Risk factors for osteoporotic fractures in black South African men: A case control study.

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I dedicate this dissertation to my father, Dr. D.W. van der Zel.

'Fear not, for I am with you. Do not be dismayed. I am your God. I will strengthen you; I will help you; I will uphold you with my victorious right hand.'

ISAIAH 41:10

OPSOMMING

Die fokus van navorsing op beenverlies en Osteoporose (OP) was tot dusver hoofsaaklik beperk tot vroue, maar OP kom al hoe meer voor by ouer mans en die impak van heupfrakture op die mortaliteit van mans kan groter wees as op vroue. OP is die hoofoorsaak van morbiditeit en mortaliteit in ontwikkelende lande, teen onkostes meer as $10 biljoen per jaar in die Verenigde state alleen. Osteoporotiese frakture affekteer 50% van vroue, 20-30% van blanke mans en 4% van swart mans ouer as 50. Hierdie persentasies mag moontlik verhoog namate die lewensverwagting verhoog. Baie min studies is nog tot op datum gepubliseer oor spesifiek swart mans en navorsing ten opsigte van die etiologie en voorkoms van OP en frakture onder die ouer swart Suid Afrikaanse populasie is egter nog beperk. Na aanleiding van bogenoemde inligting is dit duidelik dat OP van uiterste kliniese en ekonomiese belang is. Sonder inligting oor patrone en faktore betrokke by beenverlies, is die formulering van rasionele voorkomings- en behandelings programme in hierdie teikengroepe nie moontlik nie.

Die doel van hierdie studie was om die verband tussen dieetfaktore (yster, vitamien C, en proteïen) en lewenstylfaktore (alkohol en tabakrook) op osteoporotiese frakture en beenmineraaldigtheid in ouer Suid Afrikaanse swart mans te ondersoek in 'n kruiskontrole studie . Die gevalle het bestaan uit sestien swart mans met frakture van die proksimale femur, die proksimale humerus of die distale radius en wat voldoen het aan die insluitings- en uitsluitingskriteria van die studie . Die kontrole groep het bestaan uit ewe veel mans met ouderdomme binne 2 jaar vergelykbaar met die gevalle, sonder siektes en vorige breuke van die proksimale femur, die proksimale radius en die distale radius. Die kwantitatiewe ultraklank densitometer was gebruik om die beendigtheid van die werwels en die heup te doen. Vraelyste is gebruik om die demografiese en mediese inligting, data oor fisiese aktiwiteit en dieetinnames in te samel. Antropometriese meetings en bloedmonsters is geneem. Toepaslike biochemiese analises is volgens standaardmetodes gedoen.

Na aanleiding van die gemiddelde spinale beenmineraaldigtheid kon beide die gevalle en kontroles as osteoporoties geklassifiseer word. Die beenmineraaldigtheid was slegs effens laer in die gevalle as in die kontroles. Dit is nie statisties beduidend nie. Die gemiddelde tabak pakkie jare van die gevalle (13.29) [95% CI: 4.44; 22.14] was dubbel so veel as die van die kontroles (7.43) [1.83; 13.03], maar nie statisties beduidend nie (p=0.55). Tabak pakkie jare was negatief geassosieer met die beenmineraaldigtheid van die werwels (p=0.08), selfs na dit gekontroleer was vir moontlike bydraende faktore (p=0.001). Die

gemiddelde proteïen inname van die gevalle (56.11g) [46.49; 65.74] was baie laag in vergelyking met die voorgestelde inname van 63g per dag. Hierdie lae proteïen inname was ook statisties beduidend minder as die kontroles (73g) [58.28; 88.31]. Die wanvoeding, veroorsaak deur verlaagde innames van energie, proteïen, vitamien C, yster en lae BMI kon 'n groot rol gespeel het in die laer beenmineraaldigtheid van die gevalle. Daar was 'n geneigdheid van die ysterinname van die gevalle om laer te wees as die van die kontroles (p=0.09). Ysterinname was nie geassosieer met beenmineraaldigtheid nie, maar in die stapsgewyse regressie analise het ysterinnname uitgekom as 'n moontlike voorspeller van beenmineraaldigtheid van beide die heup en die werwels, alhoewel dit nie statisties betekenisvol was nie. Die BMI was < 19 kg/m^2 in 50% van die gevalle en kontroles. Die s-GGT, 'n merker van alkohol inname, was beduidend verhoog in die gevalle met 'n gemiddelde waarde van 65.88U/L teenoor die 36.33U/L in die kontrolegroep. S-GGT was die belangrikste voorspeller van beenmineraaldigtheid in beide die werwels en die heup. Daar was 'n statistiese betekenisvolle ooreenkoms tussen die GGT-waarde en die beenmineraaldigtheid van die werwels (p=0.04) en heup (p=0.02).

Wanvoeding het 'n belangrike rol gespeel in die lae beenmineraaldigtheid wat vererger was deur die rook van tabak van jongs af en die oormatige alkoholverbruik oor naweke. Intervensieprogramme moet veral fokus op alkoholmisbruik, tabakrook en die verbetering van die voedingstatus van die populasie. Kinders moet aangemoedig word om nie te rook nie en ingelig word oor die nadelige gevolge van alkoholmisbruik. Dit is ook belangrik om dieetfaktore wat die risiko vir OP verhoog te verander, naamlik om die voedingstoestand van die Suid Afrikaanse swart man te verbeter met lae koste proteïene en kalsiumprodukte. Rooi vleis moet ook deel uitmaak van die dieet om aminosure en yster te verskaf. Vitamien C verhoog yster absorpsie en siende dat dit laag was in albei groepe is dit noodsaaklik om die inname daarvan te verhoog met vrugte in seisoen sodat die beenkollageen daarby kan baat.

Sleutelwoorde: osteoporose, beenmineraaldigtheid, swart mans, GGT, alkohol, tabakrook

SUMMARY

The main focus of bone loss and Osteoporosis (OP) research has been limited almost entirely to women, but OP has become increasingly common in older men and the impact of hip fracture on mortality may actually be greater in men. OP is a major cause of morbidity and mortality in developed countries, at a cost that currently exceeds $10 billion per year in the United States (US) alone. Osteoporotic fractures affect 50 % of women and 20-30% of white men and 4% of black men over the age of 50 years. These statistics may even increase because of increasing life expectancy. Few studies focusing on Blacks have been published to date and very little is known regarding the bone health and the aetiology and prevalence of OP and fractures among older South African blacks. From the above information it is clear that OP is of considerable clinical and economic importance. Without information on the patterns and determinants of bone loss, the formulation of rational prevention and treatment strategies in these groups is not possible.

The aim of the study described in this thesis was to investigate the influence of the dietary factors (iron, vitamin C, and protein) and lifestyle factors (alcohol and tobacco smoking) on osteoporotic fractures and bone mineral density in older South African black men using a case-control study design. Sixteen black male patients with fractures of the proximal femur, the proximal humerus or the distal radius and who conformed to the inclusion and exclusion criteria were included in the study. An equal amount of age-matched (±2 years), apparently healthy black men with no previous fracture (of the proximal femur and humerus and distal radius), were recruited as a control group. Dual energy X-ray absorptiometry (DEXA) was used for the measurement of the lumbar vertebrae and the proximal femur (hip). Questionnaires were used to gather demographic and medical information, data on physical activity and dietary intakes. Anthropometric measurements and blood samples were taken. Appropriate biochemical analyses were done with standard methods.

Both the cases and controls were osteoporotic according to the mean lumbar spine BMD determined in both groups. The BMD was only marginally lower in the cases than in the controls and therefore not statistically significant. The mean tobacco pack years of the cases (13.29) [95% CI: 4.44; 22.14] were almost double that of the controls (7.43) [1.83; 13.03] but it was not statistically significant (p=0.55). Tobacco pack years were negatively associated with BMD of the lumbar spine (p=0.008) even after controlling for possible confounding

factors (p=0.001). Malnutrition, as indicated by the low dietary intakes of energy, protein, vitamin C, iron and low BMI, could play a role in the lower bone mineral density (BMD) observed in the cases. The mean protein intakes of the cases (56.11g) [46.49; 65.74] were very low compared to the recommended 63g per day. This low protein intake was also significantly less compared to the controls (73g) [58.28; 88.31]. Iron intake tended to be lower in the cases compared to the controls (p=0.09). Iron intake was not associated with BMD, however, in the stepwise regression analysis; iron intake came out as a possible predictor of BMD of both the lumbar spine and hip, although it was not statistically significant. The BMI was < 19 kg/m^2 in 50% of the cases and the controls. S-GGT, a marker of alcohol intake, was significantly increased in the cases with a mean value of 65.88u/L opposed to the 36.33U/L in the control group. S-GGT was the most important predictor of BMD in both the hip and the lumbar spine. There was a significant statistical correlation between lumbar spine BMD (p=0.04); hip BMD (p=0.02) and s-GGT.

In conclusion it can be said that malnutrition played a vital role in the low BMD aggravated by the use of tobacco from a young age and alcohol in excessive amounts over weekends. From the results of this study it can be recommended that any intervention programme should focus on alcohol abuse, tobacco smoking and improvement in nutritional status. Children should be encouraged not to smoke and be educated on the detrimental effects of alcohol. It is important to address dietary risk factors associated with OP, namely to increase the overall nutrition of the South African black male with low cost protein and calcium products. Vitamin C enhances iron absorption and may be beneficial for bone collagen. The increased intake thereof by using seasonal fruit can therefore be recommended.

Keywords: osteoporosis, bone mineral density, black men, GGT, alcohol, tobacco smoking

PREFACE

This research report follows growing interest in the prevention and treatment of osteoporosis by researchers, health professionals and the general public. Furthermore, it provides long overdue information about the possible risk factors for osteoporosis in South African black men. I hope that the findings of this study may contribute towards insight into the local setting and that the recommendations may be useful in the planning of appropriate strategies and programmes for the prevention of a possible future epidemic of osteoporosis in South African black men.

First and foremost, I can only thank God for giving me the health, strength, wisdom and perseverance to complete this study. I would like to convey my gratitude to those people and institutions that supported and assisted me in this study:

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LIST OF ABBREVIATIONS

%	percentage
$	dollar
1,25 $(OH)_2 D_3$	1,25-dihydroxyvitamin D
25-OHD	25-hydroxyvitamin D
AA	ascorbic acid
ADH	alcohol dehydrogenase system
AI	adequate intake
AIDS	acquired immunodeficiency syndrome
AGP	alpha-1 acid glyco protein
AHA	American Heart Association
ATP	adenosine triphosphate
BIA	bioelectrical impedance analysis
BCP	bromcresol purple
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BUA	broadband ultrasound attenuation
cAMP	cyclic adenosine mono phosphate
CD4	cell differentiation count 4
CD8	cell differentiation count 8
CDT	carbohydrate deficient transferrin
CI	confidence interval
CRP	cellular reactive protein
DEXA	dual energy X-ray absorptiometry
DRI	dietary reference intakes
EDTA	ethylenediaminetetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
et al	et alii
FBD	femoral bone density
FFQ	food frequency questionnaire
FNB	Food and Nutrition Board
FR	free radical
g	gram

g/L	gram per litre
GGT	gamma-glutamyltransferase
HIV	human immunodeficiency virus
IGF-1	insulin-like growth factor
IU	international units
kg	kilogram
kg/m^2	kilogram per meter squared
L2-4	lumber spine 2-4
m	meter
MEOS	microsomal ethanol oxidizing system
mg	milligram
mmol/L	millimol per litre
MJ	megajoules
ms	miss or mrs
N	number
NADH	nicotinamide adenine dinucleotide hydrogenase
NHANES iii	third national health and nutrition examination survey
nmol/L	nannomol per litre
NTx	cross-linked N-telopeptides of type 1 collagen
OP	osteoporosis
OSWAMA	**O**steoporose in **SW**art **Ma**ns
oz	ounce (30grams)
PAI	physical activity indicator
PBM	peak bone mass
PINI	prognostic inflammatory and nutritional index
Prof.	professor
PTH	parathyroid hormone
PU for CHE	Potchefstroom University for Christian Higher Education
RBD	radial bone density
RDA	recommended dietary allowances
SAMA	South African Medical Association
SD	standard deviation
S-Fe	serum iron
S-Ca	serum calcium
TIBC	total iron binding capacity
U/L	units per litre

µmol/L	micromol per litre
US	United States of America
W/H	waist to hip ratio
WHO	World Health Organisation

CHAPTER 1

1. INTRODUCTION

1.1 Background

About sixty years ago, Fuller Albright defined Osteoporosis (OP) as a disease where there is "too little bone in the bone, but what bone there is, is normal". By this he meant that, although some bone is lost, the chemical composition of the remaining bone is normal. At that time, we knew little about OP and had no means to treat or prevent it, other than mending the fractures (Ilich & Kerstetter, 2000). OP literally means "porous bone" (Bacon *et al.*, 1996). OP 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 (which is difficult to assess). There is a consequent increase in bone fragility and susceptibility to fracture, which typically involves the wrist, spine or hip (South African Medical Association (SAMA) Osteoporosis Working Group, 2000). It is a silent disease (O'Brien, 2001) but later clinical manifestations are back pain, loss of height, spinal deformity and fractures of the vertebrae, hips, wrists, and, to a lesser extent, other bones (Cohen & Roe, 2000).

Although the entire skeleton may be involved in OP, bone loss is usually greatest in the spine, hips and distal radius, as seen in Figure 1.1. Since these bones bear a great deal of weight, they are more susceptible to fracture. Hip fractures lead to death (both directly and indirectly as a result of long-term hospital stays) in 12 to 20 percent (%) of cases and precipitates long-term nursing home care for half of those who survive and very few return to independent living. Nearly one-third of all women and one-sixth of all men will fracture their hips in their lifetime (Lindsay, 1995). Serious fractures in adults relate less to the frequency of forceful accidents and more directly to the loss of bone in middle-aged and older people (Bell *et al.*, 1995). It was estimated that at least 90% of all hip and spine fractures among elderly white women are attributed to OP. Regardless of fracture type, attribution probabilities were less for men than women and generally less for non-whites than whites (Melton *et al.*, 1997). In the African population the female/male ratio of hip fractures are nearly equal, this suggests that Africans may not experience the same degree of accelerated

postmenopausal bone loss that has been documented in white populations. OP and fracture risk vary dramatically among racial and ethnic groups (Melton *et al.*, 2002). Therefore, information derived from studies of bone homeostasis in white populations cannot be simply extrapolated to other ethnic populations (Luckey *et al.*, 1996). Thinness, for example, has been clearly identified as a risk factor for hip fracture in African-Americans. Both black people and oriental people have shorter hip axis lengths. Differences in hip geometry alone could account for a 32% lower rate of hip fracture in African-Americans than in Caucasians (Heany, 2002).

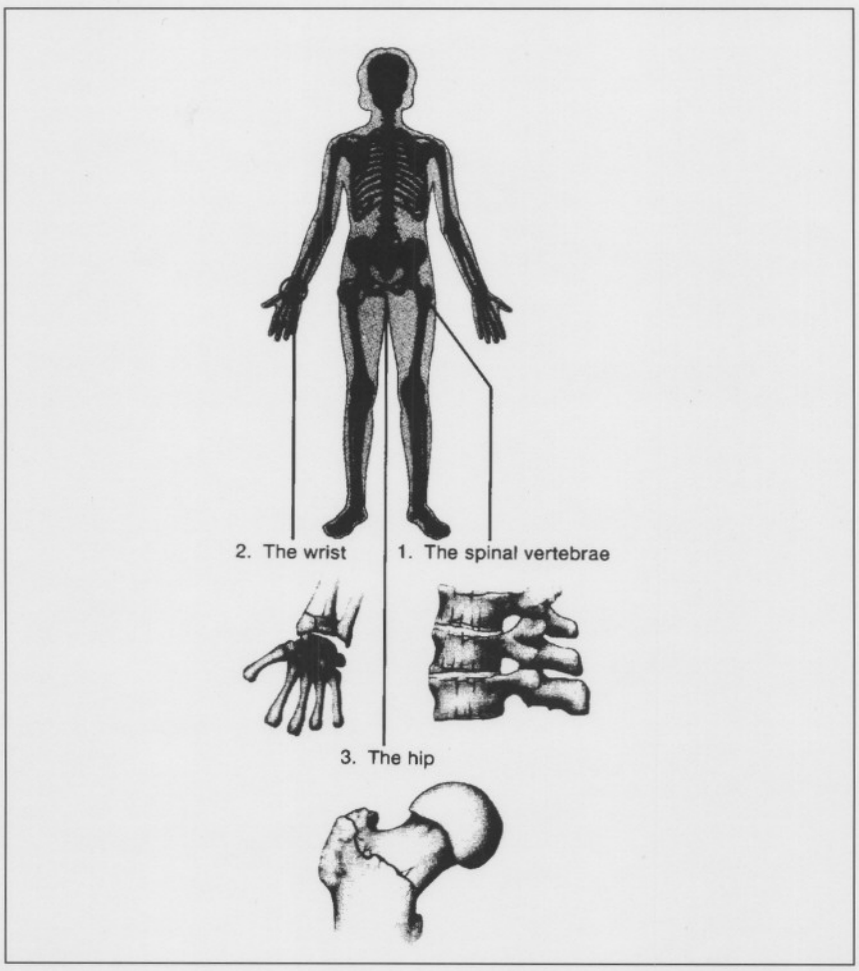

Figure 1.1: Three principle sites of osteoporosis fractures (Williams, 1999)

OP is a major cause of morbidity and mortality in developed countries, at a cost that currently exceeds $10 billion per year in the United States (US) alone. Annual economic implications of hip fracture in Canada are $50 million and are expected to rise to $2,4 billion by 2041 (Wiktorowicz *et al.*, 2001). In Austria, where osteoporotic hip fracture rates are increasing, the length of hospital stay for women was 8.5-27

days and 16-23 days for men (Koeck *et al.*, 2001). The average cost per patient for hospital treatment in the US was estimated at $9 097 with a total amount of $103 509 800 (Koeck *et al.*, 2001). Osteoporotic fractures affect 50% of women, 20-30% of white men and 4% of black men over the age of 50 years (Prince, 1997, Kirchengast *et al.*, 2001, National Osteoporosis Foundation, 2002). These statistics may even increase because of increasing life expectancy. The Southern region of Africa (Botswana, Lesotho, Namibia, Swaziland, Mozambique and Zimbabwe) has the continent's highest percentage of older inhabitants. The 1996 census data estimated that 2.8 million South Africans are aged 60 and older (7% of the population). This percentage is projected to increase to 11% or 6.3 million of the population (Charlton, 2000).

