

**THE EFFECT OF ULTRADISTANCE RUNNING
ON PREMENOPAUSAL WOMEN OF
DIFFERENT ETHNIC GROUPS**

By

AVRIL MCGREGOR

**Submitted in partial fulfillment of
the requirements for the degree of**

Master Of Medical Science



**In the
Department of Physiology
Nelson R Mandela School of Medicine
Faculty of Health Sciences
University of KwaZulu - Natal
Durban**

2005

DEDICATION

This thesis is dedicated to my husband and children, who have always believed and encouraged me to face my fears, live my dreams and achieve my goals.

“Carpe Diem”, seize the day.

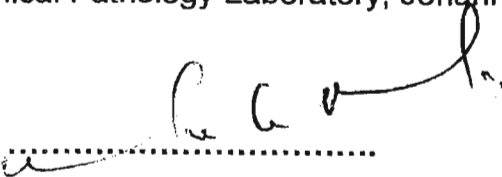
DECLARATION

This study represents the original work by the author and has not been submitted in any form to another university. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in the study was carried out in:

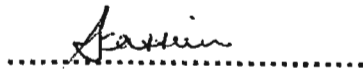
- The Department of Physiology, Nelson Mandela School of Medicine; under the supervision of Professor M Mars.
- The Department of Medicine, Nelson Mandela School of Medicine; under the supervision of Professor B Cassim.
- X – Ray Department, King Edward VIII Hospital.
- Haematology and Chemical Pathology Laboratories, King Edward VIII Hospital.
- SAIMR Chemical Pathology Laboratory, Johannesburg General Hospital.

Supervisor :


.....

Professor M Mars

Co- supervisor:


.....

Professor B Cassim


.....

Avril McGregor

I hereby certify that the above statement is correct.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to the following people for their invaluable help, assistance and support enabling me to carry out and complete this thesis.

Professor M Mars, for his supervision and expert help with statistical analysis and for proof reading the thesis.

Professor B Cassim, for her valuable time, expertise, guidance, patience, and continual encouragement throughout the study.

My colleagues, in the various clinical laboratories, for their support and assistance with sample storage and analysis.

The Scientific Group for donating the bone marker kits, without which the study would have been incomplete.

Mr. A Naidoo, for his expertise, guidance and patience in the use of the densitometer.

My fellow ultra distance runners, who volunteered to participate in the study, in the interest of science and research.

My husband Stuart, my beloved children Kirsty, Vaughan and Shannon for their support and constant encouragement to complete my thesis and not allowing me to give up.

Mangosuthu Technikon for providing funds, encouragement and allowing their staff time to pursue further academic qualification.

Summary

Introduction

Bone is a dynamic tissue and undergoes constant modelling and remodelling during life. Bone metabolism is influenced by a complex interplay of genetic, hormonal and environmental factors.

Exercise influences bone mass throughout life. In early childhood and adolescence exercise is an important determinant of peak bone mass. In adult life and especially older age, moderate, regular weight-bearing exercise appears to maintain bone mass and prevent bone loss. However, excessive exercise associated with menstrual abnormalities and nutritional deficiencies predisposes to bone loss.

Methods

Following approval from the Ethics committee of the University of KwaZulu-Natal (UKZN), 55 South African premenopausal ultradistance runners and 47 premenopausal sedentary controls between the ages of 30 - 50 years were recruited for the study.

Bone mineral density and percentage body fat was measured by dual energy X-ray absorptiometry (DEXA) using the Hologic QDR 4500A densitometer. Venous blood samples were drawn and urine samples collected for measurement of gonadal hormones and biochemical markers of bone turnover. The concentration of lutenizing

hormone (LH), follicle stimulating hormone (FSH), oestradiol and progesterone were measured using an enzyme linked immunosorbent assay (ELISA).

A direct ELISA was used for measuring bone specific alkaline phosphatase (BSAP) (n = 55 runners; n = 18 controls), a competitive ELISA for deoxypyridinoline crosslinks (Dpd) (n = 55 runners; n = 18 controls) and osteocalcin (OC) (n = 26 runners; n = 18 controls) and competitive inhibition ELISA for N-telopeptide of type 1 collagen (NTx) (n = 55 runners; n = 18 controls).

Student's t - test, Kruskal-Wallis and one-way analysis of variance (ANOVA) tests were used to compare the runners and controls and the different ethnic groups. The results are presented as the mean and standard deviation of the mean.

Results

1. The mean bone mineral density (BMD) was a significant higher at the total hip area ($0.936 \pm 0.132 \text{ g/cm}^2$ vs. $0.881 \pm 0.125 \text{ g/cm}^2$; $p < 0.05$) and total legs ($1.126 \pm 0.119 \text{ g/cm}^2$ vs. $1.014 \pm 0.180 \text{ g/cm}^2$; $p < 0.001$) in the runners compared to the controls. In contrast the BMD for the total arms was significantly lower ($0.722 \pm 0.050 \text{ g/cm}^2$ vs. $0.923 \pm 0.154 \text{ g/cm}^2$; $p < 0.0001$).

2. The percentage fat ($21.6 \pm 5.7 \%$ vs. $38.2 \pm 7 \%$; $p < 0.001$) and body weight (55.1 ± 6.3 kg. vs. 60.3 ± 11.7 kg; $p < 0.01$) were significantly lower in the runners compared to controls.
3. There was a significant positive correlation between the percentage body fat and the BMD at the total trunk ($r = 0.61$; $p < 0.01$); femoral neck ($r = 0.64$; $p < 0.005$); trochanter ($r = 0.71$; $p < 0.001$) and intertrochanteric region ($r = 0.76$; $p < 0.001$) in the controls. While there was a positive correlation between the percentage body fat and BMD at the total leg area in the controls, this did not reach statistical significance ($r = 0.46$; $p = 0.0506$). In contrast there was a significant negative correlation between percentage body fat and the BMD at the leg area ($r = -0.38$; $p = 0.005$).
4. No difference was noted in the menstrual history index (MHI) and follicle-stimulating hormone (FSH) concentration between runners and controls. However, the lutenizing hormone (LH) concentration was significantly higher in the runners compared to controls (9.7 ± 13.9 mIU/ml vs. 5.5 ± 4.0 mIU/ml; $p < 0.0001$).
5. There was a significant increase of mean oestradiol concentration in the runners (357.9 ± 362.2 pg/ml vs. 172.8 ± 161.8 pg/ml; $p < 0.05$), but the mean progesterone concentration was significantly lower in the runners compared to controls (7.8 ± 13.6 ng/ml vs. 10.3 ng/ml; $p < 0.05$).

6. The biochemical marker of bone formation, osteocalcin (OC) was significantly lower in the runners compared to the control (1.1 ± 0.5 nmol/L vs. 2.0 ± 1.9 nmol/L; $p < 0.05$) group and deoxypyridinoline (Dpd) a marker of bone resorption was also significantly lower in the runners compared to the controls (4.9 ± 1.9 nM Dpd/mM creatinine vs. 6.3 ± 3.5 nM Dpd/mM creatinine; $p < 0.05$). There was no difference however in the mean urinary N - telopeptide (NTx) concentration or bone specific alkaline phosphatase (BSAP).
7. In 18 % (10 out of 55) of the runners a history of stress fractures were reported, but there was no significant difference in the BMD measurement at all sites between those who sustained a stress fracture and those who did not. However, there was a significantly lower percentage body fat in the runners with stress fractures compared to those without (22.3 ± 5.9 % vs. 18.6 ± 3.5 %; $p < 0.05$). Additionally, the mean NTx concentration was lower in the runners with stress fractures (38.2 ± 9.7 nM NTx/mM creatinine vs. 59.6 ± 83.9 nM NTx/mM creatinine; $p < 0.0001$) compared to the runners without stress fractures. No significant differences were reported in MHI and gonadal hormones.
8. Ethnic differences were determined in 30 White, 12 Indian and 10 Coloured ultradistance runners. The White runners were on average significantly older than the Indian runners (41 ± 4 years vs. 35.3 ± 3 years; $p < 0.01$) but no significant difference in the weight, height and BMI was seen. There was also no significant

difference in the BMD at all sites in the three groups. However, a significantly higher percentage body fat was found in the Indian runners compared to the Whites ($29.8 \pm 5.0\%$ vs. $19.8 \pm 5.0\%$; $p < 0.001$) and Coloured ($20.1 \pm 5.7\%$ vs. $29.8 \pm 5.4\%$; $p < 0.001$) runners. The average total distance run in training of the White runners was significantly more than the Indian runners, (62.7 ± 18.6 km/week vs. 46.5 ± 17.2 km/week; $p < 0.05$) and the Coloured runners had been training for a significantly longer duration than the Indian runners (10 ± 5 years vs. 5.6 ± 2.2 years; $p < 0.05$).

Discussion and conclusion

1. In this study endurance running at sub-elite standard in premenopausal women was not shown to be detrimental to BMD.
2. Endurance running had beneficial effects on BMD in premenopausal women and the increase in BMD was site specific with an increase at hips and legs.
3. In the controls there was a significant positive correlation between percentage body fat and BMD supporting the hypothesis that body fat may be a protective factor against low bone mass. The runners had a lower percentage body fat compared to controls and there was a significant negative correlation between percentage body fat and BMD in the runners. These findings suggest that a low percentage body fat is not an independent risk factor in premenopausal women

runners with a normal menstrual history and that physical activity may have a stronger effect on bone mass than does percentage body fat.

4. In this cohort of runners the absence of significant evidence of menstrual irregularities despite the lower percentage body fat suggests that there may be additional factors for the occurrence of exercise induced amenorrhoea. The normal menstrual history in the runners may also explain the lack of a detrimental effect on BMD.
5. The reduced bone turnover rate demonstrated in the runners is consistent with the normal menstrual status and the lack of a detrimental effect on the BMD.
6. Stress fractures were recorded in 18 % of the runners. There was a lower percentage body fat in the runners with stress fractures compared to those without, however there was no difference in BMD and no menstrual irregularities. Stress fractures only occurred in the White runners, and may have been caused by other biomechanical factors.
7. In the cohorts recruited for the study, there were no differences in the BMD in White, Coloured and Indian runners.

TABLE OF CONTENTS

DECLARATION		i
ACKNOWLEDGEMENTS		ii
SUMMARY		iii
TABLE OF CONTENTS		ix
LIST OF TABLES		xvi
LIST OF FIGURES		xviii
ABBREVIATIONS		xix
CHAPTER 1	NORMAL BONE TISSUE	1
1.1	Structure and Function	1
1.1.1	Cortical bone	2
1.1.2	Trabecular bone	2
1.1.3	Bone marrow	3
1.1.4	Bone cells and their function	3
1.2	Bone Metabolism	6
1.2.1	Regulation of bone metabolism	9
1.2.1.1	Mechanical stimuli	9
1.2.1.2	Systemic hormones	9
1.2.1.2.1	Sex hormones	10
1.2.1.2.2	Calcitropic hormones	11
1.2.1.2.3	Leptin	12
1.3	Bone changes with age	14
1.3.1	Peak bone mass	15
1.3.1.1	Genetic factors	16
1.3.1.2	Ethnic differences	16

1.3.1.3	Gonadal status	17
1.3.1.4	Nutrition	17
CHAPTER 2	OSTEOPOROSIS	19
2.1	Definitions	19
2.2	Epidemiology	20
2.3	Pathogenesis of osteoporosis	21
2.3.1	Increased turnover	22
2.3.2	Remodelling imbalance and uncoupling	22
2.4	Classification of osteoporosis	23
2.5	Causes of osteoporosis	24
2.5.1	Genetic factors	24
2.5.2	Age-related bone loss	26
2.5.3	Hormonal deficiency	26
2.6	Causes of secondary osteoporosis	27
2.6.1	Lifestyle factors	28
2.6.2	Nutritional causes of osteoporosis	29
2.6.3	Anorexia nervosa	31
2.6.4	Drug induced osteoporosis	32
2.6.5	Endocrine causes of osteoporosis	33
2.6.6	Mechanical factors / exercise	34
2.7	Consequences of osteoporosis	35
2.7.1	Stress fractures	36
2.8	Diagnostic evaluation of osteoporosis	37
2.8.1	Clinical risk factor analysis	37
2.8.2	Bone mineral density	38

2.9	Biochemical assessment	44
Chapter 3	BONE MASS CHANGES IN ATHLETES: REVIEW OF LITERATURE	48
3.1	Introduction	48
3.2	Benefits of exercise on bone	49
3.3	Adverse effects of exercise	53
3.3.1	Exercise-induced amenorrhea	54
3.3.2	Nutrition and body composition	56
3.3.3	Training regimen	59
3.3.4	Osteoporosis	60
3.4	Summary	63
3.5	Hypothesis	65
3.6	Aims of the study	65
Chapter 4	METHODOLOGY	66
4.1	Ethical approval	66
4.2	Subject selection and recruitment	66
4.3	Collection and storage of blood and urine samples	68
4.3.1	Blood	68
4.3.2	Urine	68
4.4	Assessment of subjects	69
4.4.1	Questionnaire	69
4.4.2	Menstrual index	69
4.5	Bone mineral measurement	70
4.5.1	Instrument (QDR 4500)	70

