Oswama study

The relationship between calcium, vitamin D status, anthropometry, physical activity and bone density in black men – a case control study.

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Dedication

I dedicate this dissertation to my father (C A Groenewald) and mother (P M Groenewald).

Well, whatever you do, whether you eat or drink, do it all for God's glory.

1 Corinthians 10:31

Summary

Background

Osteoporosis literally means "porous bone" and is characterized by an increase in bone fragility and susceptibility to fracture, which typically involves the wrist, spine and hip (South African Medical Association (SAMA) Working Group, 2000). In South Africa osteoporosis and fractures are more common in whites than in blacks. African-American men experience hip fractures at a rate of only half of that of Caucasian men. The bone mass in Africans were found to be 6 – 12 % higher than in Caucasians at all ages. A higher peak bone density at skeletal maturity in African-Americans were found, so that despite comparable age related bone loss, African Americans reach the fracture threshold less frequently than whites. Age-related bone loss that begins later, is less severe, or occurs in different skeletal sites in African-Americans than whites (Luckey *et al.,* 1996). American whites have a higher bone turnover than American blacks, but in contrast to this American data, South African blacks may have a higher bone turnover and lower bone density than whites (Daniels *et al.,* 1995). If it is compared with Caucasians a lower rate of hip fracture in South African blacks were found, despite lower bone density at all ages (Villa, 1994). The lower fracture rate in blacks than in whites is because of greater bone mass and higher bone turnover leading to more frequent renewal of damaged bone. Blacks excrete less urinary calcium, and show no skeletal sensitivity towards the parathyroid hormone. Few studies focus on older black South African men and osteoporosis.

Objectives

The aim of this study was to investigate the relationship of calcium intake, vitamin D status, anthropometry and physical activity and bone density in black South African men.

Methods

A case-control study design was used, in which variables associated with bone density were compared. The case group were men with fractures of the proximal femur, the proximal humerus or the distal radius and an equal number of age-matched healthy black men (with not more than a 5-year age difference) with no fracture (the proximal femur and humerus and distal radius) previously, was recruited as a control group.

Bone density was measured with DEXA. Fat percentage was measured with a Tanita scale. Biochemical analyses were done. Questionnaires were used to gather demographic, activity and dietary information. To our knowledge, this is the first case-control study on osteoporotic fractures in South African black men.

Results

Both the groups' bone mineral densities were lower than recommended. The bone density of the case group for lumbar and hip regions was 0.86 and 0.88 and the control group's bone density for lumbar region was 0.95 and hip region 0.91. The control group was more physically active and had a better nutritional status than the case group. The control group's calcium intake was higher but the vitamin D status was lower than the case group. Both calcium and vitamin D status were not statistically significant ($p<0.5$), between the two groups. Body mass indices of the groups were the same. The serum albumin was higher in the control group than in the case group. The case group serum calcium was higher than the control group. Both serum albumin and serum calcium were statistical significant between the two groups. There were no statistically significant differences in any of the other biochemical variables between the two groups. Serum phosphate and serum vitamin D were statistical significant for bone density of the hip and lumbar regions.

Conclusion

To conclude it seems logical to suggest a healthy diet with optimal macro- and micro nutrient intake. Maintain ideal body weight and body fat percentage and recommend regular but moderate-weight-bearing exercise from a young age throughout adult life, as part of a strategy to prevent and treat osteoporosis. In the present study black South African men present with low bone mineral density, but other studies indicated a lower rate of hip fracture in South African blacks, despite lower bone density at all ages. It can be recommended that other factors may play a role in black South African men with osteoporosis. Factors such as serum phosphorus, 25-hydroxy-vitamin D, body mass index (BMI), physical activity index (PAI), animal protein, total fat intake and dietary calcium are important determinants of BMD in older South African blacks, as shown in the present study. Osteoporosis is a multi factorial problem and must be treated that way.

Keywords: black men, osteoporosis, dietary calcium intake, vitamin D status, and physical activity.

Opsomming

Agtergrond inligting

Osteoporosis beteken letterlik "sponsagtige bene" en die voorkoms van beenswakheid en beenbreuke neem toe, veral van die heup, gewrig en lumbale werwels (South African Medical Association (SAMA) Working Group, 2000). Die voorkoms van osteoporose en beenbreuke is meer algemeen onder blankes as swartes, in Suid-Afrika. Die voorkoms van heupbreuke by Afrika-Amerikaner mans is helfte die van blanke mans. Beenmassa in swartes is 6 – 12 % hoër as blankes by enige ouderdomme. 'n Hoër piekbeenmassa is gevind by volwasse Afrika-Amerikaners. Afrika-Amerikaners bereik nie so maklik hul beenbreuk drumpel waarde soos Blankes nie. Beenverlies is stadiger en beenverlies begin op 'n later ouderdom as by blankes (Luckey *et al.*, 1996). Amerikaanse blankes het 'n hoër beenomset as Amerikaanse swartes, maar in teenstelling met die Amerikaanse data, het Suid-Afrikaanse swartes 'n hoër beenomset en 'n laer beendigtheid as blankes (Daniels *et al.,* 1995). Die voorkoms van heupbreuke is laer in Suid-Afrikaanse swartes, ten spyte van hul laer beendigtheid by alle ouderdomme (Villa, 1994). Die laer beenbreuke in swart mans is as gevolg van hoër beenmassa en hoër beenomset wat aanleiding gee tot vernuwing van ou been. Minder urinêre kalsium word uitgeskei en swartes se skelet is nie sensitief vir paratiroied hormoon nie. Min studies het gefokus op ouer swart Suid-Afrikaanse mans en osteoporose.

Doelwit

Die doewit van die studie was om die verband tussen kalsiuminnname, vitamien D status, antropometrie en fisieke aktiwiteit en beendigtheid in swart Suid Afrikaanse mans te bepaal.

Metodes

Die studie was 'n gevalle-kontrolestudie, waar verskillende veranderlikes, wat verband hou met beendigtheid vergelyk is. Die eksperimentele groep was mans met beenbreuke van die proksimale femur, proksimale humerus en distale radius, terwyl die gelyke aantal in die kontrole groep geen beenbreuke (proksimale humerus, proksimale femur en distale radius) gehad het nie en hulle ouderdomme het met minder as 5 jaar verskil. Die twee groepe was ewe groot. Beendigtheid is gemeet met DEXA skandering.

Vetpersentasie is met die Tanitaskaal gemeet. Biochemiese data is geanaliseer. Vraelyste is gebruik om demografiese, fisieke aktiwiteit en dieetinligting in te samel. Ons dra nie kennis van 'n soortgelyke studie op swart Suid-Afrikaanse mans nie.

Resultate

Beide groepe se beendigtheid was laer as die aanbeveling. Die beendigtheid van die eksperimentale groep vir die lumbale en heup areas was 0.86 en 0.88 onderskeidelik. Die kontrole groep se beendigtheid was 0.95 en 0.91 vir die lumbale en heup areas, onderskeidelik. Die kontrole groep was meer aktief en het 'n beter voedingsstatus as die eksperimentele groep gehad. Die kontrole groep se kalsiuminname was hoër as die eksperimentele groep maar die vitamien D status was laer. Beide van die verskille was nie betekenisvol nie. Liggaamsmassa-indeks was dieselfde in beide groepe. Serumalbumien was hoër in kontrole groep as in die eksperimentele groep. Die eksperimentele groep se serumkalsium is hoër as in die kontole groep. Die verskille in die serumkalsium en serumalbumien tussen die twee groepe was staties betekenisvol. Daar was geen statistiese betekenisvolle verskille in enige van die ander biochemiese veranderlikes, tussen die twee groepe nie. Serumfosfaat en serum-vitamien D is statisties betekenisvol ten opsigte van beendigtheid van die heup en lumbale areas.

Gevolgtrekking

Om saam te vat word 'n gebalanseerde dieet met voldoende makro- en mikronutriente aanbeveel. Ideale liggaamsmassa en vetpersentasie moet gehandhaaf word. Gewigdraende fisieke aktiwiteit is noodsaaklik vir beengesondheid. In die studie is gevind dat swart Suid Afrikaanse mans wel 'n lae beendigtheid het, terwyl ander studies 'n lae verwantskap tussen lae beendigtheid en heupfrakture toon. Dit wil voorkom of ander faktore 'n rol speel by die voorkoms van osteoporose in swart mans in Suid-Afrika. In die studie is gevind dat serumfosfaat, serum-vitamien D, liggaamsmassa-indeks, fisieke aktiwiteit, dierlike proteiene, totale vetinname en dieetkalsium 'n belangrike rol speel in bepaling van beendigtheid. Osteoporose is 'n multi-faktoriale probleem en moet so voorkom en behandel word.

Sleutelwoorde: swart mans, osteoporose, dieetkalsium, vitamien D status, en fisieke aktiwiteit.

Preface

This research report follows after an increased interest by researchers and health professionals in the prevalence of osteoporosis in black men. It provides information on the risk factors as well as the prevalence of osteoporosis in a group of South African black men. The findings will contribute to strategies for prevention and further research in South Africa.

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List of abbreviations

BGP	Bone glutamic acid protein
BMI	Body mass index
BMC	Bone mineral content
BMD	Bone mineral density
BMD L2-4	Bone mineral density of the lumbar region
BMD hip	Bone mineral density of the hip region
BMU	Basic multicellular unit
c-AMP	Cyclic adenosine3-5 monophospate
CHO	Carbohydrate
CI	Confidence interval
DHEA-S	Dehydroepiandrosterone sulphate
DEXA	Dual energy x-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
FNF	Femur neck fracture
g	Gram
LBM	Lean body mass
OSWAMA	Osteoporosis "in swart mans"
PAI	Physical activity index
PBM	Peak bone mass
PTH	Parathyroid hormone
N	Number of subjects
RDA	Recommended daily allowance
25-OH-vit D	25-hydroxy vitamin D3
$1,25(OH)_2D$	1,25-dihydroxyvitamin D

Chapter 1

Introduction

1.1 Background information on osteoporosis

Osteoporosis is a major health problem, especially in elderly Caucasian women (Prince, 1997). Osteoporotic fractures are claimed to affect 50 % of women and 30 % of men aged over 50 years (Cohen & Roe, 2000). The study of osteoporosis has until recently, been limited almost exclusively to women, and the results that were derived from those studies cannot be simply extrapolated to other racial and ethnic populations (Luckey *et al.*, 1996). In this study the focus will be on the relationship between calcium and vitamin D intake as well as activity level and body composition and bone mineral density in black men. In order to understand current theories on how osteoporosis develops, the literature study will briefly review normal bone physiology and calcium metabolism as well as some of the risk factors like: inactivity, age, low body weight and gender. Important underlying causes of osteoporotic fractures in men include low body weight and reduced physical activity (Compston, 2001). Among the various factors that contribute to the development of osteoporosis in elderly subjects, nutritional deficiencies are clearly pivotal (Rizzoli *et al.*, 2001). The last of the risk factors are genetic and ethnic factors. Data obtained from twins and families indicate that as much as 80% variance in bone mass within a population is genetically determined. African American men experience hip fractures less frequently than Caucasian men and age related bone loss is less severe and begins later in life (Luckey *et al.*, 1996). In addition, nutritional, hormonal, lifestyle and environmental factors account for 20 % of variance in bone mass within a population. Many of the risk factors are considered to be weakly correlated with osteoporosis, although when combined they could impact significantly on bone health (Cohen & Roe, 2000).

1.2 Background to this thesis

Few studies have focused on older black South African men and osteoporosis. Prof. NGJ Maritz from Pretoria Academic Hospital (Orthopaedics) noticed, while collecting

preliminary data (from clinical practice for personal interest) on deep vein thrombosis after hip fractures between 1992 to 1993, that more than 50 % of the patients with hip fractures were black men. Together with Prof. Davidson, Bradley University, USA they initiated the present study with the objective to investigate the possible role of dietary and environmental factors in osteoporotic fractures in black men in South Africa. The study is named OSWAMA meaning "**O**steoporose in **SWA**rt **MA**ns". We hope that this study will play a valuable role in expanding the current knowledge regarding osteoporsosis and bone fractures in black South African male persons. The study is divided into two parts. One part investigated the relationship of calcium, vitamin D status and physical activity and bone density in black South African men. The other part, reported separately by M Leach, investigated iron overload, hipovitaminosis C, tobacco and alcohol and bone density in black South Africa men (Leach, 2003).

1.3 Aims of the study:

- To describe the relationship between a low dietary calcium intake and bone density in black South African men.
- To describe the relationship between a low vitamin D status and bone density in black South African men.
- To describe the relationship between physical activity and bone density in black South African men.

1.4 Hypothesis:

- A low calcium intake is negatively associated with bone density and may be a risk factor for bone fracture caused by non-traumatic accidents in black men in South Africa.
- A low vitamin D status is negatively associated with bone density and may be a risk factor for bone fracture caused by non-traumatic accidents in black men in South Africa.
- Physical activity is positively associated with bone density in black men in South Africa.

1.5 Structure of dissertation

In the introduction the background of the problem of osteoporosis and the limitations of available information on men and especially from racial groups other than whites are mentioned. This is followed by a discussion of the background, aim and hypothesis of the study. In the literature study (Chapter 2) a review of normal bone physiology, calcium metabolism and risk factors for osteoporosis is given. Chapter 3 describes the design and method of the study and provides background information on the population. The data obtained in the study is described in Chapter 4. In Chapter 5 and 6 the results are discussed, conclusions are drawn and recommendations made with regard to risk factors and bone mineral density in black South African men.

Chapter 2

Literature survey

2.1 Introduction

The objectives of this study were to describe the association between dietary and lifestyle factors and BMD in black South African men. In this chapter, background information on bone anatomy, bone physiology, calcium metabolism, bone loss and risk factors influencing peak bone mass will be briefly discussed.

2.2 Prevalence of osteoporosis

Osteoporosis (as seen in figure 2.1) can be defined as a systemic skeletal disease characterised by low bone mass (readily measured as bone mineral density (BMD) and micro-architectural deterioration of bone tissue (difficult to asses), with a consequent increase in bone fragility and susceptibility to fracture, which typically involves the wrist, spine or hip (SAMA, 2000).

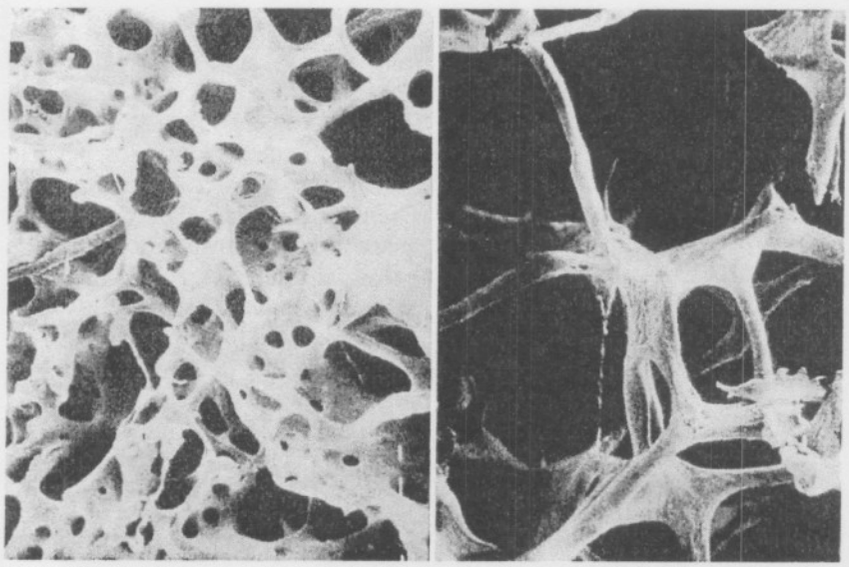

Figure 2.1: Scanning electron micrograph of normal (left) and osteoporotic (right) vertebral trabecular bone (Marcus *et al.,* 1996)

Osteoporosis, a disease affecting several million people in the world, should be prevented from childhood by achieving maximal bone mass compatible with individual genetic background (Branca & Vatuena, 2001). The numbers of people affected are on the rise because of increasing life expectancy (Ilich & Kerstetter, 2000). The average cost per patient in Austria in 2000 for hospital treatment was US$ 9097 and in total US$ 103 509 800, in the U.S. The current cost exceeds $10 billion per year. The economic importance of this development and its impact on the health care system must be considered as significant (Koeck *et al.,* 2001).

African-American men experience hip fractures at a rate only half that of Caucasian men. The incidence of fractures increases with ageing in black and white men (see Figure 2.2) and reflects an increasing prevalence of skeletal fragility (Marcus *et al.,* 1996). A 4% of non-Hispanic black men aged 50 years and older are estimated to have osteoporosis and 19 percent are estimated to have low bone mass (NOF, 2002). In 2000 Schnaid reported an incidence of 12 femur neck fractures (FNF) per 100 000 black men and women patients in Johannesburg per annum. The incidence in Caucasians in Johannesburg has been reported as 100 per 100 000 for the same period. The incidence of FNF in blacks has doubled in the last ten years. Census figures were used for the calculation (Schnaid *et al.,* 2000). Osteoporosis is responsible for more than 1,5 million fractures annually, including 300 000 hip fractures, 250 000 wrist fractures, 700 000 vertebral fractures and 300 000 fractures at other sites (NOF, 2002).

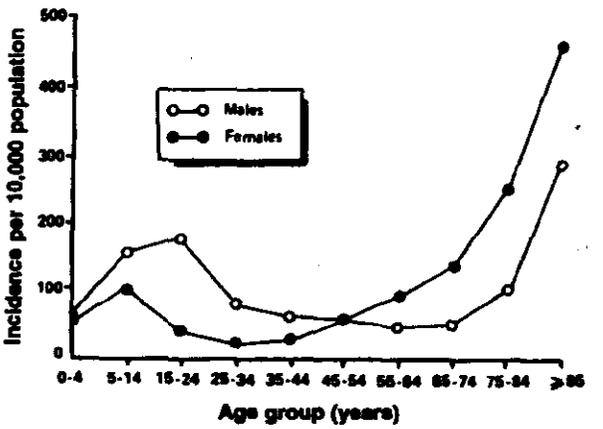

Figure 2.2: Average annual fracture incidence in male and female per 10 000 populations per age (Marcus *et al.,* 1996).