The main focus of bone loss and OP research has been limited almost entirely to women, but OP has become increasingly common in older men and the impact of hip fracture on mortality may actually be greater in men (Diamond *et al.*, 2001). The incidence of osteoporotic fractures in men is increasing as their life expectancy increases, and the incidence of hip fractures worldwide is predicted to increase in non-white populations (Raisz, 1997). Important underlying causes of osteoporotic fracture in men include glucocorticoid therapy, low body weight and reduced physical activity (Dempster & Lindsay, 1993). Tobacco and alcohol use has been consistently identified as risk factors for vertebral fracture but there is less evidence that they contribute to hip fracture (Compston, 2001).

Little is known about the aetiology and prevalence of OP and fractures among South African black men. From the above information it is clear that OP is of considerable clinical and economic importance. Without information on the patterns and determinants of bone loss, the formulation of rational prevention and treatment strategies in these groups is not possible (Melton *et al.*, 2002).

1.2 Background to the specific aim of this thesis

Few studies focusing on Blacks have been published to date (Heaney, 2002) and very little is known regarding the bone health of older South African blacks. A study conducted almost 30 years ago by Seftel *et al.*, (1966) reported that the prevalence of hip fractures in the South African black population was more than ten fold lower than

that seen in their black American counterparts (Charlton, 2000). Prof. N.G.J. Maritz from Pretoria Academic hospital (Orthopaedics) noticed, while conducting a study on deep vein thrombosis after hip fractures during the period April 1992 to June 1993, that more than 50% of the patients with hip fractures were black men. After discussing these findings with Prof. J. Davidson, Bradley University, USA, they initiated the current study with the main objective to investigate the possible role that dietary and lifestyle factors may play on the prevalence of osteoporotic fractures in this group of South African black men. The name for the study, OSWAMA, was decided on after the Afrikaans words for OP in black men (**O**steoporose in **SWA**rt **MA**ns). This study could play a very valuable role in expanding the current knowledge regarding OP and hip fractures among South African black men and the knowledge could also be used in the planning of appropriate strategies and programmes for the prevention of fractures in this group.

1.3 Aim of the study

The aim of the study described in this dissertation was to investigate the influence of some dietary factors (iron, vitamin C and protein) and lifestyle factors (alcohol and tobacco smoking) on osteoporotic fractures and BMD in older South African black men using a case-control study design.

Merensia Groenewald will, in her MSc dissertation report on the influence of physical activity, anthropometric variables (weight, body mass index (BMI), and body fat distribution) and dietary factors (calcium, vitamin D and phosphorous) on osteoporotic fractures in the same cases and controls.

1.4 Structure of the thesis

In this introductory chapter, the background of the problem, globally as well as locally, is discussed. The aims and motivation for the investigation follow in this chapter. A literature survey on OP is given in Chapter 2, including background information on the physiology of bone, pathogenesis as well as the diagnosis of OP. Relevant risk factors as well as nutrients that have an effect on BMD and bone turnover are discussed. This review also contains information on the effect of ethnicity and lifestyle factors on osteoporotic fractures. The methodology used in the empirical part of the

study is discussed in Chapter 3, including a description of the statistics used. The results are described in Chapter 4 with the discussion and recommendations of risk factors for osteoporotic fractures in older South African black men in Chapter 5.

CHAPTER 2

2. LITERATURE SURVEY

2.1 Introduction

The major objectives of this study were to describe the association between dietary and lifestyle factors and osteoporotic fractures and BMD among older South African black men. Therefore in this chapter background information on bone structure, the physiology of bone, calcium metabolism, bone markers and factors influencing peak bone mass as well as osteoporotic fractures will be briefly discussed.

2.2 Bone structure

Bone is a term used to mean both an organ, such as the femur, and a tissue, such as trabecular bone tissue. Each bone (organ) contains bone tissues of two major types, trabecular and cortical. These tissues undergo bone modelling during growth (height) and bone remodelling after growth ceases (Mahan & Escott-Stump, 2000).

2.2.1 Composition of bone and types of bone tissue

Bone consists of an organic matrix or osteoid, primarily collagen fibres, 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).

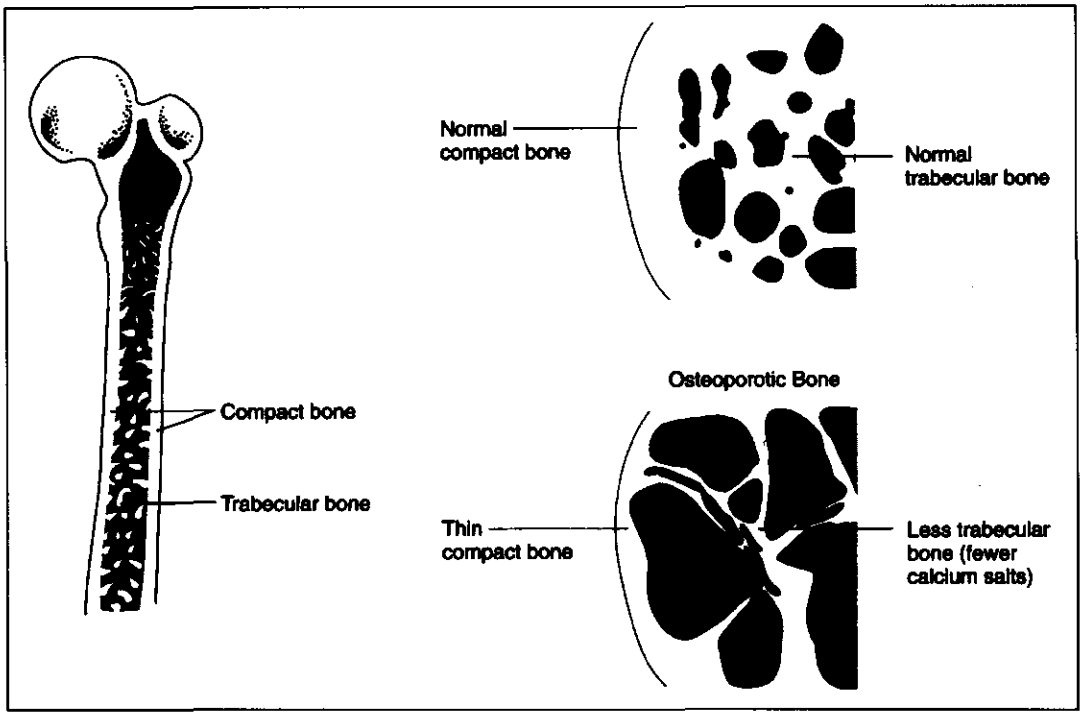

Figure 2.1: Trabecular and compact bone (Williams, 1999)

Approximately 80% of the skeleton consists of compact or cortical bone tissue. The remaining 20% of the skeleton is trabecular, or cancellous bone tissue, which exists in the knobbly ends of the long bones, the iliac crest of the pelvis, the wrists, scapulas, vertebrae, spine and in the regions of bones that line the marrow. This part of the skeleton is also referred to as the axial skeleton (Notelovitz, 1993, Mahan & Escott-Stump, 2000). Trabecular bone is less dense than cortical bone tissue as a result of its open structure of interconnecting bony spicules that resemble a sponge in appearance. Thus, trabecular bone is also called spongy bone or spongiosa. The elaborate interconnecting components (columns and struts) of trabecular bone tissue add support to the cortical bone tissue shell of the long bones as well as provide a large surface area that is lined by a larger number of cells than in cortical bone tissue (Mahan & Escott-Stump, 2000).

Trabecular bone tissue is, therefore, much more responsive to estrogens or the lack of estrogens than is cortical bone tissue. The loss of trabecular bone tissue late in life is largely responsible for the occurrence of fractures. Because of the anatomic variability in these compartments, bone loss occurs more rapidly in trabecular bone increasing its vulnerability to fracture. Figure 2.1 and 2.2 show the difference in normal trabecular bone and osteoporotic trabecular bone. This explains why

osteoporotic fractures tend to occur in the vertebrae (75% trabecular), the femoral neck and at the ends of the long bones (90% trabecular) (Notelovitz, 1993).

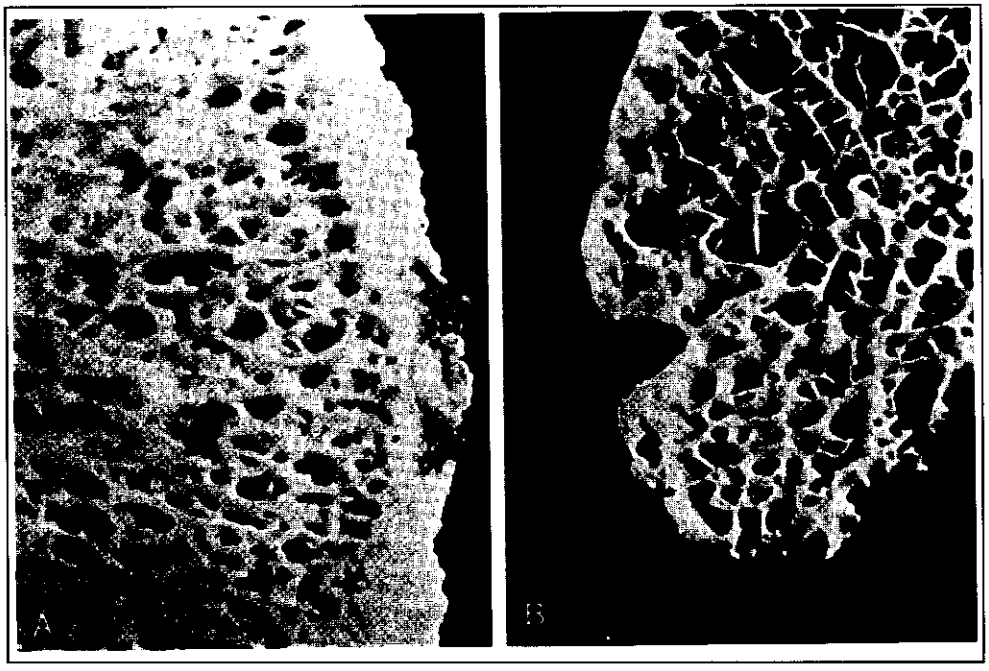

Figure 2.2: Difference between normal bone and osteoporotic bone (Mahan & Escott-Stump, 2000)

2.2.2 Structural anatomy

2.2.2.1 Cortical bone

Cortical bone has three surfaces. Each has different anatomic features, but similar cell types and a similar bone remodelling cycle. The three surfaces are:

- Endosteum envelope: The surface facing the marrow.
- Periosteum envelope: The outer surface of the bone.
- Intracortical envelope: Bony tissue between the endosteum and periosteum.

The activity of the bone remodelling cycle (discussed in more detail in 2.3.3) varies for each envelope depending on age and reproductive status, as follows (Figure 2.3):

- Childhood: New bone formation on the periosteum exceeds endosteum bone breakdown. A net increase in the outer diameter of bone results.
- Adolescence: Bone formation occurs on both the endosteum and periosteum surfaces with an increase in total bone mass.

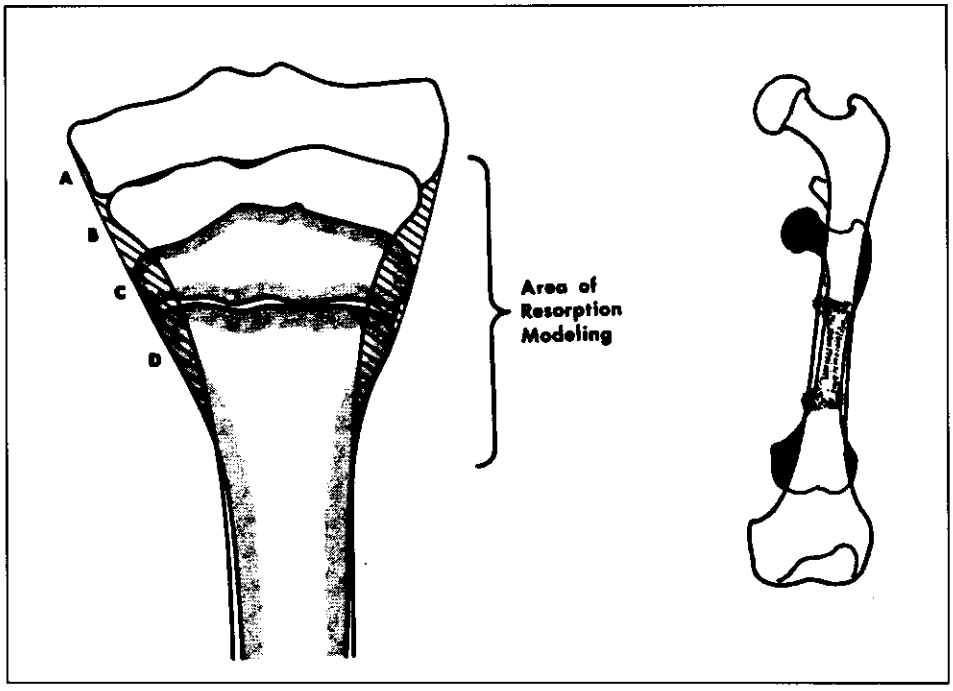

Figure 2.3: Remodelling cycle (Alford & Bogle, 1982) A: Early adulthood B: Adolescence C: Childhood D: Birth to five years

Early adulthood: Endosteum bone loss increases and begins to exceed periosteum bone apposition, indicating the beginning of age/menopause-related decrease in bone mass, with a narrowing of the intracortical envelope as result. The marrow cavity expands (Notelovitz, 1999).

2.2.2.2 Trabecular bone

Trabecular bone has a honeycomb-like arrangement of horizontal and vertical plates that are interconnected. This ensures mechanical strength. Bone remodelling takes place on the inner and outer envelopes of each trabecular plate. Excessive bone remodelling results in thinning of plates with eventual dissolution of tissue and loss of structural continuity. This occurs initially in the horizontal trabeculae and leads to a decrease in mechanical strength, with an increased liability to fracture due to physical stress (Notelovitz, 1999).

2.3 Bone physiology

2.3.1 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 almost totally reliant on this source of calcium when the diet is inadequate. Bone tissue is also dynamic - although a slow dynamic because it undergoes both modelling early in life and remodelling after skeletal growth or height gain ceases. Although 99% of the body calcium is found in the skeleton, the remaining 1% is critical to a great variety of dispensable life processes (Mahan & Escott-Stump, 2000). The concentration of calcium in blood and other extracellular fluids is regulated by complex mechanisms that balance calcium intake and excretion with bodily needs as demonstrated in Figures 2.4 and 2.5 (Williams, 1999, Whitney *et al.*, 2002).

Adaptation of the homeostatic mechanism regulating blood calcium concentration is achieved through two calcium-regulating hormones, parathyroid hormone (PTH) and the hormonal form of vitamin D: 1,25-dihydroxyvitamin D (1,25 $(OH)_2 D_3$) also known as calcitriol (Nordin, 1997). This calcium-regulatory system works more efficiently early in life, especially during the first few decades, but the efficiency undergoes a gradual decline in later life. PTH activity, which directly contributes to bone loss, increases in most individuals during the seventh decade of life, even though PTH measurements typically remain within the normal range but at the high end. Calcitriol, also plays an adaptational role by increasing the efficiency of intestinal calcium absorption in the lower half of the small bowel when dietary calcium is inadequate (Mahan & Escott-Stump, 2000).