4.5.2	Scan sites	72
4.5.3	Subject preparation for examination	74
4.5.4	Positioning for scanning	74
4.5.5	Quality control	77
4.5.6	Reproducibility	78
4.5.7	Measurement of percentage fat	78
4.6	Laboratory assessment	79
4.6.1	Blood	79
4.6.2	Urine	80
4.7	Bone markers	80
4.7.1	Bone-specific alkaline phosphatase	81
4.7.1.1	Principle	81
4.7.1.2	Equipment	82
4.7.1.3	Materials	82
4.7.1.4	Reagents	83
4.7.1.5	Method	83
4.7.1.6	Standard curve and calibration	84
4.7.2	Osteocalcin (OC)	84
4.7.2.1	Principle	85
4.7.2.2	Equipment	85
4.7.2.3	Materials	85
4.7.2.4	Reagents and preparation	85
4.7.2.5	Method	87
4.7.2.6	Standard curve and calculation	88
4.7.3	Deoxypyridinoline crosslinks (Dpd)	89

4.7.3.1	Principle	89
4.7.3.2	Required specimen	89
4.7.3.3	Equipment	90
4.7.3.4	Reagents and preparation	90
4.7.3.5	Method	91
4.7.3.6	Standard curve and calibration	92
4.7.4	Cross-linked N-telopeptide of type 1 collagen (NTx)	93
4.7.4.1	Principle	93
4.7.4.2	Equipment	94
4.7.4.3	Materials	94
4.7.4.4	Reagents and preparation	94
4.7.4.5	Storage of reagents	95
4.7.4.6	Method	95
4.7.4.7	Standard curve and calibration	98
4.8	Statistical analysis	99
Chapter 5	RESULTS	100
5.1	Demographics of study population	100
5.2	Comparison of total cohort of runners with sedentary controls	102
5.2.1	BMD measurement and body composition	102
5.2.1.1	BMD T score runners and controls	103
5.2.2	Biochemical parameters	107
5.2.2.1	Gonadal hormones and reproductive status	107
5.2.2.2	Markers of bone turnover	108

5.3	Comparison between runners with stress fractures and total cohort of runners	110
5.3.1	Site of stress fractures	110
5.3.2	Demographics	110
5.3.3	Comparison of BMD measurements and body composition in runners with and without stress fractures	111
5.3.3.1	Gonadal hormones and reproductive status in runners with and without stress fractures	113
5.3.3.2	Bone markers in runners with and without stress fractures	114
5.4	Comparison of ethnic groups	115
5.4.1	BMD and body composition	115
5.4.2	Comparison of markers of bone turnover in The different ethnic groups	117
Chapter 6	DISCUSSION	119
6.1	Exercise induced osteoporosis	119
6.2	BMD in ultra-distance runners and controls	121
6.3	Body composition and bone mass	123
6.4	Menstrual function and bone mass in athletes	127
6.4.1	Menstrual history	127
6.4.2	Gonadal hormones	128

6.5	Bone turnover in ultra-distance runners and healthy sedentary volunteers	130
6.6	Stress fractures	135
6.7	Ethnic differences in BMD in ultra- distance runners	137
6.8	Conclusion	140
6.9	Limitations and future studies	141
	Bibliography	143
Appendix		
	1. Informed consent	171
	2. Subject questionnaire	172

LIST OF TABLES

Chapter 2		Page
Table 1	WHO Classification of osteoporosis	20
Table 2	Secondary causes of osteoporosis	28
Chapter 5		
Table 3	Demographic data of runners and sedentary controls	101
Table 4	Bone mineral densities at various sites and percentage body fat in runners and controls	104
Table 5	Correlation between percent body fat and BMD in runners and controls	105
Table 6	BMD T scores runners and controls	106
Table 7	Gonadal hormone concentration in runners and controls	108
Table 8	Makers of bone turnover in runners and controls	109
Table 9	Demographic of runners with stress fractures and total cohort	111
Table 10	BMD and body composition of runners with stress fractures and without stress fractures	112
Table 11	Gonadal status in runners with or without stress fractures	113
Table 12	Bone markers in runners with and without stress fractures	114

Table 13	BMD, body composition, duration of running and kilometres run and MHI in the different ethnic groups	116
Table 14	Markers of bone turnover in the different ethnic groups	118
Table 15	Studies reporting effect of exercise on makers of bone turnover	132

LIST OF FIGURES

Chapter 1		Page
Figure 1	Bone turnover in a remodelling unit in adults	8
Figure 2	Bone changes with age	14
Chapter 4		
Figure 3	AP Lumbar spine position	75
Figure 4	Proximal hip position	76
Figure 5	Spine phantom	78

ABBREVIATIONS

α , alpha;
ALP, alkaline phosphatase;
AP, antero - posterior;
B, "bad" vitamin D receptor allele B;
b, "good" vitamin D receptor allele B;
 β , beta;
BAP, bone alkaline phosphatase;
BCE, bone collagen equivalents;
BGP, bone gla protein;
BMD, bone mineral density;
BMI body mass index;
BMP, bone morphogenic protein;
BMU, basic multicellular unit;
BSAP, bone specific alkaline phosphatase;
BSU, bone structural unit;
BUA, broadband ultrasound attenuation;
C cells, parafollicular cells;
CT, computed tomography;
CTx, C – telopeptide;
DEXA, dual energy X – ray absorptiometry;
DPA, dual photon absorptiometry;
Dpd, deoxypyridinoline;
EDTA, ethylenediamine tetra – acetic acid;
EFA, essential fatty acid;
ELISA, enzyme – linked immunosorbent assay;
ER, oestrogen receptor;
FBC, full blood count;
FGF, fibroblast growth factor;
FSH, follicle – stimulating hormone;
 g/cm^2 , grams per centimetre squared;
GH, growth hormone;
GnRH, gonadotropic – releasing hormone;

HDL, high-density lipoprotein;
ICTP, carboxyterminal cross – linked telopeptide of type 1 collagen;
IGF, insulin - like growth factor;
IL, interleukin;
IL - 1ra, interleukin - 1 receptor antagonist;
km/wk, kilometre per week;
Lep, leptin;
LH, lutenizing hormone;
LS, lumbar spine;
 μ L, micro litre;
mL, milli litre;
m/sec, millimetre per second;
mg, milligram;
MHI, menstrual history index;
ng/mL, nano gram per millilitre;
nM, nanomoles
NaOH, sodium hydroxide;
NHANES, National Health And Nutrition Examination Survey;
NIH, National Institute of Health,
Ntx, N – telopeptide;
Ob, obese;
OC, osteocalcin;
OD, optical density;
PA, prostatic antigen;
PDGF, platelet - derived growth factor;
PICP, procollagen 1 carboxyterminal peptide;
PMB, peak bone mass;
pNPP, para – nitrophenyl phosphatase substrate;
PTH, parathyroid hormone;
PTHrp, parathyroid hormone receptor gene;
Pyr, pyridinoline;
QC, quality control;
QCT, quantitative computed tomography;
QUI, quantitative ultrasound index;

QUS quantitative ultrasound;
RA, radiographic absorptiometry;
RDA, recommended dietary allowance;
ROI, region of interest;
SAOS - 2, osteosarcoma cells - 2;
SD, standard deviation;
SERM, selective oestrogen receptor modulator;
SOS, speed of sound;
SPA, single photon absorptiometry;
SXA, single energy X – ray absorptiometry;
TGF, transforming growth factor;
TNF, tumour necrosis factor;
UK, United Kingdom;
UL, unit per litre;
US, United States;
VDR, vitamin D receptor;
vs, versus;
WHO, World Health Organisation;
X – ray, radiograph.

CHAPTER ONE

1.0 NORMAL BONE TISSUE

1.1 STRUCTURE AND FUNCTION

The skeletal system is made up of bone, a specialized connective tissue and cartilage that together provide mechanical support and a site of muscular attachment for locomotion. They also protect vital organs and bone marrow, and serve as a reserve of ions, especially calcium and phosphate, for the maintenance of serum homeostasis.

The essential elements of bone are the organic matrix, a mineral phase and specialized cells. The organic matrix constitutes 90 % of the skeletal weight and is composed mainly of type 1 collagen fibres, and noncollagenous proteins including osteocalcin or bone GLA protein, osteopontin and bone sialoprotein. The collagen fibres have a high content of the amino acids and extend in all directions, but most prominently along the lines of tensional forces. The matrix is impregnated with calcium, phosphate and carbonate in the form of hydroxyapatite crystals, which provide a rigid framework for the body and specialized bone cells, namely the osteoblasts, osteoclasts and osteocytes (Baron, 1996).

In the adult, two types of bone tissue are present, cortical and trabecular bone.

1.1.1 Cortical bone

Cortical bone is also referred to as compact bone, and makes up approximately 80 % of the skeletal mass. It fulfils mainly a mechanical and protective function and is found in the shafts of long bones and the surface of flat bones. Cortical bone has a slow turnover rate and a high resistance to bending and torsion, and is composed of compact bone laid down in a series of adjacent and overlapping 'bull's eye' formations called osteons or Haversian systems. Each osteon is composed of a central vascular canal surrounded by interlinking passageways, called Haversian canals. The central canal contains capillaries, arterioles, venules, nerves as well as lymphatics. Between each osteon are interstitial lamellae (concentric layers of mineralised bone), embedded with osteocytes. Lamellar bone acquires its strength from its structure, which is made up of parallel layers of bone alternating in orientation by 90° (Marieb, 1987; Baron, 1996).

1.1.2 Trabecular bone

Trabecular bone or cancellous bone makes up the balance of 20 % of the bone mass and is largely responsible for the metabolic function of bone. It is found mainly in the epiphyseal and metaphyseal regions of the long bones, and the inner parts of flat bones. Trabecular bone constitutes most of the bone of the axial skeleton i.e. bones of the skull, ribs, and spine. It forms the interior structure, and helps bone maintain its shape despite compressive forces.

Trabecular bone is made up of interconnecting plates and rods within which lie haematopoietic or fatty marrow, lymph and blood vessels and nerves. It is less dense, more elastic and has a higher turnover rate than cortical bone. The thickness and number of trabeculae are determined at the growth plate during childhood and depend on the osteon or wall thickness (Schnitzler, 2001). Trabecular osteons correspond to Haversian systems in cortical bone and consist of parallel layers of lamellar bone with embedded osteocytes.

1.1.3 Bone marrow

The central cavities of bone are filled with the bone marrow, which is a source of blood cell precursors, growth factors, cytokines, fat cells, macrophages and fibroblasts.

1.1.4 Bone cells and their functions

There are three principle types of bone cells, namely osteoblasts, osteocytes and osteoclasts. The osteocytes and osteoclasts are derived from precursors originating in the bone marrow. The precursors of osteoblasts are multipotent mesenchymal stem cells, which also give rise to bone marrow stromal cells, chondrocytes, muscle cells and adipocytes, while the precursors of osteoclasts are haematopoietic cells of the monocyte / macrophage lineage (Manolagas and Weinstein, 1999).

Osteoblasts are bone-forming cells responsible for the synthesis of collagen and are abundant in areas of high metabolism. They are responsible for the formation and subsequent mineralization of bone matrix and the synthesis of growth factors secreted into the matrix. After erosion of lacunae by the osteoclast, the osteoblasts lay down replacement bone matrix, termed osteoid. Osteoblasts appear as cuboidal cells when actively involved in bone formation along the newly formed osteoid. Some osteoblasts are trapped in the bone matrix where they become osteocytes, whilst others become lining cells – thin flattened cells, which cover inactive bone surfaces.

Osteocytes are often considered to be metabolically inactive; however, they may have an important role in initiating the bone cycle, by detecting microfractures or other disturbances in bone structure and then signalling osteoclasts of these defects. The osteocytes are interconnected to osteoblasts and other osteocytes by fine intercellular projections running within bone canalicular network (Christenson, 1997). Osteocytes secrete enzymes, influence bone mineral content by regulating calcium release from bone tissue to the blood, and control the remodelling process by functioning as mechanotransducers. In the canaliculi, osteocytes are bathed in interstitial fluid, which undergoes pulsatile fluid flow, generated by loading. The osteocytes have the ability to sense certain stimuli from the fluid flow, namely, fluid shear stress and streaming electrical potentials, referred to as mechanosensing. Once the appropriate signal has been received, the lining cells retract and remodelling can begin (Burger and Klein-Nulend, 1999). Apoptosis, or programmed cell death, of osteocytes surrounding damaged bone also stimulates activation of

bone remodelling, with the aim of removing damaged bone (Burger and Klein-Nulend, 1999).

Osteoclasts are large multinucleated cells derived from fusion of cells of monocyte lineage. They are responsible for the resorption of bone and are active early in the bone remodelling cycle. Osteoclasts preferentially degrade fully mineralised bone by attaching themselves onto the bone surface. The osteoclast's apical pole has a fenestrated membrane that is orientated toward the bone matrix and is the site for secretion of lysosomal enzymes and acids into the space provided between their apical surface and the mineralised bone surface (Arden and Spector, 1997).

The membrane of the osteoclast's basolateral pole faces toward the local environment. The fenestrated outside edge of the osteoclasts attaches to the mineralised matrix of the bone's surface, thereby isolating a microenvironment. This isolated extracellular microenvironment provides a bone-resorbing compartment where the pH is lowered and where potent enzymes such as acid phosphatase, aryl-sulphatase, metalloproteinases, β - glucuronidases, cysteine-proteinases, and β - glycerophosphatases are released (Christenson, 1997). The attachment of osteoclasts to mineralised bone is mediated by integrins – adhesion molecules on the cytoplasmic membranes that bind to bone matrix molecules (Arden and Spector, 1997).

1.2 BONE METABOLISM

Bone is a metabolically active and dynamic tissue that is subject to growth, repair and functional (mechanical) adaptation. Histologically two types of bone reconstruction are distinguished: modelling and remodelling.