In conclusion, the risk of fractures increases exponentially with advancing age in both black and white populations, so that a dramatic increase in osteoporosis-related fractures is expected in both ethnic groups as the population over 65 years continues to grow. Little is known about the effects of aging on skeletal physiology in the US populations; however, without information on the patterns and determinants of bone loss, the formulation of rational prevention and treatment strategies in these groups is not possible. With the high prevalence and morbidity of hip fractures, ethnic group-specific data on the determinants and rates of bone loss at the hip are urgently needed (Luckey *et al.,* 1996).

2.3 Bone anatomy

2.3.1 Bone anatomy and classification of bone

In order to understand current theories on how osteoporosis develops, it is necessary to briefly review bone anatomy, normal bone physiology and calcium metabolism (Murray & Pizzorno, 1998). The three principle sites of osteoporosis fractures are hip, wrist and spinal vertebrae. The femur is the single bone of the thigh and is the largest, longest strongest bone in the body. Proximally the femur articulates with the hip bone. The humerus is the sole bone of the arm and is a typical long bone. At the proximal end of the humerus is its smooth, hemispheral head. The radius is one of the parallel long bones of the forearm (Marieb, 1995). Indications of age specific incidence rates of different skeletal sites are shown in figure 2.3.

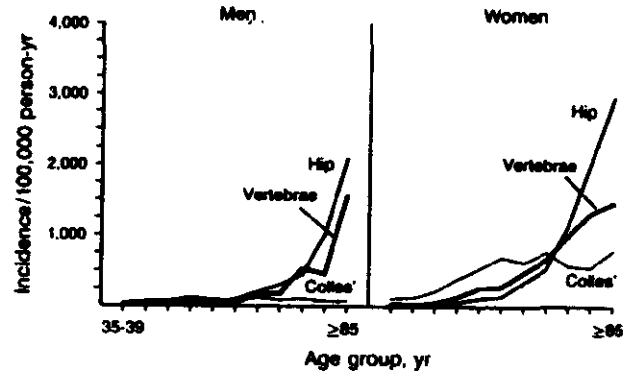

Figure 2.3: Age specific incidence rates of hip, vertebral, and Colle's fracture (radius fracture) in Rochester, Minnesota, men and women (Marcus *et al.,* 1996).

Bone contains bone tissues of two major types, trabecular and cortical bone (Maher *et al.,* 1998). The skeleton consist of 80 % cortical bone or compact bone. Shafts of the large bones are primarily cortical bone. Trabecular or cancellous bone tissues which exist in the knobby ends of the long bones make up the remaining 20 % (Mahan & Escot-Stump, 2000). Trabecular bone is more spongy and less dense, more open structure of interconnecting bone spicules and so more vulnerable to bone loss and osteoporotic fractures. Trabecular bone tissue adds support to the cortical bone tissues and bone remodelling is more active than in cortical bone, 40 % is recycled annually in trabecular bone versus 10 % in cortical bone (Marieb, 1995).

2.3.2 Composition of bone cells and bone matrix

Three types of bone cells: osteocytes, osteoblasts and osteoclasts are found in bone. Osteoblasts are responsible for bone formation or production of bone tissue. Osteoclasts (see figure 2.4) are responsible for resorption of bone (Maher *et al.,* 1998). Other important cell types are osteocytes and bonelining cells, both of which are derived from osteoblast. Bone mass is maintained when the resorption and formation phases are balanced. Negative bone balance results from overactive osteoclasts and impaired osteoblasts (Medscape, 1997).

Bone consists of an organic matrix or osteoid, primarily collagen fibers, in which salts of calcium and phosphate are deposited, in combination with hydroxyl ions in crystals of hydroxyapatite. The tensile capacity of collagen and the hardness of hydroxyapatite combine to give bone its great strength. Other components of the bone matrix include osteocalcin, osteopontin and several other matrix proteins (Mahan & Escott-Stump, 2000).

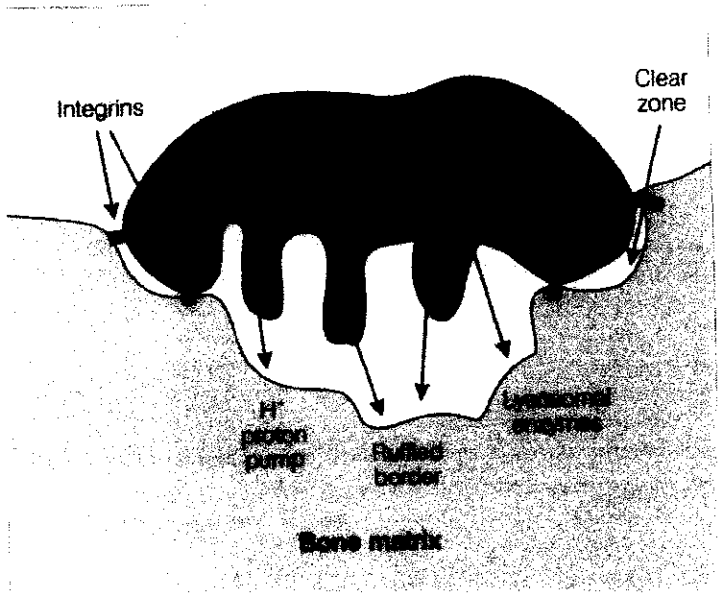

Figure 2.4: Process of bone resorption (Arden & Spector, 1997).

2.4 Bone physiology

2.4.1 Physiological process in the skeleton

Growth is a process that results in an increase in tissue volume. Bones become longer, stronger and thicker and more bone tissue is laid down on the existing bone (Maher *et al.,* 1998). Bone modelling is the term applied to the growth of the skeleton until mature height is achieved. In modelling, the process of formation of new bone tissue occurs first and is followed by the resorption of old tissue (Mahan & Escott-Stump, 2000). Bone remodeling takes place after skeletal growth is completed. It is a continuous process that ensures bone health and strength by coupling the removal of old bone (bone resorption) with synthesis of new bone matrix and later mineralization (bone formation). Old bone is "weak" and new bone is stronger. About 4 % of the total bone surface is involved in remodelling at any given time as bone is renewed continually at specific sites throughout the skeleton. There are four steps in the bone remodelling cycle: activation, resorption, reversal and formation. This takes place in two stages. The first involves the synthesis of bone matrix and then bone mineralization. Calcium precipitates out of body fluids and

is packed in collagen fibers. The latter also contains trace amounts of magnesium, potassium, sodium and carbonate (Maher *et al.,* 1998).

Osteoblasts and osteoclasts are primarily responsible for bone turnover. Bone metabolism is modulated by a variety of systemic hormones. Parathyroid hormone (PTH) increases the number and activity of osteoclasts (Marcus *et al.,* 1996). Low dietary calcium intake results in greater osteoclastic resorption than formation of osteoblasts, because of a persistently elevated PTH concentration in blood. PTH acts directly on osteoblasts, which increases the production of interleukin-6 and other cytokines that in turn stimulate osteoclasts to resorb bone. PTH increases serum calcium levels of the blood and is released by the parathyroid gland in response to hypocalcemia. PTH increases bone resorption, frees stored calcium and increases distal tubular reabsorption of calcium in the kidney (Maher *et al.,* 1998). The action of PTH in promoting activity of the osteoblasts is countered by oestrogen, which reduces the response of osteoblasts to PTH. Impaired production of this hormone could occur in the elderly, which could contribute to age-related bone loss, but no data has been published to support this possibility (Notelvitz, 1999).

Vitamin D is the precursor of cholecalciferol. Cholecalciferol is converted into its biologically active from, calcitriol. Calcitriol's main effect is on the intestinal tract to promote calcium absorption. Calcitriol is necessary for proper PTH function and efficient mobilization of calcium from bone (Maher *et al.,* 1998).

Calcitonin is produced by the C-cells in the thyroid gland. Its main physiological function is to inhibit osteoclast activity. Pharmacologic use of calcitonin results in reduced bone turnover (Medscape, 1997). Hyperthyroidism is associated with hypercalcemia (Maher *et al.,* 1998). Impaired production of this hormone could occur in the elderly, which could contribute to age-related bone loss (Mahan & Escott-Stump, 2000). Thyroxine (T4) and triidothyronine (T3) affect bone cells directly and indirectly via local growth factors, eg, insulin-like growth factor-1 (Medscape, 1997).

Bone cells have glucocorticoid receptors. Excess corticosteroid activity results in inhibition of osteoblasts and, therefore, inhibition of matrix formation and decrease in

calcium absorption with secondary hyperparathyroism. Long-term corticosteriod treatment will decrease bone mass (Medscape, 1997).

Bone is living tissue and serves three important functions: scaffolding for the muscoskeletal system, protection of vital internal organs and metabolic reservoir serving calcium homeostasis. Bone modelling and remodelling is part of physiologic processes in the skeletal. Osteoblast and osteoclast are responsible for bone turnover and bone metabolism is modulated by a variety of systemic hormones. The calcium homeostasis will be discussed in the following section.

2.4.2 Calcium homeostasis

Bone tissue serves as a reservoir of calcium and other minerals that are used by other tissues of the body. Calcium homeostasis is reliant on this source of calcium when the diet is inadequate. Although 99 % of the body calcium is found in the skeleton, the remaining 1 % is critical to a great variety of life processes. When calcium intake is not adequate, homeostasis is maintained by drawing on mineral from the bone to keep the serum calcium ion concentration at its set level. Depending on the amount of calcium required, homeostasis can be accomplished by drawing from two major skeletal sources: readily mobilized calcium ions in the bone fluid or, through the process of osteoclastic resorption from the bone tissue itself. Two calcium-regulating hormones, PTH and 1,25-dihydroxyvitamin D (calcitriol) regulate blood calcium concentration. PTH activity, which directly contributes to bone loss, increases in most individuals during the seventh decade of life. Calcitriol increase the efficiency of intestinal calcium absorption when dietary calcium is inadequate (Mahan & Escott-Stump, 2000).

Rising blood calcium signals to the thyroid gland to secrete calcitonin. Calcitonin inhibits the activation of vitamin D, prevents calcium reabsorption in the kidneys and limits calcium absorption in the intestines. Calcitonin inhibits osteoclast cells from breaking down bone, preventing the release of calcium. All these actions result in lower blood calcium levels, which inhibit calcitonin secretion. Falling in blood calcium signals to the parathyroid glands to secrete PTH. PTH stimulates the activation of vitamin D. Vitamin D and PTH stimulate calcium reabsorption in the kidneys and enhance calcium absorption in the intestines. Osteoclast cells are stimulated to break down bone and

releasing calcium into the blood. All these actions result in higher blood calcium levels, which inhibit PTH secretion (Whitney *et al.,* 2002).

The best recognised of the nutritional effects of calcium, and the only currently accepted functional indicator for calcium status, relates to skeletal mass. For bone mass, calcium is what is termed a "threshold nutrient", which means that bone mass increases as calcium intake rises, up to some level (the threshold), above which further increases in intake produce no further increase in bone mass. The reason for the plateau above threshold value is that calcium is not stored in bone but as bone. Bone mass is regulated by a mechanical feedback loop that is responding to applied mechanical loads. In the face of dietary calcium abundance, the body maintains only as much skeletal mass as is needed for current levels of work or exercise. Any bone loss with age is undesirable, but if the loss may be occurring because of non-nutritional causes, increasing calcium intake further will not alter bone balance (Heaney, 2002). Net intestinal absorption is only about 10 % at contemporary intakes, there is a possibility, at the gut alone, to adapted adequately to even very low intakes (Heaney, 2002).

There are differences in sensitivity of the effector mechanisms in different racial groups. African–Americans' bone mass values adjusted for weight are 6 – 12 % higher than Caucasians at all ages. This information gathered from 16 studies also show calcium intakes from 10 % - 30 % below that of Caucasians. The rate of spine bone loss in African-American women was one-third lower than in Caucasians, despite reported calcium intakes of about 25 % lower. The only logical explanations for those disparities are that African-Americans utilise dietary calcium more efficiently than Caucasians or that their dietary calcium intakes are substantially under-reported. While the latter cannot be conclusively excluded, it seems an unlikely explanation, particularly since the under-reporting would have to be very large (at least 50 %), and there is, in fact, a compelling body of evidence indicating more efficient utilisation of dietary calcium in Blacks (Heaney, 2002).

To conclude calcium is probably the most studied nutrient in the area of bone health. Calcium homeostasis, regulated by hormones, is reliant on dietary calcium intake and if that is not adequate, homeostasis is maintained by drawing on minerals from the bone to keep the serum calcium ion concentration at its set level. Despite the abundance of

evidence supporting the positive effects of dietary calcium on bone, calcium intakes in all ages are lower than the current recommendations (Heaney, 2002).

2.5 Bone mass

Bone mass is a generic term that refers to bone mineral content (BMC), but not to bone mineral density (BMD). BMC is more appropriate in assessing the amount of bone accumulated before the cessation of growth or height gain, whereas BMD is used to describe bone after the developmental period is completed. These measurements are often used interchangeably, but BMD is more useful in studies of adults (Mahan & Escott-Stump, 2000).

Blacks have a greater bone mass and a lower incidence of osteoporosis and hip fractures than whites (Daniels *et al.,* 1995). Weinstein and Bell (1988) performed biopsies of the iliac crest in 12 blacks and 13 whites to determine whether histomorphometric differences between blacks and whites could be identified. The static measurements of cortical and cancellous bone architecture were not significantly different in the two groups. In contrast, the dynamic measurements, determined with tetracycline markers, showed that the mean rate of bone formation in blacks was only 35% of that in whites. They conclude that the rate of bone turnover is lower in blacks than in whites, since bone resorption and bone formation are closely coupled in the steady state. If reconstitution of previously resorbed cavities at remodeling sites is incomplete in osteoporosis, a reduction in the rate of skeletal remodeling could provide a means for maintaining and preserving bone mass in blacks (Weinstein & Bell, 1988). A decline in BMD is associated with the highest risk for hip fractures (Marcus *et al.,* 1996).

In conclusion bone mineral content is appropriate in assessing the amount of bone accumulated before the cessation of growth or height gain, whereas BMD is used to describe bone after the developmental period is completed. Blacks have a greater bone mass and a lower incidence of osteoporosis and hip fractures than whites (Luckey *et al.*, 1996).

2.5.1 Measurement of bone mineral content and bone mineral density

Bone densitometry measures bone mass on the basis of tissue absorption of photons produced by one or two mono-energetic x-ray tubes. Dual energy x-ray absorptiometry (DEXA) is used to measure total body and regional skeletal sites of interest, such as proximal femur (hip) (Mahan & Escott-Stump, 2000). The Dual-Energy x-ray Absorptiometry (DEXA) quantifies bone mass in terms of BMC and BMD, both of which are influenced by bone size. True bone density may be underestimated in smaller bones and overestimated in larger ones (Bachrach, 1999). Results of BMC measurements are expressed as grams of mineral per centimetre of bone. BMD is expressed as grams per centimetre squared and is calculated from the BMC divided by the width of the bone at the measurement site (Mahan & Escott-Stump, 2000).

2.5.2 Peak bone mass

Peak bone mass (PBM) is reached around the age of 35 years and it is the greatest amount of bone accumulated at any age. In men PBM is greater than in women because of their large frame size (Mahan & Escott-Stump, 2000). Bone mineral density is also greater in African Americans than in Caucasian Americans. A strong hereditary component (70 % - 80 %) is related to the development of bone mass and contribution of environmental factors is about 20 – 30 %. Peak bone mass is related to both dietary calcium intakes and weight-bearing physical activity. The age when BMD acquisition ceases varies, depending not only on diet but also on physical activity and strain on the skeleton (Mahan & Escott-Stump, 2000). Factors influencing peak bone mass are: genetic make-up, nutrition, exercise and hormonal status (Medscape, 1997). After PBM is achieved, generalized loss of bone mass and density gradually occurs with ageing, and results in an increasing risk of osteoporotic fracture. As bone mass in later life is determined by both PBM and subsequent rate of loss, the relative contributions of these two factors are important (Sambrook *et al.*, 1993).

2.5.3 Loss of bone mass and fractures

Bone mass is the major determinant of bone strength and the relative risk of osteoporotic fractures. Physiologically BMC is a function of two factors: PBM achieved at skeletal maturity and the subsequent rates and duration of bone loss. Thus the ethnic

disparity in bone mass and fracture incidence could result from higher PBM at skeletal maturity in African-Americans, so that despite comparable age related bone loss, African Americans reach the fracture threshold less frequently than whites. Another reason can be that age-related bone loss that begins later, is less severe, or occurs in different skeletal sites in African-Americans than whites (Luckey *et al.,* 1996). In a large population–based fracture survey, among blacks, the female predominance in hip fracture risk seen in whites is either absent or much reduced. Thus, among blacks, oestrogen deficiency may not play as prominent a role in osteoporosis as it does for whites. Alternatively, black women may be more heavily exposed than black men to some protective factors (Baron *et al.,* 1994).

The incidence of proximal femur fracture increase dramatically with increasing age (Kirchengast *et al.,* 2001). Studies done on fracture rates confirm the past impression that blacks who survive into the older ages are a biological elite, more able to maintain bone strength than whites of either sex, although by no means being exempt from bone loss with age. Fractures are more common in women than in men, because men accumulate more bone than women, women lose more bone minerals with ageing than men do, and elderly men fall less frequently than women do (Marcus *et al.,* 1996).

From a societal perspective it is appropriate to formulate risks and intervention thresholds in populations. In vertebral and hip fractures it is of interest that the 10 year risks are similar in men than women for *T*-scores close to the diagnostic threshold. This confirms that the use of *T*-scores derived from women are applicable to men, and therefore support the view that diagnostic thresholds should be the same in men as in women (Kanis *et al.,* 2001). The bone loss rate associated with the process of ageing is approximately 1 % per year in men and women. Therefore having a larger bone capital and spending less, reducing bone loss delays the attainment of a bone density level at which fracture risk is high. Fracture incidence in individuals whose bone density is greater than 1 SD above the mean is 50 % lower at 80 years (Branca & Vatuena, 2001).

2.6 Risk factors for osteoporosis

Despite the vast number of risk factors that apparently predispose to the development of osteoporosis, I will only focus on age, activity, low body weight, gender, calcium and

vitamin D status, genetic factors and ethnic group. Marthie Leach focuses on factors like iron overload, alcohol and smoking (Leach, 2003). Age and BMD are the strongest known risk factors for hip fracture (Kanis *et al.,* 2001). Since rapid skeletal mineral acquisition occurs relatively early in life, the exogenous factors that might optimise peak bone mass to its genetic potential need to be identified (Ilich & Kerstetter, 2000).