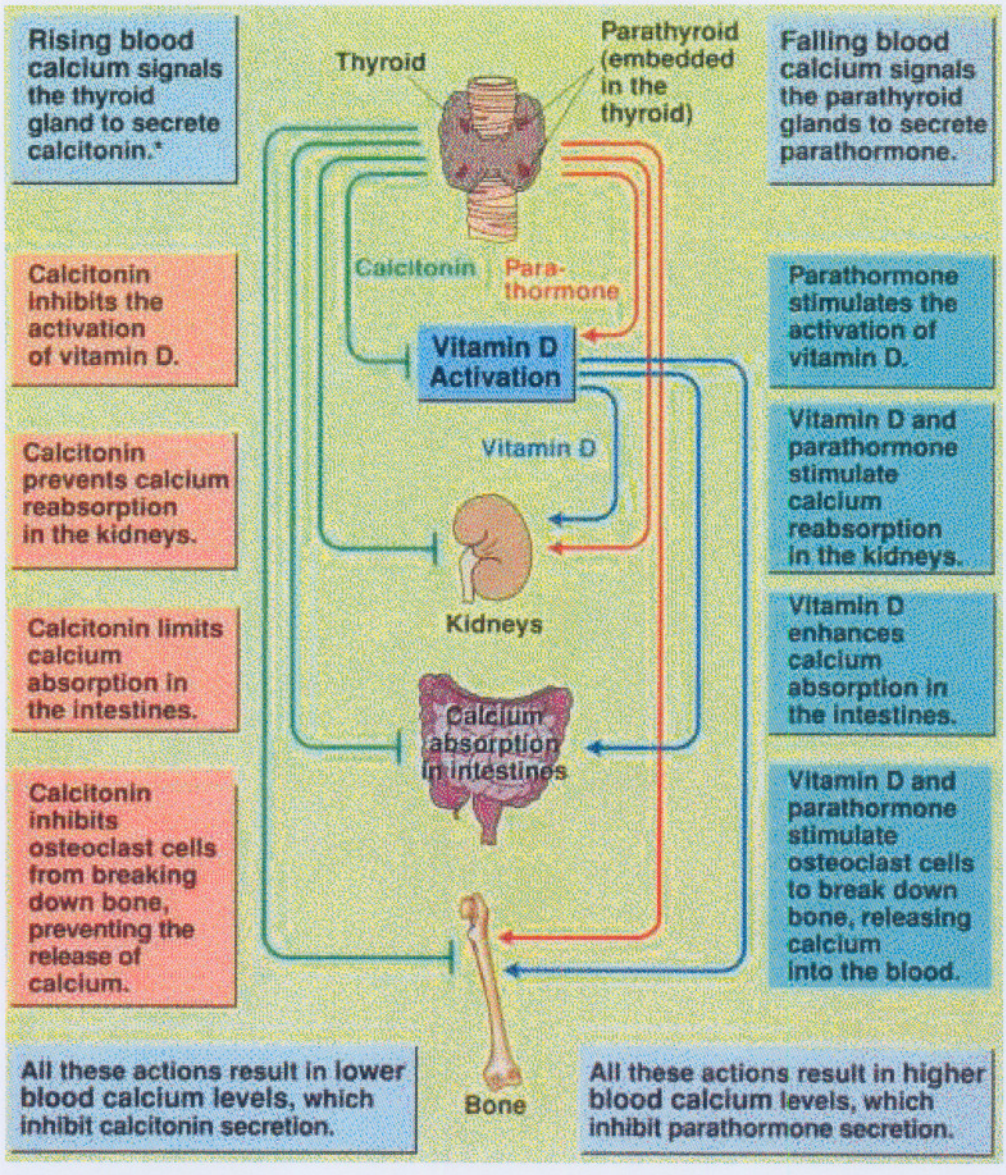

Figure 2.4: Calcium homeostasis (Whitney et al., 2002)

Because net intestinal absorption is only about 10% at contemporary intakes, there is a possibility, at the gut alone, of adapting adequately to even very low intakes (Heany, 2002) (Figure 2.5). African–Americans, for example, exhibit bone mass values, adjusted for weight, 6-12% higher than Caucasians at all ages, from infancy to old age. In fact, a compelling body of evidence indicates more efficient utilization of dietary calcium in black people (Heany, 2002).

In a review, Heany (2002) sets forth black-white differences in the components of the calcium economy and in the calciotrophic hormone levels that regulate them, from several published studies. 25-Hydroxyvitamin D (25-OHD) is almost universally found

to be substantially lower in blacks than in whites, reflecting, at least in part, the damping effect of skin pigmentation on dermal synthesis of vitamin D at most US latitudes. At the same time, serum parathyroid hormone (PTH) is generally elevated, and with it, serum $1,25(OH)_2D_3$ and nephrogenous cyclic adenosine monophosphate (cAMP). Urine calcium has been found to be significantly lower in black people in 19 of the 20 studies in which it was measured. Intestinal absorption efficiency (as illustrated in Figure 2.5) was higher in three of the four studies of black children and adolescents, but has not been found to be significantly different from that of adult Caucasians. In all of these studies, serum calcium did not differ significantly between the different ethnic groups (Heany, 2002).

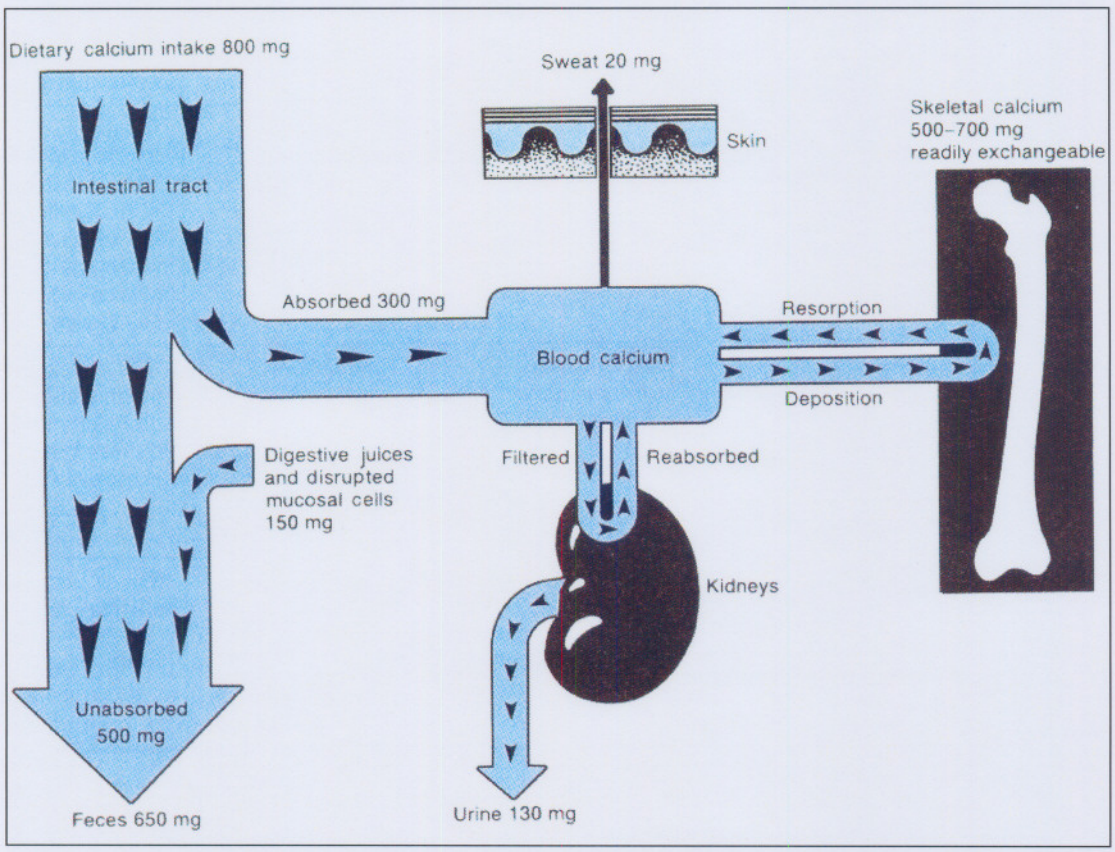

Figure 2.5: Intestinal absorption efficiency of dietary calcium (Williams, 1999)

2.3.2 Bone modelling and re-modelling

Bone modelling is the term applied to the growth of the skeleton until mature height is achieved. For example, during bone modelling long bones elongate and widen by undergoing internal changes as well as external expansions in their structures. In modelling, the process of formation of new bone tissue occurs first and it is followed

by the resorption of old tissue. Bone modelling is typically completed in girls by 16-18 years of age and in boys by18-20 years. After growth in height ceases, gains in bone tissue may continue by the process known as bone consolidation. The major activity of the skeleton in early life is growth and gain in bone, whereas in later life it is the loss of bone. This concept underlines the inevitable decline of bone mass in the late stages of life (Mahan & Escott-Stump, 2000).

Bone remodelling takes place after skeletal growth is completed. Bone continuously undergoes remodelling in response to strains on the skeleton, adapts to changes in lifestyle factors and dietary intakes, maintains the set calcium concentration in extracellular fluids, and repairs microscopic fractures that occur over time to remove old bone and form new bone. This process ensures bone health. 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. Even in the mature skeleton, bone remains a dynamic tissue (Notelovitz, 1999; Mahan & Escott-Stump, 2000). The process of the formation and breakdown of bone will be discussed in the following section.

2.3.3 Bone turnover

Two types of cells are primarily responsible for bone turnover, namely osteoblasts and osteoclasts, as can be seen in Figure 2.6. The origin of the osteoblasts and osteoclasts is from primitive precursor cells found in bone marrow (Mahan & Escott-Stump, 2000). When dietary calcium intake is low, osteoclastic resorption becomes greater than the formation by osteoblasts, because of a persistently elevated PTH concentration in blood. The action of PTH in promoting activity of the osteoclasts is countered by estrogen, 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 (Notelovitz, 1999).

These cells are derived from the bone marrow mononuclear cells (pre-osteoclasts) that line the bone-forming surfaces. The characteristic feature is a ruffled border where active resorption takes place. The resorption process is rapid and completed within a few days; whereas the refilling of these cavities by osteoblast is slow, in the

vicinity of 3-6 months. The main function of osteoclasts is to dissolve bone mineral and digest bone matrix. The differentiation, recruitment and inhibition of osteoclasts are controlled by numerous hormonal and growth factors. Osteoclasts have estrogen receptors and the primary effect of estrogen and other antiresorptive drugs is to inhibit osteoclast recruitment (Notelovitz, 1999).

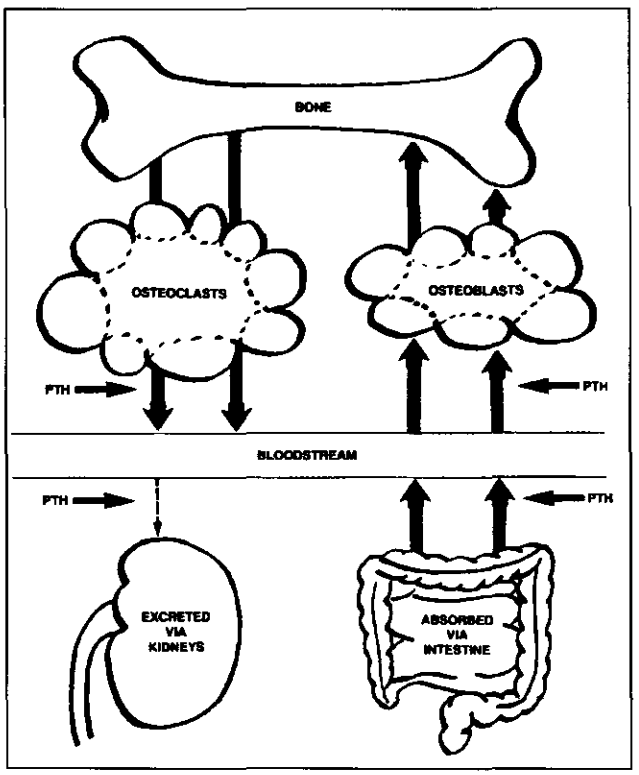

Figure 2.6: Osteoclast and osteoblast function (Marieb, 1995)

- Osteoblasts are attracted into the resorption cavity and, under the influence of various hormones and growth factors, mature to refill the resorptive cavity with "new" bone. This takes place in two stages:
  - The first stage involves the synthesis of bone matrix and 90% is made up of type 1 collagen. During the conversion from pre-collagen to collagen, extension peptides are removed.
  - In the second stage, the newly formed osteoid is now mineralised with calcium hydroxyapatite crystals. The latter also contain trace amounts of magnesium, potassium, sodium and carbonate. Two clinical points of note: (1) 1,25 $(OH)_2$ D_3 is essential for this process. In its absence mineralization is defective, leading to osteomalacia; (2) the orientation and composition of the crystals and their resistance to osteoclast activity is altered by sodium fluoride. If sodium

fluoride is used clinically, it is essential that adequate osteoid be stimulated, for example, by prior and/or concomitant estrogen and calcium therapy (Notelovitz, 1999).

2.4 Markers of bone remodelling

Bone markers exist for both bone formation and bone resorption. Plasma bone-specific alkaline phosphatase is a marker of bone formation, although total plasma alkaline phosphatase may also be used. Markers of bone resorption include plasma cross-linked collagen telopeptides, urinary N-telopeptides, and plasma tartrate-resistant acid phosphatase. Osteocalcin, considered a bone formation marker, is also released from resorbed bone matrix, and therefore interpretation of its blood values is not clear under most conditions. Serum osteocalcin appears to be the most sensitive marker for bone turnover and subtle changes of bone formation (Delmas, 1993). The other bone formation marker, osteonectin, connects the calcium hydroxyapatite crystals but it is not an effective marker because it is also found in other tissues (Mahan & Escott-Stump, 2000)

2.4.1 Bone mass

Bone mass is a generic term that refers to bone mineral content (BMC), but not to 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). Optimal bone mass depends on three essential supports: sex hormones, building materials (nutrition) and mechanics (physical load). If one of these supporters is weakened, bone quality is impaired (Ziegler *et al.*, 1995).

2.4.2 Peak bone mass

Peak bone mass (PBM) is reached around the age of 35 years. PBM is the greatest amount of bone accumulated at any age. PBM is greater in men than in women because of their larger frame size. The long bones stop growing in length before age 18 in girls and age 20 in boys, but bone mass continues to accumulate for a few more

years by a process known as consolidation. The age when BMD acquisition ceases varies and it depends not only on diet but also on physical activity and strain on the skeleton (Mahan & Escott-Stump, 1996).

Bone mass is the major determinant of bone strength and the relative risk of osteoporotic fractures. BMC is a function of two factors: peak bone mass achieved at skeletal maturity and the subsequent rates and duration of bone loss (Daniels *et al.*, 1995). Both BMC and BMD levels are normally lower in women than in men. BMD is also higher in African Americans and Hispanics than in whites and Asians (Bell *et al.*, 1995). Environmental determinants of bone mass, including dietary and other lifestyle factors, increase in importance prior to puberty in both boys and girls, perhaps most significantly during the two years immediately preceding puberty. Johnston *et al.* (1992) observed a greater increase in bone mass at three different skeletal sites in prepubertal twins receiving a calcium supplement of 1000mg per day for two years than in similarly treated post pubertal twins. This increase was independent of sex and age of puberty. Clearly, by age 18 all females and by age 22 practically all males in nations with adequate food supplies have completed their growth in height. Maximum growth is dependent on sufficient amounts of energy and protein in the diet (Anderson & Pollitzer, 1994). 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).

2.4.3 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 available for the measurement of the total body and regional skeletal sites of interest, such as the lumbar vertebrae and the proximal femur (hip). The 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. The values are presented in a graph that helps to diagnose the patient (Figures 2.7 and 2.8)

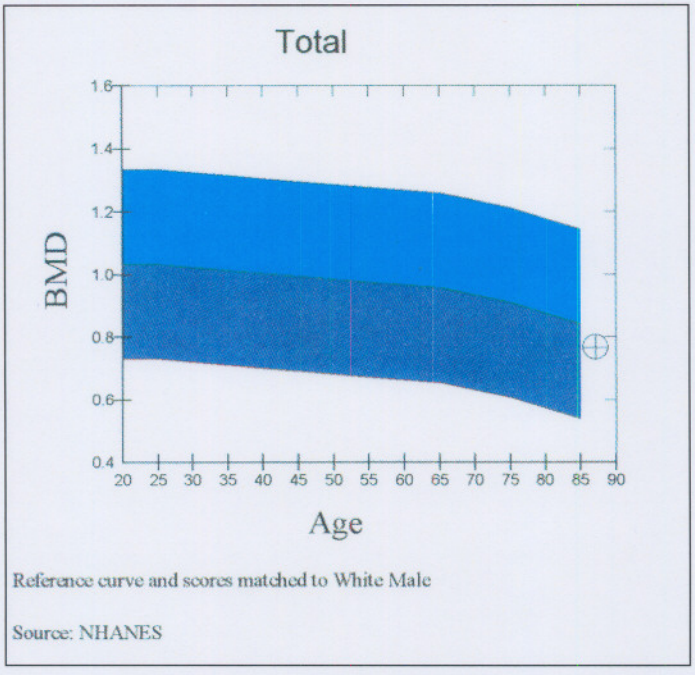

Figure 2.7: Reference curve for total hip BMD in white males (DEXA software) (light blue:osteopenic; dark blue: osteoporotic)

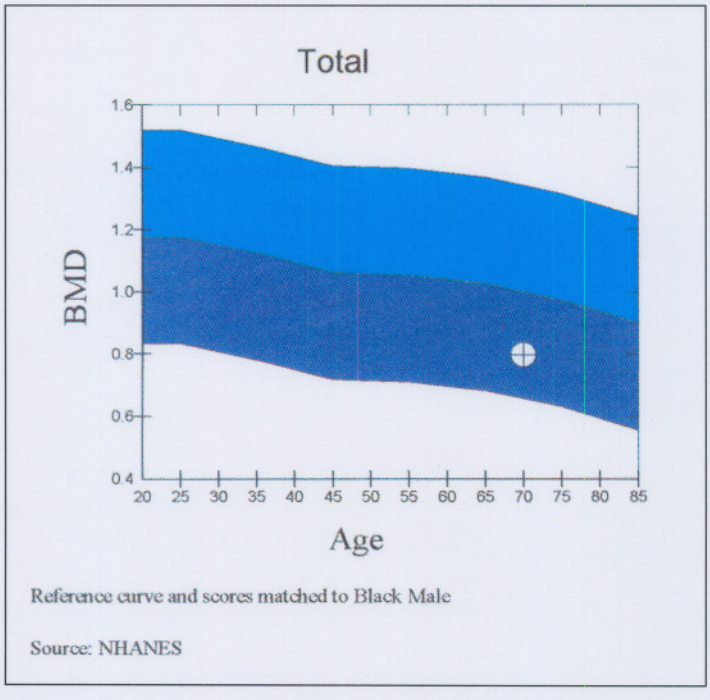

Figure 2.8: Reference curve for total hip BMD in black males (DEXA software)(light blue:osteopenic; dark blue: osteoporotic)

The information given visually in Figures 2.7 and 2.8 can also be expressed numerically by using Z-scores. The Z-scores are defined by the equation:

$$\text{Z-score} = \frac{\text{Measured BMD - Age matched BMD}}{\text{Population standard deviation (SD)}}$$

This expresses by how many SD's a subject differs from the mean value for an age, sex and race matched population. Thus, a subject lying on the central curve in Figure 2.7 has a Z-score of zero, while subjects on the upper and lower curves have Z-scores of +2 and –2 respectively (Arden & Spector, 1997). The T-score is similar to the Z-score except that the mean and SD of the young adult age group (20-35 years) are used as the reference range. T-scores compare a given subject with the sex and race adjusted expected maximum BMD achieved in life. The T-score is defined by the equation:

$$\text{T-score} = \frac{\text{Measured BMD-young adult mean BMD}}{\text{Young adult SD}}$$

(Arden & Spector, 1997).

