1	0	
Did you feel healthy enough to carry out the things you like to do during the past month?	definitely so	for the most part	health problems limited me	I was only healthy enough to take care of myself	I needed some help in taking care of myself	I needed someone to help me with most things	**V54**
	5	4	3	2	1	0	
Have you felt so sad, discouraged, or hopeless or had so many problems that you wondered if anything was worthwhile?	extremely so	very much so	quite a bit	some, enough to bother me	a little bit	not at all	**V55**
	0	1	2	3	4	5	
I woke up feeling fresh and rested during the past month	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V56**
	0	1	2	3	4	5	
Have you been concerned, worried or had any fears about your health during the past month?	extremely so	very much so	quite a bit	some but not a lot	practically never	not at all	**V57**
	0	1	2	3	4	5	
Have you had any reason to wonder if you were losing your mind, or losing control over the way you act, talk, think, feel or of your memory?	not at all	only a little	some but not enough to be concerned	some, and I am a little concerne d	some and I am quite concerned	very much so and I'm very concerned	**V58**
	5	4	3	2	1	0	
My daily life was full of things that were interesting to me	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V59**
	0	1	2	3	4	5	

Did you feel active vigorous or dull, sluggish during the month	very active everyday	mostly active, never really dull	fairly active, seldom sluggish	fairly sluggish, dull	mostly dull, sluggish	very dull, sluggish everyday	**V60**
	5	4	3	2	1	0	
Have you been anxious, worried, or upset during the past month?	extremely so	very much so	quite a bit	some, enough to bother me	a little bit	not at all	**V61**
	0	1	2	3	4	5	
I was emotionally stable and sure of myself during the past month	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V62**
	0	1	2	3	4	5	
Did you feel relaxed, at ease OR high strung, tight or keyed up during the past month?	relaxed and at ease all month	relaxed and at ease most of the time	generally relaxed	generally high strung	high strung most of the time	felt high strung the whole month	**V63**
	5	4	3	2	1	0	
I felt cheerful, lighthearted during the past month	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V64**
	0	1	2	3	4	5	
I felt tired, worn out, used up or exhausted during the past month	none of the time	a little of the time	some of the time	a good bit of the time	most of the time	all of the time	**V65**
	5	4	3	2	1	6	
Have you been under or felt you were under strain, stress or pressure during the past month?	more than I could bear	quite a bit of pressure	more than usual	some, but about usual	a little	not at all	**V66**
	0	1	2	3	4	5	

This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling *during the past two weeks*, including today. If several statements in the group seem to apply equally well, choose the highest number for that group. Be sure that you do not choose more than one statement for any group, including the items on Changes in Sleeping Pattern and Changes in Appetite.

Sadness	I do not feel sad	I feel sad much of the time	I am sad all the time	I am so sad or unhappy that I can't stand it	V67
	0	1	2	3	
Pessimism	I am not discouraged about my future	I feel more discouraged about my future than I used to be	I do not expect things to work out for me	I feel my future is hopeless and will only get worse	V68
	0	1	2	3	
Past failure	I do not feel like a failure	I have failed more than I should have	As I look back, I see a lot of failures	I feel I am a total failure	V69
	0	1	2	3	

Loss of pleasure	I get as much pleasure as I ever did from the things I enjoy	I don't enjoy things as much as I used to	I get very little pleasure from the things I used to enjoy	I can't get pleasure from the things I used to enjoy	V70
	0	1	2	3	
Guilty feelings	I don't feel particularly guilty	I feel guilty over many things I have done or should have done	I feel guilty most of the time	I feel guilty all of the time	V71
	0	1	2	3	
Punishment feelings	I don't feel I am being punished	I feel I may be punished	I expect to be punished	I feel I am being punished	V72
	0	1	2	3	
Self-dislike	I feel the same about myself as ever	I have lost confidence in myself	I am disappointed in myself	I dislike myself	V73
	0	1	2	3	