In a prospective analysis done in white men the following possible risk factors appeared to be predisposed: lack of exercise, a history of smoking, lower body mass and height, a preference for salty foods and fat distribution around the waist (Blaauw *et al.*, 1994). Dietary calcium, phosphorous, protein and caffeine intakes were similar in osteoporosis and control subjects, but alcohol consumption was clearly higher in both osteoporosis males and females (Blaauw *et al.*, 1994).

An Australian study by Nguyen et al. (1996) determined the risk factors for osteoporosis in men. Higher risks of fracture were associated with lower femoral neck BMD, quadriceps weakness, higher body sway, falls in the preceding 12 months, a history of fractures in the previous five years, lower body weight and shorter current height. Higher dietary calcium intake was associated with higher BMD, but neither of these relationships translated into a higher risk of fracture (Nguyen *et al.,* 1996). Dietary pattern is associated with BMD. A study done by Tucker et al. shows that a high fruit and vegetable intake appears to be protective in men, while high candy consumption was associated with low BMD in both men and women (Tucker *et al.,* 2002). In a study done on urban and rural communities in Australia, the older rural population had a lower fracture rate than the urban population. Environmental factors could have a different impact on bone health (Sanders, 2002). For the purpose of this thesis only the following factors will be discussed in more detail.

2.6.1 Inactivity

Physical activity has different effects on bone depending on the intensity, frequency and duration of exercise and the age at which it is started. A sustained level of activity leads to greater peak bone mass, as demonstrated by a 15 year longitudinal study in which physical activity was correlated with BMD at the lumber spine at age 27, especially when initiated well before puberty (Branca & Vatuena, 2001). The anabolic effect on bone is

greater in adolescence as a result of weight-bearing exercise. Adequate intakes of calcium appear necessary for exercise to have its bone stimulating action (Branca & Vatuena, 2001). Activity must be continuously maintained in order to be effective over long periods of time. If exercise is decreased, the rate of bone loss will increase for a period of time, perhaps as long as a year, until a new steady state is achieved (Anderson & Pollitzer, 1994). In older populations, the effects of resistance training may make a difference in being able to climb stairs, carry groceries, or rise from a chair. In addition, resistance training may have a significant impact in maintaining bone health. Recently, progressive resistance training principles have been applied to a large and growing population of older men and women, for whom the relationship of muscle strength and balance is critical in maintaining functional independence and resistance to falls and for decreasing risk factors associated with osteoporosis (Layne & Nelson, 1999). After skeletal maturity is reached, bone remodels itself according to the functional demands placed upon it, and bone strength is related to its material properties (density), geometry and loading conditions (the force applied to any bone). Boys that are physically active also have up to 5 % increased PBM compared to their sedentary counterparts (Sambrook *et al.,* 1993).

Despite the fact that physical activity plays an important role in bone health, no studies on the effects of physical activity on the bone mass in adult black populations have been reported. This area is ripe for new experimental efforts (Anderson & Pollitzer, 1994). There is consistent evidence that an increase in physical acitivity leads to an increase in forearm and lumbar spine BMD, as shown in figure 2.5 (Arden & Spector, 1997).

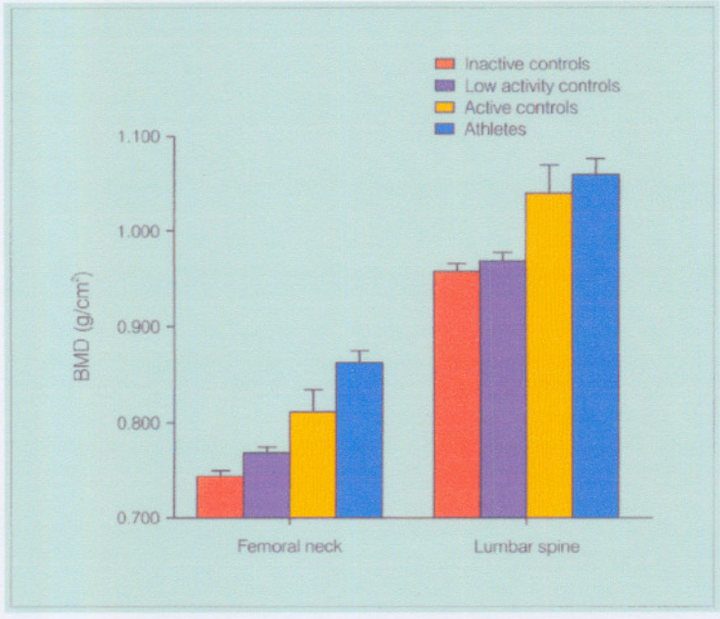

Figure 2.5: Differences in bone mineral density by physical activity levels.

2.6.2 Age

Age is an important determinant of BMD, at approximately the age of 40, BMD begins to diminish gradually in both sexes. Men continue to have bone loss also, but at a much slower rate than the women of the same age, until age 70, when the loss rates are about the same for both genders. Loss of bone mass is the result of changes in the hormone-directed mechanisms that govern bone remodelling. The processes of resorption and formation are uncoupled to the degree that it interferes with the ability of osteoblastic activity to keep up with the resorptive activities of osteoclasts to maintain balance. Trabecular bone begins to diminish in both sexes as early as at 40 years of age. The normal bone loss that occurs with aging in both sexes is related to impaired calcitriol activity in target tissues and the decline of osteoblastic function (decreased level of growth-factor 1 that stimulate osteoblasts to increase bone formation), such as the reduced production of type 1 collagen, osteocalcin, osteopontin, and other matrix proteins. As a result of the uncoupling of the remodelling process, resorption exceeds formation with an increasing differential. Reasons for bone loss in men has not yet been

established, but it may be related to the decline in androgen production by the gonads or the adrenal cortex (Mahan & Escott-Stump, 2000).

In a study performed by Fatayerji *et al.* the hormonal influence on age-related changes in calcium homeostasis was evaluated in 178 healthy men aged between 20-79 (Fatayerji *et al.,* 2000). The study showed that there was no change in serum calcium with age, but there was a decrease in serum phosphate, urinary calcium and creatinine clearance with age, while the calcium intake remained unchanged. PTH increased with age and there was a linear increase in 25-OH-vit D with age that persisted after correcting for seasonal variation. Serum insulin-like growth hormone was positively associated with creatinine clearance, serum calcium, and phosphate and negatively associated with PTH. In this cross-sectional study of otherwise healthy, normally aging men, age-related decreases in insulin–like growth hormone seem to have a greater impact on mineral absorption than does vitamin D status (Fatayerji *et al.*, 2000).

In Figure 2.6 the change in BMD with age for men and women is demonstrated. Age related bone loss probably commences during the fourth decade and continues throughout life. Bone losses in men are slower with age than women (Arden & Spector, 1997).

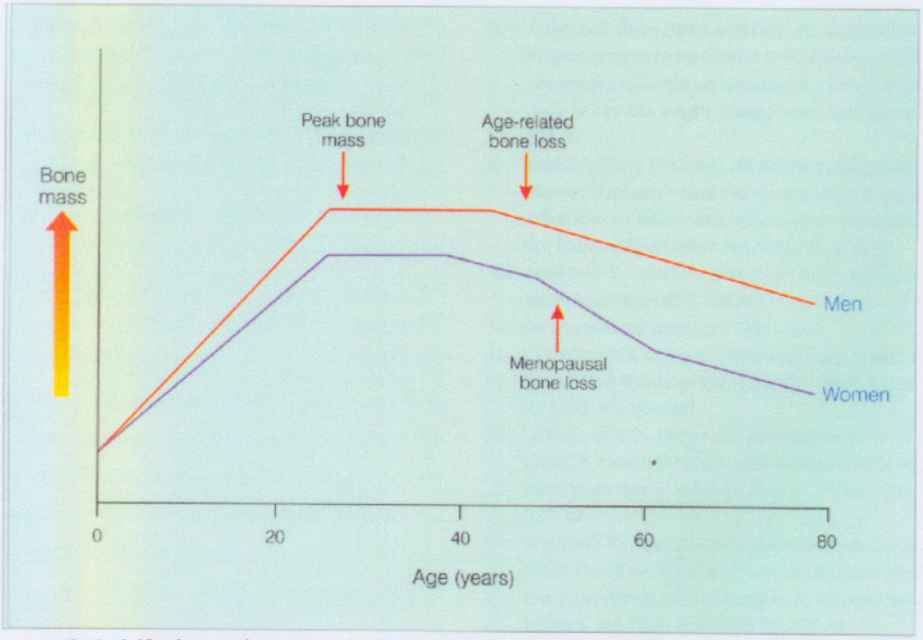

Figure 2.6: Lifetime changes in bone mass (Arden & Spector, 1997)

To conclude bone density decreased with age, in men at a slower rate than women. The bone loss with age is related to increase in PTH, impaired calcitriol activity and a decline in osteoblastic function.

2.6.3 Low body weight

The importance of malnutrition as a risk factor in osteoporosis is emphasized by the evidence that patients with fractures of the proximal femur are often undernourished. In underweight subjects, low levels of albumin (<35g/l) were associated with higher femoral bone loss. Other factors occurring in malnutrition, besides body composition changes, such as protein deficiency, could be involved in the association between underweight and osteoporosis (Coin *et al.,* 2000). A lower body fat in African Americans and a higher 17B-estradiol attribute to increased aromatase. The enzyme aromatase is found predominnantly in fat tissue. Serum 17B estradiol is a major determinant of growth hormone secretion through a process called aromatization. Greater production of growth hormone and 17B-estradiol contribute to greater bone mass in African Americans (Bell, 1997). Higher 17B-estradiol may play a protective role despite the lower body fat in African Americans. Body weight may increase mechanical stress on bone that may stimulate bone remodeling and preserve bone minerals in both men and women (Baumgartner *et al.,* 1996).

2.6.4 Gender and sex hormones

On average males have larger bone sizes and male bone size at the spine and hip increase with age (Deng *et al.,* 2002). The bone size of the hip, spine and wrist in humans is significantly influenced by genetic factors and peak bone mass is higher in men than women (Deng *et al.,* 2002). The gender difference in bone measurements are due to differences in body size (height and weight) and peak strain activity. The peak strain activity indicates that women do less high peak strain sport than men and do not benefit from their sports activity (Neville *et al.*, 2002). Net bone loss is less in men than women. Sex hormone deficiencies contribute to abnormalities in skeletal size and mass during growth, remodelling imbalance and bone loss during ageing in men. The larger peak bone size and greater bone size with ageing in men is most likely to be androgen-dependent in Caucasians and Asians. Androgen deficiency may partly account for

reduced bone formation and negative bone balance at the basic multicellular unit. Oestrogen deficiency during growth is associated with reduced bone mass and increased leg length in male and females. Oestrogen deficiency during ageing may account for trabecular bone loss in men by increasing remodelling rate (Duan & Seeman, 2002). Because elderly men have low serum bioavailable oestrogen and testosterone levels, and because recent data suggest that oestrogen is the main sex steroid regulating bone metabolism in men, oestrogen deficiency may also be the principal cause of bone loss in elderly men (Riggs, 2002).

A study done by Evans and Davie on 81 male subjects, with idiopathic vertebral fracture, investigated the associations of sex hormone levels with fracture. Sex-hormone binding globulin was higher in the osteoporotic subjects and 24-hr urinary creatinine (an index of lean body mass) was lower. High levels of sex-hormone binding globulin have previously been described in men with idiopathic osteoporosis and reflect lower free testosterone levels. The role of sex hormones in the genesis of vertebral fracture in men is uncertain (Evans & Davie, 2002).

Males have larger bone sizes and higher peak bone mass, because of more peak strain activities, than women and bone loss with age are not so rapid.

2.6.5 Calcium intake

From observational retrospective and cross-sectional research, it appears that calcium intake is a determinant for bone mass acquisition from early childhood to pre-pubertal stages in healthy children with no hormonal imbalances. During infancy and puberty, calcium absorption is maximised to meet the increased needs through hormonal mechanisms and, as long as calcium intake is above a minimum threshold around the recommended daily allowance (RDA), its influence on bone mass gain is widely overwhelmed by genetic factors. The above is especially true for the weight-bearing sites of the skeleton, which are more likely to be affected by nutritional influences. This may explain why studies linking current calcium intakes with BMD at the forearm in adolescents fail to find an association, while investigators considering calcium intake during childhood, or for long periods of time during childhood and adolescence, report

significant correlations with BMD at the hip and lumbar spine in children, adolescents and adults (Branca & Vatuena, 2001).

The gain in bone density throughout the first several decades translates to lower risk of fracture later in life, and there are two epidemiologic studies that support this contention (Matkovic *et al.,* 1979; Hu *et al.,* 1993). They examined bone mass in populations accustomed to different calcium intakes over a lifetime. Both studies were cross-sectional: one in a Croation and another in a Chinese population. Differences in bone mass in both men and women living in high and low calcium regions were present during young adulthood and continued into old age. These studies indicate that calcium is an important agent for skeletal formation affecting PBM and subsequent rates of bone fractures. Retrospective studies in adults support the above conclusions (Matkovic *et al.,* 1979; Matkovic *et al.,* 1995). Dietary calcium from the distant past (childhood and adolescence) was a significant predictor of current adult bone mass. Overall, it is likely that variations in calcium nutrition early in life can account for as much as a 5 % to 10 % difference in peak adult bone mass. Such a difference, although small, could potentially contribute more than 50 % to the hip-fracture rates later in life (Matkovic *et al.,* 1995). Supplementation with 500mg/d of calcium in children and adolescents with either low intakes or intakes close to the RDA, significantly increases BMD at weight-bearing sites, at least initially. In any case, usual consumption levels (around 50 % of the RDA) are insufficient for maximal PBM achievement (Branca & Vatuena, 2001).

Recently the National Academy of Sciences released calcium requirements for North Americans. Adequate intake for individuals over the age of 50 years was set at 1200mg/day. The evidence used to determine calcium requirements was accumulated primarily in whites. The panel recognised that more data is needed to determine the calcium requirements of other racial and ethnic groups and in disease state (Weaver, 1998).

Some investigators support the concept that dietary calcium intakes and exercise patterns play important roles in the development of PBM during the early to mid-periods of adolescence. In young adult males, environmental factors appear to influence bone development as well as bone maintenance after completion of PBM. During the early years of the third decade of life in males, the level of dietary calcium has a significant

impact on radial bone mass. During middle adult life other environmental factors may affect the loss of bone mass in males (Anderson & Polltzer, 1994).

Although modest differences in nutrient intakes exist between blacks and whites in the USA, these differences cannot explain the greater bone mass of blacks. It is especially noteworthy that blacks have a significantly lower calcium intake throughout the lifecycle (Anderson & Polltzer, 1994). Minerals and trace elements other than calcium are involved in skeletal growth, some of them as matrix constituents, such as magnesium and fluoride, others as components of enzymatic systems involved in matrix turnover, such as zinc, copper and manganese. Vitamins also play a role in calcium metabolism (e.g. vitamin D) or as co-factors of key enzymes for skeletal metabolism (e.g. vitamins C and K)(Branca & Vatuena, 2001).

It has been suggested that lactose malabsorption may contribute to osteoporosis, either through a direct effect or because lactose intolerant people tend to consume less calcium from dairy products, but the evidence is inconsistent. In a study the association between lactose malabsorption and lower bone density were reported (Honkanen *et al.*,1996). This factor should not be overlooked because our sample group could be more prone to lactose intolerance.

There are different aetiological factors in black men but alcohol abuse appears to be important in the pathogenesis of fracture. Alcohol is a strong inhibitor of bone formation and is toxic to bone cells (osteoblasts) (Schnaid *et al.,* 2000). Chronic alcoholism leads to lower BMD and higher fracture risk due poor nutrition and malabsorption of critical nutrients, particularly calcium, magnesium and zinc, abnormal vitamin D metabolites and parathyroid function. Increased risk to fall thereby increases chances for fractures (Ilich & Kerstetter, 2000). Alcohol increased urinary calcium concentration (Cohen & Roe, 2000).

Vitamin C is required for the synthesis of type I collagen (the main organic compound of bone), for the subsequent extracellular modifications that allow formation of collagen crosslinks, and for the synthesis of other important matrix constituents, such as glucosamineglycans. Patients affected by scurvy are also osteoporotic. The anti-oxidant role of vitamin C might also be important to modulate skeletal metabolism (Branca &

Vatuena, 2001). The administration of ascorbic acid in black subjects with siderosis, vitamin C deficiency and osteoporosis significantly reduced urinary calcium excretion. While this could be a direct renal effect, it was more likely to be a reflection of improved calcium retention due to increased bone formation and decreased bone resorption induced by ascorbic acid repletion. It was concluded that osteoporosis is not primarily a disease of calcium deficiency. In this study the evidence rather suggests that the bone disease was due to ascorbic acid deficiency, and that changes in calcium metabolism are secondary (Lynch *et al.*, 1970).

Dietary salt is claimed to be the main determinant of urinary calcium excretion. Although studies have shown a relationship between increased sodium intake and increased urinary hydroxyproline, no consistent effect has been seen with respect to the more reliable biomarkers of bone resorption (urinary pyridinoline and deoxypyridinoline) (Cohen & Roe, 2000). Some of the studies indicate poor correlation between dietary sodium and urinary calcium execretion, no correlation with PTH, calcitriol and calcium absorption and no change in serum calcium. The conclusion from review studies show that the relationship between salt intake and osteoporosis is still controversial, and that the possible relation between salt intake and fracture risk should be addressed in future research (Burger *et al.*, 2000).

Too little or too much protein in the diet can adversely affect the calcium balance. Hip fractures are more common in people with low energy intake, low serum albumin and muscle weakness (Rizzoli *et al.*, 2001). Protein, especially animal protein, increases urinary calcium loss and because it does not increase calcium absorption itself, protein produces an unbalanced additional loss of calcium (Heaney, 2000). Urinary calcium excretion increase by 0,85mg per day for each extra gram of protein ingested. Whether this effect results in actual negative calcium balance depends heavily on the amount of calcium in the diet. It will be more useful to evaluate the protein-calcium ratio in the diets (Heaney, 1998). High plant diets have an alkaline load and have been proposed as a major factor in overall calcium balance (Massey, 1998).