A World Health Organization (WHO) Technical Report advocated an interpretation of bone densitometry measurements based on T-score values in which subjects are divided into four categories as follows.

Normal: A BMD value of not more than 1SD below the young adult mean value (T>-1.0).

Osteopenia: A BMD value that lies between 1 and 2.5 SD below the young adult mean (-1.0>T>-2.5).

OP: A BMD value more than 2.5 SD below the young adult mean value (T<-2.5)

Established OP: A BMD value more than 2.5 SD below the young adult mean value (T<-2.5) in the presence of one or more fragility fractures (WHO, 1994).

Caution may be needed in the interpretation of T-scores in the older population, Z-scores are probably more appropriate (Arden & Spector, 1997). Fracture incidence in individuals whose bone density is greater than 1 SD-above the mean is 50% lower at 80 years (Branca & Vatuena, 2001). Computed tomography may also be used to measure BMD (a true volumetric density) of the spine. This technique has now been developed to measure the limbs as well (Mahan & Escott-Stump, 1996).

2.4.4 Ultrasound measurements of bone

The quantitative ultrasound measurement of the kneecap and heel bone (calcaneus) provide information on two properties: the elasticity and strength of the bone. The ultrasound values are not equivalent to the BMD measurements because ultrasound assesses the properties of collagen in the organic matrix rather than the mineral phase of bone tissue. Ultrasound instruments actually measure the velocity of sound waves transmitted through bone and broadband ultrasound attenuation (BUA). Measurements at the calcaneus correlate well with BMD measurements at this same skeletal site, meaning that low values of BUA typically mirror low values by DEXA. Therefore, ultrasound is about as good as DEXA in predicting the risk of fracture (Mahan & Escott-Stump, 1996).

2.5 Factors influencing bone mass, osteoporosis and fractures

From a societal perspective it is appropriate to formulate risks and intervention thresholds in populations (Kanis, 2001). Data obtained from twins and families indicated that as much as 80% variance in bone mass within a population is genetically determined (Sambrook *et al.*, 1993). Bone density in later life depends on the peak achieved at skeletal maturity and on subsequent age-related bone loss, peak bone density being strongly determined by genetic factors (Stewart & Ralston, 2000). Dietary factors such as calcium intake, vitamin D status, protein intake, fruit and vegetable consumption and iron overload and lifestyle factors such as physical activity, smoking and alcohol intake as well as long-term corticosteroid therapy play contributory roles in the development of this multifactorial disease. These factors account for ±20% of the variance in bone mass within a population. Many of the risk factors are considered to be weak, although when combined they could impact significantly on bone health (Cohen & Roe, 2000). These risk factors that apparently predispose to the development of OP have not been accurately identified and given relative priority (Blaauw *et al.*, 1994). Nordin (1997) summarised the different risk factors and how they may predispose to OP (Figure 2.9). For the purpose of this thesis only the factors that will be examined as well as the role of ethnicity will be discussed in more detail.

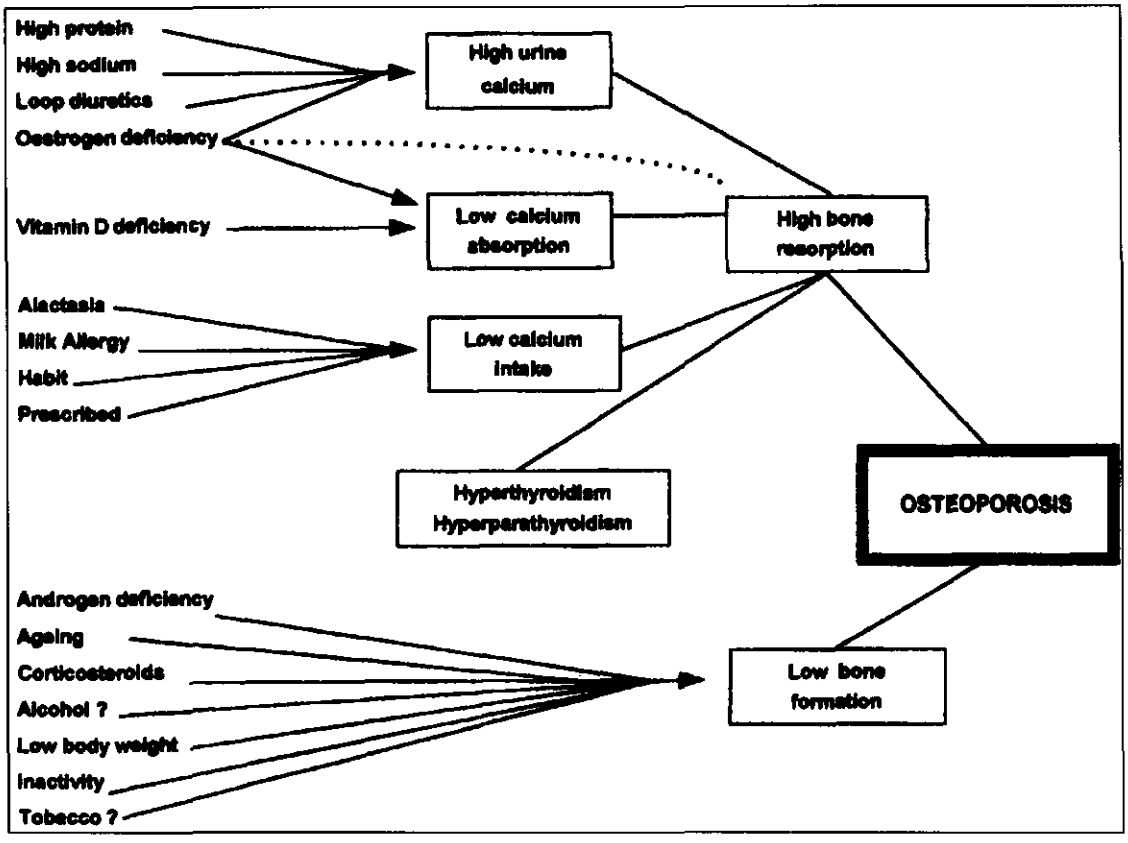

Figure 2.9: Diagrammatic representation of the pathways leading to osteoporosis (Nordin, 1997)

2.5.1 Protein

Dietary protein affects bone in a variety of ways. Approximately one third of the mass of bone is protein, and, as such, bone is one of the most protein-dense tissues of the body. Dietary protein, with its content of essential amino acids, is necessary for new bone matrix synthesis (Heany, 1998). Essential amino acids (lysine, methionine, tryptophan, arginine and threonine) can stimulate bone matrix formation and could represent useful agents for the prevention and therapy of OP (Conconi *et al.*, 2001). Essential amino acids stimulate alkaline phosphatase activity and collagen synthesis. Alkaline phosphatase is involved in the mineralisation (see 2.5), allowing calcium deposition into the bone matrix. The effects of the essential amino acids are probably mediated by insulin-like growth factor 1 (IGF-I), which stimulates osteoblast proliferation and differentiation, type 1 collagen synthesis, osteocalcin production and alkaline phosphatase activity. Moreover, IGF-I is considered an important factor for bone longitudinal growth and plays a role in trabecular and cortical bone formation (Conconi *et al.*, 2001).

Bone growth is stunted in protein-energy malnutrition, and the outcome of hip fracture is dramatically improved with protein supplements in the typical elderly victim of osteoporotic fractures (Heaney, 1998). The importance of malnutrition is emphasised 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 being underweight and OP (Coin *et al.*, 2000). Additionally, dietary protein comes in the form of foods that contain associated nutrients also important for bone building. Clearly these effects of protein on bone are all positive and underscore the importance of ensuring an adequate protein intake throughout life (Heaney, 1998).

In contrast, high animal protein intake may increase urinary calcium loss and increase glomerular filtration rate (Massey, 1998). These changes may be ascribed to changes in acid load, commonly associated with oxidation of sulphur-containing amino acids, the relatively large number of inorganic ions associated with meat or to increased insulin concentrations (Barzal & Massey, 1998). High plant diets have an alkaline load and have been proposed as a major factor in favour of overall calcium balance (Massey, 1998). Diets high in fruit and vegetables produce more alkaline urine by contributing a variety of compounds that accept hydrogen ions during their metabolism (Tucker *et al.*, 1999). Results from metabolic ward studies showed that for each gram of ingested animal protein ±40mg of calcium may be lost in the urine (Nordin, 2000). Studies done under free living conditions, however, reported little evidence that high animal protein intake reduce bone mass and increase fractures. Where high animal protein intakes are associated with high calcium intakes, protein had no effect on the bone status (Heaney, 1998; Heany, 2000b). Studies are therefore inconclusive about the relationship between calcium absorption efficiency and protein intake (FNB, 2002). It may be useful to evaluate the protein-calcium ratio in the diets (Heaney, 1998). The Food and Nutrition Board, in 1997, recommended a calcium: protein intake ratio (mg:g) of 20:1 (FNB, 1998).

2.5.2 Alcohol

Alcohol abuse appears to be an important factor in the pathogenesis of femur and neck fractures in black men (Schnaid *et al.*, 2000). Alcohol abuse is associated with a reduction in bone volume and trabecular thickness, and mild demineralisation (Schnaid *et al.*, 2000). Alcohol is a strong inhibitor of bone formation as reflected by decreased serum levels of osteocalcin (Laitinen *et al*, 1994), and consumption over a long period is toxic to bone (Illich & Kerstetter, 2000), specifically the osteoblasts (Schnaid *et al.*, 2000). In chronic alcoholics, poor nutrition and malabsorption of critical nutrients, particularly calcium, magnesium, zinc, (Illich & Kerstetter, 2000) as well as the deficiency of active metabolites of vitamin D were observed (Medra & Jankowska, 2000). Drinkers showed a higher 24-hour urinary calcium excretion and lower plasma alkaline phosphatase than non-drinkers. Alcohol may therefore act by depressing bone formation and increasing urinary calcium. The effect of alcohol on calcium excretion is greater than that of smoking, but in combination with smoking probably the most significant effect (Cohen & Roe, 2000).

Alcohol can also cause liver disease and influence PTH function. Alcohol intake may furthermore increase the propensity to fall, thereby increasing the chances for fractures (Illich & Kerstetter, 2000). Hoidrup *et al.*, as discussed by Illich and Kerstetter (2000), showed the increased risk for hip fractures in 18 000 men who consumed more than 27 drinks/week, particularly in those who preferred beer over wine or other spirits. In a prospective study by Schnaid *et al.* (2000) in black patients, alcohol abuse in the men appeared to be one of the important etiological factors in the pathogenesis of femur neck fracture.

Moderate alcohol consumption appears to be beneficial for bone. In the Framingham Heart Study cohort, both men and women who consumed 420g and 210g of alcohol per week, respectively, had higher BMD in the femur, spine and forearm, compared to those who consumed < 30g of alcohol /week (Tucker *et al.*, 2002). On a similar note, a positive association between moderate alcohol intake and BMD was also revealed from the Copenhagen Centre for Prospective Population Studies (Hoidrup *et al.*, 1999). The possible explanation for why moderate alcohol intake improves bone status may be that alcohol stimulates androstenedione conversion into estrone (Turner & Sibonga, 2001). The aromatization of androgens to estrogens in

postmenopausal women is the only source of their estrogen (Illich & Kerstetter, 2000). Because elderly men have low serum bioavailable estrogen and testosterone levels, and because recent data (Bachrach, 1999) suggest that estrogen is the main sex steroid regulating bone metabolism in men, estrogen deficiency may also be the principal cause of bone loss in elderly men (Riggs, 2002).

2.5.3 Tobacco smoking

A review of 48 cross-sectional, cohort or case control studies regarding the effect of smoking on bone density and hip fracture rates concluded that the hip fracture rate after age 50 was higher and the bone density was significant lower in smokers compared to non-smokers, independent of thinness or physical activity. A review of 13 studies on BMD in smokers and non-smokers concluded that smoking did not have an important influence on peak bone density but was associated with an increased rate of bone loss (Cohen & Roe, 2000).

Tobacco smoking is found to be associated with a low bone mass, increased bone loss and an increased risk of osteoporotic fractures (Brot *et al.*, 1999). Several hypotheses have been put forward concerning the mechanisms by which smoking affects bone, the main focus being on the anti-estrogenic effect. Smokers are often lean, have early menopause and have reduced levels of circulating estrogens due to an increased hepatic turnover. All these factors contribute to a reduced exposure to estrogen, resulting in an increase in early bone loss. Other lifestyle risk factors for OP are regarded as more prevalent among smokers compared to non-smokers such as less physical activity, increased alcohol intake, or associated nutritional deficiencies, all of which may play a role (Szulc, *et al.*, 2002). A direct toxic effect of tobacco smoking on bone cells is also a possibility (Brot *et al.*, 1999).

Men who had smoked more than 7120 packs (third quartile) per year had lower BMD of total hip and distal forearm compared with men who never smoked. Current smokers had a higher prevalence of vertebral deformities after adjustment for age and body weight. In moderate smokers with low body weight increased bone resorption, not matched by increased bone formation, resulted in decreased BMD and an increased prevalence of vertebral deformities. As discussed earlier PTH and vitamin D metabolites are crucial in the regulation of calcium homeostasis and bone

metabolism (Brot *et al.*, 1999). The finding of lower 25-OHD concentrations in smokers in a Boston study was consistent with previous reports in humans and may be the effect of nicotine (Harris *et al.*, 2000). According to a large, cross-sectional study by Szulc *et al.* (2002), current smokers were younger, thinner, and drank more coffee and more alcoholic beverages. Low serum 25-OHD and secondary hyperparathyroidism explained, at least partly, the effect of tobacco on bone turnover in this study. In former smokers, bone resorption was not increased, but BMD remained lower compared with that in never-smokers (Szulc, *et al.*, 2002).

2.5.4 Vitamin C status

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, but there is no information about optimal intakes. The anti-oxidant role of vitamin C might also be important to modulate skeletal metabolism (Branca & Vatuena, 2001).

The administration of ascorbic acid (AA) in black subjects with siderosis and osteoporosis significantly reduced urinary calcium excretion in those black subjects who were initially deficient in the vitamin. While this could be a direct renal effect, it is more likely to be a reflection of improved calcium retention due to increased bone formation and decreased bone resorption induced by AA repletion (Lynch *et al.*, 1970).

In a recent cross-sectional study on women, high intakes of vitamins E and C significantly decreased the odds ratio for hip fracture in current smokers. The study supports the hypothesis that certain anti-oxidant vitamins are protective against oxidant–mediated bone loss in smokers (Illich & Kerstetter, 2000).

2.5.5 Iron

2.5.5.1 Iron overload

Strachan first described iron overload or excessive deposition of iron in the organs

and tissues of Africans in 1929 and it has been the subject of extensive research. Iron overload can be diagnosed with a plasma iron of more than 30 μmol/L and a transferrin saturation of more than 60% (Beard *et al.*, 1996). Cirrhosis, portal fibrosis, OP, and scurvy are associated with iron overload. In 1953 it was hypothesised that iron overload was due primarily to excessive iron intake derived from food and drinks prepared in iron vessels (Walker & Segal, 1999). Sorghum beer is brewed in iron containers in rural areas. The acidic beer from the containers leaches iron. It is then absorbed and deposited in the reticuloendothelial system (Schnaid *et al.*, 2000).

In some patients with primary haemochromatosis, hyperparathyroidism has been described. Conversely, the accumulation of iron in the parathyroid glands has resulted in hypoparathyroidism (Eyres *et al.*, 1992). Eyres *et al.* (1992) described the first case report of osteoporotic fractures as a presenting feature of haemochromatosis occurring in a eugonadal (no hyper- nor hypoparathyroidism) patient with normal liver function, although it was possible that alcohol may have contributed in part.

Ebina *et al.*, as discussed by Eyres *et al.* (1992), have distinguished the effects of iron and aluminium on bone formation. Whereas aluminium was deposited at the interface between osteoid and mineralised bone, iron was deposited in the osteoblasts and osteoclasts. They suggested that iron had a cellular effect on osteoblastic activity by altering lipid peroxidation. Even moderate iron retention in the absence of gonadal or hepatic impairment appeared to induce significant bone disease in susceptible patients (Eyres *et al.*, 1992). Recently in 1992 it was advanced that a gene may also play a role. The candidate gene is yet to be characterised. The condition is characterised by excessive iron absorption, increased plasma iron concentration and transferrin saturation, and increased iron stores in parenchymal cells. This condition has only been described in populations of European ancestry (Kasvosve *et al.*, 2000).

Schnaid *et al.*, (2000) reviewed many studies that have concluded that iron overload is responsible for considerable morbidity and mortality. However, there are numerous limitations in the evidence. There are also problems in interpretation, since levels of iron in the serum are affected additionally by a variety of factors: inflammation, infection, certain cancers and alcohol intake. These considerations complicate

attempts to assess to what extent the associations described denote causation, and whether iron overload has significant ramifications for ill in the general African population (Schnaid *et al.*, 2000).