Bone modelling refers to the alteration of size, shape and spatial location of bones through the synthesis of new bone on some surface, and resorption of bone at other surfaces in response to factors, such as mechanical strain. Thus bone is formed without prior resorption. This process is not dependent on the coupling between osteoclasts and osteoblasts (Baron, 1996). Growth, surface drift, and functional adaptation are various forms of modelling (Smit and Burger, 2000). Bone modelling occurs primarily during the growing years although limited modelling can occur following skeletal maturation (Bass and Myburgh, 2000).

Bone remodelling, replaces fatigued or damaged bone and continues throughout life where old bone is replaced by new bone. This occurs without changing bone geometry, so that the skeleton can adapt to ongoing mechanical stresses, which might weaken the skeleton (Schnitzler, 2001). The remodelling sequence consists of a biologically coupled event, involving activation, resorption, reversal matrix formation and mineralization and quiescence and requires the action of different bone cells, in a specific sequence. The group of cells responsible for remodelling, or the basic multicellular unit (BMU), consists of pre-osteoclasts, osteoclasts, mononuclear cells, pre-osteoblasts and osteoblasts in a sequential order (Schnitzler, 2001).



Remodelling begins in a quiescent phase. During activation the resting bone surface is prepared for resorption by the retraction of lining cells and removal of the thin collagenous membrane covering the mineralised bone surface. Matrix metalloproteinases, produced by osteoblasts are involved in the latter process (Arden and Spector, 1997). Thereafter, osteoclasts are attracted to the new BMU site where they erode the bone matrix, forming lacunae. In the process requiring about 10 days, osteoclasts resorb bone until the lacunae are approximately 100 μm in diameter and 40 - 60 μm deep. Resorption is then halted and osteoblasts are recruited to the BMU site. The last phase of the remodelling cycle is formation when osteoblasts lay down the osteoid matrix. When the lacunae are filled with osteoid, a process requiring about 80 days, the newly formed matrix is mineralised with hydroxyapatite, giving the BMU tensile strength. The remodelled area then passes into the inactive phase to complete the 60 to 120 day bone cycle (Christenson, 1997). The new bone so formed is referred to as a bone structural unit (BSU) (Dunitz, 1995; Dempster, 1995) (Figure 1).

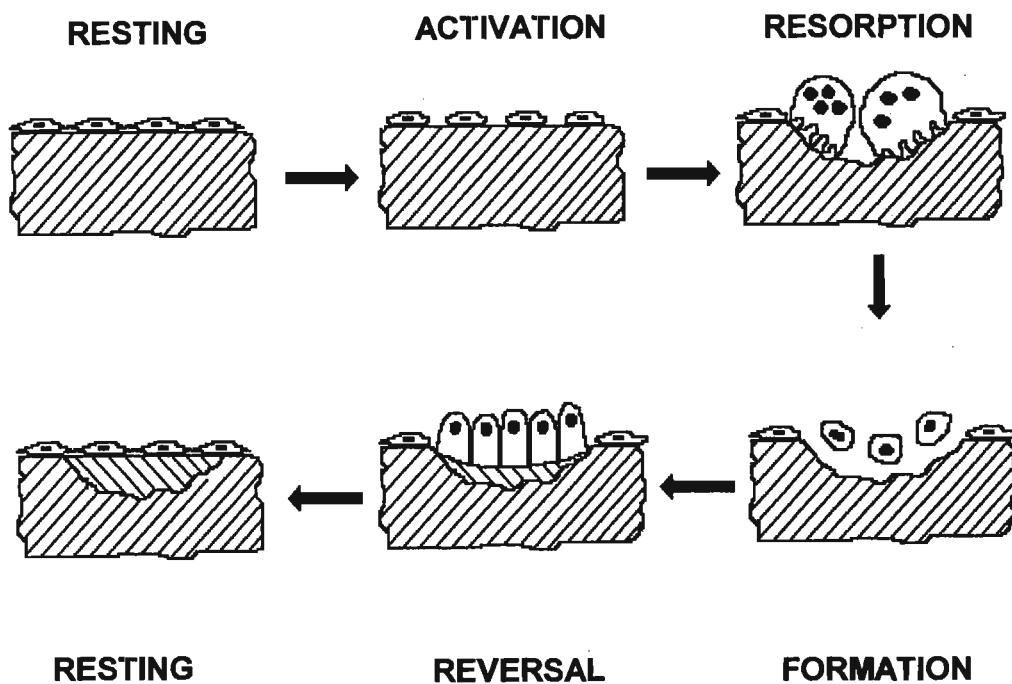


Figure 1: Bone turnover in a remodelling unit in adults

In the healthy adult, there is coupling of bone resorption and formation i.e. bone resorption is followed by bone formation and the amount of bone resorbed is equal to that formed. The net result is that roughly 5 to 10 % of tissue is replaced each year (Van de Graaf *et al*, 1997). Normal coupling is under the influence of 'coupling factors' generated by the increasing depth of the resorption cavity and the rising mechanical strain. These include growth factors namely; transforming growth factor - beta (TGF - β), bone morphogenic proteins (BMPs), insulin-like growth factors I and II (IGF I and II), fibroblast growth factors (FGFs) and platelet-derived growth factors (PDGFs) (Schnitzler, 2001).

1.2.1 Regulation of bone metabolism

Bone metabolism is regulated by the complex interaction of mechanical stresses, systemic hormones and locally produced cytokines, prostaglandins, and growth factors in an autocrine or paracrine manner. Other factors that importantly influence bone growth and development are general health and nutrition, adolescent exercise and calcium intake (Kanis, 1994; Dempster, 1995).

1.2.1.1 Mechanical stimuli

Bone tissue has the ability to adapt to the mechanical demands by altering its mass and distribution of mass. Mechanical stimuli are therefore the major determinants of the size, shape and microarchitecture of bones and skeletal growth. Physical stimuli and muscle action influence the bone remodelling process by creating torsion, shearing, bending and compressional forces on bone (Hughes *et al*, 1995; Burger and Klein-Nulend, 1999; Smit and Burger, 2000). Increased mechanical load e.g. weight bearing exercise results in bone gain. In contrast, decreased mechanical loading e.g. complete immobilization, results in bone loss (Arden and Spector, 1997; Zerwekh *et al*, 1998).

1.2.1.2 Systemic Hormones

Systemic hormones influence bone modelling remodelling by their effect on progenitor cells, osteoblasts and osteoclasts and include the sex steroids, calcitropic

hormones, tri-iodothyroxine, growth hormone (GH) and corticosteroids. These hormones influence bone mass by their effect either on bone formation and resorption or on bone turnover (Christenson, 1997).

1.2.1.2.1 Sex hormones

The sex hormones, principally oestrogen, play a crucial role not only in the adolescent growth spurt and in skeletal maturation, but also in the rate of bone loss.

Oestrogen, also referred to as a bone-remodelling inhibitor, (Dempster, 1995) specifically plays an important role in bone metabolism. However the exact mechanism is not known. The presence of oestrogen receptors on both the osteoblasts and osteoclasts suggests that this hormone acts directly on bone cells. Oestrogens may also exert their effect on bone via a number of mediators and growth factors by

- Stimulating the release of IGF 1, which is osteogenic.
- Activating TGF β , which has been shown to reduce bone resorption.
- Inhibiting prostaglandins, which stimulate bone resorption.
- Down-regulating interleukin 6 (IL-6) expression. IL-6 recruits and activates osteoclasts thereby increasing bone resorption.
- Decreasing monocyte production of the bone resorbing cytokines, IL-1 and tumour necrosis factor alpha (TNF α).

- Increasing intestinal absorption of calcium, reducing urinary excretion of calcium, and facilitating the retention of calcium by bone (Lindsay, 1995).

Androgen receptors have also been demonstrated in osteoblasts, and have direct receptor-mediated effects on bone cells and skeletal metabolism comparable to oestrogen (Kanis, 1994).

1.2.1.2.2 Calcitropic hormones

Since bone acts as a reservoir for calcium and stores 99 % of the body's calcium, the hormones regulating calcium homeostasis influence bone metabolism. The serum concentration of calcium is maintained at a relatively constant level and is regulated by the calcitropic hormones. Parathyroid hormone (PTH) is synthesized as a precursor, preproparathyroid hormone and stored in the secretory granules of the parathyroid gland. The PTH released in response to hypocalcaemia increases calcium absorption from bone and decreases urinary calcium excretion, thereby maintaining normal serum calcium concentration (Kanis, 1994; Cormier *et al*, 1997).

Vitamin D (cholecalciferol), produced in the skin by ultraviolet light during solar exposure, is converted in the liver to 25 - hydroxy-cholecalciferol and then in the kidney to the active form, 1,25 - dihydroxy-cholecalciferol. The last step requires adequate amounts of 1 - alpha-hydroxylase. Vitamin D, maintains the plasma calcium concentration by increasing absorption of dietary calcium from the small

intestine and enhancing the mobilization of calcium stores in bone at times when dietary calcium is inadequate to maintain plasma calcium (Cormier *et al*, 1997).

Calcitonin is secreted by the parafollicular cells (C cells) of the thyroid gland in response to an acutely rising plasma calcium concentration. This is a physiological effect to maintain calcium homeostasis. The main biological effect of calcitonin is to inhibit osteoclastic bone resorption (Kanis, 1994; Cormier *et al*, 1997).

1.2.1.2.3 Leptin

Recently, a newly discovered protein, leptin, has been linked with bone metabolism. In 1994 leptin was discovered as a product of the *obese* Ob (Lep) gene located on the mouse chromosome 6. In humans this gene is located on chromosome 7q 31.3. Leptin is a 16-kilodalton adipocyte derived protein hormone, whose crystal structure suggests that it belongs to the cytokine family. It circulates in serum in both the free and bound forms (Mantzoros, 1999). The circulating concentration of leptin is directly proportional to the amount of body fat or adipose tissue and reflects not only the amount of fat stored but also energy imbalance. Women have a higher leptin concentration due to a higher percentage of body fat compared to men. Leptin concentrations have been shown to positively correlate with body mass index (BMI) in humans (Maeta *et al*, 2001; Warren and Perlroth, 2001) and appears to function as an important signal between peripheral fat mass and the brain's central control of metabolism, namely food intake and regulation of energy expenditure, body weight,

the initiation of puberty and maintenance of menstrual cycles, reproductive ability (Sundaresan *et al*, 2000) and regulation of bone mass (Takeda *et al*, 2002).

In animal studies the anorexigenic and antiosteogenic actions of leptin appear to be mediated centrally through the hypothalamus, by two distinct pathways. The anorexic function of leptin is mediated through neuropeptides and leptin may function as an adaptive mechanism in an environment where food availability is limited (Grinspoon *et al*, 2001; Takeda *et al*, 2002). The decreased concentration of leptin in anorexia nervosa and women athletes who exercise strenuously reflect the abnormal metabolic and neuroendocrine changes that occur in these individuals. In addition amenorrhoea is associated with low body fat and leptin concentration (Mantzoros, 1999). The effect of leptin on bone metabolism is mediated via the sympathetic nervous system and bone formation is inhibited via the beta adrenoceptors on osteoblasts (Takeda *et al*, 2002).

Other mediators involved in the regulation of bone remodelling include prostaglandins and prostacyclins, nitric oxide, oxygen-derived free radicals, constitutively produced receptor antagonist (IL-1 α), and cell to cell contact. A large number of cytokines and growth factors have been shown to affect bone cells and may act interdependently with other factors resulting in multiple actions on both osteoblasts and osteoclasts. Thus, they influence both bone resorption and bone formation (Arden and Spector 1997).

1.3 BONE CHANGES WITH AGE

Bone mass accumulates during childhood and adolescence until peak bone mass (PBM) is attained. During the fourth decade of life age-related bone loss commences and continues throughout life, accelerated in women during the years around menopause (Figure 2). The rate of bone loss varies according to the skeletal site. Losses in cancellous bone are greater than the loss of bone in cortical bone. Approximately 50 % of cancellous and 35 % cortical bone mass are lost over a lifetime in women, with losses in men around two-thirds these amounts (Kanis, 1994; Arden and Spector, 1997).

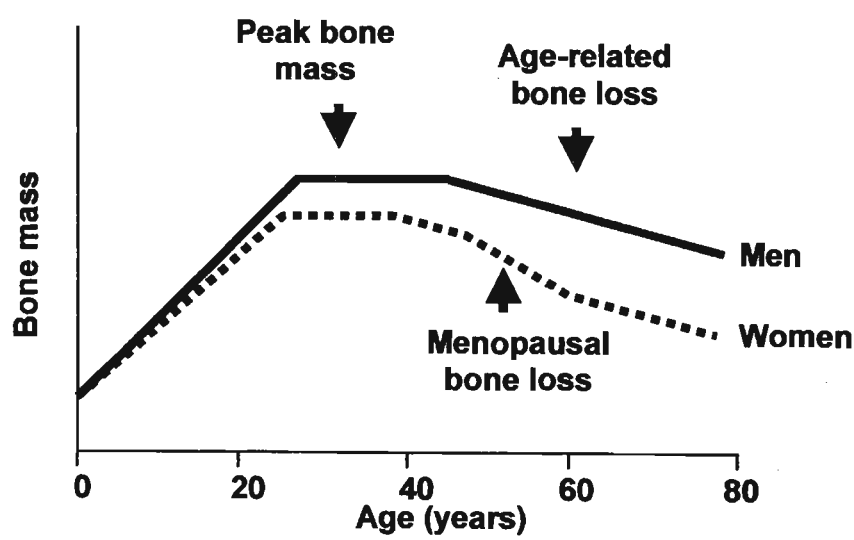


Figure 2: Bone mass changes with age (Adapted from Arden and Spector 1997 and Cassim, 2001).