In conclusion dietary calcium is important especially in the pre-puberty stage of life to ensure optimal peak bone mass. It is necessary to meet the RDA recommendations during the life stages. Except for calcium other factors such as exercise, genetic factors

and other vitamins and minerals are necessary for calcium metabolism and optimal nutrition play an important role in bone health. High alcohol, salt and protein intake may have a negative effect on calcium status in the body.

2.6.6 Vitamin D status

Vitamin D is involved in bone and calcium metabolism, having effects on calcium absorption from the intestine and resorption from the kidney. Vitamin D is obtained from two sources: dietary intake and cutaneous production. Vitamin D exist in two forms vitamin D2 and vitamin D3. All vertebrates, including humans, obtain most of their daily vitamin D requirement from exposure to sunlight. During exposure to sunlight, the solar ultraviolet B photons penetrate into the skin where they cause the photolysis of 7-dehydrocholesterol to precholecalciferol. Once formed, precholecalciferol undergoes a rearrangement of its double bonds to form cholecalciferol (Holick, 1995a). Once vitamin D3 is formed in the skin or ingested in the diet, it must be hydroxylated in the liver and kidney to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Figure 2.7 illustrates vitamin D metabolism in the body. There is evidence that 25-OH-vit D, the major circulating form of vitamin D, is directly metabolised in prostate, breast, colon and skin cells to its active form $1,25(OH)_2D$ (Holick, 2000). The vitamin D active form, $1,25(OH)_2D$, stimulates intestinal calcium absorption and mobilizes stem cells to mobilize calcium stores from bone (Holick, 1995b). Factors that strongly influence the cutaneous production of vitamin D are: melanin pigmentation, latitude, time of day, sunscreen use and ageing (Holick, 2000). An increase in skin pigmentation, ageing and the topical application of a sunscreen diminishes the cutaneous production of cholecalciferol. Latitude, season and the time of day as well as ozone pollution in the atmosphere influence the number of solar ultraviolet B photons that reach the earth's surface, and thereby, alter the cutaneous production of cholecalciferol. It is now recognised that vitamin D insufficiency and vitamin D deficiency are common in elderly people, especially in those who are infirm and not exposed to sunlight or who live at latitudes that do not provide them with sunlight-mediated cholecalciferol during winter months. Vitamin D insufficiency and deficiency exacerbate osteoporosis, cause osteomalacia, and increase risk of skeletal fractures (Holick, 1995a). People with increased skin pigmentation have decreased body levels of vitamin D (Scragg *et al.,* 1995).

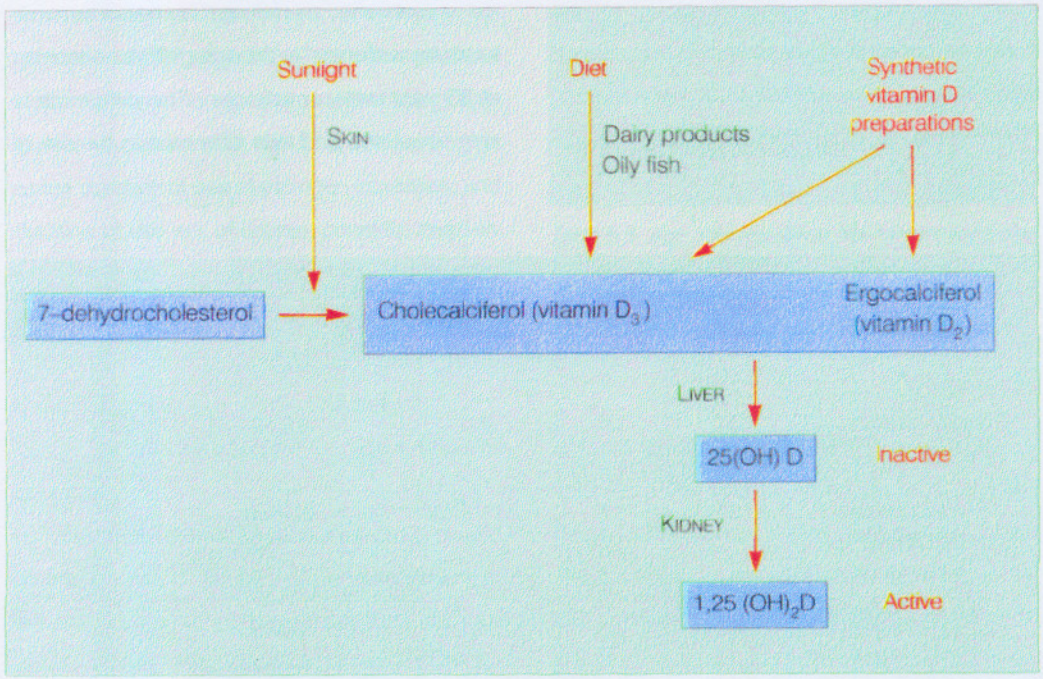

Figure 2.7: Illustration of vitamin D metabolism in the body (Arden & Spector, 1997).

African-Americans are at higher risk of vitamin D deficiency because of reduced skin synthesis of vitamin D precursors. Harris *et al.* (2000) found a lower mean plasma 25-OH-vit D and a higher mean PTH in black than in white older men and women (64 years to 100 years) living in Boston. Low 25-OH-vit D concentrations clearly contributed to reduced serum calcium and increased PTH in both blacks and whites. PTH increases more rapidly with age in blacks than in white adults (Harris *et al.,* 2000). The parathyroid glands of healthy blacks are larger than those of healthy whites, and this difference is not explained by differences in body size. In the study they demonstrated that the PTH difference is not entirely due to differences in current vitamin D status, but their data does not provide an explanation for the residual difference. It may be due to a relatively lower skeletal sensitivity to the resorption for the residual differences in calcium and sodium intake and handling. Another interesting suggestion is that long-term vitamin D deficiency may cause parathyroid hyperplasia that results in elevated PTH even after vitamin D status has been improved. It will be important to test theories because elevated PTH has been linked not only to bone loss, but also to hypertension, a condition that is highly prevalent in elderly blacks (Harris *et al.,* 2000).

A study describing the prevalence of hyperparathyroidism in black and white subjects concluded that 38 % of the 144 black subjects and 20 % in the 111 white subjects had hyperparathyroidism. Over short periods and in younger adults, an acute increase in PTH causes less skeletal calcium release in blacks than in whites. But in elderly blacks the effect of elevated PTH on bone turnover and bone density are as strong or stronger than in whites of similar age and socio-economic background. This indicate that the adaptive skeletal response of blacks is reduced with ageing or that it is reduced with sustained hyperparathyroidism, but long–term studies would be needed to clarify these hypotheses (Harris *et al.*, 2001). Blacks have decreased skeletal sensitivity to PTH. It has been suggested that 30 % of the inter-racial variation in BMD between Caucasian and Africans might be explained on the basis of polymorphism in the vitamin D receptor gene (Hough, 1998).

When compared to whites, blacks have a lower urinary calcium and phosphorus secretion (Wright *et al.*, 2002). Results of some but not all studies provide evidence for secondary hyperparathyroidism in normal young black adults. Bell et al found an increase in serum PTH, circulating $1,25(OH)_2D$ and urinary cyclic adenosine 3'5'-monophosphate (cAMP) and lower urinary calcium, phosphate, potassium and magnesium in blacks. Urinary sodium and creatinine clearance were the same in black and white men. Serum-Gla protein (bone marker) increased in response to higher PTH, but was lower in the blacks than in the whites despite increase PTH. They hypothesised that intestinal absorption of calcium is enhanced in blacks because of increased circulating $1,25(OH)_2D$, the major determinant of intestinal absorption of calcium in man. On the other hand the mean serum 25-OH-vit D was lower in blacks than in whites. This difference could result from feedback inhibition of hepatic synthesis of 25-OH-vit D by the increased circulating $1,25(OH)_2D$ in the blacks. A more likely explanation could be the damping effect of skin pigmentation on dermal synthesis of vitamin D from 7-dehydrocholesterol because of absorption of photons of light energy by skin pigment in the blacks. This would then indicate that the vitamin D endocrine system is ideally programmed to spare the skeleton (Bell *et al.,* 1985). Four studies done by Bell et al. (1993), Abrams *et al.* (1995), Bryant *et al.* (2000) and Pratt *et al.* (1996) indicate that intestinal absorption efficiency was higher in three of the four studies of black children and adolescents, but has not been found to be appreciably different from that of Caucasians in adults. In all of these studies, serum calcium was not appreciably different

between the races (Bell *et al.,* 1993; Abrams *et al.*,1995; Bryant *et al.,* 2000; Pratt *et al.,*1996). The higher PTH, with its expected effects on nephrogenous cAMP and renal calcium clearance, is nevertheless not producing an elevation of serum calcium in blacks (Heaney, 2002). 25-OH-vit D was unrelated to body mass index, serum lipids, blood pressure or cigarette smoking, but people doing vigorous (aerobic) leisure physical activities and moderate activities had a higher 25-OH-vit D level (Scragg *et al.,* 1995).

Smoking has a significant effect on calcium and vitamin D metabolism. Smokers had small but significant reductions in bone mineral density when compared to non-smokers. Smokers (510 healthy Danish women) had significantly reduced levels of serum 25-OH-vit D, $1,25(OH)_2D$ and PTH. It may seem paradoxical that, whereas $1,25(OH)_2D$ levels and BMD were inversely related to each other, smokers had at the same time decreased $1,25(OH)_2D$ levels and BMD when compared to non-smokers. Brot *et al.* 1999 hypothesised that it was a compensatory mechanism for an underlying cause leading to decreased bone mineralization, like for instance low calcium intake. This may in part account for the decreased bone mass and increased fracture risk seen among smokers later in life (Brot *et al.,* 1999). The finding of lower 25-OH-vit D concentrations in smokers in the Boston study is consistent with previous report in humans and may be the effect of nicotine (Harris *et al.,* 2000).

In conclusion blacks have higher PTH and $1,25(OH)_2D$, with lower 25-OH-vit D and urinary calcium. The high $1,25(OH)_2D$ is responsible for better calcium absorption. The low 25-OH-vit D might be because of increased skin pigmentation and lower vitamin D 3 production. It seems that blacks have a decreased skeletal sensitivity to PTH, but in the elderly blacks the influences of high PTH are as strong as in whites on bone turnover and bone density. The reasons may be that the adaptive skeletal response of blacks is reduced with ageing or that it is reduced with sustained high PTH. Long–term studies would be needed to clarify these hypotheses.

2.6.7 Ethnicity

The higher bone fracture rate in whites compared to blacks in the United States may be related to lower bone density (Daniels *et al.,* 1995). According to a study done by Solomon in 1979, bone density reaches a peak about fifteen years later in African men

than in Caucasian men. Bone loss during the period between 30-50 years is not so rapid in Africans than in Caucasians (Solomon,1979).

American whites have a higher bone turnover than American blacks and this may be associated with a loss of bone in adults. In contrast to the American data, South African blacks may have a higher bone turnover and lower bone density than whites (Daniels *et al.,*1995). When compared to Caucasians, South African blacks had a lower rate of hip fracture, despite lower bone density at all ages (Villa, 1994). The lower fracture rate in African Americans compared to whites is because of greater bone mass, skeletal modelling and because the mineral apposition rate is also lower (Bell, 1997).

Peak bone density and radius bone density was similar in blacks and whites even though blacks reach their peak bone mass at a later age than whites. The later sexual maturation that was observed in black compared with white subjects may partially account for this delay in peak bone density in blacks. The ethnic difference in femur bone density only became apparent in the fourth decade. This suggests that the factors responsible for the higher peak femur bone density in blacks exert their influence during early adulthood. Weight accounted for 24 % of the variation in peak femur bone density in blacks whereas it was not associated with peak femur bone density in whites (Daniels *et al.,*1995).

Schnitzer et al. (1996) found that South African blacks had, apart from a greater volume of trabecular bone and thickness, higher values for osteoid and erosion variables than whites. The authors postulate blacks may therefore have greater bone turnover leading to more frequent renewal of fatigue-damaged bone. This would result in better bone quality which, together with sturdier bone microarchitecture, would make blacks less prone to fatigue fractures (Schnitzer *et al.,* 1996).

Black children and adults excrete less urinary calcium than whites on essentially the same diets and consequently retain more calcium in their skeletons. Better calcium retention is commensurated with the faster rate of bone growth in black children (Anderson & Pollitzer, 1994). The issue of the calcium requirement in blacks is correspondingly less clear. The intake of calcium is lower in black populations than in white populations. Bone density seems to be lower but the differences tended to

disappear when statistical adjustments were made for height and weight, suggesting that some of the black deficit was due to growth stunting, possibly reflecting the generally poorer nutrition of blacks in these African countries (Heaney, 2002).

The skeletal advantage in blacks during young adulthood is not explained by bone size (Henry & Eastell, 2000). Black men have a higher BMD and lower incidence of osteoporosis and fractures than whites. Serum 17 beta-estradiol level, growth hormone and BMD values for the total body, forearm, trochanter and femoral neck are greater in black than in white men. As oestrogen is known to increase growth hormone secretion and growth hormone to increase bone mass, increases in circulating 17 beta-estradiol may contribute to the higher growth hormone secretion and bone mass in black men (Wright *et al.*, 1995). Blacks have a greater skeletal calcium content, but also greater total body potassium and muscle mass (Pollitzer & Anderson, 1989).

In conclusion, South African blacks reach their peak bone mass fifteen years later than whites. Bone loss between ages 30-50 years is not so rapid in black as in Caucasian men. South African blacks have a thicker and greater volume of trabecular bone as well as higher bone turnover leading to more frequent renewal of damaged bone, this is in contrast with African Americans. Blacks excrete less urinary calcium and lower bone density was found in black children but if statistical adjusted for height and weight were made, differences disappeared. Blacks' lower fracture rate may not be related to low bone density but higher bone mass.

2.6.8 Genetic risk factors

Genetic factors are responsible for an estimated 70 – 80 % of bone mass during the first 20 years of life, the remainder is determined by environmental and lifestyle factors including nutrition. These estimates are based on different lines of research, mostly on white female subjects. No known estimates have been published on black, Asian and other ethnic groups (Anderson & Pollitzer,1994).

The study of Gilsanz et al. (1991) showed that puberty is the crucial time of bone formation in both black and white children and in particular, supports the strong contribution of genetic determinants of increases in BMD during pubertal development,

independent of dietary intake. Genetic control of osteoid synthesis, rather than intestinal calcium absorption and/or renal reabsorption of calcium, must be considered as the dominating factor governing adolescent bone development (Gilzanz *et al.,* 1991).

The mechanisms responsible for black-white differences in bone mass have not yet been elucidated, but two possible explanations have been put forth. One deals with calcium and bone metabolism, and the other with reproductive hormones. Bell and his co-workers (1991 and 1992) support the concept of a difference in calcium-regulating hormones that favours retention of calcium in blacks. These researchers showed that black children 7-13 years old had a lower urinary calcium excretion than whites (Bell *et al.,* 1991; Bell *et al.,* 1992). Bell *et al.* (1985) found a lower plasma concentration of 25-OH-vit D, a higher concentration of parathyroid hormone, and a lower urinary calcium excretion in older black women than in whites (Bell *et al.,* 1985). Also, there were indications that blacks have a higher threshold for $1,25(OH)_2D$ action on the intestine, resulting in a lower efficiency of calcium absorption. Although these studies, on the control of calcium metabolism, do not fully explain black-white differences in bone mass, they support the concept, based on evidence for a lower blood concentration of osteocalcin and a lower urinary excretion, that lower bone turnover by blacks contributes to the accumulation of a greater bone mass (Anderson & Pollitzer, 1994). A report by Peacock *et al.* (1992) suggests that genetic factors are operative in the regulation of several aspects of calcium metabolism, including intestinal calcium absorption and parathyroid hormone secretion, and thereby may have an effect on bone density. The genes that govern these processes, however, have no known serum markers (Peacock *et al.,* 1992). The genetic regulation of vitamin D-binding proteins and the calcium-binding proteins, especially those found in the small intestinal mucosa, recently has been identified. Over the next several years it can be anticipated that new genes related to bone tissue will be identified that will enhance our understanding of the role of hereditary factors in bone development (Anderson & Polltzer, 1994).

Osteoporosis can occur as the result of mutations in a single gene. Examples are the osteoporosis-pseudoglioma syndrome, caused by inactivating mutations in the lipoprotein receptor-related protein 5 gene and the high bone mass syndrome, caused by activating mutations of the same gene. A great deal of research has been done on candidate genes; among the best-studied are the vitamin D receptor and the collagen

type I alpha I gene. Polymorphisms of vitamin D receptors have been associated with bone mass in several studies, and there is evidence to suggest that this association may be modified by dietary calcium and vitamin D intake. An important problem with most candidate gene studies is small sample size, and this has led to conflicting results in different populations (Ralston, 2002).

In support of the hormone concept, Richards *et al.* (1992) found greater serum concentrations of oestradiol and lower levels of androstenedione in pubertal black males and females than in whites (Richards *et al.,* 1992). Buchanan and co-workers (1988) observed that higher levels of circulating androgens in females were associated with increased trabecular, but not cortical bone density. The implications of these differences in steroid hormone levels for bone mass are not yet clear, but higher oestrogen levels are considered to favour the retention of mineral by the skeleton of either sex (Buchanan *et al.,* 1988).

As reviewed by Anderson & Pollitzer (1994) studies on twins and parent-offspring pairs have provided much of our current knowledge of the contribution of hereditary and environmental factors to bone mass and density. Smith and co-workers(1973), Christian and colleagues (1989), Pocock *et al.* (1987), Eisman *et al.* (1991) and Slemenda *et al.* (1992) (as reviewed by Anderson & Pollitzer) have found a significantly higher correlation between bone density at different skeletal sites in monozygotic twins than in dizygotic twins and less intrapair variance in bone measurements. In a continuation of a 16-year study on men by Christian *et al.* (1989) the value for radial bone mass correlation between monozygotic twins continued to be much greater than that for dizygotic twins (Anderson & Pollitzer, 1994). Studies in different sex twins did not indicate any significant difference in peak lumbar spine or femoral neck bone density between the sexes. It is now well established that there are strong familial and genetic factors that determine peak adult bone mass, with genetic factors estimated to contribute around 80 % of the total population variance in bone mineral density (Sambrook *et al.,* 1993).