Vitamin C deficiency also stimulates bone erosion and adding smoking to the iron overload as a risk factor (Walker, 1999), the combination of these factors can cause OP (Schnaid *et al.*, 2000). Earlier studies found no positive correlation between iron and erosion variables in bone but the number of iron granules in the bone correlated with the erosion depth found in subjects with iron overload and hypovitaminosis C (Schnitzler *et al.*, 1994). To get an understanding of the mechanistic role iron plays in oxidative damage, interpretation of the fact that plasma concentration of several anti-oxidants are decreased in the presence of disease is offered (Crawford, 1995). Although less well studied than ascorbic acid, several other organic acids appear to have comparable enhancing effects on iron absorption in single-meal studies. The high absorption of iron from maize and sorghum beers in sub-Saharan Africa is due to the presence of lactic acid (Lynch, 1997). The combination of citric acid and ascorbic acid (a synergistic pair of strong enhancers) is instrumental in causing a deleterious increase in iron load in ageing populations (Crawford, 1995).

However, in Johannesburg, within recent years it became apparent that both the prevalence and severity of iron overload in urban African men have decreased markedly. This may be attributed largely to a change in drinking habits, with Western liquors having partially replaced traditional beverages (Walker & Segal, 1999). While the adverse sequelae of iron overload may be of less significance than many believe, the precise pathogenicity of the phenomenon will remain uncertain until further investigations, including prospective studies, are undertaken (Walker & Segal, 1999).

2.5.5.2 Low iron status

Not much could be found in the literature regarding low iron status and osteoporosis. Anderson *et al.* (1996) summarised that iron plays a role in collagen maturation and low iron status can lead to insufficient cell energetics in collagen maturation.

2.6 Ethnicity

Because this study was done on black men it is appropriate to discuss the role that ethnicity plays on osteoporosis. Race may have a significant and differential effect on the bones in the axial and appendicular skeletons (Henry *et al.*, 2000). In the axial skeleton, black children had greater cancellous bone density, but similar cross-sectional areas of the vertebral bodies. In contrast, in the appendicular skeleton, black children had greater femoral cross-sectional areas, but similar cortical bone areas and cortical bone density. Compared to white children, black children at sexual maturity had on average 10.7% and 5.7% higher vertebral bone density and femoral cross-sectional areas, respectively. Such significant variations may contribute to the racial differences in the prevalence of osteoporosis between black and white adults (Gilsanz *et al.*, 1998), but the skeletal advantage in blacks during young adulthood is not explained by bone size (Henry *et al.*, 2000). Wright and his co-workers reported that the greater BMD in adult black men compared with white men was associated with a higher secretion of growth hormone (Wright *et al.*, 1995). However, in prepubertal boys the higher hip BMD in black compared to white boys could not be explained by differences in secretion of growth hormone (Wright *et al.*, 2002).

Blacks have a greater bone mass and a lower incidence of OP and hip fractures than whites (Weinstein & Bell, 1988). In the US, age-adjusted hip fracture incidence was 50% lower in African-American than in white women (Luckey *et al.*, 1996). 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 black people was only 35% of that in whites. The rate of bone turnover is probably lower in blacks than in whites, since bone resorption and bone formation are closely coupled in the steady state. Bell (1997) discussed five studies from 1985 – 1996 that have shown that serum osteocalcin, an indicator of bone formation rate, was lower in African Americans than in white men and women.

Although bone loss occurs universally with age in all populations, the incidence of age-related osteoporotic fractures varies widely among ethnic groups (Luckey *et al.*, 1996; Zerbini *et al.*, 2000 & Melton *et al.*, 2002). Bone density reaches a peak about fifteen years later in African men than in Caucasian men (Bell *et al.*, 1995; Daniels *et*

al., 1995). Bone loss during the period between 30-50 years is not as rapid in Africans as in Caucasians (Solomon, 1979). Bell (1997) confirmed that a major factor responsible for the lower fracture rate in African Americans is greater bone mass. Thus the ethnic disparity in bone mass and fracture incidence could result from higher peak bone mass 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 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, Baron *et al.* (1994), noted that among blacks, the female predominance in hip fracture risk seen in whites is either absent or much reduced (Baron *et al.*, 1994). Even in Australia with Aboriginals, the indigenous females develop osteoporotic type fractures of the femoral neck at a later age than do non-indigenous females. This may reflect a genetic difference in bone mineral density or a healthy lifestyle in earlier days (Macintosh & Pearson, 2001).

Findings of lower appendicular bone mineral content in Gambian children compared with white British children and marginally (2%) higher bone mineral content in Gambian females compared with white British adult females have been reported. A study of South African school children up to eighteen years of age using single photon absorptiometry, did not find any significant difference in appendicular bone mass between blacks and whites (Daniels *et al.*, 1995).

Schnitzer *et al.*, found (as discussed by Schnaid *et al.*, 2000) that South African blacks had, apart from a greater volume of trabecular bone and thickness, higher values for osteoid and erosion variables compared to whites. The authors postulated that blacks might 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 (Villa, 1994). Blacks do not only have greater skeletal calcium content, but also greater total body potassium and muscle mass (Pollitzer & Anderson, 1989).

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. Schnitzler *et*

al. (1998) found in their study on black teenagers with slipped capital femoral epiphysis that many of the children were osteopenic. This was due to hypogonadism, prolonged growth spurt or dietary calcium deficiency. 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 may be due to growth stunting, possibly reflecting the generally poorer nutrition of Blacks in these African countries. It seems that the urine calcium levels are lower in Blacks than in Caucasians (Heaney, 2002).

CHAPTER 3

3. METHOD

3.1 Introduction

In this chapter general methodology and details on the experimental methods will be discussed. The problems experienced during the study are also pointed out. A multi-disciplinary team of researchers conducted the study at the Pretoria Academic and Kalafong hospitals in Pretoria. The research team and their different contributions to the study are given at the end of this chapter. The laboratory work was executed at the laboratories of Niehaus Ungerer and The Institute of Pathology, University of Pretoria.

The results reported in this study are preliminary data that was obtained from cases recruited to the study from May 2001 until August 2002. The study is ongoing and cases are recruited as they are admitted to the hospitals.

3.2 Ethics

The ethics committee of the Medical Faculty of the Pretoria University approved the protocol and the Superintendent of the Pretoria Academic hospital gave ethical approval for HIV testing (Ethics no: HC 652). Pre- and post-test counselling was given to each subject following the guidelines of the South African Department of Health. The objective of pre-test counselling was to ensure that the decision to take the test is fully informed and based on an understanding of all the implications of a positive result. Post-test counselling involved breaking the news. The Constitution of SA, Act no 108 of 1996, stipulates that all persons with HIV/Aids have the right to privacy, including privacy concerning their HIV/Aids status (Evian, 1993 & WHO, 2000). The subjects signed informed consent forms before inclusion in the study.

3.3 Subject details

Sixteen black male patients who were admitted to the Pretoria Academic and Kalafong hospitals with fractures of the proximal femur, the proximal humerus or the

distal radius between the period of May 2001 and August 2002 and who conformed to the inclusion and exclusion criteria were included in the study.
Inclusion criteria were black men older than 40 years with fractures of the proximal femur, the proximal humerus or the distal radius.

The following exclusion criteria were used:

- Men with pathological fractures from causes other than OP;
- Multiple trauma patients;
- Patients with identified chronic diseases such as tuberculosis, acquired immunodeficiency syndrome (AIDS) (Cell differentiation (CD 4) count of less than $200mm^3$), metabolic disorders, disorders of the thyroid, etc;
- Patients on any medication with the potential of influencing bone mineralisation, including calcium salts, bisphosphinates, vitamin D, fluoride etc; and
- Mentally disturbed individuals.

An equal amount of age-matched (±2 years), apparently healthy black men with no previous fracture (of the proximal femur and humerus and distal radius), were recruited as a control group. A lot of difficulty was encountered with the recruitment of the controls mainly because they had to be hand picked and aged-matched. They came from the community, townships, local schools and shopping centres. One of the controls was a patient admitted to the hospital for other reasons than a fracture. The control subjects were rewarded with R100.

It was also difficult to determine age correctly. Some of the subjects had no identity document and did not know their date of birth. One of the subjects even told us that he was cheated with three years on his identity document. From this situation we tried to age- match the subjects as best we could.

3.4 Study design

A case-control study design was used. After admission to the hospitals the patients were screened for inclusion in the study. They were then stabilised for 3-4 days to minimize the effect of the acute phase response on blood values.

Thereafter, blood samples were collected and sent to Nieuhaus Ungerer Laboratories or to the Institute of Pathology where the biochemical analyses were done. Patients were sent to the DEXA scanner at Pretoria East hospital for measurements of BMD on Wednesdays because of the availability of the DEXA scanner.

- The heights and weights of the subjects were taken without shoes and while wearing light indoor clothing. The body fat percentage and body mass index (kg/m^2) was calculated with the Tanita body composition analyser, model TBF 300.

- Three structured, standardised questionnaires were administered on days 3-5 after the injury during individual interviews to yield demographic (Addendum A), physical activity (Addendum B) and dietary information (Addendum C). Hospital staff was asked for assistance where language was a problem.

- Physical activity information was obtained using a validated quantitative questionnaire (Addendum B) (Kruger *et al.*, 2000) measuring physical activity index (PAI), based on the Baecke physical activity questionnaire (Baecke *et al.*, 1982). This questionnaire also took into account daily tasks such as climbing of stairs, lifting heavy objects and travelling.

For the control subjects, blood samples were drawn and DEXA scanning was done on the same day as well as the completion of the three questionnaires. The control group was done in groups of up to 6 patients per day, mainly on a Wednesday, because of the availability of the DEXA scan.

3.5 Dietary intakes

Two registered dieticians, during individual interviews, obtained dietary information from the subjects with the help of questionnaires (Addendum C). Habitual dietary intakes were measured by taking a dietary history in combination with a validated quantitated food frequency questionnaire (FFQ) (Macintyre, 1998). 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. It was previously validated against 7-day weighed records and reproducibility tested.

Spaced duplicate questions and an additional diet history of daily meal patterns assessed reliability of reported intakes (Macintyre *et al.*, 2001a, Macintyre *et al.*, 2001b). The computer programme, FoodFinder 2 (MRC, 2000) based on the South African Food Composition Tables was used to do the nutrient analysis.

3.6 Blood sampling

The patients were asked to hold a soft fist and the area where the sample was to be taken was cleaned. Non-fasting venous blood samples (14 ml in total) were taken using a 21-gauge needle infusion set (bulldog) without exerting pressure on the vein. The gel barrier red top tubes were filled completely and the blood allowed clotting for 30 minutes. Blood was also collected into ethylenediaminetetra-acetic acid (EDTA) lavender top tubes. The EDTA tubes were filled completely and gently inverted for 5 times immediately after collection to avoid clotting. The gel barrier and EDTA tubes were marked with generic labels provided and sent ambiently to Niehaus Ungerer Laboratories or the Institute for Pathology. The blood of the cases and the one control from the hospital were analysed at the Institute for Pathology, University of Pretoria and the other controls were analysed at Niehaus Ungerer Laboratories.

3.7 Experimental methods

The experimental methods are summarised in Table 3.1. Only the variables that are investigated in this thesis are included.

Table 3.1: Experimental methods

Variable	Analytical system	Experimental method
Serum albumin	Beckman Access Immunoassay from Beckman Coulter, delta CX76766541	The Beckman LX 20 analyser 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.
Serum transferrin Serum iron Transferrin saturation	Beckman Access Immunoassay from Beckman Coulter, delta CX76766541	Using a turbidimetric method the laboratory used the Beckman LX20 analyser to determine the serum transferrin. In the reaction, transferrin combines with specific antibodies to form insoluble antigen-antibody complexes. The same analyser is used for the reaction where iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxile amine and thioglycolate. The ferrous iron is immediately complexed with ferrozine iron reagent by means of a bichromatic end-point methodology. Total iron binding capacity is then calculated with the Beckman LX20 analyser. The transferrin saturation was calculated with the serum iron divided by total iron binding capacity and multiplied by 100.
CD4 count	Beckman Access Immunoassay from Beckman Coulter, delta CX76766541	The Coulter Epex MCLX analyser was used to count the CD4. The differentiated cells combine with specific coloured antibodies and can then be counted. 10 units of antibodies and 100 units of blood were used.
HIV status	Abbot Axsym Immunoassay system, Abott 1836	The Abbott axsym analyser was used to determine the HIV status with the ELISA method.
Serum GGT	Beckman Access Immunoassay from Beckman Coulter, delta CX76766541	The Beckman LX 20 analyser was used for this analysis.

GGT: gamma-glutamyltransferase; CD: cell differentiation; HIV: human immunodeficiency virus

3.8 DEXA scan

The DEXA scan was used to do the lumbar spine (L2-4) BMD and the total BMD of the hip. Although DEXA is the method of choice for measuring bone mass, its use

has been associated with errors in measuring fat content. There are different DEXA models, data collection modes, and software, and each may be a source of error. The model that was used at the Pretoria East hospital did not have the software to do body fat measurements. As new DEXA techniques are developed and validated, it may become the method of choice for body composition evaluations that include bone density (Cummings *et al.*, 2002). Nevertheless, DEXA is a valuable technique and highly effective in increasing awareness of osteoporosis (Anastasopoulou & Rude, 2002) and mass utilization may decrease the cost of DEXA analysis (Williams, 1999). The DEXA scan was done at the rooms of Dr. De Weerd (Pretoria East hospital) on Wednesday afternoons.

3.9 Bioelectrical impedance analysis

Total body fat percentage was measured with the bioelectrical inpedance analysis (BIA), using the Tanita scale. This is a more expensive method than skinfolds, but a good practical technique to assess body composition (Williams, 1999). The BIA scan was done on Wednesday afternoons. BIA is based on the principle of resistance to an electrical current that is applied to the body. The smaller the recorded resistance to the electrical current, the greater the water content and hence the greater the body density. Early research with BIA revealed large standard errors in predicting lean body mass, so it was not considered to be very valid. However, body composition researchers have developed newer techniques and prediction equations with lower standard errors (3-4%), comparable to the skin fold technique. The patients were not allowed to drink coffee, alcohol or do exercise four hours prior to the test to minimise dehydration and ensure accuracy.

3.10 Statistical analyses

The statistical analyses were carried out with the aid of the Statistica® computer software package. Descriptive statistics were performed for each group (cases and controls) and are reported in the dissertation as means and [95 % confidence intervals (CI)]. The variables were tested for normality using the Shapiro-Wilk's W-test. Data that was not normally distributed was logarithmically transformed and again tested for normality. The case and the control groups were tested for statistical differences regarding the following categorical variables: cookware, tobacco use,

income and qualification by using the Chi-square test. Instances where the Chi-square test could not be performed due to limited expected counts, the Fisher's exact test was done. Significant differences in continuous variables between cases and controls were estimated by using the T-test for independent samples for normally distributed data and the Mann-Whitney U test for non-normally distributed data. The relationship between BMD and the different variables (for cases and controls combined) were estimated by using Pearson correlation coefficients. Partial correlations were also calculated with BMD as dependent variable, to control for possible confounding factors (age, BMI, PAI, tobacco pack years, dietary calcium and energy intake). Because of the acute phase response that can influence the iron measurement after a fracture, the group (case or control) was controlled for when the relationship between BMD and iron status was calculated. When partial correlations between BMD and tobacco pack years were calculated, tobacco pack years was left out of the list of confounding factors. The most significant determinants of BMD were estimated by using a stepwise regression analyses procedure. A p value of $p \leq 0.05$ was considered to be of statistical significance.

3.11 Research team

The study was planned and executed by a team of researchers. The contributions of each of the researchers are summarised in Table 3.2.

Table 3.2: Research team

PERSON	FUNCTION
Prof. J. Davidson (Bradley University, Peoria, IL, USA	Together with Prof. N.G.J. Maritz, initiated the study and acquired funding from Bradley University for the study.
Prof. N.G.J. Maritz (Orthopaedic department, Pretoria Academic Hospital, Pretoria	Supervisory physician in the study. Together with Prof. J. Davidson, initiated the study-design. Obtained ethical approval for the study.
Dr's. L. de Villiers and C Oosthuizen (Orthopeadic department, Pretoria Academic hospital, Pretoria	Dr L. de Villiers was the consulting physician in the study. Dr L. de Villiers and Dr. C. Oosthuizen shared the responsibility for co-ordination of subjects, especially the cases, including recruitment of subjects, transport to Pretoria East hospital for DEXA scanning, blood sampling and signing of informed consent forms. Dr L. de Villiers organised analysis of blood samples.

PERSON	FUNCTION
Ms. M.E. Leach (Author of this dissertation, registered dietician) Ms. M. Groenewald Msc student in nutrition, PU for CHE, registered dietician)	Ms. M.E. Leach and Ms. M. Groenewald shared the responsibility for completion of questionnaires, anthropometrical measurements, recruitment of controls to Pretoria East hospital, nutrient analysis and computerisation of data.
Dr. A.J. de Weerd (Osteoporosis clinic, Pretoria-East hospital	Responsible for DEXA scanning.
Prof. W. Oosthuizen (School of Physiology, Nutrition and Consumer Science, PU for CHE)	Study-leader of the author of this dissertation. Acquired funding from the PU for CHE for the study.
Prof. J.C. Jerling (School of Physiology, Nutrition and Consumer Science, PU for CHE)	Study-leader of Ms. M. Groenewald. Assisted with the statistical analysis of the data.
Prof. H.S. Steyn (Statistical Consultation Service, PU for CHE)	Statistical analysis of data

PU for CHE: Potchefstroom University for Christian Higher Education; DEXA: dual energy X-ray absorptiometry

CHAPTER 4

4. RESULTS

4.1 Introduction

In this chapter the characteristics, habitual mean 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 alcohol drinking, tobacco smoking, hypovitaminoses C, dietary iron and protein on osteoporotic fractures and BMD are reported.