1.3.1 Peak bone mass

Peak bone mass is the maximal lifetime amount of bone tissue attained in individual bones and therefore, in the entire skeleton. It is the consequence of the net accumulation of bone mass during childhood and adolescence, and the balance between bone gained and resorbed prior to the adult premenopausal period. Approximately 80 - 85 % of PBM has been accrued by the time of menarche. At puberty bone mass and bone mineral content increases coincide with the growth spurt in puberty. In girls this occurs at between 10 to 12 years and in boys at ages 12 to 13 years. Growth causes progressive increases in bone mass until ages 16 to 17 years in girls and 18 to 19 years in boys. Although linear bone growth then stops, mineralization of cortical bone is not completed or at peak (normal adult) values until approximately the age of 20 - 25 years and up to as late as 35 years (Bonjour, 1991).

Peak values for vertebral trabecular bone density and forearm cortical bone mineral content are 25 - 20 % higher in males. In the spine and femur, the difference in bone mass is mostly attributed to the greater cross-sectional bone area in males than females. These male-female differences in PBM have been shown in male-female twins and are thought to be due to gonadal hormones, rather than some other male-female genetic or environmental differences (Neer, 1995) because the acquisition of PBM that occurs during the latter stages of puberty, coincides with the maximum growth hormone, high serum IGF-I concentrations, and rising concentrations of oestradiol and testosterone. Skeletal growth and development continues over several decades (Rosen, 2002). Peak bone mass is biologically

determined by factors such as gender, race, genetics, mechanical loading, hormonal and nutritional background (Kanis, 1994; Dempster, 1995).

1.3.1.1 Genetic factors

Twin studies with monozygotic and dizygotic twins suggest that up to 80 % of the variance in bone density at both axial and appendicular sites is determined genetically (Heaney and Matkovic, 1995; Kannus *et al*, 1999). In addition daughters of women who have had an osteoporotic fracture have a significantly lower PBM than their peers (Kanis, 1994; Heaney and Matkovic, 1995). A number of factors may account for these genetic differences. Studies in Australian women suggest that up to 75 % of genetic effects on BMD could be accounted for by polymorphisms in the vitamin D receptor (VDR) gene (Morrison *et al*, 1994). In contrast, in a study of a population-based cohort of young healthy men and women where environmental factors together with polymorphisms in the VDR and oestrogen receptor (ER) candidate genes only explained an 18 % variance in PBM in women and 14 % in men, and the authors suggest that the bulk of the unexplained variance was probably caused by an as yet undefined allelic variation in candidate genes (McGuigan *et al*, 2002).

1.3.1.2 Ethnic differences

Ethnic differences in PBM have also been reported. Higher values of PBM have been reported in black people compared to Caucasians, even when adjusted for

body weight and height (Kumar *et al*, 1997). The lowest values of PBM are found in Asians (Vidyashankar, 2002) and Japanese (Arden and Spector, 1997). However, these low values could also be attributed to environmental factors, which may have been underestimated.

1.3.1.3 Gonadal status

Gonadal hormones play an important role in achieving PBM and puberty is associated with a growth spurt in both sexes. A delay in puberty and particularly menarche is associated with decreased height and a lower PBM. Malnutrition, excessive exercise and low body weight are associated with a delayed menarche (Frisch *et al*, 1980) and therefore a lower PBM (Kanis, 1994).

1.3.1.4 Nutrition

An adequate and balanced diet is important for normal growth and development. Protein calorie malnutrition as well as a dietary excess of protein, salt and caffeine may have a negative influence on PBM.

Calcium is important for the mineralization of bone and achieving PBM. The Recommended Dietary Allowance (RDA) of calcium varies from country to country and ranges from 400 to 1500 mg daily, with increased intakes recommended in adolescence, pregnancy and postmenopausal women. Although the importance of calcium for mineralization and regulation of bone remodelling (through the calcitropic

hormones) is well known, the results of studies correlating calcium intake with PBM are equivocal. However, epidemiological studies have shown that a lifetime low calcium intake is associated with low bone density and an increased fracture risk (Kanis, 1994; Arden and Spector, 1997).

Excess salt, protein and caffeine intake is associated with increased urinary calcium loss (Morgan, 2001).

CHAPTER TWO

2.0 OSTEOPOROSIS

2.1 DEFINITIONS

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture (National Institutes of Health Consensus Development Conference Statement, 2002). Bone strength reflects a combination of bone density and quality. The latter is not readily measurable and diagnosis of osteoporosis is generally based on BMD. Thus the World Health Organisation (WHO) proposed a classification of osteoporosis based on bone mineral density (BMD) and fractures (Table 1) (Frost *et al*, 2001).

Osteopenia describes too little bone in the absence of spontaneous fractures.

Table 1 WHO Classification of Osteoporosis:

Definition	Criteria
Normal	A value for BMD or bone mineral content (BMC) within 1 SD of the young adult reference mean
Low bone mass (osteopenia)	A BMD or BMC value of more than 1 SD, but less than 2.0 SD below the young adult reference range
Osteoporosis	BMD or BMC more than 2.5 SD below young adult reference mean
Established (severe) Osteoporosis	BMD or BMC more than 2.5 SD below young adult reference mean plus one or more fragility fractures.

Adapted from the WHO Study Group. WHO Technical Report Series 843. World Health organisation, Geneva, Switzerland, 1994.

2.2 EPIDEMIOLOGY

Osteoporosis is a common health problem and in 1986 it was estimated that 75 million people were affected by osteoporosis in Europe, Japan and the USA (Riggs and Melton III, 1986). Because of its association with fracture, osteoporosis is a major economic burden on society. It has been estimated that the costs for direct medical treatment of osteoporosis in the United States is in excess of \$13.8 billion annually. Osteoporosis will have a substantial effect on health spending with a predicted rise in the number of osteoporotic hip fractures occurring in the world each year, rising from 1.66 million to 6.26 million by the year 2050 (Iqbal, 2000).

The prevalence of osteoporosis varies with age, gender and ethnicity. The prevalence of osteoporosis increases with age and women are more affected than men due to a lower PBM and accelerated bone loss with the onset of menopause. The WHO estimated prevalence of osteoporosis in western women (adjusted to 1994 US white women) at any site is 14.8 % in women aged 50 - 59, 21.6 % for ages 60 - 69, and 38.5 % for ages 70 - 79, rising to 70 % in women over 80 (Arden and Spector, 1997; Walker-Bone *et al*, 2001).

A higher PBM has been reported in Blacks compared to Whites and Asians. In addition there may be a lower rate of bone loss in Blacks. In South Africa, the prevalence of osteoporosis in White, Asians and mixed race populations appears to be similar to that of the UK and USA. While there is substantial difference in vertebral BMD in Black and White South Africans (Daniels *et al*, 1995), there is a significantly lower fracture rate in Blacks (Solomon 1979). This difference may be due to differences in bone quality and turnover rates, age, genetic predisposition and the propensity to fall (Hough 2004; Kruger *et al*, 2004).

2.3 PATHOGENESIS OF OSTEOPOROSIS

Intensive investigation in the recent past has greatly increased the understanding of osteoporosis. Two factors, which affect the probability of developing osteoporosis in later life, are the PBM and the rate of bone loss. PBM is largely genetically determined, but may be influenced by a number of other factors (refer to chapter 1). Bone loss may be due to an increased turnover of bone or a remodelling imbalance.

2.3.1 Increased turnover

Increased turnover refers to the increase in activation frequency of bone remodelling i.e. the number of remodelling units on the bone surface, which are undergoing resorption at any given time are increased. Essentially, there is a primary increase in bone resorption followed by a coupled increase in formation. During the process of remodelling there is a net deficit of bone (until the resorption cavities are completely infilled with osteoid) so that the skeletal volume missing at any one time increases proportionately with the number of functional bone resorption units. In addition, together with the increase in frequency, there is an increase in the number of osteoclasts. Thus the extent of resorption and the depth of the resorption cavity increase. If the depth of the erosion is sufficient, perforation of the trabecular plate will occur. Further, with increased turnover, a greater proportion of bone volume is occupied by young rather than old BSUs. Consequently the proportion of immature and incompletely mineralised bone increases. Osteoporosis due to an increased turnover typically occurs in postmenopausal osteoporosis, immobilization and hyperparathyroidism (Kanis, 1994; Khosla and Melton III, 1995).

2.3.2 Remodelling imbalance and uncoupling

The other mechanism resulting in bone loss, which may operate independently or in conjunction with increased activation frequency, is remodelling imbalance. Here, the amount of bone formed within individual bone remodelling units is less than that resorbed due to an increase in erosion depth and/or a

reduction in the amount formed, with a small but finite deficit of bone. The formed bone loss is irreversible within the remodelling unit once the remodelling cycle is complete (Kanis, 1994; Arden and Spector, 1997). This may be accompanied by uncoupling. Uncoupling implies the dissociation of bone resorption and bone formation; either resorption cavities are created without subsequent attraction of osteoblasts as seen in neoplasia, or new bone is deposited at sites other than sites of previous resorption, seen in, for example prostatic bone disease (Kanis, 1994).

2.4 CLASSIFICATION OF OSTEOPOROSIS

Osteoporosis is divided into two categories: primary osteoporosis and secondary osteoporosis.

Primary osteoporosis is further divided into three types.

- Postmenopausal osteoporosis (Type 1) refers to the peri and post - menopausal osteoporosis (hypogonadal) bone loss that occurs between the ages of 45 and 65 years. It is characterized by the disproportionate loss of trabecular bone and is associated with fractures at sites rich in cancellous bone such as the vertebral body and distal radius.
- Senile osteoporosis (Type 2), or age-associated osteoporosis is a result of the age related loss of between 0.7 – 1.0 % in both men and women annually and affects all skeletal sites with both cortical and cancellous bone such as the proximal femur (Riggs and Melton III, 1986).
- Idiopathic osteoporosis (Type 3) affects premenopausal women as well as middle-aged and young (Riggs and Melton III, 1986).

2.5 CAUSES OF OSTEOPOROSIS

The prevalence of osteoporosis differs in the various ethnic groups and between men and women. This is as a result of variance in a number of risk factors such as genetics, age, and gonadal status as well as lifestyle influences such as physical activity and general health (Kanis, 1994).

2.5.1 Genetic factors

Osteoporosis is a complex disease, which is thought to be mediated by an interaction between environmental factors and several different genes that individually have modest effects on BMD and other aspects of fracture risk. The best example of this is osteogenesis imperfecta, which is a rare Mendelian inherited osteoporotic disease. The disease severity can vary markedly within and between families that have identical mutations in the collagen genes, presumably due to the influence of other genes on bone mass and bone fragility (Ralston, 1999; Stewart and Ralston, 2000). The strong correlations between spine, femur and radial bone density in monozygotic twins indicate that a single gene or gene cluster controls skeletal growth at all anatomical sites, both axial and appendicular (Garnero *et al*, 1996; Kannus *et al*, 1999).

The active metabolites of vitamin D play an important role in regulating bone cell function and maintenance of serum calcium homeostasis by binding to the Vitamin D receptor (VDR) and regulating the expression of a number of response genes. Morrison *et al* (1994) reported a significant association between

polymorphisms in the 3' region of the VDR and BMD in a twin study and a population-based study. Here, the VDR alleles accounted for 75 % of the total genetic effect of bone density. The "good" VDR allele b is associated with higher BMD than the "bad" allele B. Individuals with genotype BB have lower peak bone density at age 25 - 30 years than those with genotype Bb or bb. Other genetic markers which have been shown to be associated with low bone mass are polymorphisms of the oestrogen receptor gene (Kobayashi *et al*, 1996), collagen type 1 alpha gene (Grant *et al*, 1996,) PTHrp receptor gene (Ralston, 1999) and IL 6 promoter (Zmuda *et al*, 2000).

Genetic factors may also account for the ethnic differences in BMD. The lifetime risk of bone fracture for postmenopausal Caucasian American women is about 40 %, which is double the risk for African American women who have a higher BMD than Mexican American women, who have a higher BMD than white non-Hispanic women (NIH Consensus Development Panel on Osteoporosis, 2001). In South Africa the incidence of osteoporotic fracture is lower in Black populations than in Whites. In a South African study by Daniels *et al* (1995), the peak bone density was higher at one anatomical site (femur) compared to the Whites. Additionally the Blacks were shorter, than the Whites and their body weight, body mass index and skin fold thickness increased with age, suggesting that environmental factors play an important role other than genetics in the ethnic differences in BMD (Daniels *et al*, 1995).

2.5.2 Age-related bone loss

The term involutional or 'age-related' bone loss describes the continued bone loss that occurs in men and women throughout life, which contributes to the development of osteoporosis. The age-related loss of bone mass appears to be caused in large by reduced number and activity of osteoblasts resulting in impaired bone formation (Kanis, 1994; Schnitzler, 2001). Beyond the third decade, with each remodelling cycle, new bone formation does not quite compensate for bone loss, resulting in a gradual attrition of bones. Elderly persons are more likely to have a low calcium intake, either due to decreased dietary intake or decreased absorption of calcium by the gut. The negative calcium balance results in secondary hyperthyroidism and increased bone resorption. Additionally, a mild vitamin D deficiency, due to decrease sun exposure and dietary intake may result in secondary hyperparathyroidism. A decline in muscle mass with advancing age is associated with osteoporosis (Kanis, 1994). Other lifestyle factors, such as lack of physical exercise, poor nutrition, smoking, high alcohol intake/abuse, poor calcium intake, hormonal deficiency in both men and women, the use of toxic drugs, or certain medical diseases are superimposed on this age related bone loss, clinically significant osteoporosis may ensue (Kanis, 1994).