Little information exists on the genes that determine differences in bone mass, density and loss among individuals and ethnic and racial group. To date only one gene has been identified for a specific bone molecule, namely the one for osteocalcin or bone glutamic

acid protein, which behaves as an autosomal dominant gene. The occurrence of a specific gene for osteocalcin was predicted by Kelly and co-investigators (1991) on the basis of measurements of blood concentrations of osteocalcin in monozygotic and dizygotic twins. Another bone protein, alpha2-HS-glycoprotein, has also been reported to be under strong genetic influence. Other bone markers that may be under genetic control are bone-specific alkaline phosphatase, collagen cross-link pyrilinodine peptides, tartrate-resistant acid phophatase, and other bone proteins such as osteonectin and bone sialoprotein II (Anderson & Pollitzer, 1994).

With low dietary calcium intake, the response to physical activity is small and there is little difference between those with high and low genetic potential. As dietary calcium increases, the ability to increase bone mineral density in response to exercise is increased and can augment the underlying genotype. With low levels of physical activity, the effect of dietary calcium intake and genetic potential may be masked. As physical activity increases, the ability to increase bone density is determined by dietary calcium intake and genetic potential. A threshold effect will be reached in individuals with high calcium intakes and high levels of physical activity. Further increases in those parameters would have little additional effect (Sambrook *et al.,* 1993).

In conclusion, genetic factors are responsible for 70 – 80 % of bone mass during early life. The mechanisms responsible for black-white differences in bone mass have not yet been elucidated. Factors that might contribute to greater bone mass in blacks are: lower urinary calcium excretion, higher PTH levels and lower skeletal sensitivity to high PTH levels, lower 25-OH-vit D concentration and higher threshold for $1,25(OH)_2D$. Higher concentrations of hormones such as androgens in females and estrogen in both sexes are considered to favour retention of minerals by the skeleton. A great deal of research has been done on genes effecting the vitamin D receptor, over the next several years it can be anticipated that new genes related to bone tissue will be identified and that osteoporosis might be caused by a singly gene.

2.7 A summary of the aims of the study

One of the aims of this study was to describe the relationship between low dietary calcium intakes and bone density in black South African men. Dietary calcium intake

plays an important role in bone health, despite the fact that blacks' calcium intake is 10 - 30 % lower than whites' their bone mass is greater and rate of bone loss is slower. There are differences in sensitivity of the effector mechanisms in different racial groups. The only logical explanations for those disparities are that Africans may utilize dietary calcium more efficiently than Caucasians. Black children and adults excrete less urinary calcium than whites on essentially the same diets and consequently retain more calcium in their skeletons. Better calcium retention is commensurate with the faster rate of bone growth in black children (Anderson & Pollitzer 1994). Blacks have a greater skeletal calcium content, but also greater total body potassium and muscle mass (Pollitzer & Anderson, 1989). High calcium intake seems not to be the solution.

The second aim of the study is to describe the relation of low vitamin D intake and bone density in black South African men. Blacks have higher PTH and $1,25(OH)_2D$, with lower 25-OH-vit D and urinary calcium. The high $1,25(OH)_2D$ are responsible for better calcium absorption. The low 25-OH-vit D might be because of increased skin pigmentation and lower vitamin D 3 production. It seems that blacks have a decreased skeletal sensitivity to PTH, but in the elderly blacks the influences of high PTH are as strong as in whites on bone turnover and bone density. The reasons may be that the adaptive skeletal response of blacks is reduced with ageing or that it is reduced with sustained high PTH. Long–term studies would be needed to clarify these hypotheses.

The third aim of this study was to describe the relationship between physical activity and bone density in lack South African men. Physical activity has different effects on bone depending on its intensity, frequency, duration and the age at which it is started. If exercise is decreased, the rate of bone loss will increase for a period of time, perhaps as long as a year, until a new steady state is achieved (Anderson & Pollitzer, 1994). In conclusion, South African blacks reach their peak bone mass fifteen years later than whites. Bone loss between ages 30-50 years is not so rapid than in Caucasian men. South African blacks have a thicker and greater volume of trabecular bone (greater bone mass) as well as higher bone turnover leading to more frequent renewal of damaged bone. Blacks excrete less urinary calcium and lower bone density were found in black children but if statistical adjustment for height and weight were made, differences disappear. A strong hereditary component (70 %) is related to the development of bone mass and contribution of environmental factors is about 30 % (Luckey *et al.*, 1996).

Much of the research on mineral homeostasis and bone mass was done on white women and men. Other studies that focused on black subjects have been conducted on African-Americans in the USA. The origins of the majority of African Americans are from West Africa. Do the black people of South Africa have similar origins, and thus have similar genetic backgrounds? Should we apply data obtained from African-Americans to the Black Population in South Africa? The bone homeostasis in black South African males is an area of research that offers many exciting opportunities. The following chapter will focus on the methods used in this present study.

Chapter 3

Method

3.1 Introduction

The present study was a case-control study, where different hypotheses were tested on the same subjects. One part investigated the influence of calcium, vitamin D status and physical activity on bone fracture in black South African men and the other part, reported separately by M Leach, iron overload, hipovitaminosis C, tobacco and alcohol on bone fracture in black South Africa men. To our knowledge, this is the first case-control study on osteoporotic fractures in South African black men.

3.2 Method

In this chapter general methodology and details regarding experimental methods used in the study of the influence of calcium, vitamin D and physical activity on bone density will be discussed.

The duties of the researcher (registered dietician) were to complete the three questionnaires with the case and control group. To recruit subjects for the control group. Measure height, weight and body fat percentage of the subjects. To collect all data and analysed it on computer programs. Captured the data into an excel computer program before it was statistically analysed.

3.3 Settings

A case control study design was used. Subjects used in the case group were from orthopedic wards in Pretoria Academic –and Kalafong Hospitals in Pretoria. The control group was from the same environment or community (Pretoria area), most of them were not admitted to the hospitals. The orthopedic wards of the two hospitals admit 12 000 patients per year.

3.4 Ethical approval

The Ethics Committee of the Medical Faculty of the Pretoria University approved the protocol and gave the ethical approval for human immunodeficiency virus (HIV) testing, after the head of the orthopedics department of the Pretoria University discussed and motivated the reasons, counselling was given to each subject in compliance with the guidelines of the Department of Health. Pre-test and post-test counselling was done with each individual. The objective of pre-test counselling is to ensure that any decision to take the test is fully informed and based on an understanding of personal, medical, legal, social and psychological implications of a positive result. All the subjects signed an informed consent form before the tests were done. The Constitution of SA, Act no 108 of 1996, stipulates that all persons with human immunodeficiency virus/Acquired immunodeficiency syndrome (HIV/AIDS) have the right to privacy, including privacy concerning their HIV/AIDS (WHO Aids Centre, SAMR, 2000).

3.5 Subjects

Thirty-two black men were included in the study, 16 in a case group and 16 in a control group. Black men aged 40 years or more, with fractures of the proximal femur, the proximal humerus or the distal radius were included in the study. An equal number of age-matched healthy black men (with no more than 5-year age difference) with no fracture (the proximal femur and humerus and distal radius) previously, was recruited as a control group. An amount of R100,00 cash was paid to the control group, to cover their expenses.

Inclusive criteria were as follows:

1. Patients older than 40 years.
2. Fractures of the proximal femur, humerus and distal radius caused by minor trauma such as falls.

Exclusion criteria for the case group were as follows:

1. Men with pathological fractures caused by serious trauma such as an accident on duty, where an arm or leg was broken by heavy objects.
2. Multiple trauma patients.

3. Patients with identified chronic diseases such as tuberculosis, Acquired immunodeficiency syndrome metabolic disorders, disorders of the thyroid, etc.
4. Patients on any medication with the potential of influencing bone mineralization, including calcium salts, bisphosphinates, vitamin D and fluoride.
5. Mentally disturbed individuals.

3.6 Study design

All the black male patients admitted to the hospitals between 2001-05-01 and 2002-09-30 were screened by a general practitioner and then included in the study if they met the criteria. The following procedures were performed on each patient after 3-4 days in hospital. Blood samples were collected under the supervision of the general practitioner and sent to registered pathology laboratories in Pretoria where the biochemical analyses were done. Bone mineral density was measured in the lumbar spine and femoral neck at the Pretoria East hospital. The Dexa method was used under the supervision of a physician at the osteoporosis clinic. The control group consisted of healthy black men of the same age as the case group and was recruited by registered dieticians. The same procedures were followed for samples collected and analyses performed in the control group.

3.6.1 Dual energy x-ray absorptiometry

The Dexa technique used two different energy levels to measure the bone mineral content of the body. X-rays are used as the energy source. The increased output of energy with a x-ray tube has led to increased resolution, improved precision, and reduced scanning time. Dexa measures the density of the skeleton. In addition, the bone area can be determined from the image by identifying each pixel as either bone, fat, or lean tissue, thus allowing a calculation of bone mass in grams. Because the scan is obtained in two dimensions, certain assumptions regarding bone geometry are made within the computer software. The total body scans provide measurements of total bone mineral content, bone area, and therefore bone mineral density but also measure fat and lean mass as well, if the software is adapted (Specker, 1999). The Dexa scan at Pretoria East could not measure fat percentage. The Dexa scans were done on the patients 3-4 days after admission to hospital. Figure 3.1 shows how the Dexa scans were done.

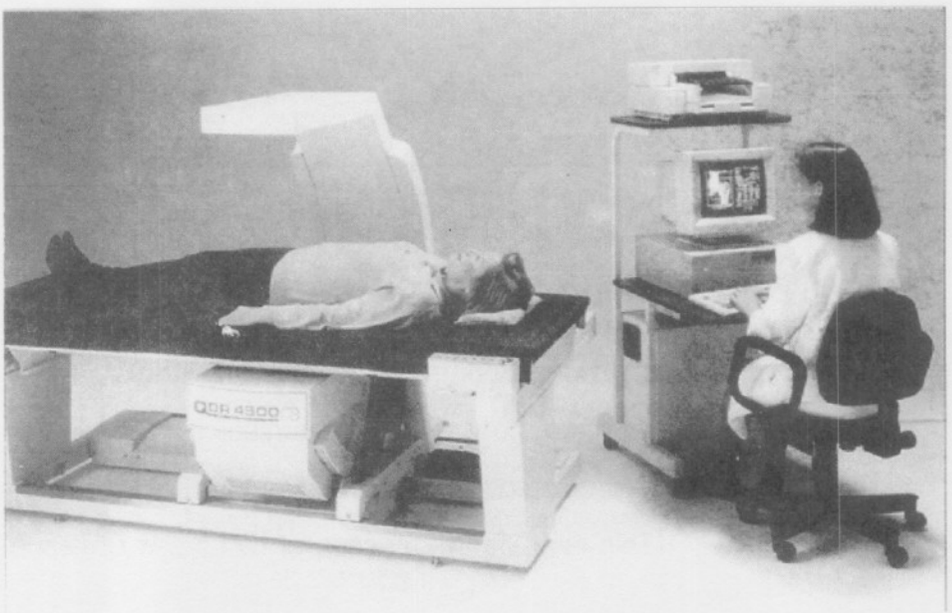

Figure 3.1 shows how the Dexa scan was done:

3.6.2 Structured questionnaires

The three structured questionnaires (dietary information, physical activity and demographic questionnaires) were completed on day 3-5 after injury during individual interviews in the language of the subject's choice with translators if necessary. Registered dietitians did the individual interviews with the subjects to complete the questionnaires. The three questionnaires completed were firstly for demographic information, including type of cooking utensils, alcohol and smoking habits. The second questionnaire includes dietary information, data was obtained with quantitative food frequency questionnaire during individual interviews. Habitual dietary intakes were measured by taking a dietary history in combination with a validated food frequency questionnaire (Macintyre *et al.,* 2001a). A laminated portion-photo book was used to reflect portion sizes (Venter *et al.,* 2000). The questionnaire was developed for use in South African inter-ethnic populations (Macintyre *et al.,* 2001b). It was previously validated against 7 day weighed records and reproducibility tested. The data provided by the questionnaire was analysed on a computer programmed based on the South

African Food Composition Tables (Food Finder, MRC 2000). Physical activity information was obtained with a third quantitative questionnaire measuring physical activity as well as daily tasks such as climbing of stairs, lifting heavy objects, traveling etc. Since little is known about the physical status and health of black South Africans there was a need for an instrument to measure physical activity in this population. This physical activity questionnaire was based on the Baecke questionnaire (Baecke et al., 1982). Adaptations were made by Kruger and co-workers to be more appropriate for South Africa populations (Kruger *et al.*, 2000). The reliability and reproducibility of the questionnaire was assessed by test-retest design. The significant positive correlations between physical activity index score and energy cost of physical activities from the 24-hour recall confirm the conclusion that this questionnaire is valid (Kruger *et al.*, 2000). See appendix A for the three questionnaires.

3.6.3 Anthropometry

The height and weight of the subjects were taken by registered dieticians with them being bare foot and wearing light indoor clothing. The body fat percentage and body mass index were measured by a Tanita body composition analyser (bioelectrical impedance analysis), model TBF 300 (Tokyo, Japan). The Bioelectrical impedance analysis is based on the principle of resistance to an electrical current that is applied to the body. The greater the water content, the greater the body density. Early research with bioelectrical equipment revealed large standard errors in predicting lean body mass, so it was not considered to be usually not valid. However, body composition researchers have developed better techniques and prediction equations with lower standard errors (3 – 4 %), comparable to skin fold techniques. Researchers recently noted that BIA is a good practical method to assess body composition (Williams, 1999).

3.7 Blood sampling

The following blood values were measured and the experimental methods are summarized in Table 3.1.

***Table 3.1*: Blood values and experimental methods**

Variable	Analytical system	Experimental method
Serum albumin	Beckman Access Immunoassay system from Beckman, Beckman Coulter, delta CX76766541	The Beckman LX 20 analyzer was used with the SYNCHRON LX system to determine the albumin concentration. This system determines albumin concentration by means of a bichromatic digital endpoint methodology using bromcresol purple (BCP) reagent.
Total lymphocyte count	Advia 120, Bauer IRO 3349811	This count was done on the Advia 120 Haemocytometer
HIV status (if the patient gave consent)	Abbot Axsym Immunoassay system, Abott 1836	The Abbott axsym analyzer was used to determine the HIV status with an ELISA method, with positive or negative result.
Serum calcium	Beckman Access Immunoassay system Beckman Coulter, delta CX76766541	The Beckman LX20 analyzer was used with the SYNCHRON LX system to determine total calcium concentration by indirect potentiometry utilizing a calcium ion selective electrode in conjuction with a sodium reference electrode.
Serum phosphate	Beckman Access Immunoassay system Beckman Coulter, delta CX76766541	The Beckman LX20 analyzer was used with the SYNCRON LX system to determine total phosphate concentration.
Serum vitamin D	Radio immuno-assay, DiaSorin for the United States of America	A radio immuno-assay was used to measure vitamin D and the test is done with a nickle kit.

The blood sample was taken using a 21-gauge needle infusion set without exerting pressure on the vein. Non vasting venous blood, 14 ml in total, was drawn after the area was cleaned. The gel barrier red top tubes were filled with 10ml blood, allowed to clot for 30 minutes and marked with generic labels provided and sent ambiently (immediately in cool bag) to the pathology laboratories. Another 4.5ml blood sample was also collected in lavender top tubes coated with anticoagulant ethylenediaminetetraacetic acid (EDTA). The EDTA tube was filled completely and gently inverted for 5 times immediately after collection to avoid clotting. The EDTA tubes were marked with generic labels provided and sent ambiently to pathology laboratories. From the gel barrier red top tube the following blood values were measured serum albumin, HIV status, serum calcium, serum phosphate and serum vitamin D. The total lymphocyte count was measured from the EDTA tube.

3.8 Statistical analyses

The Statistica® computer programme was used to carry out the statistical analyses. Descriptive statistics were performed for each group. The case and control group descriptive statistics of normally distributed variables are reported as means and 95% confidence intervals (CI). Non-normally distributed variables are reported as medians, 75 and 25th percentiles. Stepwise multiple regression is the method used to study the relationship between the variables in the hope that any relationship that is found can be used to assist in making estimates or predictions of a particular variable (Hoel & Jessen, 1984).

T-tests were performed to test for differences in continuous variables between cases, for normal distributed data. The Mann-Whitney test was performed to test for differences between cases for non-normally distributed data. The Pearson correlation coefficients were used to describe the relationship between weight and BMD while controlling for possible confounding factors (age, pack years, physical activity index, energy, calcium). Multiple regression analyses were performed in an attempt to find the best set of predictors for BMD. A p value of less than 0.05 was considered to be of statistical significance. The following chapter will be the presentation of the results of the study.

Chapter 4

Results

4.1 Introduction

In this chapter a summary of all the characteristics of the study participants is given. The mean habitual daily nutrient intakes and mean blood values of the cases and controls are described and compared to desirable levels. The results with regard to the influence of age, body weights, activity, calcium- and vitamin D status on fractures and BMD are reported in the 32 subjects.

4.2 Descriptive statistics

The characteristics of the case and control groups are given in Table 4.1. The mean age of the case group was 66.2 years and the control group 63.5 years (5 year age match control). The BMI of the case group ranged between 14.9-29.4 kg/m^2 (mean 20.4) and the control group's BMI ranged between 14.2 – 28.3kg/m^2 (mean 20.4). The normal range for BMI in men is 19-24kg/m^2 (Williams, 1999). The BMI of 8 subjects was higher than the normal and 16 subjects were lower than normal in both groups. The normal range for percentage of total body fat for men is between 6 % and 15 % (Williams, 1999). The mean fat percentage of the case group was 16.7 ranging between 3.7% and 33.2%. In the control group the mean fat percentage was 15.0 % ranging between 4.2% and 30.5%. Due to the nature of their injuries three subjects in the case group could not stand on the Tanita scale to measure fat percentage. Only one subject in the case group and two in the control group had a fat percentage below 6 %. Five subjects in the case group and six in the control group had a fat percentage higher than 15 %. The bone mineral density was lower in the case group than in the control group at all the sites (L2-4 and hip), but the difference did not reach statistical significance. The differences in age, BMI and body fat percentage were not statistically significant between the two groups, because the p-values were higher than 0.05. Physical activity levels were low in both groups, with a physical activity index lower than 4.0 (on a scale

of 1.0-10.0). The control group (mean = 2.60) were significantly more active than the case group (mean = 1.93) ($p<0.005$).