4.2 Characteristics

The characteristics of the subjects are summarised in Table 4.1. Cases and controls did not differ significantly with regard to age, height, weight, BMI, body fat percentage, number of tobacco smokers and alcohol drinkers, income and education. The mean tobacco pack years of the cases were almost double that of the controls but it was not statistically significant. The control group was significantly more active compared to the group of cases.

The African population examined in this study spoke six of the nine official languages of South Africa as their first language (Zulu, Swati, Sepedi, Tswana, Ndebele, and Shangaan). Looking at the cases and controls presented in this study, the characteristics of the populations in the two groups can be summarised as follows: There were two main groups amongst the cases. The cases consisted of pensioners on a government pension living in townships with family members or on farms in brick or sink houses. These subjects were very poor with a low educational level. Water and electricity were available but sometimes as communal facility. Families lived in brick or sink houses provided by white farmers on the land. The second group was still working but their living conditions were unfavourable as a result of their low income. They lived in townships and on farms with water and electricity that are not always freely available.

Some of the controls lived in the city of Pretoria in apartments at their working place

and went home to rural areas when money was available for transport. Some of them lived under bridges, in the streets or in the neighbouring townships. Most of these controls were employed and with exception to the street dwellers, water and electricity were available.

This group of subjects were relatively old with a mean age of 66 [58, 74] for the cases and 64 [57, 69] for the controls. The eldest of the cases could not remember whether he was 91 or 81 years old and we used 91 because the hospital records decided on that date. This one individual increased the mean age substantially.

Table 4.1: Characteristics of subjects

Variable	Cases (n=16)		Controls (n=16)		P VALUE
	Mean	**95% CI**	**Mean**	**95% CI**	
Age (years)	66.19	58.13, 74.24	63.50	57.63, 69.37	0.57
Height (m)	1.69	1.66, 1.72	1.65	1.62, 1.69	0.11
Weight (kg)	58.24	51.99, 64.50	55.03	48.16, 61.89	0.47
BMI (kg/m^2)	20.45	18.19, 22.71	20.09	17.79, 22.40	0.79
Body fat (%)	16.69	10.80, 22.57	15.01	11.07, 18.94	0.82
PAI	1.93	1.58, 2.27	2.60	2.28, 2.91	0.005*
Smokers n/16	13/16		12/16		
Tobacco pack years**	13.29	4.44, 22.14	7.43	1.83, 13.03	0.55
Alcohol drinkers n/16	12/16		10/16		
Income ≤ R1000	13/16		11/16		
Income R1000-R3000	3/16		5/16		
Education ≤ Gr 7	14/16		16/16		
Education Gr 7-Gr 12	2/16				

*: Statistically significant (p≤0.05); **: Pack years = packs (20 per pack) per year x amount of years smoked; PAI: physical activity index; BMI: body mass index; n: number of subjects; CI: confidence interval

The normal range for BMI in men is 19-25 kg/m^2 (Williams, 1999). The BMI of the subjects ranged from 14.20 to 29.40 kg/m^2. Seven cases had BMI values under the

normal range and seven men had BMI values within the normal range. Only two subjects had BMI values above the normal range. Eight men in the control group had BMI levels under the normal range and five had BMI levels within the normal range. Three men had BMI levels above the normal range.

The average percentages of body fat for U.S. male's range between 15% and 18% and minimal percentages of total fat for men range between 6% and 15% (Williams, 1999). Only two of the cases had body fat percentages in the average range. Six men had body fat percentages in the minimal range and one subject was below the minimal range. Five subjects had body fat percentages above the normal body fat range. Three cases could not stand on the Tanita scale long enough to measure the fat percentage. In the control group only one subject had a body fat percentage within the average range. Eight subjects had fat percentages in the minimal range and two below. Five subjects had body fat percentages above the normal range.

The cases were significantly less active than the controls. According to Kruger *et al.*, (2000) the cases can be categorised as being low active (1.14-2.25) and the controls as being moderately active (2.25-2.81). Merensia Groenewald discusses the effect of exercise on fractures in more detail in her dissertation.

The tobacco pack years were calculated for all the subjects who smoked, including those who stopped smoking. Eight cases reported pack years of less than 20, and five cases reported pack years of greater than 20. Nine controls reported pack years of less than 20; three reported pack years of greater than 20, and one reported 140 pack years. We asked the question in different ways and at different times but this particular subject was adamant that he smoked 100 cigarettes per day for 28 years from 1948 until 1970. Because this did not seem possible, the pack years of this particular subject was omitted from the data.

Four cases and 6 controls abstained from any alcohol use. The recommendation for alcohol intake is less than 30g alcohol per day (AHA, 2000). Eight of the cases consumed less than 30g, four consumed greater than 30g and one consumed 162g of alcohol per day. Seven of the controls consumed less than 30g, four consumed more than 30g, with the highest alcohol intake of 74g per day. Alcohol was not consumed daily but mainly during weekends or when money was available.

The subjects in this study had a low level of education. Only two of the cases and none of the controls had an education level higher than Grade 7.

The main income of the cases was a government pension of R500 per month. Only three of the cases had an income of more than R1000 per month. The income of most of the subjects in the control group was less than R1000 per month. Five of the controls had an income of more than R1000 per month with a maximum income of R3000 per month.

4.3 Bone mineral density

Figure 4.1 illustrates the difference in BMD between the cases and the controls. Although the mean BMD of the cases tended to be lower than the controls the differences were not statistically significant (p= 0.40 for the hip and p=0.13 for the lumbar spine). The normal values for BMD of the lumbar spine (L2-4) range between 1.2 and 1.4 g/cm^2 and of the hip in black males range between 1.0 and 1.5 g/cm^2 (Looker *et al.*, 1997). The rapid turnover of metabolically active trabecular bodies makes the spine the optimum site for monitoring loss or gain of bone mineralisation (Slosman *et al.*, 1990). Much to our surprise, most of the controls, especially if we look at the lumbar spine BMD, had OP.

According to the WHO classification (see 2.4.3) of OP, 14 of the 16 cases had established osteoporosis and two had osteopenia. In the control group, and specifically if we look at the lumbar spine BMD, 13 of the 16 had OP and 3 of the 16 had osteopenia. The BMD in 100% of the subjects was below the normal range.

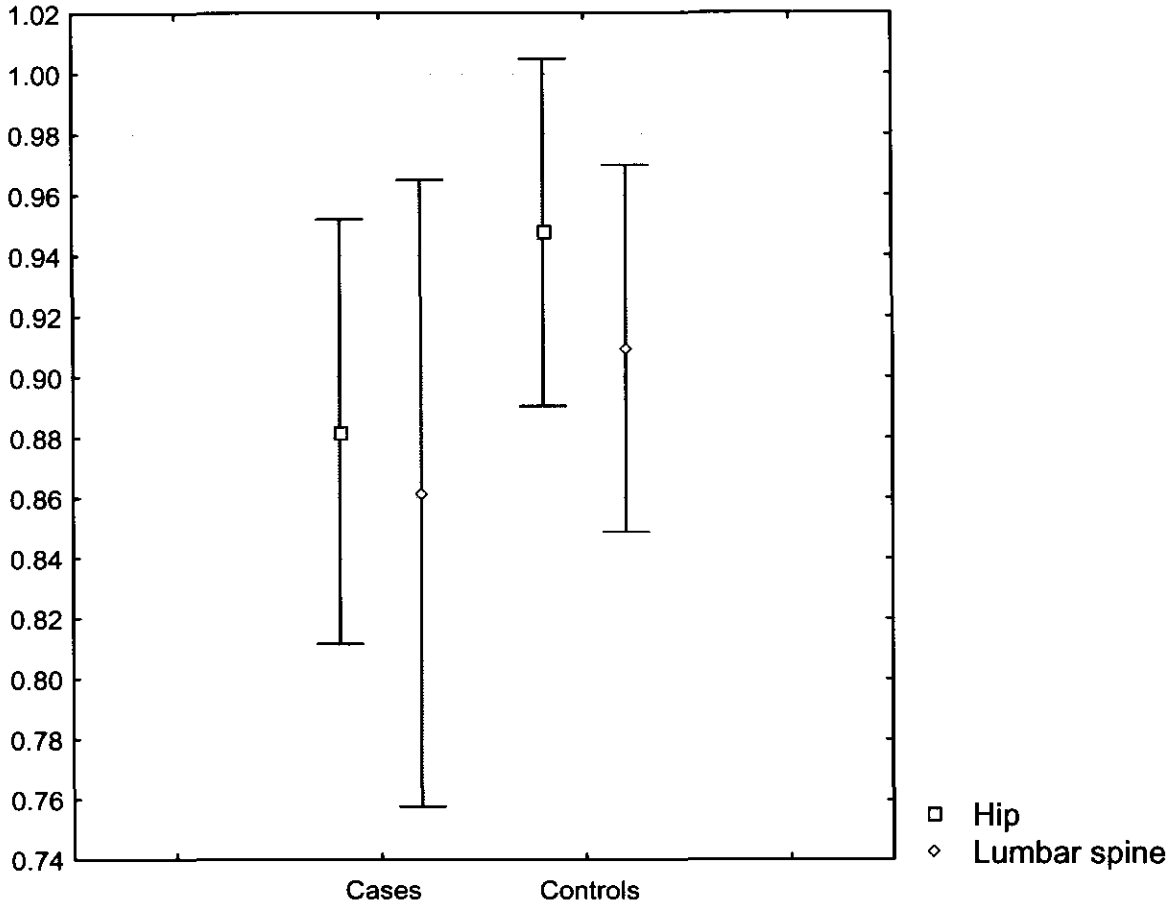

Figure 4.1: Mean [95% CI] bone mineral densities (g/cm^2) of the hip and lumbar spine of cases and controls

4.4 Diet

The habitual nutrient intakes of selected nutrients of the cases and the controls are summarised in Table 4.2. Although not statistically significant, the cases tended to have a lower total energy intake (8.43 MJ) compared to the control group (9.87 MJ). Except for total protein intake that was significantly lower in the cases compared to the control group, no statistically significant differences between cases and controls were seen for any of the other macronutrients. Iron intake tended to be lower in the cases compared to the controls (p=0.09). Compared to the recommended dietary intakes (Williams, 1999) (Table 4.2), cases consumed inadequate amounts of energy, protein, fibre, calcium, iron and vitamin C. Both controls and cases had low-fat diets and consumed only half of the recommended fat intakes of 30% of total energy intake. Alcohol intake made up the other half of the recommended fat intake. Intake of total protein by the control group met recommended amounts but the percentage of total energy provided by protein was inadequate. Controls also had low fibre, calcium and vitamin C intakes compared to recommended intakes.

Table 4.2: Habitual mean daily nutrient intakes and comparisons with DRI/RDA

Nutrient	Cases		Controls		DRI/RDA#	P VALUE
	MEAN	95% CI	MEAN	95% CI		
Total energy(MJ)	8.43	7,08, 9,78	9.87	8,05, 11,70	9.66	0.19
Total protein (g)	56.11	46.49, 65.74	73.29	58.28, 88.31	63	0.05*
Plant protein (g)	33.10	26.84, 39.36	38.76	28.73, 48.79	-	0.32
Animal protein (g)	22.54	14.67, 30.42	29.99	18.99, 41.00	-	0.22
% Energy: protein	11.39	10.15, 12.63	12.63	11.01, 14.24	15-20%	0.21
Carbohydrate (g)	302.71	257.42, 348.04	358.98	286.49, 431.47	-	0.17
%Energy: carbohydrate	61.31	56.64, 65.99	60.78	56.11, 65.44	55-65	0.86
% Energy: added sugar	8.18	4.53, 11.83	7.58	5.06, 10.10	<10%	0.77
Fibre (g)	15.68	13.20, 18.17	19.88	14.44, 25.32	30	0.31
Fat (g)	37.91	26.88, 48.93	46.55	36.22, 56.88	-	0.23
% Energy: fat	16.90	13.10, 20.70	18.53	14.59, 22.47	<30	0.53
Alcohol (g)	22.71	1.5, 43.91	16.0	4.84, 27.16	<30	0.82
% Energy: alcohol	6.59	1.65, 11.52	4.21	1.80, 6.64	-	0.65
Calcium (mg)	361.00	258.77, 463.23	503.69	261.95, 745.42	1200**	0.47
Iron (mg)	6.95	5.93, 7.97	8.7	6.82, 10.58	8	0.09
Vitamin C (mg)	15.13	9.35, 20.9	27.94	10.01, 45.86	90	0.11

Williams, 1999; *: Statistically significant ($p \leq 0.05$); ** AI: Adequate intakes; DRI: Dietary reference intakes; RDA: Recommended dietary allowances; CI: Confidence interval

4.5 Biochemical variables

Table 4.3 gives a summary of the blood values of the subjects. Mean serum albumin, transferrin, transferrin saturation and iron levels were significantly lower in cases compared to controls. All these variables were also lower than the desirable levels

while the controls had normal serum albumin, transferrin, transferrin saturation and iron levels. Serum GGT, a marker of alcohol use (Peterson *et al.*, 1983; Kumar & Clark, 1987 & Akkus *et al.*, 1997), was significantly higher in the cases with a mean level of 65.88U/L compared to 36.33U/L in the control group. The normal range for GGT values is 10-40 IU/L.

Table 4.3: Mean blood values of cases and controls

Blood value	Cases (n=16)		Controls (n=16)		Desira-ble Range**	P Value
	MEAN	**95% CI**	**MEAN**	**95% CI**		
S-Albumin (g/L)	28.75	25.43, 32.08	37.31	35.93, 38.70	32-51	0.0002*
S-GGT (U/L)	65.88#	33.76, 98.00	36.33	21.97, 50.70	11-49	0.03*
S-Transferrin (µmol/L)	23.64	19.64, 27.64	31.54	28.98, 34.10	26-52	0.001*
S-Transferrin saturation (%)	16.69	10.46, 22.92	28.88	19.71, 38.04	20-50	0.008*
S-Iron (µmol/L)	7.30	4.22, 10.37	17.61	12.23, 23.00	12-26	0.00006*

S-GGT: serum gammaglutamyltransferase; ** Niehaus Ungerer Laboratories; # n=8; *: statistical significant ($p \leq 0.05$)

4.6 Correlations of BMD with nutrient intakes, lifestyle factors and biochemical variables

Table 4.4 summarises the correlations between dietary factors, lifestyle factors, biochemical variables and BMD. Significant positive correlations between lumbar spine BMD and tobacco pack years could be seen even when controlled for possible confounding factors (age, body mass index, physical activity index, dietary calcium and energy intake and group in the case of irons status variables). Significant positive correlations between lumbar spine BMD and s-GGT ($p= 0.02$) and transferrin ($p=0.03$) could be seen, but when controlled for possible confounding factors (age, BMI, PAI, alcohol intake, tobacco pack years, dietary calcium, energy intake and group) no significant positive correlations could be found. Significant positive correlations between the hip BMD and s-GGT, transferrin and protein intake could be seen but when controlled for possible confounding factors (age, body mass index, physical activity index, alcohol intake, pack years, dietary calcium, energy intake and group), statistically significant correlations could be found only with s-GGT and a

tendency for protein intake (p=0.07).

Table 4.4: Pearson correlation coefficients between bone mineral density, nutrient intakes, lifestyle factors and biochemical variables of the total group (n=32)

Variable	BMD - Lumbar spine		BMD - hip	
	r- value	p-value	r-value	p-value
Tobacco pack years	-0.46 (-0. 60)#	0.008* (0.001)#	-0.16 (0.13)#	0.40 (0.54)#
s-GGT	-0.47 (-0.46)#	0.02 (0.17)#	-0.58 (-0.52)#	0.005 (0.04)#
Alcohol intake	-0.20 (0.06)#	0.27 (0.83)#	-0.07 (-0.27)#	0.70 (0.33)#
Vitamin C intake	0.03 (0.12)#	0.87 (0.60)#	-0.09 (-0.11)#	0.62 (0.63)#
Protein intake	0.27 (0.08)#	0.14 (0.73)#	0.42 (0.6)	0.02 (0.07)
s-Transferrin saturation	-0.16 (-0.23)#	0.40 (0.12)#	-0.05 (-0.03)#	0.81 (0.91)#
s-Transferrin	0.39 (0.09)#	0.03 (0.66)#	0.48 (0.16)#	0.007 (0.76)#
s-Iron	0.12 (-0.38)#	0.52 (0.06)#	0.21 (0.05)#	0.27 (0.85)#
Iron intake	0.20 (-0.03)#	0.27 (0.52)#	0.28 (-0.02)#	0.12 (0.63)#

*: Statistically significant ($p \leq 0.05$); # Controlled for possible confounding factors (age, body mass index, physical activity index, tobacco pack years, dietary calcium and energy intake, plus group (case or control) in the case of iron status); GGT: gamma-glutamyltransferase (n=24); s: serum; BMD: bone mineral density

4.7 Multiple regression analysis for dependant variable in BMD (L2-4 &hip)

In Table 4.5 it can be seen from the multiple regression analysis that 38% (r^2=0.38) of the variance in BMD of the lumbar spine could be explained by the variables entered in the equation. A forward stepwise multiple regression analysis revealed that 30% (r^2=0.30) of the variance in BMD of the lumbar spine could be explained by the s-GGT levels and iron intake combined. For BMD of the hip, 52% (r^2=0.52) of the variance could be explained by the variables entered in the equation. The forward stepwise multiple regression analysis revealed that 48% (r^2=0.48) of the variance in BMD of the hip could be explained by s-GGT levels, iron intake and protein intake combined. Of all the variables entered into the equation, s-GGT levels were the most significant predictor of BMD of both the lumbar spine and the hip as can be seen from the p-values ($p<0.05$) and the beta coefficients. The magnitudes of the beta coefficients allow one to compare the relative contribution of each independent variable (all the variables entered into the equation) in the prediction of the dependent

variable (BMD: lumbar spine and hip). Therefore, in these subjects, high levels of s-GGT were associated with lower BMD of the lumbar spine and hip.