2.5.3 Hormonal deficiency

Hormonal deficiencies play a crucial role in the development of osteoporosis, especially in postmenopausal women. The effects of oestrogen on bones appear to be mediated, at least in part, by regulation of cytokines that affect

the bone. Postmenopausal osteoporosis is primarily due to an increase in turnover and increased resorption. Decreased oestrogen levels are associated with increased production of IL-1 by monocytes, which in turn stimulate increased production of IL-6 by osteoblasts. IL-6 recruits and activates osteoclasts, thereby causing increased resorption of bone. Recent evidence suggests that oestrogen deficiency may also lead to decreased synthesis of cytokines and growth factors that promote bone formation (Arden and Spector, 1997). Thus oestrogen deprivation, either spontaneous or by oophorectomy is characterised by an increase in the number of new resorption sites and consequent bone loss as well as a decrease in bone synthesis (Jilka, 1998; Gallagher 2001). Hysterectomy is followed by accelerated bone loss due to diminished blood supply to the ovaries (Siddle *et al*, 1987) and resultant reduction in hormone production.

Unlike women, men do not undergo a natural menopause, but with age the production of gonadal steroids decreases. The low concentrations of androgens are associated with low concentrations of calcitriol, decreased intestinal absorption of calcium resulting in accelerated bone loss (Kanis, 1994).

2.6 Causes of secondary osteoporosis

A secondary cause for osteoporosis is associated with up to 30 % of the cases of vertebral fractures in women and 57 % of cases in men. Several chronic diseases and drugs can cause secondary osteoporosis (Reid and Harvey, 1997) (Table 2).

Table 2 Secondary causes of osteoporosis

Endocrine diseases	Cushing's disease, hyperparathyroidism, hyperthyroidism, Hypogonadism
Drugs	Corticosteroids, heparin, anticonvulsants, lithium, excess thyroid hormone replacement
Gastrointestinal and liver disease	Subtotal gastrectomy, malabsorption syndromes, primary biliary cirrhosis, haemachromatosis, Haemosiderosis
Hypogonadism	Turner's syndrome, Klinefelter's syndrome, hypopituitarism, anorexia nervosa, exercise induced amenorrhoea
Bone marrow disorders	Multiple myeloma, disseminated carcinomatosis, leukaemias and lymphomas
Miscellaneous	Immobilization, chronic alcoholism, rheumatoid arthritis, chronic obstructive pulmonary disease, smoking, organ transplantation

Adapted from Kanis, 1994 and Reid and Harvey, 1997

2.6.1 Lifestyle factors

Alcohol and osteoporosis

Alcohol has a direct affect on bone metabolism by reducing bone cell proliferation and functional activity. Alcoholics have decreased bone formation, which is reflected by reduced markers of bone formation, as well as uncoupling of resorption and formation and increased bone resorption. Intakes of alcohol in excess of four whiskeys or two litres of beer a day results in numerous metabolic disturbances, which enhance bone loss. There is a decline in intestinal calcium absorption increased calciuresis, decreased vitamin D production by the liver, a

decline in gonadal hormone concentration and a rise in serum cortisol concentration. Additional risk factors such as a sedentary lifestyle, smoking, alcoholic myopathy and muscle weakness may be present (Schnitzler *et al*, 1994; Reid and Harvie, 1997).

Smoking

Compared to non-smokers the risk of osteoporosis is increased in smokers. Heavy smoking has been shown to be associated with a reduction in gonadal hormone concentration. Premenopausal smokers are known to have an altered oestrogen metabolism thereby inducing early menopause by approximately 1 - 2 years. The lower bone mass in heavy smokers is associated with additional risk factors such as ethanol and caffeine intake, chronic obstructive airways disease and inactivity (Schnitzler, 1996; Arden and Spector, 1997).

2.6.2 Nutritional causes of osteoporosis

Calcium

There is no compelling evidence to suggest that on a mixed diet, calcium plays a critical role in the attainment of peak bone mass. The precise role of calcium deprivation in the causation of bone loss remains unclear, but lifelong high calcium intake has been reported to be associated with lower hip and wrist fracture rates than low calcium intake (Matkovic *et al*, 1979). Pharmacological doses of

calcium have also been shown to delay the rate of bone loss in postmenopausal women who had low calcium diets (Nordin and Morris, 1989).

Vitamin D

Vitamin D is integrally involved in bone and calcium metabolism. Adequate amounts are required for its important role as regulator of calcium absorption from the gut, calcium excretion in the urine, the deposition of calcium in bone and other tissues and the synthesis of bone matrix. Vitamin D also exerts a direct effect on osteoblasts (Arden and Spector, 1997). In vitamin D deficiency, insufficient calcium is absorbed, consequently more parathyroid hormone is secreted and bone erosion increases (Schnitzler, 1996).

Essential fatty acids (EFAs)

Essential fatty acids (EFAs) are vitamin-like substances, which must be provided in the food because they cannot be manufactured in the body. There are two types of EFAs, the n - 6 and the n - 3 series. Linoleic acid is the parent dietary compound of n - 6 found particularly in vegetable oils while the parent n - 3 compound is alpha-linolenic acid, found in dark green vegetables. In an early study, animals deprived of EFAs were shown to develop severe osteoporosis with increased urinary calcium excretion leading to renal stones and renal calcification (Borland and Jackson, 1931). Subsequent studies have confirmed that EFA deficiency was associated with loss of normal synthesis of bone connective tissue matrix, loss of normal cartilage and bone demineralisation. The mechanism for

the bone loss appears to be mediated by Vitamin D in that the actions of vitamin D on calcium require the presence of EFAs. Thus in EFA deficiency the absorption of calcium is substantially reduced (Horribin, 1996).

Marked calorie and protein malnutrition and prolonged vitamin D deficiency during childhood have a significant effect on skeletal development and bone mass attainment due to a delay in puberty. In the elderly, under nutrition may contribute to bone loss, the risk of falling and response to injury (Kanis, 1994).

In contrast, **high protein** intake may increase the risk of osteoporosis, because the high intake of protein usually equals a high phosphate intake. Phosphates bind calcium in the gut and so reduce its absorption; phosphates also increase urinary calcium loss. High dietary sodium intake also leads to urinary calcium-wasting (Heaney, 1988).

2.6.3 Anorexia Nervosa

Osteoporosis is typically found in women with low body weight. The protective effect of body fat is most likely related to the ability of fat cells to convert androgens synthesised by adrenal gland and the ageing ovary to oestrogens. Adipose tissue could thus be regarded as an "extra" ovary without menopause (Heath, 1985). An increased body mass in Black females appeared to protect them against rapid postmenopausal bone loss and there is a positive relationship between body mass and BMD in postmenopausal Blacks. These findings

suggested that adiposity might have a role in maintaining bone density (Daniels *et al*, (1995).

Anorexia nervosa is an eating disorder affecting approximately 1 % of adolescent females. It is associated with low body weight, low oestrogen levels, and elevated cortisol concentration, all of which may cause increased bone loss. A low BMD and increased fracture rate has been demonstrated in anorexic individuals. Studies have shown that BMD is most closely related to the duration of amenorrhoea or the duration of the disease (Reid and Harvie, 1997).

2.6.4 Drug induced osteoporosis

Many drugs such as steroids, anticonvulsants and heparin have been associated with an increased risk of osteoporosis.

Corticosteroids were first used therapeutically in 1948 and hypercortisolism has been associated with the development of osteoporosis. The extent of bone loss depends on dose and duration of treatment and also the age of the patient (< 15 years appear to lose bone more rapidly). Postmenopausal women and individuals with a low BMD prior to commencement of therapy are at higher risk for the development of corticosteroid – induced osteoporosis. Corticosteroids appear to have a greater effect on trabecular than cortical bone, demonstrated by low BMD in the spine and proximal femur (Reid and Harvie, 1997). Several mechanisms are thought to contribute to corticosteroid-induced osteoporosis. These include secondary hyperparathyroidism and increased bone

resorption due to decreased intestinal absorption of calcium and increased urinary excretion of calcium, a direct negative effect on follicle stimulating hormone (FSH) induced oestrogen and testosterone production and reduced adrenal production of androstenedione, a direct inhibitory effect on osteoblast function, modulation of cytokines and other hormones which influence bone metabolism, proximal myopathy and the underlying disease process (Lukert and Raisz, 1990).

The prolonged use of **heparin** therapy has been associated with osteoporosis and spontaneous fractures. Heparin has a direct toxic effect on osteoclast development and activity leading to bone loss (Kanis, 1994).

Anticonvulsants induce microsomal enzymes and increase the catabolism of Vitamin D metabolites, particularly 25 - hydroxyvitamin D and β - oestradiol. High doses of anticonvulsants decrease vitamin D mediated absorption of calcium and may decrease the response of target tissue to calcitriol. While anticonvulsants are more commonly associated with osteomalacia, they may also cause osteoporosis particularly in patients with chronic liver disease (Kanis, 1994).

2.6.5 Endocrine causes of osteoporosis

A number of endocrine disorders are also associated with osteoporosis. Hypogonadism from congenital disorders such as Turners syndrome; Klinefelter's syndrome or acquired defects that occur post puberty e.g. hypopituitarism, hyperprolactinaemia, and anorexia nervosa are associated with bone loss (Reid and Harvie, 1997).

In primary hyperparathyroidism, there is increased activation of bone remodelling, resulting in an increase in bone turnover.

Long standing thyrotoxicosis accelerates bone loss by increasing the rate of bone remodelling. Thyroid hormones increase the bone turnover by increasing osteoclast recruitment and resorption sites. Over-zealous treatment of patients suffering from hypothyroidism with thyroxine therapy has also been associated with a decreased BMD (Reid and Harvie, 1997).

2.6.6 Mechanical factors/ Exercise

Mechanical factors, particularly weight bearing, are important stimuli for normal remodelling of bone. Reduced physical activity is associated with accelerated bone loss, demonstrated by bone loss in paralysed or prolonged, immobilised extremities. In older adults the general sedentary life style undoubtedly contributes to the progression of osteoporosis (Chestnut III, 1993; Kirchner *et al*, 1995). There is little evidence that normal variations in daily physical activity are responsible for variations in rates of bone loss. However female athletes, especially runners who become amenorrhoeic as a result of strenuous exercise (Heath, 1985) may develop stress fractures and later progress to osteoporosis (Lloyd *et al*, 1986).

2.7 CONSEQUENCES OF OSTEOPOROSIS

Osteoporosis is a common disease with high health related costs due to its association with fracture (Kanis 1994; Erickson *et al*, 1997; Iqbal, 2000). While osteoporosis is currently defined in terms of the BMD because of the ease with which it can be measured, bone quality is an important component of bone strength. However, this is not readily measurable. The qualitative properties contributing to bone strength are bone geometry, trabecular connectivity, presence of vertebral fractures, increased bone turnover and impaired fatigue damage repair. The reduced effectiveness of trabecular connectivity and loss of the cross-linkage of the bone matrix and that occurs with osteoporosis contributes to bone fragility and increased fracture risk (Kanis, 1994).

The distal radius, spine and hip are the common sites for fractures (Cooper, 1997). In White women over the age of 50 years the lifetime risk of sustaining any osteoporotic fracture is 40 % compared to 13 % in men (Melton III, 1992). Distal forearm fractures are the most common fractures below age 65 years while the incidence of hip and vertebral fractures rises exponentially with increasing age. Hip fractures are the most serious because of the high morbidity and mortality and the associated high social and economic costs. Five to twenty percent of patients with hip fractures die within one year and over 50 % of the survivors are incapacitated. More recently, it has been recognised that there is a high morbidity and mortality with a fracture at any site including vertebral and forearm fractures (Kanis, 1994).

In South Africa, osteoporotic fracture rates in white, Asian and 'mixed race' populations are similar to those reported from North America and Europe with the lowest occurrence of hip fracture in the world reported in South African Blacks (Council of the National Osteoporosis Foundation, 1996).

2.7.1 Stress fractures

Stress fractures occur when there is a failure of the skeleton to withstand submaximal forces over time. Stress fractures occur as a result of one of two entities. Firstly those described in military recruits and runners called 'fatigue fracture', in which normal bone is exposed to repeated abnormal stresses and secondly 'insufficiency fracture' where normal stress is applied to abnormal bone found for example in osteoporosis or Paget's disease (Sinha, 2002). Bone reacts to stress by increasing bone density at the site of stress through increasing osteoblastic activity. However, there is a limit to this adaptability. With continuous or repeated trauma to the same site, osteoclastic activity can exceed osteoblastic activity, and trabecular microfractures can result. With the persistence of the traumatic forces, the trabecular microfractures progress to small cortical fractures, termed stress fractures. If the trauma persists, a complete fracture can result. A commonly associated condition, which some call shin splints. These are believed to result from periosteal reaction caused by microperiosteal tears from abnormal stress mediated by Sharpey fibres, which connect the tendons to the bones. Shin splints usually do not progress to further damage to the bone (Sinha, 2002).

2.8 DIAGNOSTIC EVALUATION OF OSTEOPOROSIS

Since a low BMD is the most important risk factor for fractures, the diagnosis of osteoporosis is currently based on the measurement of BMD. However clinical risk factors, qualitative abnormalities and the risks of trauma and falls also influence the fracture risk.

2.8.1 Clinical risk factor analysis

Clinical risk factor analysis will identify patients at high risk of low bone mass who will require BMD measurement to confirm the diagnosis of osteoporosis. It is equally important to assess the risk factors for falls as these may predispose the osteoporotic patient to fractures. Clinical risk factors include:

- Advanced age
- White, Asian or mixed race
- Premature menopause (before 45 years of age) and other causes of low sex hormone levels
- Strong family history of osteoporosis
- Low body weight
- Lifestyle factors such as poor nutrition, lack of exercise, alcohol or tobacco abuse
- Use of bone toxic drugs
- Presence of a medical disease known to affect bone
- Prevalent vertebral fracture

Risk factors for falls include mental impairment, gait and balance disorders, muscle weakness, visual impairment, the presence of environmental hazards and a past history of falls.