The mean 25-OH-vit D ($p=0.04$) and lymphocyte ($p=0.01$) levels were statistically significantly higher in the control group than in the case group, but within the normal range for both groups. The median for serum calcium and phosphate was higher in the case group than in the control group. Serum albumin was significantly lower in the case group compared to the control group ($p<0.005$). The median for serum albumin levels was below the normal range in the case group. Serum calcium and phosphate were within the normal range for both groups. Serum calcium was significantly higher in the case group than in the control group ($p=0.01$).

In both groups the education levels were low. Only two subjects had an education higher than grade 7. Most of the subjects received government pension of R500,00 per month while only eight of them were employed, with the highest income of R3000,00 per month (data not shown).

Table 4.1: **Descriptive statistics of participants in the trial groups**

		Cases			Controls			
Variable	Reference range	N	Mean	95%CI	N	Mean	95%CI	p-value
BMD L2-4 (g/ cm^2)	1.2-1.4g/cm^2	16	0.88	0.81, 0.95	16	0.95	0.89, 1.00	0.13
BMD hip (g/ cm^2)	1.0-1.5g/cm^2	15	0.86	0.76, 0.97	16	0.91	0.85, 0.97	0.39
PAI	1.0-10.0	16	1.93	1.58, 2.27	16	2.60	2.28, 2.91	<0.005*
BMI (kg/m^2)	19-24kg/m^2	16	20.4	18.2, 22.7	16	20.4	17.8, 22.4	0.79
Body fat %	6-12%	13	16.7	10.8, 22.6	16	15.0	11.1, 18.9	0.82
25-OH-vit D ng/mL	9.00-37.6	12	14.8	10.0, 19.4	16	20.1	10.1, 31.8	0.04*
Lymphocytes %	20.0-45.0	16	24.7	17.3, 32.0	16	36.9	18.0, 56.9	0.01*
Variable	Reference range	N	Median	25, 75 th	N	Median	25, 75 th	p-value
Albumin (g/L)	32-51g/l	16	28.0	24.5, 33.5	16	37.5	36.0, 39.0	<0.005*
Serum calcium (mmol/L)	2.18-2.60	16	2.45	2.41, 2.54	16	2.38	2.34, 2.42	0.01*
Serum phosphate (mmol/L)	0.80-1.40	16	1.08	0.99, 1.21	16	0.95	0.89, 1.12	0.10

N = number of subjects, CI = confidence interval, BMD L2-4=bone mineral density of lumbar, BMD hip = Bone mineral density of hip, PAI = physical activity index, BMI= Body mass index, 25-OH-vit D= 25-hydroxy vitamin D3, 25^{th} and 75^{th}= 25 and 75 th percentile. Physical activity index between 1.0-4.0 inactive, 4.01-6.0 medium active and 6.01-10.0 very active. * Statistically significant p-value < 0.05

4.3 Habitual nutrient intake

The characteristics of the case and control groups are given in Table 4.2. The mean (9874kJ) energy intake of the control group was higher than the recommended (9660kJ) and higher than the case group. The mean (8431kJ) energy intake for the case group was lower than the recommended (9660kJ) (Williams, 1999). The difference in energy intake between the two groups was not statistically significant. Protein intake was higher in the control group (73.3g) than the recommended (63g) and in the case group (56.1g). The difference in the groups was statistically significant (p=0.05). Both groups had a higher intake of plant protein than animal protein.

The macronutrient distribution for fat (18.6 % of total energy) was lower than the recommended (30 % of total energy) and the carbohydrate (61,5 % of total energy) was higher than the recommended (55 – 65 % of total energy) in the control group. In the case group the macronutrient distribution was also lower for fat (17.0 % of total energy) and higher for carbohydrate (62 % of total energy) than the recommended (William, 1999). Although the control group tended to have a higher intake of macronutrients, distribution was similar in the two groups. The percentage for carbohydrate intake was within the normal limits for the control group and case group, but the fibre intake was lower than the recommendation of 30 gram per day (data not shown) (Williams, 1999). The macro-nutrients DRI (AMDR – acceptable macro-distribution range) recommendations for CHO are 45 – 65 % and protein 10 – 35 %. Fibre recommendation are the same as for the prudent diet (30 gram per day) (NICUS, 2003).

Mean calcium intakes were lower than the recommended adequate intake of 1200mg/day in both groups. The mean calcium intake was higher in the control group (504mg) than in the case group (361mg). Both groups' vitamin D intakes were lower than the recommended amount (10ug). The case group vitamin D intake (1.78ug) was higher than the control group (1.3ug) but the difference was not statistically significant. The adequate intake for calcium intake are the same as the RDA, 1200mg per day (NICUS, 2003).

Table 4.2: **Descriptive data for dietary intake.**

				Cases			Controls	
Variable	Reference range	N	Mean	95%CI	N	Mean	95%CI	p-value
Energy intake (kJ)	9660	16	8431	7076, 9784	16	9874	8054, 11694	0.19
Total protein intake (g)	63	16	56.1	46.5,65.7	16	73.3	58.3, 88.3	0.05*
Plant protein intake (g)		16	33.1	26.8, 39.4	16	38.8	28.7, 48.8	0.32
Animal protein intake (g)		16	22.5	14.7,30.4	16	30.0	19.0, 41.0	0.22
Total fat intake (g)		16	37.9	26.9, 48.9	16	46.6	36.2, 56.9	0.23
% Energy: fat	<30%	16	17.0	13.2, 20.8	16	18.6	14.7, 22.6	0.53
Carbohydrate intake (g)		16	303	257, 348	16	359	286, 431	0.17
% Energy: CHO	55-65%	16	62.0	57.3, 66.7	16	61.5	56.8, 66.2	0.86
Calcium intake(mg)	1200	16	361	258, 463	16	504	262, 745	0.47
Phosphorus intake (mg)	700	16	1031	851, 1211	16	1290	1006, 1575	0.11
Variable	Reference range	N	Median	25,75 percentile	N	Median	25,75 percentile	p-value
Vitamin D intake (ug)	10ug	16	1.78	0.91, 2.74	16	1.30	0.67, 2.31	0.41

N = number of subjects, CI = confidence interval and CHO=Carbohydrate

4.4 Correlations

Table 4.3 shows the correlation coefficients between lifestyle factors, nutrient intake biochemical variables and bone mineral density of the lumbar and hip regions. Correlations were used, because of several variables studied simultaneously and to determine the interrelationship between variables. Simple Pearson correlation coefficient between BMD and other factors as well as correlation coefficient adjusted for age, physical activity, pack years, energy intake, dietary calcium and BMI are reported.

A significant positive correlation between BMI ($p=0.05$), total fat intake ($p=0.04$) and lumbar spine BMD was found. Fat percentage and PAI showed a trend towards a positive correlation ($p=0.06$) with lumbar BMD. When adjusted for age, PAI, pack years, energy, dietary calcium intake and BMI statistically significant correlations between weight ($p<0.005$), body fat percentage ($p<0.005$), BMI ($p<0.005$) total fat intake ($p=0.04$), animal protein ($p=0.05$), dietary calcium ($p=0.03$) and lumbar spine BMD were found.

Significant positive correlations between weight ($p<0.005$), body fat percentage ($p<0.005$), BMI ($p<0.005$), total energy intake ($p=0.05$), total protein intake ($p=0.02$), total fat intake ($p=0.05$) and hip BMD were found. There was a positive trend towards a correlation between plant protein and carbohydrate and hip BMD ($p=0.06$). When adjusted for age, PAI, pack years, energy, dietary calcium intake and BMI a statistically significant correlation was found between weight ($p<0.005$), body fat percentage

(p<0.005), BMI (p<0.005) and BMD of the hip. There was a positive trend towards a correlation between total protein intake and hip BMD (p=0.07).

***Table 4.3:* Pearson correlation coefficients between bone mineral density, nutrient intakes, lifestyle factors and biochemical variables of the total group.**

	BMD L2-4					BMD Hip total				
Variables	**N**	**r^2**	**p-value**	**Adjusted r^2**	**p-value**	**N**	**r^2**	**p-value**	**Adjusted r^2**	**p-value**
Age (years)	32	-0.67	0.72	0.44	0.03	31	-0.28	0.13	-0.10	0.62
Height (cm)	32	-0.20	0.27	0.08	0.70	31	0.24	0.20	0.45	0.03
Weight (kg)	32	0.29	0.11	0.52	<0.005*	31	0.67	0.00*	0.69,	<0.005*
Fat percentage (%)	29	0.35	0.06	0.60	<0.005*	29	0.64	0.00*	0.65	<0.005*
PAI	32	0.34	0.06	0.56	0.003*	31	0.22	0.24	0.17	0.42
BMI (kg/m²)	32	0.35	0.05*	0.54	<0.005*	31	0.61	0.00*	0.58	<0.005*
Energy intake (kJ)	32	0.20	0.28	-0.08	0.71	31	0.35	0.05*	0.22	0.28
Total protein intake (g)	32	0.27	0.14	0.15	0.45	31	0.42	0.02*	0.36	0.07
Plant protein intake(g)	32	0.78	0.67	-0.13	0.50	31	0.34	0.06	0.27	0.18
Total fat intake (g)	32	0.36	0.04*	0.39	0.04*	31	0.35	0.05*	0.28	0.17
Total fat percentage (%)	32	0.23	0.21	0.34	0.08	31	0.12	0.51	0.66	0.75
Vitamin D intake (ug)	32	-0.06	0,98	-0.43	0.83	31	0.67	0.72	-0.13	0.53
Percentage protein (%)	32	0.13	0.49	0.30	0.14	31	0.20	0.29	0.22	0.29
Animal protein intake (g)	32	0.28	0.12	0.38	0.05*	31	0.24	0.19	0.98	0.64
Calcium intake (mg)	32	0.03	0.88	-0.41	0.03*	31	0.11	0.56	-0.23	0.26
Phosphorous intake (mg)	32	0.10	0.60	-0.01	0.96	31	0.26	0.16	0.08	0.71
25-OH-vit D (ng/L)	28	0.47	0.12	0.36	0.10	27	0.99	0.63	0.07	0.78
Lymphocytes (%)	32	0.79	0.67	-0.10	0.65	31	0.10	0.61	0.03	0.89
Albumin (g/L)	32	0.26	0.15	-0.06	0.77	31	0.43	0.02*	0.20	0.34
Serum calcium (mmol/L)	32	0.05	0.81	0.20	0.32	31	-0.18	0.33	-0.15	0.47
Serum phosphate (mmol/L)	32	-0.5	0.78	-014	0.51	31	0.17	0.36	0.26	0.20

N= number of subjects, r^2= Pearson correlation coefficient, PAI= physical activity index, BMI= body mass index, 25-OH-vit D=25-hydroxy vitamin D3. *Statistical significant p< 0.05

4.5 Regression analysis

Regression analysis helps the researcher to select the best possible set of predicting variables for a particular variable. The R^2 field contains the coefficient of multiple determination, which measures the reduction in the total variation of the dependant variable due to the (multiple) independent variables. Adjusted R^2 is interpreted similarly to the R^2 value except the adjusted R^2 takes into consideration the number of degrees of freedom. Adjusted R^2 is adjusted by dividing the error sum of squares and total sum of squares by their respective degrees of freedom. The magnitude of the Beta coefficients allows one to compare the relative contribution of each independent variable in the prediction of the dependent variable.

In Table 4.4 it can be seen that 53 % of the variance in BMDL2-4 could be explained by the variables entered into the equation. A forward stepwise multiple regression analysis revealed that 53 % of the variance in the BMD lumbar spine could be explained by 25-OH-vit D, total fat, calcium, BMI and PAI.

Table 4.4: **Regression summary for dependant variables for BMD L2-4**

Multiple regression analysis (r^2= 0.53)			Stepwise forward multiple regression (r^2=0.53)		
Variable	Beta	p-value	Variable	Beta	p-value
Serum phosphate (mmol/L)	-0.07	0.66	25-OH-vit D (ng/mL)	0.41	0.02
25-OH-vit D (ng/mL)	0.41	0.02	Total fat intake (g)	0.48	0.02
BMI (kg/m²)	0.17	0.32	Calcium intake (mg)	-0.53	0.02
PAI	0.37	0.06	PAI	0.36	0.04
Animal protein intake (g)	0.06	0.89	BMI (kg/m²)	0.17	0.29
Total fat intake (g)	0.47	0.08			
Calcium intake (mg)	-0.57	0.11			

N=28

N= number of subjects, BMI= Body mass index, 25-OH-vit D=25-hydroxy vitamin D3, PAI = physical activity index.

In Table 4.5 it can be seen that 49 percent of the variance in BMD total hip could be explained by the variables entered into the equation. A forward stepwise multiple regression analysis revealed that 46 percent of the variance in the BMD of the hip could be explained by BMI, serum phosphate and PAI, with the highest contributing factor being BMI with a Beta value of 0.62.

***Table 4.5*: Regression summary for dependant variables for BMD Total Hip**

Multiple regression analysis (r^2= 0.49)			Stepwise forward multiple regression (r^2=0.46)		
Variable	Beta	p-value	Variable	Beta	p-value
Serum phosphate (mmol/L)	0.14	0.40	BMI (kg/m²)	0.62	0.00
25-OH-vit D (ng/mL)	0.03	0.86	PAI	0.17	0.29
BMI (kg/m²)	0.59	0.00	Serum phosphate (mmol/L)	0.16	0.32
PAI	0.22	0.27			
Animal protein intake (g)	0.15	0.73			
Total fat intake (g)	0.15	0.58			
Calcium intake (mg)	-0.31	0.39			

N=27

N= number of subjects, 25-OH-vit D=25-hydroxy vitamin D3, BMI= Body mass index, PAI = physical activity index.

4.6 Summary of the main findings of the study

4.6.1 Bone density

BMD Lumbar spine	The bone mineral density of the lumbar spine was lower in the cases than that of the controls, but the difference between the groups was not of statistical significance (p = 0.13).
BMD hip	Bone mineral density of the hip was lower in the cases than that of the controls, but the difference between the groups was not of clinical relevance (p =0.39). According to the WHO classification all the subjects were classified as osteoporotic with high risk for fractures or osteopenia with increased risk of fracture.

4.6.2 Characteristics of the two groups

Age	The groups were of comparable age. Statistically significant correlation between age and BMD of the lumbar spine (p=0.03) but not with BMD of the hip (p=0.62).
Height, weight	Both groups were of comparable height and weight. Weight correlated significantly with both BMD lumbar and hip. Height correlated only significantly with BMD in the hip.
BMI	No difference in BMI between the two groups (p=0.79). BMI correlated significantly with both BMD lumbar and hip (p<0.005)
Body fat %	The mean body fat was higher in the cases (16.7) than in the controls (15.0) but the difference was not statistically significant (p=0.82). Body fat percentage correlated significantly with both BMD lumbar and hip (p<0.005)
PAI	The control group was significantly more active than the case group with only 2 subjects with a PAI of lower than 2.00 in the control group (p<0.01). Activity correlated positively with BMD in the lumbar region (p=0.003) but not the hip region

4.6.3 Biochemical values

Albumin	The mean serum albumin was lower in the cases (28g/L) than in the control group (37.5g/L) (p<0.005), but the acute phase response must be taken into account. No statistically significant correlation could be found between albumin and BMD of lumbar spine or hip.
Serum calcium	The mean serum calcium was higher in the case group (2.45mmol/L) than in the control group (2.38mmol/L) (p=0.01). No statistically significant correlation could be found between serum calcium and BMD of lumbar spine or hip.
Serum phosphate	The mean serum phosphate for the cases is higher than for the controls but was not statistically significantly different (p=0.10). No statistically significant correlation could be found between phosphate and BMD of lumbar spine or hip
25OH-Vit D	25OH-Vit D was significantly lower in the cases than the controls (p= 0.04). No statistically significant correlation could be found between 25OH-Vit D and BMD of lumbar spine or hip.
Lymphocytes	Lymphocyte count as a percentage of total white blood cells was significantly lower in the cases than the controls (p=0.01). No statistically significant correlation could be found between lymphocyte count and BMD of lumbar spine or hip.

4.6.4 Dietary intake

Total energy intake	The cases (8 431kJ) tended to have a lower total energy intake compared to the control group (9874kJ), but the differences were not statistically significant (p=0.19). No statistically significant correlation could be found between total energy intake and BMD of lumbar spine or hip.
Total protein intake	Total protein intake was statistically significantly lower in the cases (mean = 56.1) than in the control (mean =73.3) (p=0.05). No statistically significant correlation could be found between total protein intake and BMD of lumbar spine or hip.
Plant protein intake	The control group's (38.8) mean intake was higher than the case group's (33.1) but the difference was not significant. No statistically significant correlation could be found between plant protein intake and BMD of lumbar spine or hip.
Animal protein intake	The control group's (30.0) mean intake was higher than the case group's (22.6) but the difference was not significant. A positive correlation could be seen between animal protein intake and BMD of the lumbar spine (p=0.05) but not the BMD of the hip.
Total fat intake	The control group's (46.6) mean intake was higher than the case group's (37.9) but the difference was not significant. A positive correlation could be seen between total fat intake and BMD of the lumbar spine (p=0.04) but not the BMD of the hip.
Total carbohydrate intake	The control group's (359) mean intake was higher than the case group's (303) but the difference was not significant.
Dietary calcium intake	In both groups calcium intakes were low and no statistical difference could be seen between the two groups (p=0.47). A positive correlation could be seen between total calcium intake and BMD of the lumbar spine (p=0.03) but not the BMD of the hip.
Dietary vitamin D	No statistical difference could be seen between the intake of vitamin D between the two groups (p=0.41). No statistically significant correlation could be found between vitamin D intake and BMD of lumbar spine or hip.
Dietary phosphorus	No statistical difference could be seen between the intake of

	phosphorous of the two groups (p=0.11) No statistically significant correlation could be found between dietary phosphorus intake and BMD of lumbar spine or hip.

All reference to correlations are made to the adjusted correlations which were adjusted for age, pack years, BMI, PAI, energy and dietary calcium.

4.6.5 Predictors of BMD

Variables were entered into stepwise multiple regression.

	Significant predictors
BMD L2-4	25-OH-vit D, total fat intake, dietary calcium, PAI, BMI
BMD Hip	BMI, PAI, serum phosphate

BMD =Bone mineral density, 25-OH-vit D= 25-hydroxy vitamin D3, PAI= physical activity index, BMI=Body mass index.

In the next chapter the focus will be on the discussion of the results, possible explanations for specific results and recommendations.