Table 4.5: Multiple regression analysis for dependant variable BMD L2-4 & hip

BMD – Lumbar spine (L2-4)					
Multiple regression analysis (r^2 = 0.38)			Stepwise forward multiple regression analysis (r^2 = 0.30)		
Variable	Beta	p-value	Variable	Beta	p-value
S-GGT	-0.50	0.04*	S-GGT	-0.50	0.02*
Tobacco pack years	-0.11	0.65	Iron intake	0.27	0.16
Protein intake (%E)	-0.37	0.38			
Fat intake (%E)	0.25	0.54			
Alcohol intake	-0.06	0.81			
Iron intake	0.33	0.22			
Vitamin C intake	-0.11	0.61			
BMD – hip					
Multiple regression analysis (r^2 = 0.52)			Stepwise forward multiple regression analysis (r^2 = 0.48)		
Variable	Beta	p-value	Variable	Beta	p-value
S-GGT	-0.53	0.02*	S-GGT	-0.54	0.005*
Tobacco pack years	-0.08	0.69	Iron intake	0.29	0.10
Protein intake (%E)	0.16	0.69	Protein intake (%E)	0.25	0.16
Fat intake (%E)	0.06	0.87			
Alcohol intake	-0.15	0.50			
Iron intake	0.38	0.13			
Vitamin C intake	-0.12	0.55			

*: Statistically significant ($p \leq 0.05$); %E: percentage of energy intake; s-GGT: serum gamma-glutamyltransferase

4.8 Main findings of the OSWAMA study

The results can be summarised as follow in Table 4.6:

Table 4.6: Summarised results of the Oswama study

Tobacco pack years	Most of the subjects (cases and controls) smoked. The mean tobacco pack years of the cases (13.29) were almost double that of the controls (7.43) but it was not statistically significant (p=0.55). Tobacco pack years were negatively associated with BMD of the lumbar spine (p=0.008) even after controlling for possible confounding factors (p=0.001).
BMD of the lumbar spine and hip	The BMD of the hip and lumbar spine tended to be lower in the cases compared to the controls, but the differences were not statistically significant (p=0.40 and 0.13, respectively). Both the cases and controls were osteoporotic.
Dietary intake	There was no statistically significant difference in the dietary intakes of the cases and controls; the only exception was the protein intake that was lower in the cases. The nutritional status of both the cases and the controls were inadequate seen in the light of the low fat, vitamin C, protein, iron(cases) and total energy(cases) intakes. The fact that 50% of the cases and controls had a low BMI (lower than the recommended 19kg/m^2) confirms the inadequate nutritional status in both groups.
Total energy intake	The cases tended to have a lower total energy intake (8.4MJ) compared to the control group (9.9 MJ), but the differences between the groups were not statistically significant (p=0.19).
Total protein intake	The mean protein intakes of the cases (56.11g) were very low compared to the recommended 63g per day. The protein intake was also lower in the cases compared to the controls (73g). The total protein intake was significantly positively associated with BMD of the hip and after controlling for possible confounding factors it tended to be positively associated (p=0.07).
Alcohol	Alcohol intake between cases and controls did not differ significantly although the mean intake of alcohol per day was higher in the cases (22.71g) than in the controls (16g). The alcohol intake reported by the subjects fell within recommended guidelines of <30g/day. These reported amounts might not be a true reflection of actual alcohol intakes (discussed in more detail in Chapter 5). Furthermore, the alcohol was not consumed daily but mainly during weekends. There seems to be a decrease in traditional alcohol preparation and consumption. Commercial beers were mostly used by the cases and controls in this study.
Vitamin C intake	Vitamin C intake was very low in both the cases (15.13mg/day) and the controls (27.94mg/day) and tended to be lower in the cases compared to the controls (p=0.11). Vitamin C intake was not associated with BMD probably because of the low intakes (95% CI < DRI).
Iron intake	The mean iron intake was below the RDA for cases (6.95mg/day)

	and slightly higher than the RDA in the control group (8.7mg/day). Iron intake tended to be lower in the cases compared to the controls (p=0.09). Iron intake was not associated with BMD, however, in the stepwise regression analysis; iron intake came out as a possible predictor of BMD of both the lumbar spine and hip, although it was not statistically significant.
Biochemical variables	
Albumin	The mean serum albumin concentrations in the cases were significantly lower (28.75g/L) than in the controls (37.3g/L) and also below the desirable range of 32-51g/L. The low albumin levels in the cases may, however, be a consequence of the metabolic response to the injury (fracture) (Visser & Labadarios, 2002) (discussed in more detail in Chapter 5).
GGT	Mean serum GGT levels were significantly higher in the cases with a mean value of (65.88U/L) compared to the control group (36.33U/L). Serum GGT levels were also significantly negatively associated with BMD of both the hip and the lumbar spine even when controlled for possible confounding factors of the hip (p=0.04). Among the factors examined in this study, serum GGT was identified as the most important predictor of BMD in this group of subjects.
Iron status	The mean serum transferrin, transferrin saturation and iron levels were significantly lower in the cases compared to the controls. The levels in the cases were also below the recommended desirable ranges. As in the case of albumin, these low levels may be a consequence of the metabolic response to the injury (fracture) (Visser & Labadarios, 2002) (discussed in more detail in Chapter 5). Serum transferrin saturation and iron were significantly positively associated with lumbar spine and hip BMD, but when controlled for possible confounding factors no statistically significant correlations could be found.

CHAPTER 5

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

The main objective of this study was to investigate the influence of some dietary factors (vitamin C, iron, and protein) and lifestyle factors (tobacco smoking and alcohol consumption) on osteoporotic fractures and BMD in older South African black men using a case-control study design.

5.2 Limitations of the study

There were several limitations in this study and it is important to keep these limitations in mind when interpreting the results. The limitations were as follow:

- Age-matched controls were difficult to find in Pretoria because of the non-existance of old age homes for black people and therefore the fact that older people move out to the townships and rural areas. The other difficulty was to determine the exact age of the individual as discussed in Chapter 4.
- Controls were also osteoporotic and factors other than low BMD could probably have contributed to the fractures. This could have resulted in an underestimation of the results when cases were compared to controls.
- Two different laboratories were used because of the involvement of the two different hospitals and availability and cost of the laboratories.
- The small group of subjects probably did not provide sufficient statistical power to detect small but possibly important effects. It was difficult to find cases and controls that complied with the inclusion and exclusion criteria. Many of the possible candidates had either a low CD4 count, were on medication, had infections or diseases for example diabetes.
- GGT analyses were only done for 8 cases, but it was analysed for all the controls.
- Albumin and iron variables were affected by the acute phase response.
- The intake of alcohol and tobacco pack years was probably underestimated. There were difficulties in defining and measuring lifetime "exposure" to tobacco and alcohol use because of the language barrier and cultural differences between the female researchers and the black male subjects. These difficulties were further

compounded by the possibility that the elderly individuals may have had impaired memory with regard to the counts of alcohol after the person was inebriated. They can sit down and drink continuously for two days, sometimes even without taking any food. On another weekend however, with no money available the binge drinking can be absent or less. To determine the tobacco pack years, questions were asked in different ways but no consistent answers were given.

- It is difficult to test for all possible confounding factors and unknown factors could have influenced the results.

5.3 Risk factors for osteoporosis and BMD

Among the risk factors examined in this study, serum GGT, a marker of alcohol intake, was identified as the most important predictor of BMD and fractures. Other possible factors that could have played a role in fractures and BMD in this group of subjects were tobacco pack years and malnutrition. The poor dietary intakes (protein, vitamin C and dietary iron) of both the cases and controls measured in this study confirm the malnutrition in both groups. Osteopenia and osteoporosis were present in 100% of the subjects (cases and controls) and malnutrition with aggravating risk factors (alcohol abuse and tobacco smoking) played a vital role in the low BMD. The cases were, however worse off with regards to most of these risk factors. Of these risk factors, GGT were however, more pronounced in the cases.

A number of factors have been implicated in age-related bone loss in men, including heredity, androgen status, physical inactivity, tobacco smoking, alcohol consumption, poor dietary intakes, low vitamin D levels, and decreased calcium absorption (Arden & Spector, 1997). Studies on black men (Schnaid *et al.* 2000, Schnitzler *et al.*1994; Solomon, 1979) confirmed the results in this study regarding the s-GGT, hypovitaminoses C and alcohol use. No studies on black men have reported negative associations between tobacco smoking or protein intake and BMD.

OP can also be classified into primary or secondary OP, depending on the absence or presence of an underlying condition known to cause the disease. Underlying secondary causes of bone loss may accelerate the development of OP. Although the

distinction between risk factors for age-related bone loss and underlying secondary causes of OP may be arbitrary, secondary causes of OP was found in up to 30% of women and 54% of men attending a bone clinic in the United Kingdom with symptomatic vertebral crush fractures (Arden & Spector, 1997). (See Figure 5.1).

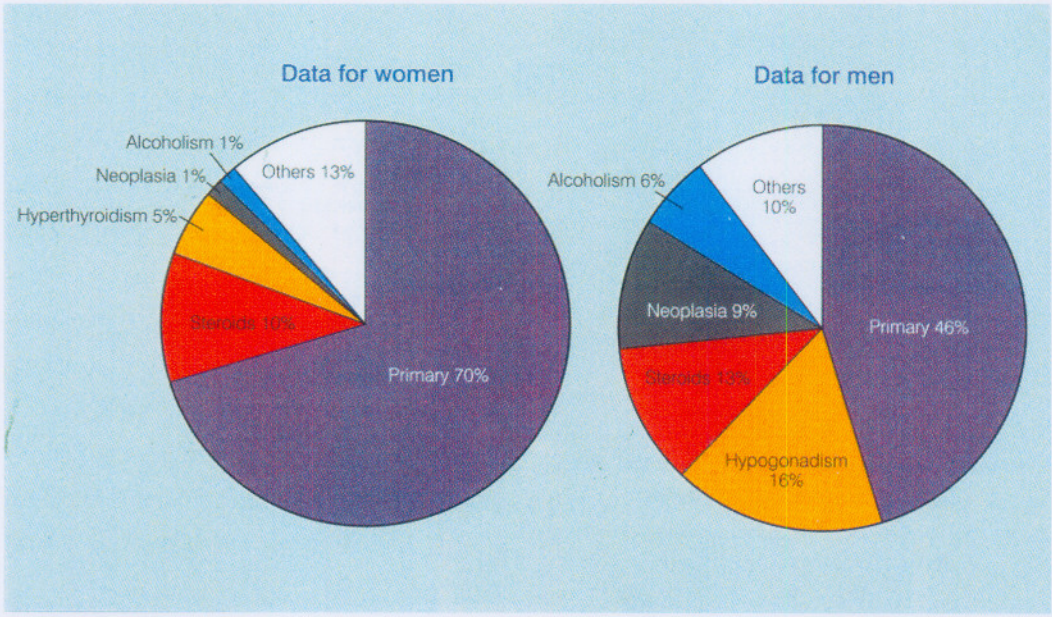

Figure 5.1: Prevalence of secondary osteoporosis in women and men (Arden & Spector, 1997).

From Figure 5.1 and other studies (Seeman *et al*, 1983; Scane *et al*, 1996; Medra & Jankowska, 2000)) we can see an increased risk of vertebral fractures with alcohol consumption, tobacco smoking and underlying secondary causes of OP (Arden & Spector, 1997). From this study on black South African men it can be seen that 30% of the variance in BMD of the lumbar spine could be explained by s-GGT and iron combined. The two main risk factors to decrease BMD of the hip and lumbar spine in this study where malnutrition was present in both the cases and controls were a high s-GGT (a marker of alcohol use) and tobacco smoking.

5.3.1 Lifestyle risk factors

The prevention of fractures requires attention to risk factors influencing bone density. To warrant study from a public health point of view, potential risk factors for OP should occur commonly and be amenable to safe and low cost intervention (Marcus *et al.,* 1995). Modifying a risk factor that is common and has a large effect (such as

estrogen deficiency) may help the individual and the public health problem. Modifying a risk factor that is uncommon but has a large effect (such as corticosteroid treatment) may help the individual, but not the public health problem. Modifying a risk factor that is common but has a small effect may not help the individual much, but may help the public health problem of OP (Marcus *et al.*, 1995). Many individuals, during a large proportion of their lives, use tobacco and alcohol. The effect may be small over the short term but may become clinically important when exposure is prolonged. These are risk factors that can be modified at little or no cost.

5.3.1.1 Tobacco smoking

Tobacco was used by most of the subjects in the study (cases and controls) making comparisons between cases and controls with regard to tobacco use difficult. Tobacco pack years were higher in the cases compared to the controls but the number of subjects in the study was probably not enough to detect a significant difference. The observation in this study ,that tobacco pack years were significantly negatively associated with BMD, is in accordance with the literature (summarised by Brott *et al.*, 1999). Brott *et al.* (1999) showed that when tobacco is used by individuals for a large proportion of their lives, it was associated with an increased risk of fractures. This risk emerges in advanced age and reduces the skeletal protective effects of obesity and estrogen exposure. The effect is mediated, in part, by a reduction in bone density, which is probably due primarily to increased bone loss. Decreased bone formation and increased bone resorption are responsible for bone loss. Increased bone resorption associated with tobacco smoking is, in part, due to a reduction in the production and acceleration in the degradation of estrogen. Briggs suggested that carbon monoxide might inhibit testosterone formation due to the blockade of 17 alpha hydroxilation of progesterone. The enzyme catalysing this reaction requires a co-factor related to cytochrome P450. Carbon monoxide inhibits mycrosomal cytochrome P450 (Marcus *et al.*, 1995).

The consequence of tobacco use on bone density are usually undetectable until late in adulthood because smoking one pack per day on average results in a deficit in spinal bone density of 2% per decade. However, a deficit in BMD of 0.5 to 0.8 standard deviations directly attributable to tobacco use may be incurred over the three decades from age 20 to age 50, a change that may double fracture risk (Marcus

et al., 1995). Quite a number of the cases (4/13) started tobacco use when they were still young, the youngest being 5 years old.

Centenarians are a rapidly growing segment of our society. Within the southern African region, South Africa has the highest proportion of older population. The 1996 census data estimate that 2.8 million South Africans are aged 60 years and older, which constitutes 7% of the population. This percentage is projected to increase to almost 11% of the population over the next 20 years (Charlton, 2000). The Census Bureau numbers and projections for centenarians in the US in 1990 was 37 306, for 2003 already 59 493 and projected for 2050 is 1 149 500. This will allow for more than only three decades of tobacco smoking in a lifetime (Marcus *et al.*, 1995).

5.3.1.2 Alcohol

More than half of the subjects in the case and control groups reported alcohol consumption. The cases reported a mean intake of 22.71g per day, which met the recommendation of less than 30g of alcohol per day considered as a healthy guideline. Available data suggests that moderate alcohol intake is unlikely to be associated with lower bone density (Marcus *et al.*, 1995). In fact, moderate alcohol intake has been associated with higher bone density. The results of these studies should however be interpreted with caution. Although these results were adjusted for co-variates, moderate alcohol intake may be associated with higher socio-economic groups with better nutrition and life-style during growth and adulthood (Marcus *et al.*, 1995). Moderate alcohol consumption may well be a risk factor for OP among those with lower body weight (Marcus *et al.*, 1995). Muscle strength is weakened substantially, even before changes in occur (Koehn *et al.*, 1993) in alcoholic patients and that weakness is related to the severity of malnutrition (Anderson *et al.*, 1998). The cases and controls in this study had a mean BMI of 20kg/m^2 and a mean fat % of 17 in the cases and 15 in the controls. The subjects cannot be referred to as alcoholics but alcohol was abused certain periods of their lives depending on availability. The study of Addolorato *et al*, can explain why the fat % is not so low and even higher in the cases than the controls. They came to the conclusion that small quantities of alcohol seem to have no effect on body weight. Ingestion of heavy amounts (as was the case of our subjects over weekends) may lead to an increase in body fat in healthy subjects, probably via its lipid oxidizing supressive effects. Chronic

intake of excessive amounts in alcoholics leads to a decrease in body weight, probably via increased lipid oxidation (Addolorato *et al.*, 1998) and by altering the leptin system that regulates energy expenditure (Obradovic & Meadows, 2002).