Self-criticalness	I don't criticize or blame myself more than usual	I am more critical of myself than I used to be	I criticize myself for all of my faults	I blame myself for everything bad that happened	V74
	0	1	2	3	
Suicidal thoughts or wishes	I don't have any thoughts of killing myself	I have thoughts of killing myself, but I would not carry them out	I would like to kill myself	I would kill myself if I had the chance	V75
	0	1	2	3	
Crying	I don't cry anymore than I used to	I cry more than I used to	I cry over every little thing	I feel like crying, but I can't	V76
	0	1	2	3	
Agitation	I am no more restless or wound up than usual	I feel more restless or wound up than usual	I am so restless or agitated that it's hard to stay still	I am so restless or agitated that I have it keep moving or doing something	V77
	0	1	2	3	

Loss of interest	I have not lost interest in other people or activities	I am less interested in other people or things than before	I have lost most interest in other people or things	It's hard to get interested in anything	V78
	0	1	2	3	
Indecisiveness	I make decisions about as well as I ever did	I find it more difficult to make decisions than usual	I have much greater difficulty in making decisions than I used to	I have trouble making decisions	V79
	0	1	2	3	
Worthlessness	I do not feel I am worthless	I don't consider myself as worthwhile and useful as I used to	I fell more worthless as compared to other people	I feel utterly worthless	V80
	0	1	2	3	
Loss of energy	I have as much energy as ever	I have less energy that I used to have	I don't have enough energy to do very much	I don't have enough energy to do anything	V81
	0	1	2	3	
Changes in sleeping pattern	I have not experienced any change in my sleeping pattern	I sleep somewhat more than usual OR I sleep somewhat less than usual	I sleep a lot more than usual OR I sleep a lot less than usual	I sleep most of the day OR I wake up 1-2 hours early and can't get back to sleep	V82
	0	1	2	3	

Irritability	I am no more irritable than usual	I am more irritable than usual	I am much more irritable than usual	I am irritable all the time	V83
	0	1	2	3	
Changes in appetite	I have not experienced any change in my appetite	My appetite is somewhat less than usual OR My appetite is somewhat greater than usual	My appetite is much less than usual OR My appetite is much greater than usual	I have no appetite at all OR I crave food all the time	V84
	0	1	2	3	
Concentration difficulty	I can concentrate as well as ever	I can't concentrate as well as usual	It's hard to keep my mind on anything for very long	I find I can't concentrate on anything	V85
	0	1	2	3	
Tiredness or fatigue	I am no more tired or fatigued than usual	I get more tired or fatigued more easily than usual	I am too tired or fatigued to do a lot of the things I used to do	I am too tired or fatigued to do most of the things I used to do	V86
	0	1	2	3	
Loss of interest in sex	I have not noticed any recent change in my interest in sex	I am less interested in sex than I used to be	I am much less interested in sex now	I have lost interest in sex completely	V87
	0	1	2	3	

Appendix C

SALIVA COLLECTION: DEPRESSION AND BONE MINERAL DENSITY

1. Kindly follow the procedures **exactly**. If you do not follow the steps and keep within time limits, we will not be able to use your sample.
2. You have been given 4 vials marked as follows:
   0700; 1300; 18:00; BED
3. The numbers/letters indicate the times for collection, i.e. 7am, 1pm, 6pm and just before you go to bed.
4. Please collect your saliva for one day at the indicated times.
5. Please spit into the containers at the indicated times. **Try not to be more than half an hour early or late with your collection.**
6. You do not have to stop your normal routine for these collections. Before you collect your saliva, though please **rinse your mouth with ordinary water**. Then spit into the appropriate container.
7. The night before you collect saliva put a glass of water by your bed side. As soon as you wake, **BEFORE BRUSHING TEETH, EATING OR DRINKING,** rinse your mouth out with water.
8. You do not have to fill the container all the way – only till the black line.
9. Please place the container **into the bag provided and place the bag in a cold fridge immediately**.
10. Please return all 4 vials to me **the next day before 10am**.

Kindly keep this information safe and confidential. This form contains your unique (random) code This code must be written on ALL pages and on the labels of any investigations related to this study. The investigator does not have a list of names and correlating codes, so if you cannot remember your code, she will not be able to trace it. *It is therefore vital that you remember the code and keep it safely for further reference.*

Name:	
Code:	
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	012 319 2135 / 084 789 7464
Name of study:	Depression and bone mineral density