Chapter 5

Discussion

5.1 Introduction

Few studies have focused on the determinants of osteoporosis in black men in South Africa and very little information is available on the prevalence of osteoporosis in older black South African men. Therefore, the objective of this study was to investigate the risk factors (calcium and vitamin D intake, physical activity and anthropometry) on bone density in older black men.

5.2 Characteristics of the two groups

It is well known that bone mass decreases with age and the phenomenon is well described in the literature. In the present study the case and control groups were age matched and age was negatively correlated with bone density as expected. Possible explanations for bone loss can be a decline in osteoblastic function, lower calcium absorption from the intestinal tract, higher PTH concentrations with age and a decrease in calcitonin production. Age related bone loss commences during the fourth decade and bone loss is slower in men than in women (Arden & Spector, 1997; Sambrook *et al.,* 1993). Elevated PTH levels are linked to bone loss, but blacks seem to have a decreased skeletal sensitivity to high levels of PTH. In the elderly blacks this adaptive skeletal response reduce with age and the elevated PTH levels become a stronger risk factor for bone loss (Hough, 1998).

Although the present study was a case-control study it is interesting to note that the BMD was low in both groups. Villa *et al.* indicated a lower rate of hip fracture in South African blacks, despite lower bone density at all ages, compared with Caucasians (Villa, 1994). In contrast to the American data, South African blacks may have a higher bone turnover and lower bone density than whites (Daniels *et al.,* 1995). This leads one to speculate that there might be other more important risk factors for bone fractures than BMD in blacks.

The control group was better nourished and exercised more than the case group. Nutrition is an important modifiable factor in the development and maintenance of bone mass and the prevention and treatment of osteoporosis. Osteoporosis is a complex, multifactorial condition and for normal bone metabolism all nutrients are necessary. Besides calcium and vitamin D other nutrients such as magnesium, vitamin A, vitamin C, vitamin K, protein, zinc, copper, iron and fluoride play an important role (Ilich & Kerstetter, 2000). Patients with fractures of the proximal femur are often undernourished and other factors such as protein deficiency could be involved in the association between being underweight and osteoporosis (Coin *et al.,* 2000). A higher calcium intake together with physical activity may result in an increase in BMD (Branca & Vatuena, 2001).

Despite the fact that the BMI in both the groups were within the normal range (19-24kg/m^2), the body fat percentage of both the groups was high. A possible reason for the higher body fat percentage (in relation to their body weight) in the case group might be a high alcohol consumption. Although we do not have reliable alcohol consumption data, it was evident during questioning that alcohol was used over weekends or more toward the end of every month. Alcohol has a lipid-oxidizing suppressive effect and this favours the development of fat tissue and obesity (Addolorato *et al.,* 1998). The control groups fat percentage were lower than the case group.

In the present study weight correlated significantly with the bone density of the lumbar and hip regions. It has been reported in studies that a higher body weight had a protective effect on BMD (Baumgartner *et al.,* 1996). The reason for this might be the increase in mechanical stress on the bone that may stimulate bone remodeling and preserve bone minerals in both men and women. It could also be due to the protective effect of the higher 17B-estradiol concentration and the increase in the aromatase enzyme (found in fat tissue) and growth hormone reported in blacks (Bell, 1997).

5.3 Risk factors for osteoporosis

5.3.1 Physical activity

Both groups were classified as inactive according to the physical active index. The control group was more active than the case group and the difference was statistically significant. A sustained level of activity leads to greater BMD but the activity must be continuously maintained. Bone remodels itself according to the functional demand. Physical activity increases muscle strength and helps with balance (Layne & Nelson, 1999). It can be speculated that superior strength and balance is a very important protective factor in the control group. In the present study physical activity correlated significantly and positively with BMD in the lumbar region. The lumbar region has more active trabecular bone and bone turnover is more rapid than in the hip region (Arden & Spector, 1997) and could explain the fact that the correlation was found only for the lumbar region. According to the regression analysis, physical activity contributes significantly to BMD of hip and lumbar region. It has also been recommended to increase physical activity in order to increase BMD (Branca & Vatuena, 2001).

5.3.2 Calcium intake and serum calcium

Calcium is one of the most important constituents of bone. Variations in calcium intake and calcium status early in life can account for as much as a 5 – 10 % difference in peak adult bone mass, and such a difference could potentially contribute more than 50 % to the hip-fracture rates later in life (Matkovic *et al.,* 1979; Matkovic *et al.,* 1995). It is especially noteworthy that blacks have a significantly lower calcium intake throughout the lifecycle (Anderson & Polltzer, 1994). It has been suggested that lactose malabsorption may contribute to osteoporosis, either through a direct effect or because lactose intolerant people tend to consume less calcium from dairy products. Evidence on this issue is inconsistent (Honkanen *et al.,* 1996). There have been numerous retrospective studies of calcium intake and BMD, with apparently conflicting results (Hannan *et al.,* 2000; Prince,1997; Reid, 1996; Vorster, 1999). Hannan *et al.* (2000) examined the risk factors for BMD at the hip, radius and spine in 800 older women and men from the population-based Framingham osteoporosis study. Their BMD were assessed in 1988-1989 and again in 1992-1993. In both women and men, low body weight were associated with lower BMD and in women greater alcohol use. Bone loss was not affected by caffeine, physical activity, serum 25 OH-vit-D or calcium intake (Hannan, *et al.*, 2000). A study done by Vorster in 1999 on post menopausal black women shows a negative correlations between calcium intake and BMD. A higher

calcium intake do not contribute to a greater BMD (Vorster, 1999). Calcium supplementation of at least 1200mg per day prevent or reduce bone loss in elderly people. Raising calcium balance corrects the negative extracellular calcium balance and results in decreased parathyroid hormone – mediated bone resorption (Prince, 1997). Reid, 1996 conducted a study on post menopausal women for 4 years. The control group received 1 g of calcium supplementation and the placebo group received no supplementation. In the second to fourth year lossed of BMD was significantly less than the placebo group (Reid, 1996).

The present study showed a negative correlation between BMD and dietary calcium intake. A potential reason for these conflicting results is that many studies have been performed in adults whose BMD reflects a combination of peak bone mass achieved and subsequent bone mass loss (Arden & Spector, 1997). Low dietary calcium intake is a risk factor for osteoporosis and skeletal response will occur only when calcium is increased from the deficiency level to a threshold zone. Adding more calcium when the level of dietary intake already exceeds the threshold will not improve bone mass (Ilich & Kerstetter, 2000). The calcium intake of both groups was lower than the recommended amount, but the difference between the groups was not significant. In the present study dietary calcium had a negative correlation with BMD of the lumbar and hip region, but only the correlation with the lumbar region reached statistical significance. No plausible explanation for this negative correlation could be found in these groups with a low calcium intake.

Calcium ("threshold nutrient") is stored as bone and bone mass increases as calcium intake rises, up to a certain level, above which further increases in intake produce no further increase in bone mass. Calcium homeostasis is dependent on other nutrients such as magnesium. Magnesium depletion leads to derangements in bone morphology and function, and disturbances in calcium homeostasis (Welch et al., 1981). Other minerals and trace elements involved in skeletal growth and matrix constituents are fluoride and magnesium, others are components of enzymatic systems involved in matrix turnover, such as zinc, copper and manganese. Vitamins also play a role in calcium metabolism (e.g. vitamin D) or as co-factors of key enzymes for skeletal metabolism (e.g. vitamins C and K)(Branca & Vatuena, 2001). Further research must be done on the relationship between calcium and magnesium intakes in the same group

of people. One of the limitations of the study was that only calcium and vitamin D were measured and no other micronutrients. From these results it seems that calcium intake on its own is not as important as was previously thought and that other nutrients are probably as important for calcium homeostasis.

Despite the fact that both groups' dietary calcium intake was lower than the recommended value, serum calcium values were within the normal range for both groups with the case group significantly higher than the control group. A possible explanation may be that the bone fracture and inactivity after the injury lead to a decrease in mineralization (Mahan & Escott-Stump, 2000). This sample group could be more prone to lactose intolerance and it may be the reason for a low dietary calcium intake, although not proven. It seems that blacks utilize dietary calcium more efficiently than whites (Heaney, 2002). A chronic low calcium intake leads to adaptations in gut to absorb more calcium and to decrease calcium excretion as seen in blacks (Heaney, 2002). The body is more concerned with maintaining plasma calcium to protect the neuromuscular system than with preserving the integrity of the skeleton. Serum calcium levels are a poor indication of bone status because blood levels are maintained by drawing calcium from the bone (Nordin, 1996). It is thus unlikely that the differences in serum calcium are of any clinical importance in BMD in the present study. Lynch et al. (1970) reported that calcium intake, and calcium absorption and excretion in blacks do not indicate that deficiency of the mineral is of primary importance in the genesis of the osteoporosis, but rather vitamin C deficiency (Lynch *et al.,* 1970). Low dietary calcium intakes per se may not be as important a risk factor for the development of osteoporosis in this group.

5.3.3 Vitamin D status

The vitamin D intake was higher in the case group than in the control group, but the difference was not statistically significant. In both groups the vitamin D intake was lower than the recommended amount and a possible explanation for this is, that it is difficult to estimate dietary vitamin D intake since the content of most South African foods is unknown (Vorster *et al.,* 1996). Very low blood levels of vitamin D are associated with osteomalacia in adults (Arden & Spector, 1997). In this study the vitamin D intake was not significantly correlated with bone density of the lumbar and hip regions.

The 25-OH-vit D for both groups was in the normal range with the control group significantly higher than the case group. The control group was more active than the case group and it is tempting to speculate that the higher 25-OH-vit D levels could be because they were more exposed to sunlight than the case group due to physical activity. The regression analysis for both lumbar and hip regions shows that 25-OH-vit D was an important contributing factor to variance in BMD. Many elderly people in South Africa live in crowded cities with little exposure to sunlight. There are large seasonal variations in serum 25-OH-vit D concentrations with the lowest in winter months (Arden & Spector, 1997). An increase in skin pigmentation and ageing diminishes the cutaneous production of cholecalciferol and if this is the case dietary intake may be more important in black men and women (Hollick, 1995b). Melanin protects the body from excess radiation (Marcus *et al.,*1996). In black people melanin is in competition with 7-dehydrocholesterol for ultraviolet light so that it means blacks need to spend more time in sunlight to make the same amount of vitamin D than their white counterparts. Most clothing absorbs solar ultraviolet B radiation, and blacks normally wear clothes over most sun-exposed areas (long sleeves) which makes it conceivable that they might be at an increase risk for vitamin D deficiency (Holick, 1995a).

5.4 Other findings of this study

5.4.1 Albumin

The metabolic response to injury might be a reason for the albumin to be lower in the case group than the control group. Visceral proteins (albumin, transferrin, pre-albumin) have traditionally been used for assessment of nutritional status, and have been considered to reflect visceral protein stores. In the short term, it may reflect the severity of the metabolic response to stress. In many clinical situations, it may be difficult to determine whether changes in a patient's serum protein and albumin levels reflect nutritional status or are a consequence of the metabolic response to injury (Visser & Labadarios, 2002). Too little or too much protein in the diet can adversely affect the calcium balance. Hip fractures are more common in people with low energy intake, low serum albumin and muscle weakness (Rizzoli *et al.,* 2001).

5.4.2 Serum phosphorus

Serum phosphate is part of the group of variables that explain variances in BMD in lumbar and hip region. A positive correlation was found between serum phosphate and BMD of the hip region, but negative with lumbar region. No plausible explanation can be offered for these obviously contradicting correlations. The adjusted correlation coefficients were very low and were probably a chance finding. One of the functions of phosphate is to combine with calcium to form hydroxyapatite, the major inorganic molecule present in teeth and bones (Mahan & Escott-Stump, 2000). About 80 % of all phosphorus are within bone, the rest in plasma. Phosphate reabsorption from the kidney is decreased by PTH (Kumar & Clark, 1992). Chronic consumption of a low calcium high phosphorus diet can result in elevated concentration of PTH. Persistently high PTH contributes to increased bone turnover and bone mass loss (Mahan & Escott-Stump, 2000). It is thus clear that a too low or too high phosphorus intake could have significant effects on bone health.

5.4.3 Total fat intake

Total fat intake correlated significantly positively with the BMD of the hip and lumbar regions and significantly influenced the variance on BMD of lumbar and hip regions as was seen from the regression analyses. After adjustment the correlation holds true only for BMD in the lumbar region. In the literature no data is available on the relationship between total fat intake and bone mineral density. It might be speculated that the very low fat intake in the case and control groups (below 19 % energy) could have significantly influenced micronutrient metabolism and therefore also bone metabolism. This phenomenon should be researched in more detail - especially in populations at risk of undernutrition.

5.4.4 Animal protein

There was a statistically significant adjusted correlation between total protein and hip BMD and between animal protein and BMD of the lumbar regions. The regression analysis revealed that animal protein contributes significantly to the variance in BMD of

hip and lumbar regions. Too little or too much protein can adversely affect calcium balance (Rizzoli *et al.,* 2001). A high animal protein intake increases urinary calcium loss (Heaney, 2000). Whether this effect results in actual negative calcium balance depends heavily on the amount of calcium in the diet. It will be more useful to evaluate the protein-calcium ratio in the diets (Heaney, 1998). This was, however, not a problem in the present study as both groups consumed in the region of 25-30g animal protein per day. An adequate intake of dietary protein is essential for optimal bone maintenance. In M Leach's dissertation the relationship between protein intake and bone density is discussed in more detail (Leach, 2003).

5.5 Conclusion

The aim of the study was to describe the relationship between dietary calcium intake and bone density in black South African men. The hypothesis was that a low calcium intake is negatively associated with bone density and may be a risk factor for bone fracture caused by non-traumatic accidents in black men in South Africa. The results were not as expected, a negative correlation between dietary calcium intake and BMD of the lumbar and hip region were founded. The lumbar region were statistical significant. A higher calcium intake leads to a lower BMD of the lumbar region. The regression analysis yielded results that also suggest an inverse relationship between BMD and calcium intake. This can in part be explained by the fact that calcium on its own, cannot alone maintain calcium homeostasis, other nutrients are needed and that calcium on its own is not as important as was previously thought.

A second aim was to describe the relationship between vitamin D status and bone density in black South African men. The hypothesis was that a low vitamin D status is negatively associated with bone density and may be a risk factor for bone fracture caused by non-traumatic accidents in black men in South Africa. The results in this study show that 25-OH-vit D in part explains variances in BMD of the lumbar and hip region. Adequate intake of vitamin D (diet or sunlight) is necessary for maintaining BMD.

The last aim was to describe the correlation between physical activity and bone density in black South African men and the hypothesis was that physical activity is positively

associated with bone density in black men in South Africa. In the study this hypothesis was confirmed.

The present study has also shown that the following variables are predictors of BMD, serum phosphorus, 25-OH-vit D, BMI, PAI, animal protein, total fat intake and dietary calcium are important determinants of BMD in older South African blacks.

To conclude from the results of this study it seems logical to suggest a healthy diet with optimal macro- and micronutrient intake. Maintenance of ideal body weight and body fat percentage and regular but moderate-weight-bearing exercise from a young age throughout adult life is recommended, as part of a strategy to prevent and treat osteoporosis. Osteoporosis is a multi factorial problem and must be approached in that way.

Chapter 6

Summary, recommendation and conclusion

6.1 Summary

As seen in this study both groups' bone density was lower than the recommended value for black men, most of them where classified as osteoporotic or osteopenic. Bone fractures were only limited to the case group. Bone density might not be as important to bone health in blacks as in whites. Calcium intake on its own seems not to play such an important role in BMD and must be combined with other micronutrients. Osteoporosis in older black South African men is a reality and this leaves many opportunities for further research.

6.2 Recommendation

The small subject group was a limitation in this study, and this sample might not be representative of the whole black South African male population. Due to time constraints (3 years) for the degree, all subjects admitted to the two hospitals and who complied with the inclusion and exclusion criteria were included. Despite this it is recommended that this study should be ongoing. Another limitation might be the biochemical markers not included, such as urinary calcium, serum magnesium and especially bone markers such as PTH, bone specific alkaline phosphatase and osteocalcin.

6.3 Conclusion

In conclusion, osteoporosis has a formidable impact on the lives and well-being of people, it is a multi factorial problem, but a preventable condition. It is never too early to start prevention through exercise, good nutrition and healthy lifestyle and never to late to treat osteoporosis. Referring to the Oscar Wilde's adage " Truth is seldom pure and never simple", we realize that what is considered a truth now might change, but as long as we keep up with our quest, the more certain we will become that what we know is true (Ilich & Kerstetter, 2000).

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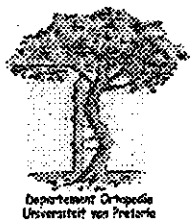
Appendixs A

(i) Demographic questionnaire

Oswama Project



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Subject number			
Date	D	M	Y
Place			
Interviewer			

Age, years			
Date of birth	D	M	Y

First Language
Second language

What is your marital status?	Never married	1
	Married	2
	Divorced	3
	Widower	4

Do you snuff?	Yes	1
	No	2
Do you smoke?	Yes	1
	No	2
If no – have you smoked regularly before?	Yes	1
	No	2
If yes – what do you smoke?	Cigarettes	1
	Tobacco/pipe	2
	Snuff	3
	Other	4
If other, please describe		
If cigarettes, how many cigarettes do you smoke?	Per day	
	Per week	
If tobacco, how many packages?	Per day	
	Per week	
If snuff, how many parcels?	per day	
	per week	
If other, describe frequency		

How long have you been smoking (years)?			
Interviewer: Calculate pack years			
Do you drink alcoholic drinks?		yes	
		no	
If yes, what do you drink?	Traditional beer, home-made		
	Tlokwe		
	Beer, commercial		
	Spirits		
	Wine		
	Liqueur		
If homemade, what kind of container is it brewed in?			
Try and quantify your alcohol use, per day/per week	Traditional beer, homemade - glass		
	Tlokwe - box		
	Beer, commercial – quart,tin,dumpy		
	Spirits – tot,bottle, half-jack		
	Wine – glass, bottle, box, can		
	Liqueur – glass,bottle		

What is your highest qualification?	None	1
	< Std. 6	2
	Std. 6-8	3
	Std. 6-8 + trade	4
	Std. 9-10	5
	Std. 9-10 + trade	6
	Std. 9-10 + academic	7

What is your occupation?	