The predictive value of risk factors to identify high-risk individuals is generally regarded as unsatisfactory. Sensitivity is low and more than 50 % of patients without risk factors may develop osteoporosis. Risk factors for different types of osteoporosis (e.g. hip vs. spine) and different populations (age, race, gender) may differ greatly. However, advanced age, a maternal history of fractures, low body weight, smoking and a prevalent fracture are important predictors for future fractures (Kanis, 1994; Cummings, 1995).

2.8.2 Bone mineral density

Bone mineral density is the principal determinant of bone strength, accounting for 75 - 85 % of the variance of bone strength measured *in vitro*. Qualitative, structural properties of bone are said to account for the remaining 15 - 25 %, but cannot readily be assessed. The exponential increase in fracture incidence with diminishing bone mass has been firmly established, and when osteoporosis is suspected, BMD measurement is the single best diagnostic tool because it helps determine fracture risk and identifies patients who are candidates for intervention. During the last two decades there have been major advances in the technology for determining bone mass and a number of techniques have been developed. The dual energy X - ray absorptiometry (DEXA) is the gold standard for the measurement of BMD at the hip and spine (Kaufmann, 2002).

Conventional radiography

Until recently the only way to estimate the bone mass was to take regular radiographs of the skeleton and a grading system was developed for the diagnosis of osteoporosis based on the appearance of trabecular patterns of the femoral neck seen on radiographs, histological grades and clinical history of the patient, referred to as 'Singh Index'. Grading is from grade six (normal) to grade one (severe osteoporosis) (Singh M, 1970; Kaufman, 2002). However conventional radiographs are insensitive and bone loss is not recognized until around 30 - 40 % of bone density has been lost. Further, about 25 % of patients with osteopaenia on radiographs have a normal BMD. The main role of X - ray today is for the diagnosis of fractures secondary to osteoporosis (Kanis, 1994; Van Kuijk and Genant, 1995; Schnitzler 1996).

Single photon absorptiometry (SPA) was developed in the 1960's and determined bone density using a radioactive isotope. A single low energy photon beam from a radionuclide source of iodine¹²⁵ is passed through bone and soft tissue and the transmitted radiation measured using a sodium iodide scintillation detector. The amount of soft tissue the beam has to penetrate must be small and this technique is only useful for measuring BMD at peripheral sites, namely the forearm. The calculation of bone mineral in the path of the beam is expressed in grams of mineral per centimeter of bone (g/cm^2). SPA measurements are accurate, precise and have a low radiation dose. The disadvantages are that the test usually takes approximately 15 minutes, there is decay of the radioactive

source with time and BMD at the hip and spine cannot be measured (Johnston Jr. and Melton III, 1995)

Dual photon absorptiometry (DPA) is a direct extension of SPA. It utilizes a radionuclide gadolinium¹⁵³ as a source, and measures bone density by determining the absorption of two beams of photons at different energies. One energy peak is absorbed by soft tissue and the other by bone. The soft tissue component is then mathematically subtracted and the BMD determined. Using DPA, BMD at the spine and proximal femur could be measured for the first time. Results are usually expressed as grams of mineral per unit area scanned (g/cm^2) This technique is accurate for predicting fracture risk, but precision is poor due to the decay of the isotope, and the machine used, has limited usefulness in monitoring BMD changes over time (Johnston Jr. and Melton III 1995).

Computed Tomography (CT) imaging was developed in the early to mid 1970's. CT imaging is fast, patient friendly and has a distinctive facility to image a combination of soft tissue, bone, and blood vessels. CT scanners use a technique that is unique in that it provides for true three-dimensional imaging and reports BMD as true volume density measurements. However the disadvantages are a higher radiation dose and cost (Kanis, 1994).

Quantitative computed tomography (QCT) is an extension of CT and is the only noninvasive three-dimensional bone mass measurement technique available and has been used primarily to measure cancellous bone of the spine. Additionally, CT has the ability to isolate the area of interest from the surrounding

tissue. It can, therefore, localize an area in a vertebral body of only one trabecular bone leaving out elements most affected by degenerative changes and sclerosis (Genant, *et al*, 1988). With QCT, the result is a volumetric density (in milligrams per cubic centimeter) in contrast to the area density (in grams per square centimeter) used by other techniques (Faulkner, 2001). However, this technique has a high precision error (3 - 5 %) due to marrow fat and a higher radiation dose than that of dual-energy X - ray absorptiometry (DEXA), and is therefore not ideal for serial measurements (Kanis, 1994).

Radiographic absorptiometry (RA) uses a standard X - ray of the hand and the use of a small aluminum alloy reference wedge to calculate bone density. Sites of measurements are the middle phalangeal bones of the 2nd (index), 3rd (middle) and 4th (ring) fingers. The aluminum wedge is referenced to measure bone mass in every pixel inside the automatically detected contour. Then, one average bone mass is calculated per bone. The BMD is calculated in arbitrary units for the three bones in each exposure. BMD results are cross-checked between two exposures and the overall average BMD index is reported. Normative databases are used to calculate the T score (number of standard deviations (SD) above or below the young adult mean) and Z score (number of standard deviations the patient's bone density is above or below the values expected for the patient's age) (Johnston Jr. and Melton III, 1995; Radiographic Absorptiometry, 2002).

Single Energy X - Ray Absorptiometry (SXA) although accurate, precise and having a low radiation, is limited by its ability to measure BMD at peripheral sites only and not at the hip or spine (Johnston Jr. and Melton III, 1995).

Dual Energy X - Ray Absorptiometry (DEXA) is the most recent, accurate and least invasive technique and is currently the gold standard for measuring BMD at the hip and spine. It is similar to DPA, but the isotope source is replaced by an X - ray source. This obviates the problem of decay of the isotope sources, but more importantly, the greater photon flux permits scan times to be speeded up considerably without the loss of precision. Beam collimation is also tighter, because BMD is measured by means of a multiple detector fan beam, with higher spatial resolution as a result.

DEXA measures BMD at the lumbar spine, proximal femur, forearm and in many cases, total body, with a precision error of about 1 - 2 %. Accuracy is comparable with DPA, ranging from 4 - 8 % depending on the skeletal site, and some improvement relative to conventional DPA may result from the capability to scan the lumbar spine laterally (Soicher, 1991). The X - ray source emits two energy peaks ("dual energy"), one measures soft tissue density and the other measures both, soft tissue and bone density. The computer subtracts the soft tissue value from that of soft tissue plus bone, and reports the resultant bone density value on a reference curve (Schnitzler, 1996).

The scanning time for DEXA is shorter than for DPA (approximately 5 minutes at each site) and the radiation dose is less than 1 mR/hour at a distance

of 2 meters from the equipment, which is one tenth of a standard chest radiograph (35 mR = 0.35 mSV) (Council of the National Osteoporosis Foundation, 1996; Training manual for Hologic QDR[®] 4500). Furthermore, the DEXA instruments also appear to be easier to use with less operator interaction and have been utilized in prospective studies to ascertain subsequent fracture risk (Johnston Jr., 1995).

Quantitative ultrasound (QUS) assesses the bone mass by measuring velocity (e.g. speed of sound, SOS) attenuation (broadband ultrasound attenuation, BUA) and reflection. SOS measures (in m/sec) the time it takes for a sound wave to traverse the bone and is directly proportional to the bone mass. BUA assesses how much sound is mopped up by the micro-architecture of bone and it too increases with the solidity of bone. By combining SOS with BUA the quantitative ultrasound index (QUI) or stiffness can be derived and from this the estimated BMD can be calculated (Wuster *et al*, 1996; Ascott-Evans, 1999).

These variables appear to correlate with fracture risk and can be measured at skeletal sites with minimal soft tissue covering e.g. the heel but not at the hip or spine. The advantages are that there is no radiation and the equipment is less expensive and portable. In addition, QUS may also provide information on the quality of bone. The exact role for QUS in the diagnosis and follow up of osteoporosis is as yet not determined (Faulkner, 2001).

2.9 BIOCHEMICAL ASSESSMENT

Biochemical evaluation of a patient suspected to have osteoporosis is aimed at excluding other causes of osteopaenia, assessing bone turnover and excluding secondary causes of osteoporosis. Serum calcium and phosphate concentrations are usually normal in osteoporosis but indicated in all patients to exclude hyperparathyroidism or osteomalacia as a cause of low bone mass. Investigations to exclude a secondary cause for osteoporosis will depend on presence of clinical clues to secondary causes (ref to Table 2).

Biochemical markers of bone turnover

Increased bone turnover is an independent risk factor for fractures and biochemical markers of bone formation and resorption can assess the rate of bone turnover.

Markers of bone formation include bone specific alkaline phosphatase and osteocalcin which are proteins secreted by the osteoblasts (Risteli and Risteli, 1993; Kanis, 1994; Caillot-Augusseau *et al*, 1998).

Alkaline phosphatase (ALP) and Bone specific Alkaline Phosphatase (BSAP).

The alkaline phosphatases belong to the group of orthophosphoric acid monoester phosphohydrolases, which split phosphoric acid monoester

hydrolytically to liberate phosphate and the corresponding alcohol. Alkaline phosphatase is widely distributed throughout the tissues of the body, e.g. in liver, bones, small intestines, kidneys and placenta with over 95 % derived from the liver and bone. As an enzyme, ALP is involved in the transport of substances from the intracellular compartment across the membrane to extracellular region. In bone, ALP may be involved in the breakdown of pyrophosphate, a potent inhibitor of calcium phosphate deposition at the extracellular level (Risteli and Risteli, 1993).

Alkaline phosphatase has at least nine recognized isoenzymes all derived from the same gene locus with varying properties of heat stability, chemical inhibition and electrophoretic mobility. They include fast (pre-liver), liver, bone, placenta, Regan, Nagao, renal, intestinal, and prostatic antigen (PA). Each of these isoenzymes is relatively specific for the respective tissues with which they are associated (Bishop *et al*, 2000).

The BSAP predominates during childhood and adolescence, whereas the liver isoform predominates during adulthood. BSAP is produced by the osteoblasts and extremely high amounts are produced during the bone cycle's formation phase and are therefore an excellent indicator of bone formation activity. Numerous methods have been developed for the measurement of BSAP, however immunoassay is considered the method of choice because of better analytical sensitivity and reported precision (Woitge, 2001).

Osteocalcin, (OC) or Bone Gla Protein (BGP)

Osteocalcin or “bone γ -carboxyglutamic acid-containing protein” (BGP) is synthesized from osteoblasts during the matrix mineralization phase. It was first identified in 1975 in chicken bones and in 1976 in cattle bones. It is a relatively small noncollagen bone protein, produced by osteoblasts and its synthesis is controlled by 1, 25-dihydroxy-vitamin D3 (Stracke and Kuhnel, 1985). OC is incorporated into the bone matrix where it is the most abundant noncollagenous protein, accounting for 20 - 25 % of the total bone protein. During bone resorption OC is degraded, however up to 70 % also enters the circulation (Risteli and Risteli, 1993). In individuals with normal renal function OC is excreted in urine because of the low molecular weight. In a number of studies, serum OC concentration has been used as a biochemical indicator of osteoblast function (Epstein, 1988; Delmas, 1995).

Markers of bone resorption

Resorption markers reflect osteoclast activity and/or collagen degradation and are an important method of assessing the rate of bone turnover.

The bone matrix is made up of fibrils of type 1 collagen, which represents 90 % of the organic matrix of bone (Delmas, 1995). Each of the fibrils contains amino terminal and carboxyterminal ends. In type 1 collagen, these ends are each linked to a helical portion of an adjacent collagen molecule by pyridinium crosslinks. The amino- and carboxy - nonhelical ends are termed N - telopeptides

(NTx) and C - telopeptides (CTx), respectively. Degradation by osteoclasts during the resorption process releases NTx and CTx fragments of various sizes, still attached to helical portions of a nearby molecule by a pyridium crosslink, into the circulation for metabolism and excretion in urine. The liver and kidney continue to degrade the fragments that are finally broken down to the pyridiniums, pyridinoline (Pyr) and deoxypyridinoline (Dpd) crosslinks, which are excreted in the urine (Delmas, 1995). Approximately 60 % of the deoxy-pyridinoline crosslinks in urine are in the form of small peptides and 40 % as free amino acids. The concentrations of NTx, CTX, Pyr and deoxypyridinoline crosslinks in urine therefore reflect bone resorption and are of value in monitoring bone turnover and the response to anti - resorptive therapy (Hanson *et al*, 1992; Delmas 1995; Clemens *et al*, 1997).

CHAPTER THREE

3.0 BONE MASS CHANGES IN ATHLETES: REVIEW OF LITERATURE

3.1 INTRODUCTION

More and more women are realizing the health benefits of exercise and are finding their achievements in different sporting disciplines, fun and rewarding. Female athletes now compete in virtually all the major sporting arenas of the world and the most important factors determining their participation and success are social and historical influences. As recently as 1972 at the Olympic Games the 1500m became a women's event and the marathon only in 1984 (Fenton, 2000).

In South Africa at present, male athletes appear to dominate distance-running events, but with the gradual change in social attitudes, women of all ethnic groups are taking up the challenge and competing in distance running. The sport has gained tremendous popularity witnessing thousands of girls and women, of all ages running harder and longer than ever before. (Comrades Marathon Association; Alexander, 1976). To demonstrate this phenomenon the number of women who have entered the Comrades Marathon during the past 32 years, although officially only since 1975, has increased from 1 runner in 1966 to 745 in 1990 and up to 1621 in 2002. The numbers of women competitors in 2000 was as high as 4312, when the finishing time for the race was extended to 12 hours.