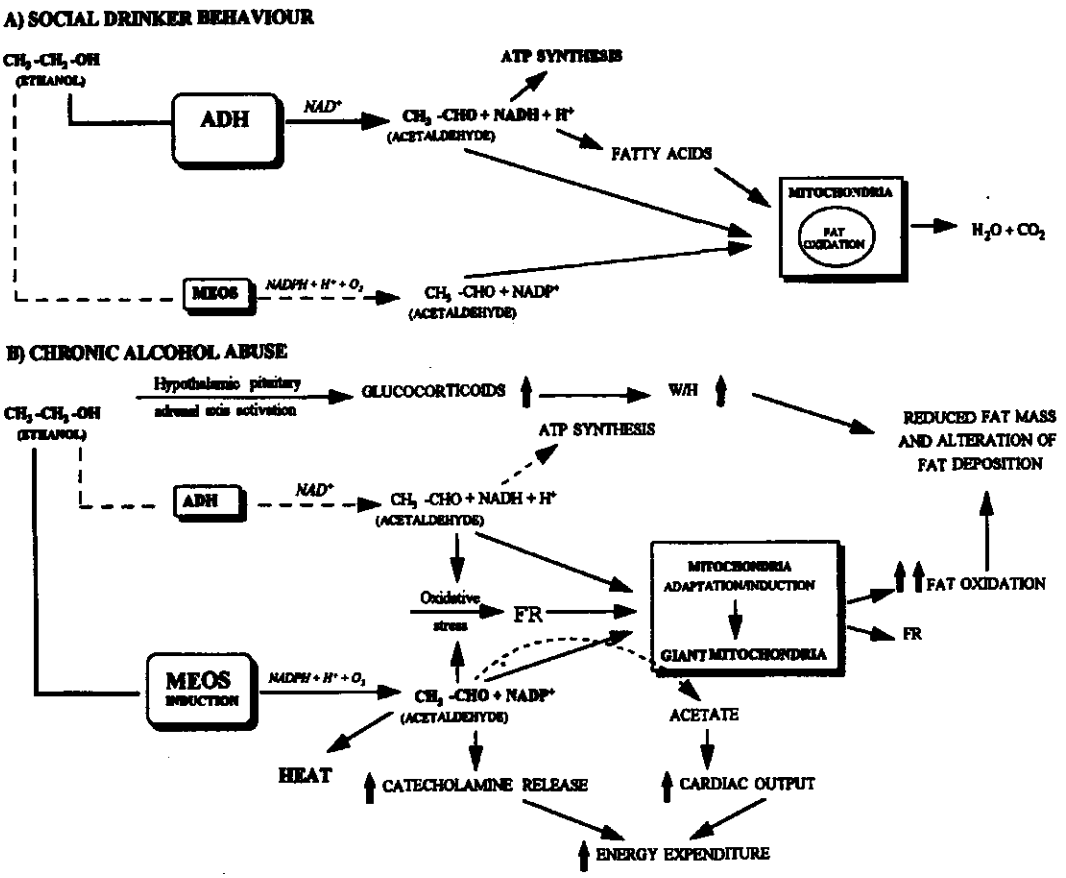

Figure 5.2: Diagram of the suggested mechanism on the basis of the increased energy expenditure and lipid oxidation in alcoholics. ADH: alcohol dehydrogenase system; MEOS: microsomal ethanol oxidizing system; FR: free radical; W/H: waist to hip; continuous line and bold: main mechanisms of ethanol oxidation; dashed line: secondary mechanisms of ethanol oxidation (Addolorato *et al.*, 1998).

The reported intakes in this study could be an underestimation of the actual amounts consumed due to the difficulty in obtaining accurate information on alcohol habits because of its emotional and moral overtones or the fact that respondents may underestimate the amount drunk. S-GGT has been widely used as an index of liver dysfunction and marker of alcohol intake (Whitfield, 2001). Mild elevations in s-GGT are common even with small amounts of alcohol. S-GGT was markedly increased in the cases (65.89 IU/L) opposed to the controls (36.33 IU/L). The normal range for GGT values is 10-40 IU/L (Peterson *et al*, 1983; Kumar & Clark, 1987 & Akkus *et al.*, 1997). In this study the high s-GGT levels in the cases were not related to the

reported alcohol intake. This may be an indication that the reported amounts of alcohol consumed by the cases may have been underestimated. Compared to other markers such as carbohydrate-deficient transferring (CDT) s-GGT, was shown to be more suitable for assessing a wide range of alcohol intakes (Steffenson *et al.*, 1997 & Aithal *et al.*, 1998) and remains the test that combines greatest convenience and sensitivity (Sharpe, 2001). Rico *et al.* (summarised by Marcus *et al.*, 1995) found a significant correlation between GGT and serum osteocalcin ($r = -0.78$, $p < 0.001$).

It is important to realise that s-GGT can be decreased by coffee consumption and increased by liver disease. Coffee consumption is inversely related to serum GGT and coffee may inhibit the inducing effects of ageing and possibly of tobacco smoking and alcohol on serum GGT in the liver (Nakanishi *et al.*; 2000a, Nakanishi *et al.*, 2000b & Nakanishi *et al.*; 2000c). The subjects did not use coffee and liver disease was one of the exclusion criteria of the subjects and therefore could not have influenced the GGT values in this study by influencing the value and masking the effect of alcohol abuse. The last few years have seen improvements in these areas of research on GGT and advances in the understanding of its physiological role in counteracting oxidative stress (caused by alcohol, ageing & tobacco smoking) by breaking down extracellular gluthathione and making its component amino acids available to the cells to protect against oxidation (Whitfield, 2001).

The way in which the alcohol intake is reported, namely daily amounts, could also have been misleading because the subjects did not consume the alcohol on a daily basis but in excessive amounts over weekends. The daily amounts were calculated because the Food Finder programme expresses nutrient intakes per day. The subjects actually consumed that amount of alcohol over a period of one or two days, which may have a significant impact on bone reduction (Schnaid *et al.*, 2000). A reduction in bone formation may be a direct toxic effect of alcohol causing a reduction in osteoblast life span or a reduction in the activity of osteoblasts (Illich & Kerstetter, 2000).

Few fractures in women are attributable to alcohol abuse, while alcohol abuse with or without concomitant hypogonadism is reported to be much more common in men presenting with fractures (Marcus *et al.*, 1995). A reduction in serum testosterone concentration may contribute, as there is an association between bone volume and

serum testosterone in alcoholic cirrhosis (Marcus *et al.*, 1995). The mean serum testosterone in this group of black South African men was below normal in the cases (7.21nmol/L [4.12; 10.32] opposed to the controls (16.54nmol/L [13.37; 19.72]. The normal values range between (7.4 –25.7nmol/L).

A marginal intake of magnesium may also be exacerbated by excessive alcohol consumption. Magnesium is a minor constituent of bone and is also required for the synthesis of 1,25-dihydroxyvitamin D, the release and periferal action of PTH and is also a co-factor for alkaline phosphatase (Bunker *et al.*, 1994). Unfortunately, serum magnesium concentrations were not analysed for these subjects.

5.3.2 Dietary factors

Before we take a look at the different dietary factors we must discuss the limitations in measuring the different factors accurately. The FFQ that was used in this study may also give rise to errors that must be taken into account. The FFQ makes use of average portion sizes and this can result in over or underestimation of the actual food consumed. In other words, the subjects just look at a picture in the picture book and say that one of them is there portion, but when you ask to show it using their hands it is much more. The subjects may be unable to recall past eating patterns, like seasonal fruit eaten in other seasons of the year (Macintyre, 1998).

5.3.2.1 Protein

The mean protein intakes of the cases (56.11g) [46.49; 65.74] were very low compared to the recommended 63g per day. This low protein intake was also significantly less compared to the controls (73.29g) [58.28; 88.31]. The inadequate intake of energy and protein by the cases is probably a lifetime problem because of availability of food and when money is available it is often spent on tobacco and alcohol and not on food. Furthermore, protein intake was positively associated with the hip BMD.

Several studies (Bonjour *et al.*, 2001; Doherty *et al.*, 2002) have strongly suggested that low protein intake per se could be particularly detrimental for both the acquisition of bone mass and the conservation of bone integrity with ageing. During growth,

malnutrition, including inadequate supply of energy and proteins, can severely impair bone development. Nethertheless, the long-term influence of dietary protein on bone mineral metabolism and skeletal mass has so far been difficult to identify. Low protein intake could be detrimental for skeletal integrity by lowering the production of IGF-I to an inadequate level. Supplemental protein significantly increased the low IGF-1 and reduced the length of inpatient rehabilitation care (Rizzoli *et al.*, 2001). The hepatic production and plasma level of this growth factor, which exerts several positive effects on the skeleton, is under the influence of dietary protein. IGF-I is considered an essential factor for bone longitudinal growth. It also plays a role in trabecular and cortical bone formation (Marcus *et al.*, 1995). In the Ranco Bernardo study of 572 women and 388 men, a positive effect of protein on IGF-1 was reported only in women and not in men. They also found a negative correlation between vegetable protein consumption and BMD. It is noteworthy that the positive association between total and animal protein consumption and BMD was greatest for women with the lowest calcium intakes (Promislow *et al.*, 2002).

5.3.2.2 Vitamin C

No significant difference was found in the vitamin C intake of the cases or the controls. Vitamin C intake was furthermore not associated with BMD. This lack of association was probably due to the low vitamin C intakes in all the subjects ranging from 9.4mg/day to 20.9mg/day in the cases and from 10.01mg/day to 45.86mg/day in the controls. The recommended 90mg per day was not reached by the cases (15.13mg/day) or the controls (27.94mg/day). Considering the above findings, it seems that in this study the very low vitamin C intakes correlated with the possible malnutrition in both groups. Vitamin C is needed for the synthesis of type 1 collagen, the main organic compound of bone and the anti-oxidant role might be important to modulate skeletal metabolism. Mild-to-moderate vitamin C depletion in weanling guinea pigs affected the collagen cross-link ratios in femur shaft and attributed to impairment of hydroxylation of collagen lysine. The same cross-link changes were not produced by total food restriction (Bates & Tsuchiya, 2003). Data collected from 13080 adults enrolled in the Third National Health and Nutrition Examination Survey (NHANES iii) during 1988-1994 showed different associations between ascorbic acid and BMD in men and women. Among men, serum ascorbic acid was associated in a non-linear fashion with BMD, and dietary ascorbic acid was associated in a non-linear

fashion with self-reported fracture. Among postmenopausal women without a history of smoking or estrogen use, serum ascorbic acid was unexpectedly associated with lower BMD (p=0.01) (Simon & Hudes, 2001). Otsuka *et al.*, (2000) have shown that ascorbic acid is essential for osteoclastogenesis and, moreover, that ascorbic acid acts in co-operation with 1,25 $(OH)_2 D_3$. Their findings suggest that ascorbic acid might play a key role in the regulation of the balance, in terms of differentiation and activation, between osteoclasts and osteoblasts. Adding an oxidant such as tobacco in this study population of black South African men with already low anti-oxidant vitamin C intakes may have increased the risk for bone mineral loss. However, in these men other dependent variables contributed more to the low BMD seen in both the cases and the controls.

5.3.2.3 Iron

The mean iron intake was below the RDA of 8mg for the cases (6.95mg) and just above (8.7mg) for the controls. Iron plays a role in collagen maturation and low iron status can lead to insufficient cell energetics in collagen maturation. Arginine supplementation in rats increased the iron content in the vertebra and suggests that iron has a physiological role in bone formation together with calcium, copper, potassium, manganese, zinc, silicon and molybdenum (Seaborn & Nielsen, 2002). In the stepwise model protein intake was shown to be a possible predictor of BMD, although not significant. The low iron intakes together with the lower dietary intakes of energy, protein & Vitamin C could be an indication of malnutrition in the cases, which may have increased the risk for lower BMD and fractures.

5.3.3 Biochemical variables

5.3.3.1 Albumin

The mean serum albumin level in the cases was lower than in the control group and also below the normal range. The viceral proteins have traditionally been used for assessment of nutritional status, and have been considered to reflect viceral protein stores. In the short term, however, they may reflect the severity of the metabolic response to surgical stress and the prognosis in critically ill patients. In many clinical

situations, it may be difficult to determine whether changes in a patient's serum protein levels reflect nutritional status or are a consequence of the metabolic response to injury. As a result of the metabolic alterations, alternative indices have been developed for the assessment of nutritional status, which take into account the changes in nutritional and inflammatory proteins. An example is the Prognostic Inflammatory and Nutritional Index (PINI). The index combines multiple serum proteins such as albumin, thyroid-binding pre-albumin (TBPA), cellular reactive protein (CRP) and alpha-1 acid glycoprotein (AGP), and is considered more valuable for assessing nutritional status than the determination of single viceral proteins (Visser & Labadarios, 2002). Other researchers (Merli *et al.*, 1987) say anthropometric parameters (including mid arm circumference) seem preferable to serum visceral proteins for the assessment of nutritional status in patients with liver disease (Mendandall *et al.*, 1995). In alcoholic cirrhotic patients the lower mid upper arm muscle circumference was significantly lower than in non-alcoholic cirrhotic patients (Narayanan *et al.*, 1999). It is difficult to determine whether the lower albumin values measured in the cases reflects nutritional status or is a consequence of the metabolic response to injury. Unfortunately these albumin values must be interpreted with caution and cannot be used to confirm malnutrition.

5.3.3.2 Iron status

The serum iron, transferrin and the saturation thereof were significantly lower in the cases compared to the controls and below the normal range in the cases and within normal limits in the controls. These low levels may, however, have been due to the consequence of the metabolic response to the fracture. Serum transferrin concentrations and transferrin saturation decreased after major and minor types of surgery while ferritin concentrations increased. Serum transferrin receptor concentrations increased only four weeks after major surgery to compensate for the acute phase response and could be a better marker for future research (Visser & Labadarios, 2002). The plasma concentration of iron falls rapidly after injury and continue to fall for several days up to 1-2 weeks. Van Iperen (reviewed by Visser & Labadarios, 2002) reported decreased serum iron concentrations to 23 and 46% after major and minor surgery, respectively, and these levels remained low for 28 days.The fall in plasma iron results from the transfer of iron from the iron-transferrin complex in the plasma to other proteins. Initially, iron is transferred to lactoferrin,

released from leukocytes at the sites of inflammation. Iron is taken up and bound to ferritin in the liver and spleen (Visser & Labadarios, 2002). The movement of iron into a storage form reduces its availability within the plasma, thereby withholding iron from bacteria (Visser & Labadarios, 2002). The serum iron was lower in the cases than in the control group. The lower serum iron in the cases does correlate with the lower intake of iron in the diet of the cases. However, iron concentrations have been documented to decrease after surgery and during infections. The blood samples of the cases were taken during this period and the effect of the acute phase response on the lower serum iron, transferrin and transferrin saturation in the cases can therefore not be ruled out. The time period of 3-5 days, before blood samples were taken, was not long enough to minimize the acute phase response. Due to financial reasons the cases could not be hospitalised for a longer period. Thus, although the lower serum iron, transferrin and transferrin saturation levels in the cases correlate with the lower intake of iron in the diet, these results should be interpreted with caution.

No incidences of iron overload or increased serum iron levels were found in any subject. Iron pots are rarely used to brew beer. Even in the rural areas, commercial beers are preferred (Walker & Segal, 1999).

5.3.4 Conclusions

This study on the risk factors involved in OP in older South African black men showed a low BMD for both the cases and the control groups. This may be an indication that OP in older black men in South Africa may be a very important public health issue that needs to be further investigated. Malnutrition played a vital role in the low BMD and was aggravated by the use of tobacco from a young age and alcohol in excessive amounts over weekends or when money was available.

5.3.5 Recommendations

In Chapter 1 one of the important outcomes envisaged from this study was the identification of lifestyle risk factors that may contribute to OP and the formulation of recommendations in order to develop a specific strategy in the prevention of OP in

South African black men. From the results of this study, it can be recommended that any intervention programme should focus on alcohol abuse, tobacco smoking and improvement in nutritional status. Children should be encouraged not to smoke and be educated on the detrimental effects of alcohol.

It is important to address dietary risk factors associated with OP, namely to increase the overall nutrition of the South African black male with low cost protein and calcium products. The following recommendations are made regarding the dietary intakes. The total protein content of the diet needs to be sufficient (0.8-1g/kg body weight), but not too much to negatively influence the protein: calcium ratio of 1:20 (FNB, 1998). An example of a very inexpensive source of protein and calcium is pilchards with their bones and sour milk. Red meat should also be included to achieve normal iron levels in the bloodstream. Although the results of this study do not allow conclusions regarding vitamin C intake and fractures and BMD, increased intake of vitamin C may be beneficial. Vitamin C enhances iron absorption and may be beneficial for bone collagen (Branca & Vatuena, 2001). The increased intake thereof by using seasonal fruit can therefore be recommended.

5.3.6 Future research

To ensure a high quality of life in old age, it is important to gain more empirical information on the incidence and the trends in the development of OP in South African black men, and all factors influencing these trends. Thus, this information may be valuable in designing appropriate and timely interventions to prevent a possible future epidemic of OP among the increasing older population of South African black men.

The results of this study showed that 100% of the subjects were osteoporotic. This finding needs further investigation with more subjects to determine the BMD of South African black men without fractures. The findings of such a study can confirm the incidence of OP and osteopenia as was observed even in the control subjects. This information is of national interest for a country in a world where people are getting older and where bone fragility is costly and increases morbidity and mortality.

This study should be followed up by a prospective study to measure the bone loss in

black South African men including the higher income groups where malnutrition does not play such a very big role.

Prospective interventional studies are needed to ascertain whether variations in protein intake within the range recorded in this study population will effect bone mass accumulation during growth. Such prospective intervention studies should delineate the crucial years during which modifications in nutrition would be particularly effective for bone mass accumulation in children and in adolescents. This kind of information is of importance in order to make credible and well-targeted recommendations for OP prevention programs aimed at maximising peak bone mass.

To improve the accuracy and outcome of future studies the following measurements can be included in a follow-up study. Osteocalcin can be done because it correlates with GGT and is a marker of bone resorption and NTx because it is sensitive to bone resorption due to vitamin C deficiency. Magnesium can be done to determine the effect of alcohol consumption on magnesium values and the effect of magnesium on bone density. The bone density measurement with the DEXA method as measured in this study is recommended because the software can be adjusted for black South African men and it is a very accurate method. The extra software to determine fat percentage with the DEXA machine is available only in the latest machines, but it should be worthwhile for a follow up study to use it. Two of our controls could not stand up long enough for the measurement of the fat percentage with the Tanita scale. Mid arm circumference can be a valuable anthropometric measurement for protein-energy malnutrition and GGT combined with other liver enzymes for liver disease. Other parameters to include for malnutrition can be CD8 or total lymphocyte counts (Mendenhall *et al.*, 1995) or the PINI index (Visser & Labadarios, 2002).

ADDENDUM A DEMOGRAPHIC QUESTIONNARE

Oswama Project

Department Orthopedie
Universiteit van Pretoria

BRADLEY UNIVERSITY

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

ADDENDUM B PHYSICAL ACTIVITY QUESTIONNARE

BRADLEY UNIVERSITY

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.

ADDENDUM C QUATITATIVE FOOD FREQUENCY QUESTIONNARE

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						0004	
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!

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