Do you have a job at the moment?	Yes	1
	No	2
If yes – what kind of job?		
On which days of the week do you work?	Irregular (piece work)	1
	Part time (1-4 days)	2
	Full time (5-6 days)	3

How much money do you earn per month?	R0-100	1
Is it between	R101-500	2
	R501-1000	3
	R1000-2000	4
	R2000-3000	5
	R3000+	6

What is the source of this income?	

Do you receive any additional pensions?	Yes	1
	No	2

How much pension do you receive per month?		
Interviewer - *Re-evaluate final income category*	R0-100	1
	R101-500	2
	R501-1000	3
	R1000-2000	4
	R2000-3000	5
	R3000+	6

Does anybody else contribute money to your household?	Yes	1
	No	2
If yes, how much?		

Does anybody else contribute other resources e.g. food, to your household?	Yes	1
	No	2
If yes, describe.		

Please name the members of your household			
Member	Age	Education	Present job

What type of house do you live in?	Traditional	1
	Mokuku	2
	Brick house	3
	Other	4
If other, specify		

Where do you get your drinking water from?	Fountain, river	1
	Communal tap	2
	Tap on premises	3
	Tap in house	4
	Other	5
If other specify		
Do you have access to electricity inside your house?	Yes	1
	No	2

Where do you eat your meals usually?	At home	1
	Dining hall	2
	Fast foods	3
	Restaurant	4
	Other	5
If other, please specify		

What type of stove do you have?	None	1
	Coal/wood	2
	Gas or paraffin	3
	Electric	4

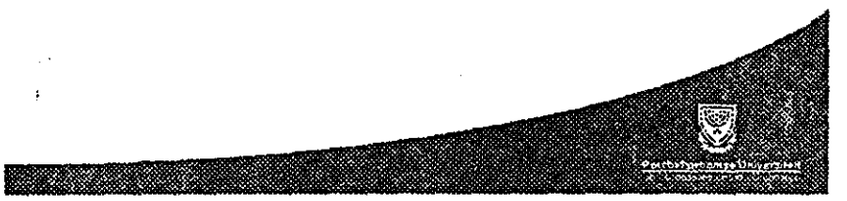
What kind of cooking pots do you use?	Stainless steel	1
	Enamel	2
	Aluminium	3
	Iron	4
	Other	5
If other, please specify		

What type of fridge do you have?	None	1
	Paraffin	2
	Gas	3
	Electric	4

How long have you been living here? (years)	

Where did you live before coming here?	Rural area	1
	Farm	2
	Squatter camp	3
	Township	4

(ii) Physical activity questionnaire



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Physical activity questionnaire

Date: Place: Interviewer:

The information on this questionnaire is confidential

1.	Subject number						(1-4)
2.	Gender	Male	1	Female	2		(5)
3.	What is your main occupation?.....						
	Low level: office work, housework, scholar				1		
	Middle level: factory work, carpentry, farming, hospital nurse, plumber				2		(6)
	High level ("sweat work"): construction work, digging, manual labour				3		
4.	At work I sit	1. never	2. seldom	3. sometimes	4.often	5.always	(7)
5.	At work I stand	1. never	2. seldom	3. sometimes	4.often	5.always	(8)
6.	At work I walk	1. never	2. seldom	3. sometimes	4.often	5.always	(9)
7.	At work I lift heavy loads	1. never	2. seldom	3. sometimes	4.often	5.always	(10)
8.	At work I am tired	1.never	2.seldom	3. sometimes	4.often	5.always	(11)
9.	At work I sweat	1.never	2.seldom	3. sometimes	4.often	5.always	(12)
10.	If you work away from home, how do you get to work/school?				walk	1	
					cycle	2	(13)
					car/taxi	3	
11.	How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station)				0-15 min	1	
					16-30 min	2	
					31-60 min	3	
					1-2 hours	4	(14)
12.	If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station)				casual strolling	1	
					fairly brisk	2	
					brisk/fast	3	(15)
13.	Do you climb stairs often?				yes	1	
					no	2	(16)
14.	If yes, how many flights of stairs do you climb each day? (1 flight = 10 steps)						(17)
15.	How many days per week do you climb steps?						(18)
16.	Do you play sport?				yes	1	
					no	2	(19)
17.	Which sport do you play most frequently?				low level: bowling, golf, billiards	1	0.76*[1]
					middle level: tennis, athletics, cycling	2	1.26
					high level: soccer, rugby, netball, boxing	3	1.76(20)
					If other, specify		
18.	How many hours per week do you practice?<1/ 1-2/ 2-3/ 3-4/ >4 (Write appropriate code in space) 0.5, 1.5, 2.5, 3.5, 4.5*[2]						(21-23)

19.	How many months per year ? (Write appropriate code in space) *[1] intensity code of sport, *[2] time code for sport, *[3] proportion of year	<1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92*[3]	.		(24-26)

20.	If you play a second sport, which is it?	low level: bowling, golf, billiards	1	0.76*[1]
		middle level: tennis, athletics, cycling	2	1.26
		high level: soccer, rugby, netball, boxing	3	1.76(27)
		Other, specify		

21.	How many hours per week do you practice? 0.5, 1.5, 2.5, 3.5, 4.5*[2]	<1/ 1-2/ 2-3/ 3-4/ >4			.		(28-30)
22.	How many months per year?	<1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92*[3]			.		(31-33)
23.	During leisure time I watch TV/ do sitting activities (read, study, play cards)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways	(34)
24.	During leisure time I walk/ do standing activities (gardening, housework)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways	(35)
25.	Other leisure-time activities:..... (leisure-time = time off from work/. school)		2.sel-dom	3.some-times	4.often	5.al-ways	(36)

Definitions and explanation of the questionnaire (interviewer's notes)

Item 1: Write in the subject number as on the name label provided at the recruitment station.
Item 2: Circle gender: male or female
Item 3. Occupation: paid job or unpaid duties for most of the day; including school, housework, childminding
Write in the occupation stated and circle 1,2 or 3 (low level, middle level or high level)

Item 4-9:
- never: ⊕: never, almost never
- seldom: ◔ one-quarter of the workday or workweek
- sometimes: ◑ half the workday or workweek
- often: ◕ three-quarters of the workday or workweek
- always: ● almost all the time

Be clear, that if they do not have a steady job, they may also do these activities. Find out what they do mostly and how often they do it.

Item 13: If the subject does not climb stairs, go on to question 16.
Item 16: If the subject does not play sport, go on to question 23.
Item 17: Circle 1/2/3
Item 18 and 21: Write time code in space, note decimal point
Item 19 and 22: Write code in space, note decimal point
Item 20: Circle 1/2/3

Item 23-25:
- never: ⊕ never, almost never
- seldom: ⊕ one-quarter of off-time, 1-2 days per week
- sometimes: ⊕ half my off-time, 3-4 days per week
- often: ⊕ three-quarters of my off-time, 5-6 days per week
- always: ⊕ almost all the time, mostly 7 days per week

Item 23: sitting activities: watch TV, listen radio, reading, writing, knitting, needlework, playing cards, visiting friends
Item 24: standing activities: gardening, walking with friends, cleaning, cooking, doing laundry, ironing, dishwashing after work at your own home

Item 25: other leisure-time activities: name any other leisure-time activities that you do and how often you do these activities.

NB: *leisure-time* is time after work, school, or housework is finished

Calculate the work-index, items 3-9: $[I_3 + (6-I_4)^* + I_5 + I_6 + I_7 + I_8 + I_9]/7$

Sum of all items' scores (maximum 5) divided by 7;

* Item 4 reversed because highest score for lowest activity level

Calculate the commuting-index, items 10-12: 0 for people who do not commute

$[(4 - I_{10}) + I_{11} + I_{12}]/3$

Calculate the stairs-index: 0 for people who do not climb stairs

$= I_{14} \times I_{15}/7$

Calculate the sport-scores (I_{16} and I_{20}, 0 for people who do not play sport)

= [intensity x time x proportion of year]; Sport index = $I_{16} + I_{20}$

Calculate the leisure-time index: $[(6 - I_{23}) + I_{24} + I_{25}]/3$

Composite physical activity (PA)-index

= Work-index + commuting-index + stairs-index + sport index + leisure-time-index

Calculate a weighted composite PAI for proportionate time spent in each activity category:

=0.47(work-index) + 0.059(commuting index) + 0.001(stairs-index) + 0.47(sport index + leisure-time-index)

Factors for the weighted index may be changed for a study population for which times spent in main occupation and leisure-time differs much from the proportions stated here.

(iii) Dietary questionnaire

Oswama Project

BRADLEY UNIVERSITY

Subject number_____ **Interviewer**_____

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

INTRODUCTION:

Greeting

Thank you for agreeing to participate in this study. Here we want to find out what kind of foods you regularly eat and drink. This information is important to know, as it will tell us whether anything you eat or drink played a role in the fracture you have experienced.

Please think carefully about the food and drink you have consumed during the past four weeks. I will now go through a list of foods and drinks with you and I would like you to tell me:

- if you eat or drink the food
- how the food or drink is prepared
- how much of the food you eat or drink at a time
- how many times a day you eat or drink it and if you do not eat it every day, how many times a week or a month you eat or drink it.

To help you describe the amount of a food you eat or drink, I will show you pictures of different amounts of the food and drinks. Please say which picture is the closest to the amount you eat or drink, or if it is smaller, between sizes or bigger than the pictures.

THERE ARE NO RIGHT OR WRONG ANSWERS.

EVERYTHING YOU TELL ME IS CONFIDENTIAL. ONLY YOUR SUBJECT NUMBER APPEARS ON THE FORM.

IS THERE ANYTHING YOU WANT TO ASK NOW?

ARE YOU WILLING TO GO ON WITH THE QUESTIONS?

INSTRUCTION

Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

Do you eat maize meal porridge? YES 1 NO 2

If YES, what type do you have at home now?

Brand name_____

Don't know_____2

Grind self_____3

If brand name given, do you usually use this brand YES 1 NO 2 DON'T KNOW 3

Where do you get your maize-meal from? (May answer more than one)

Shop ☐ 1

Employer ☐ 2

Harvest and grind self ☐ 3

Other - specify _____ ☐ 4

Don't know ☐ 5

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Maize-meal porridge	Stiff (pap)						e4225 4250	
Maize-meal porridge	Soft (slappap)						e4225 4250	
Maize-meal porridge	Crumbly (phutu)						e4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella	Soft						4082	
Oats							4032	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Breakfast cereals	Brand names of cereals at home now: (5) Don't know							

Do you pour milk on your porridge or cereal? YES 1 NO 2

If YES, what type of milk (whole fresh, sour, 1%, fat free, milk blend.)_____

INSTRUCTION: Show subject examples.

If YES, how much milk?								

Do you pour sugar on your cereal/porridge/mabella YES 1 NO 2

If YES, how much sugar?							9012	
Samp	Bought Self ground						4077 4073	
Samp and beans							A014	

Are the amounts of samp and beans the same as in the picture? YES NO

If no, do you use more beans than in the picture or less? MORE LESS

Samp and peanuts							A013	

Are the amounts of samp and peanuts the same as in the picture? YES NO

If no, do you use more peanuts than in the picture or less? MORE LESS

Rice	White Brown Maize rice						4040 4134 4043	
Pastas	Macaroni Spaghetti Other:						4062	

You are being very helpful. Can I now ask you about meat?

How many times do you eat meat, chicken or fish? Per day: _____

Per week? _____

Other? Specify: _____

CHICKEN, MEAT, FISH

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Chicken	Boiled						1521	
	Fried: in batter/crumbs Not coated						1634 1520	
	Roasted/grilled						1520	

Do you eat chicken skin ALWAYS 1 SOMETIMES 2 NEVER 3

Chicken bones stew							A003	
Chicken feet							A004 1609	
Chicken offal							1610	
Red meat:	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed						A001	
	Mince with tomato and onion						1585	
Beef Offal	Intestines: boiled, nothing added						1616	
	Stewed with vegetables							
	Liver						1515	
	Kidney						1518	
	Other specify:							
What vegetables are usually put into meat stews?								
Wors / sausage	Fried						1526	
Bacon							1501	
Cold meats	Polony						1514	
	Ham						1564	
	Viennas						1531	
	Other - specify							
Canned meat	Bully beef						1535	
	Other specify:							
Meat pie	Bought						1548	
Hamburger	Bought						A015	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Pilchards in tomato/chilli/ brine	Whole						2557	
	Mashed with fried onion						A005	
Fried fish	With batter/crumbs						2509	
	Without batter/crumbs						2523	
Other canned fish	Tuna						2547	
	Pickled fish Other:						2562	
Fish cakes	Fried						2531	
Eggs	Boiled/poached Scrambled Fired						1001 1025 1003	
Dried beans/peas/ lentils (10)	Soup Salad						3033 3508	
Soya products eg. Toppers	Brands at home now (5) Don't know_____ Show examples						3527	

WE NOW COME TO VEGETABLES

How often do you eat vegetables? **Per day?** _____

Per week? _____

Other? Specify: _____

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						8066	
	Boiled with potato and onion and fat						A006	
	Fried, nothing added						A007	
	Boiled, then fried with potato, onion						A006	
	Other: Don't know							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Spinach/morogo/ other green leafy	How do you cook spinach?							
	Boiled, nothing added						8071	
	Boiled fat added						8209	
	Boiled with onion/tomato and fat						A011	
	- onion, tomato & potato							
	- with peanuts							
	Other: Don't know							
Tomato and onion 'gravy'	Home made - with fat - without fat						A012 A016	
	Canned						8221	
Pumpkin	How do you cook pumpkin?							
	Cooked in fat & sugar						A010	
	Boiled, little sugar and fat						A009	
	Other: Don't know							
Carrots	How do you cook carrots?							
	Boiled, sugar & fat						8129	
	With potato/onion						A008	
	Raw, salad						8015	
	Chakalaka							
	Other: Don't know							
Mealies/Sweet corn	How do you eat mealies?							
	On cob						8033	
	Off cobb - creamed sweet corn - whole kernel						8034 8261	
Beetroot salad	Home made Bought						8005	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						8046	
	- without skin						8045	
	Mashed						8187	
	Roasted						8189	
	French fries						8048	
	Salad Other:						8236	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						8057	
	- without skin						8214	
	Mashed							
	Other: Don't know							
Salad vegetables	Raw tomato						8059	
	Lettuce						8031	
	Cucumber						8025	
Other vegetables, specify:								

FRUIT:

How often do you eat fruit?

Per day? _____

Per week? _____

Other? Specify: _____

Apples/Pears	Fresh Canned pears						7001 7054	
Bananas							7009	
Oranges/naartjie							7031	
Grapes							7020	
Peaches	Fresh Canned						7036 7038	
Apricots	Fresh Canned						7003 7004	
Mangoes	Fresh						7026	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Guavas	Fresh Canned						7021 7023	
If subject eats canned fruit: Do you have custard with canned fruit: YES 1 NO 2								
Custard	Home made Ultramel						0001	
Wild fruit/berries	Specify type						7070	
Dried fruit	Types:							
Other fruit								

BREAD AND BREAD SPREADS

How often do you eat bread and rolls?

Per day? _____

Per week? _____

Other? Specify: _____

Bread/Bread rolls	White						4001	
	Brown						4002	
	Whole wheat						4003	

Do you spread anything on the bread? ALWAYS 1 SOMETIMES 2 NEVER 3

Margarine	What brand do you have at home now? _____ Don't know_____ Show examples							
Peanut butter							6509	
Jam/syrup/honey							9008	
Marmite/Fray Bentos							9501	
Fish/meat paste							1512	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Cheese	Type:						0010	
Achaar							A017	
Other spreads:	Specify							
Dumpling							4001	
Vetkoek							4057	
Provita, crackers, etc.								
Mayonnaise/salad dressing	Number of spoons_____/ number in family						6573	

DRINKS:

Tea							9514	
	How is the tea prepared? What type of tea?							
Coffee							9513	
Sugar/cup tea or coffee							9012	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life whole						0006	
	Fresh/long life 2%						0069	
	Fresh/long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	
	Evaporated milk						0003	
	None							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Milk as such	What type of milk do you drink as such?							
	Fresh/long life whole						0006	
	Sour / Maas						0006	
Milk drinks Brand	Nestle_____ Milo_____ Flavoured milk_____ Other_____						0023	
Yoghurt	Drinking yoghurt Thick yoghurt						0044 0020	
Squash	SweetO SixO Oros/Lecol with sugar - artificial sweetener Kool Aid Other						9013 9013 9002 9013 9002	
Fruit juice	Fresh/Liquifruit/Ceres						0535	
	Tropica Show examples						0089	
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Mageu/Motogo							9562	
Home brew							9516	
Tlokwe							9516	
Beer							9506	
Spirits							9510	
Wine red							9508	
Wine white							9518	
Other specify								

SNACKS AND SWEETS:

Potato crisps							8049	
Peanuts	Raw Roasted						6001 6007	
Cheese curls: Niknaks etc.							4076	
Raisins							7022	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Peanuts and raisins							6007 7022	
Chocolates	Name						9024	
Candies	Sugus, gums, hard sweets						9009	
Sweets	Toffees, fudge, caramels						9014	
Biscuits	Type							
Cakes & tarts	Type							
Scones							4029	
Rusks							4160	
Savouries	Sausage rolls Samoosas Biscuits eg bacon kips Other:						1534 4196 4162	
Jelly							9004	
Baked pudding							4181	
Instant pudding							4066	
Ice cream Sorbet							6507 6516	
Other Specify:								

SAUCES / GRAVIES / CONDIMENTS

Tomato Sauce Worcester sauce							9505	
Chutney							9524	
Pickles							8176	
Packet soups							4069	
Others:								

WILD BIRDS, ANIMALS OR INSECTS (hunted in rural areas or on farms)

Wild fruit								

MISCELLANEOUS: Please mention any other foods used more than once/two weeks which we have not talked about:

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		

SALT USE:

What type of salt do you use?_____

The next few questions are to find out if you use salt, where you use it and how much you use?

Do you add salt to food while it is being cooked?

Always 1	Sometimes 2	Never 3	Don't know 4

Do you add salt to your food after it has been cooked?

Always 1	Sometimes 2	Never 3

Do you like salty foods eg. salted peanuts, crisps?

Very much 1	Like 2	Not al all 3

Do you use any of the following:

	Name of product	Amount/day
Vitamins/vitamins & minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: specify		

THANK YOU FOR YOUR COOPERATION AND PATIENCE

GOOD-BYE!