However, although physical activity can be beneficial to health and specifically bone health, numerous studies have shown that rigorous physical

activity is associated with a distinctive set of risk factors for female athletes. (Heath, 1985; Myburgh *et al*, 1990; Drinkwater, 1990; Hetland, 1993; Micklesfield *et al*, 1994; Lindique, 1996; Warren *et al*, 2001) The different features, relating to exercise-induced effects on the female athlete, important to this study, will be discussed separately.

3.2 BENEFITS OF EXERCISE ON BONE

To provide a structure that permits resistance against gravity and rigidity for locomotion, bone adapts to the mechanical demands placed on it. This was first recognised by Julius Wolff in 1892 and Wolff's law states that bone accommodates the loads imposed on it by altering its mass and distribution of mass. When loading occurs, bone mass is gained and when loading decreases bone mass is lost. The relationship between bone mass and mechanical loading is curvilinear. While the amount of bone loss with inactivity is substantial, the amount of bone gained with increased exercise is limited (Kanis, 1994).

The osteogenic effect of mechanical loading occurs as a result of the physical strain placed on bone. Types of strain that lead to reduced bone resorption and stimulation of bone formation include tension, compression and shear stresses (Madsen *et al*, 1997). This is supported by the increased numbers of osteoblasts observed at the periosteum in response to mechanical loading (Pead *et al*, 1988). The effect of exercise on bone mass may also be mediated via an increase in muscle mass (Frost, 2000) and certain local and systemic non-mechanical factors such as hormones, vitamins, nutrition, cytokines and drugs (Frost, 2000).

Physical activity is known to stimulate release of growth hormone and a correlation between growth hormone and muscle mass has been demonstrated in adult women (Kelly *et al*, 1990). In addition the administration of growth hormone to elderly men resulted in an increase in spinal bone mineral density (Rudman *et al*, 1990).

These mediators exact their effect on bone mineral density largely via an alteration in the hypothalamic-pituitary gonadal axis. Diets deficient in amino acids have been shown to alter neurotransmitter synthesis (Lytle *et al*, 1975) and this may affect the secretion of gonadotrophin releasing hormones. Nutritional deficiencies may also influence body composition and percentage body fat with subsequent amenorrhea resulting in increased bone resorption (Watkin *et al*, 1991).

The osteogenic effect of exercise has been shown to depend on the time of life that exposure to exercise occurs, the type and intensity, plus the anatomical site where the stress is applied (Wolman, 1990 and 1994).

Peak bone mass (PBM) is achieved in normal individuals throughout childhood and adolescence until the age of between 20 - 30 years. It involves a complex interplay of genetics, physical activity, nutritional intake and reproductive hormonal status (Neer, 1995), [As described in chapter 1]. Maximising the attainment of PBM is an important component of preventing osteoporosis and the risk of fracture in old age. To increase PBM exercise may be the most effective adaptable environmental factor. Moderate weight bearing exercise has been shown to

increase bone mineral density in young prepubertal boys (Bradney *et al*, 1998) and pre-menarchal girls (Morris *et al*, 1997).

In young adults several studies have demonstrated a non-linear increase in BMD with exercise. The average BMD increase is 1 - 5 % in the first year with a plateau thereafter. In a systematic review of the effect of exercise programs on bone mass in pre-menopausal and post-menopausal women positive effects were seen at the lumbar spine (Wallace and Cummings, 2000), however there were too few trials of the effect on the femur.

At menopause, with the loss of oestrogen, the effect of exercise is likely to be less marked. However, resistance training in recently postmenopausal women has been reported to maintain bone mass at the lumbar spine and femoral area (Pruitt *et al*, 1992). In contrast several studies have reported significant increases in BMD at the lumbar spine in older men and women undertaking resistance and endurance exercise. The gains of trabecular density especially at lumbar spine in older persons appear to exceed those seen in younger individuals and may be due to the relative inactivity prior to the exercise programs (Dalsky *et al*, 1988; Simkin *et al*, 1987).

Physical activity such as jogging, running or jumping, and resistance exercise such as lifting weights or using weight machines, strengthens muscles and bones. The resistance against gravitational forces is associated with an increase in bone density (Zerwekh *et al*, 1998; Burger, 1999). Swimming however, being a weight-supported activity, produces only limited effect on the skeleton

compared with other weight bearing activities (Wolman, 1994; Madsen *et al*, 1997). Several studies have shown the positive role of weight-bearing exercise on BMD in athletes at all sites compared to non-athletes (Risser *et al*, 1989; Chestnut III, 1993; Kirchner *et al*, 1994; Madsen *et al*, 1996; Vorster *et al*, 2001).

Weight-bearing exercise has been shown to exert a local effect on the skeleton at the site of maximum stress. This is demonstrated by the increase of bone density seen in professional tennis players, whose playing arm can be up to 30 % more dense than the non-playing arm, and runners, who compared with sedentary controls have a significant increase in the os calcaneus, femoral shaft, and spine, produced by the repetitive stress loading on the legs by the running action, especially in ultra-distance runners. Rowers who perform intensive upper body exercise have experienced even more considerable increases in spinal bone density (Wolman, 1994).

In contrast the lack of physical activity is associated with bone loss. Bone loss is a recognized complication of neurological disorders such as paraplegia, cerebrovascular accidents and spinal cord syndromes where as much as 40 % of their original bone mass may be lost within six months of immobilization (Kanis, 1994) and astronauts may lose up to 23 % of their bone mass after six months in space (New, 2001). However, when astronauts exercise against resistance on stationary bicycles while in orbit, BMD at weight-bearing sites is conserved, signifying the correlation between BMD, exercise and muscle strength (Frost, 1999).

3.3 ADVERSE AFFECTS OF EXERCISE

Female athletes participating in a variety of competitive sport, has become the norm rather than the exception for team sports such as soccer, softball, gymnastics and basketball (Wiggins *et al*, 1997). The pressure to excel and the athlete's constant pre-occupation to achieve or maintain unrealistic low body weight are potential risk factors for developing exercise related problems.

High intensity exercise has been associated with osteoporosis and fractures. This is thought to be primarily due to amenorrhoea and eating disorders and the term "female athlete triad" encompassing amenorrhoea, eating disorders and osteoporosis was proposed by the American College of Sports Medicine at a consensus conference in 1997 (American College of Sports Medicine, 1997).

While the negative effect of exercise on BMD is thought to be primarily due to amenorrhea and eating disorders, these in turn are inter-related and associated with a number of other factors such as the intensity of the training regimen, (Warren, 1980; Fournier *et al*, 1994) energy drain, (Warren, 1980; Slosman *et al*, 1992; Loucks, 2000) quality and quantity of diet, (Cummings *et al*, 1995; Garner *et al*, 1995) abnormal nutritional patterns, (Bonjour *et al*, 1996) and delayed menarche (Frisch *et al*, 1980; Noakes *et al*, 1988; Garner *et al*, 1995).

3.3.1 Exercise-induced amenorrhea

The normal menstrual cycle (eumenorrhea, defined as 10 menstrual cycles per year) is a complex interaction of the reproductive and endocrine systems and has three phases: the follicular phase is where the follicle matures, followed by the ovulatory phase in which the ovum is released, and the final luteal phase is that in which the endometrium prepares for implantation of a fertilized ovum. Menstruation ensues if implantation does not occur. The normal menstrual cycle starts with the pulsed release of gonadotropic-releasing hormone (GnRH) from the hypothalamus. This stimulates the release of follicle-stimulating hormone (FSH) from the pituitary. Follicle-stimulating hormone starts the follicular development in the ovary with the subsequent release of oestrogen, which in turn causes endometrial proliferation. At mid cycle lutenizing hormone (LH), released from the pituitary, allows the ovum to be released from the follicle and progesterone is produced. Progesterone changes the endometrium to a secretory pattern and if fertilization does not occur, oestrogen and progesterone concentration fall and the endometrium is shed (Van Wylsberghe *et al*, 1995).

Any disruption in this hypothalamic pituitary axis will result in oligomenorrhea or amenorrhea. Amenorrhea is subdivided into two categories. Primary amenorrhea is defined as the absence of menstruation by the age of 16 years and secondary amenorrhea the absence of menses for three or more consecutive menstrual cycles in women who had previously menstruated (Gidwani, 1999). Oligomenorrhea refers to three to six menstrual periods per year

that occur at intervals greater than every 36 days (Carlberg *et al*, 1982; Loucks, 1985; Drinkwater 1990; Pearce *et al*, 1996; Gremion *et al*, 2001).

Menstrual disturbance is the most recognizable clinical symptom of the “female athlete triad” and has been extensively studied. The prevalence of secondary amenorrhea in adult athletes ranges from 3.4 % to 66 % compared with 2 % to 5 % of women in the general population (Loucks, 1990). A number of factors influence the prevalence of exercise-induced amenorrhea and include reproductive maturity, body composition, nutrient intake and training regimen. Athletic amenorrhea may also be sport specific and appears to be higher in runners and ballet dancers than in swimmers and cyclists (Feicht *et al*, 1978; Frisch *et al*, 1980; Sanborn, 1982; Wolman *et al*, 1989; Watkin *et al*, 1991; Carbon, 1994)

Exercise-induced amenorrhea is attributed to the disruption of the hypothalamic pituitary axis (Warren and Perlroth, 2001) and occurs when the stress from athletic participation, whether psychological or physiological results in the cessation of normal menses (Harmon, 2002). Additionally women with low body weight and fat due to extensive exercise or eating disorders such as anorexia nervosa commonly develop hypothalamic amenorrhea.

Psychological stresses associated with competitive athletes have been associated with decreased levels of GnRH. Stress elevates cortisol and dopamine levels as well as endogenous opiates and other neurotransmitters with exercise leading to amenorrhea (Loucks, 1985; Harmon, 2002).

3.3.2 Nutrition and body composition

Nutrition has been also implicated in the cause of menstrual dysfunction in athletes and ballet dancers (Warren, 1983; Nelson *et al*, 1986). In 1980 Warren was the first to suggest that menstrual disorders in dancers were as a result of “energy drain’ unrelated to body fat. Diets deficient in certain amino acids have been shown to alter neurotransmitter synthesis and in amenorrheic athletes this alteration may disrupt gonadotropin–releasing hormone (GnRH) pulsatility (Loucks *et al*, 1985). It has been proposed that the cessation of reproductive function may be energy–conserving adaptation in response to inadequate diet (Warren, 1983; Loucks, 2000). A study by Meyerson *et al*, (1991) investigating resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners suggested that reduced resting metabolic rate found in the amenorrheic group was part of an adaptive syndrome to conserve and maintain stable weight in response to caloric demands of a high-level training programme that was not compensated by an increase in caloric intake.

Several studies have reported the relationship between amenorrhea and low kilojoule (kJ) intake. Nelson *et al* (1986), reported a significantly lower protein intake in 82 % of amenorrheic women runners compared to 35 % eumenorrheic women. However, in two South African studies on female runners no relationship between menstrual dysfunction and nutritional intake or BMI was reported. Watkin *et al* (1991) compared the dietary intake of macronutrients and micronutrients of 38 runners with menstrual disturbance and 38 runners without menstrual disturbance. The two groups were matched for age, BMI, menarche,

training distance and racing performance. There was no difference in the intake of macronutrients and micronutrients in the runners with and those without menstrual disturbance. However, 55% of the runners with menstrual disturbance had the menstrual disturbance before they had started running. Similarly, Vorster et al (2001) also reported no difference in nutrition between athletes and controls. However, there was a small number of subjects (9 athletes and 9 controls) and there was no difference in the BMD in the two groups and only 3 women (2 athletes and 1 non athlete).

It is well known that women with eating disorders such as anorexia nervosa and bulimia suffer from menstrual dysfunction and the practice of weight loss is common in women athletes. These conditions have been associated with amenorrhea and nutritional and psychological factors (Nattiv *et al*, 1994).

The relationship between body composition (the ratio of lean mass to fat body mass) and menstrual function has been established. Frisch and McArthur (1974) originally proposed that amenorrhea was associated with anorexia, under nutrition, a delay in menarche and adolescent growth spurt. In order for a young girl to begin menses a critical percentage of body fat (17 %) was required and a greater percentage (22 %) required for the maintenance of regular menstrual cycling. The attainment of a critical body weight and body fat lowers the metabolic rate providing a signal for the hypothalamus to become more sensitive to the changes in oestrogen and androgen levels, therefore amenorrhea would occur if body fat decreased to less than the critical percentage. With an increase in food intake and weight gain the menstrual cycle is usually resumed.

Female athletes with low fat and lean body mass tend to be amenorrheic or oligomenorrheic (Carlberg *et al*, 1983; Heath, 1985; Loucks, 1985; Vorster *et al*, 2001) and those with higher body fat levels eumenorrheic. Carlberg *et al*, (1983) studied 14 athletes with oligomenorrhea or amenorrhea and 28 athletes with normal menses and found an association between menstrual dysfunction and low body weight. Drinkwater *et al*, (1990) confirmed these findings in 1990 that suggested that body weight became more important as a predictor variable as the severity of menstrual dysfunction increased. In contrast, other investigators reported no differences in body fat content among eumenorrheic and amenorrheic athletes and suggested that the discrepancies were due to the differences in the measurement of body fat level and other concomitant factors such as changes in muscle mass (Hendrix and Lohman, 1981).

Additionally, a study by Marcus *et al* (1985) on long-distance runners found that women runners were able to sustain regular menses with body fat as low as 4 % emphasizing that body weight rather than body fat was an indicator of the risk of the developing amenorrhea particularly if weight loss was rapid.

Recent research on the role of leptin (described in chapter 1) has shown that circulating leptin concentrations are directly proportional to the amount of body fat or adipose tissue (Mantzoros, 1999) and correlate with body mass index in humans (Macut *et al*, 1998). Leptin, a hormone secreted by adipocytes, may be an important indicator of nutritional status and may also mediate reproductive function by responding to an altered metabolic state that is characteristic of athletes with exercise-induced hypothalamic amenorrhea. It has been suggested that altered

leptin secretion could be the underlying mechanism in the pathogenesis of hypothalamic amenorrhea (Laughlin, 1997). In addition to its metabolic function, leptin appears to affect neuroendocrine functions including the hypothalamic-pituitary-gonadal axis (Mantzoros, 1999). In athletic amenorrhea, low leptin concentration and neuroendocrine abnormalities are closely associated with strenuous exercise and low body fat. The body senses its own fat content through leptin and inhibits ovulation when certain nutritional reserves are not present (Mantzoros, 1999) a characteristic of exercise-associated amenorrhea. The leptin pathway may be also involved in thyroid metabolism in the presence of nutritional deficiencies and influence bone mass (Warren, 2002).

3.3.3 Training regimen

A number of independent parameters of athletic training determine a training regime and include distance, speed, frequency of exercise, duration of training session and level of performance. Surveys on runners have reported incidences of amenorrhea between 1 - 43 %, however these differences may be due to the sample methodological differences (Loucks, 1985). A study by Sanborn *et al*, (1982) on college athletes demonstrated no relationship between amenorrhea and training intensity in swimmers and cyclists, but in women runners 43 % of whom ran more than 112.6 km/wk there was a significant correlation between amenorrhea and training. In another study amenorrhea was more common in a subgroup running more than 80.4 km/wk (Lutter and Cushman, 1982). The relationship between a delay in menarche and intensity of training has

been well documented (Frisch, 1980; Warren *et al*, 1986) especially in young athletes engaged in sports that emphasize low body weight (Warren *et al*, 1986).

3.3.4 Osteoporosis

Gonadal hormones are important in normal bone metabolism. There is sufficient evidence to show that low oestrogen status in premenopausal women has an adverse effect on bone mineral density (BMD). Athletic amenorrhea results in increased bone resorption and a reduction in BMD, which can lead to the development of premature osteoporosis, stress fractures and other skeletal problems related to hypoestrogenism (Warren *et al*, 2000).

In 1984 Drinkwater *et al*, compared 14 amenorrheic to 14 eumenorrheic runners and found that in the two groups there was no difference in nutritional intake, percentage body fat, age at menarche, years of athletic participation and frequency and duration of training, however, the lumbar spine BMD was significantly lower in the amenorrheic group as was the oestradiol concentration and progesterone peak. In a longitudinal study Drinkwater *et al*, (1986) restudied nine of the original 14 amenorrheic athletes after 15.5 months and found that seven of the nine amenorrheic women had regained normal menstrual function and increased body weight. Additionally, their BMD had increased by 6.3 % but the two remaining amenorrheic runners the BMD had decreased by 3.4 %. There was no change in BMD in the eumenorrheic group.

In a cross-sectional, analytic study by Vorster *et al*, (2001) on the influence of exercise and diet on BMD in South African premenopausal athletes aged between 20 – 29 years found significantly lower forearm BMD in women with a history of amenorrhea. Fisher *et al*, (1986) studied 11 amenorrheic and 24 eumenorrheic women runners. Serum hormonal concentrations were measured and the amenorrheic women were found to have low oestrogen concentrations, lower LH pulse amplitudes and reduced BMD at the lumbar spine illustrating a positive correlation between BMD and oestradiol levels. Marcus *et al*, (1985) studied 17 elite women who were distance runners. Eleven of the athletes had secondary amenorrhea for a period of 1 to 7 years and lower BMD of the lumbar spine. Six women had no menstrual dysfunction. Exercise related fractures are more common in amenorrheic runners with reduced bone mass.

Micklesfield *et al*, (1995) conducted a study on 25 premenopausal ultramarathon runners aged between 29 – 39 years to determine the relationship of a history of menstrual disturbances to BMD. Fifteen of the athletes had normal menses compared to 10 who had a history of oligomenorrhea or amenorrhea. Lower BMD values were reported in the vertebral spine with no difference in the femur. The menstrual history index (MHI) was estimated, (MHI is the number of periods per year since the age of 13) and was higher in the group of normal menstruating women runners, which indicated a correlation between lumbar BMD and MHI. However, in a later study by Mickelsfield *et al*, (1998) investigating changes in BMD in premenopausal marathon runner with a history of menstrual irregularities found that restoration of lumbar spine BMD with the resumption of menses in women with a history of menstrual disturbances was slow and did not

return to normal adult bone mass (Micklesfield *et al*, 1998) concluding that a menstrual history was more important than current menstrual status for the prediction of low bone mass.

In another South African study, Myburgh *et al*, (1993) compared the BMD values of 12 amenorrheic to nine eumenorrheic women athletes at the axial and appendicular sites and concluded that in amenorrheic athletes a low BMD was present at other skeletal regions including appendicular weight-bearing bones. This study was confirmed by the findings of Rencken *et al*, (1996) which showed significantly lower BMD in amenorrheic athletes at multiple sites including those subjected to impact loading compared to a control group.

Hypoestrogenic amenorrhea in athletes may predispose to osteopenia and osteoporosis and an increase of the risk of scoliosis and fracture (Drinkwater *et al*, 1984; Marcus *et al*, 1985; Warren *et al*, 1986). Decreased BMD constitutes the final element of the “Female Athlete Triad” i.e. eating disorders, amenorrhea and osteoporosis. In a case-control study by Myburgh *et al*, (1990) on 25 athletes, nineteen of who had confirmed stress fractures determined that those athletes with injuries from overuse of bone weakened by osteopenia had a lower bone density than did the control group. Marcus *et al*, also found a significantly higher incidence of stress fractures in a group of elite amenorrheic runners than their eumenorrheic counterparts (Marcus *et al*, 1985). An increase in incidence of stress fracture in ballet dancers has been associated with older age of menarche (Warren *et al*, 1986).

Summary

Normal bone metabolism is dependant on a complex interaction between genetic, hormonal, mechanical and nutritional factors. Bone mass increases from childhood in a linear fashion until PBM is achieved. Thereafter bone mass is maintained until the menopause in women, when there is a rapid bone loss due to increased bone resorption with the loss of oestrogen. In males, where there is no abrupt andropause, a slower rate of bone loss occurs with age. This is most likely due to decreased formation. The bone mass in normal adults depends on the PBM and the amount of bone loss.

Mechanical stimulation is an important stimulus for bone formation. However, this is influenced by other important factors such as nutrition and gonadal status and the level of physical activity. Moderate physical activity is an important modifiable factor for attaining PBM and has been shown to increase bone mass in pre-pubertal boys and pre-menarchal girls. In early postmenopausal women moderate physical activity maintains bone mass and prevents bone loss. Physical activity in the elderly is also associated with bone gain.

Vigorous physical activity however can be detrimental to bone health and may result in the “female athlete triad” of amenorrhoea, eating disorders and osteoporosis. These factors are inter-related. The intensity and type of physical activity is also important. High performance athletes have been reported to develop exercise-induced amenorrhoea that may be due to a disruption in the hypothalamic-pituitary axis, alteration in body composition, nutritional and

psychological factors. Several studies have reported the association of amenorrhoea and lower BMD measurements in female athletes. A lower BMD has been reported specifically at the appendicular sites. As a consequence of the lower BMD, female athletes are at higher risk of overuse injuries and stress fractures. Studies on the association between body composition and BMD and menstrual disturbances have shown conflicting results.

Several studies have reported an association between amenorrhoea and low BMD in South African female athletes (Watkin et al 1991; Myburgh et al 1992, Micklesfield 1995; Micklesfield 1998). However, these were White female athletes and no data exist on the possible influence of different genetic backgrounds and cultural history in South African female athletes. There are also no data on the markers of bone turnover. Further these women had participated in the Two Oceans marathon, which is run over a distance of 56 km.

The Comrades Marathon is a longer event run over a distance of approximately 90 km, which has gained popularity over the years. This present study is the first to investigate effects of regular long distance running on bone health, including markers of bone turnover and body composition in an ethnically diverse cohort of women athletes participating in the Comrades marathon and comparing them to a sedentary control group.

Hypothesis

Female ultra-distance runners have a higher incidence of osteopenia that is related to menstrual disturbances and change in body composition, than sedentary females.

Aims of the study

1. To compare the BMD in ultra-distance runners and healthy sedentary volunteers
2. To compare body composition in ultra-distance runners and healthy sedentary volunteers.
3. To compare the concentrations of gonadal hormones of ultra-distance runners and healthy sedentary volunteers.
4. To compare the markers of bone turnover of ultra-distance runners and healthy sedentary non-obese volunteers.
5. To determine ethnic differences in BMD in ultra-distance runners.
6. To correlate the presence of a history of stress fractures with BMD, body composition, gonadal status and biochemical markers of bone formation and resorption.
7. To correlate the percentage body fat and BMD in female ultradistance runners and controls.

CHAPTER FOUR

4.0 METHODOLOGY

4.1 ETHICAL APPROVAL

Ethical approval for the study was obtained from the ethics committee of the Nelson R Mandela School of Medicine.

Informed consent was obtained from all subjects and controls (Appendix 1)

4.2 SUBJECT SELECTION AND RECRUITMENT

Premenopausal, South African women runners of different ethnic groups and body mass, between the ages of 30 – 50 years and who were in training for an ultra-distance event or events, were recruited for the study. Ultra-distance was defined, as a running event with distances including or greater than 50 kilometres. The two events of interest were the Two Oceans Marathon (56 km) and or Comrades Marathon (90 km). Runners participating in qualifying races, predominantly in KwaZulu Natal, were invited to participate and requests for volunteers were made in newspaper advertisements and radio station broadcasts.

During the months June 1997 to July 1998, subjects reported to the X - ray Department at King Edward VIII Hospital in Durban for the measurement of BMD by Dual Energy X-ray Absorptiometry (DEXA) using the Hologic QDR 4500A densitometer. Lumbar spine, hip and whole body mineral density, muscle mass and percentage body fat were measured.

Venous blood samples were drawn and urine samples collected at the time of DEXA scanning, for hormonal parameters and markers of bone metabolism.

Inclusion criteria

- (i) Women who were currently involved in training for an ultra distance running event.
- (ii) Aged between 30 – 50 years
- (iii) Not premenopausal or pregnant.
- (iv) Women on oral contraceptives were included in the subject group but excluded from portions of the analyses where appropriate.

For the control group, forty - seven (47) premenopausal sedentary healthy volunteers were included in the study. An attempt was made to find women to match the subject group. However, matching success was poor with regards to age, weight and ethnicity.

Exclusion criteria

Knowledge of being post-menopausal or pregnant or suspecting they might be pregnant.

For the control group, currently participating in regular exercise, currently menopausal or postmenopausal, and experiencing or having had any serious menstrual irregularities.

4.3 COLLECTION AND STORAGE OF BLOOD AND URINE SAMPLES

4.3.1 Blood

Whole venous blood (20 ml) was obtained by venipuncture from runners and control subjects. For the measurement of gonadal hormones, blood was drawn into vacutest tubes with no anticoagulant and haemolysis was avoided. The blood samples were allowed to clot, centrifuged and the serum separated into appropriately labelled tubes then stored immediately at -20°C for analysis at a later date.

In addition venous blood samples were drawn for estimation of bone markers. These samples were kept on ice until they were centrifuged and separate into aliquots, and then frozen at -20°C until subsequent analyses could be performed.

4.3.2 Urine

Fasting urine samples were taken between 2 - 3 hours after the first voiding and collected into screw - cap, leak-free urine containers. Labelled aliquots were stored at -20°C until analysis of urinary creatinine and bone marker measurements.

4.4 ASSESSMENT OF SUBJECTS

4.4.1 Questionnaire

All women entered into the study were invited to complete a questionnaire (Appendix 2). The questionnaire included demographic details, record keeping of the average monthly training distance, the number of years running, medical history of stress fractures and shin splints, family history of osteoporosis, smoking history, dietary calcium intake, calcium and other mineral supplementation and medication history.

4.4.2 Menstrual index

A detailed menstrual history was recorded for all subjects to calculate the modified menstrual history index (MHI). This was based on the calculation described by Grimston *et al* in 1990 and later modified by Micklesfield LK *et al* in 1995.

MHI Calculation:

$$\text{MHI} = (11.5 * R + 7 * O + 1.5 * A) / (C - 13)$$

where:

R = number of years of regular menstrual cycles per year, and where regular is equivalent to 11 – 12 periods per year with an average of 11.5 periods/year;

O = number of years of oligomenorrhea, which was defined as between 4 – 9 menstrual periods per year with an average of 7 periods / year;

A = number of years of amenorrhea which was defined as 0 – 3 menstrual periods per year and assuming an average of 1.5 periods/year;

C = current age;

13 = average age at which menarche begins.

Using the MHI, subjects were classified into three categories; eumenorrhea (10 – 12 periods/year), oligomenorrhea (4 – 10 periods/year) and amenorrhea (0 – 3 periods/year).

Body Mass Index (BMI) was calculated using the following equation:

$$\text{BMI} = \text{Mass} / \text{height}^2 \text{ (kg/m}^2\text{)}$$

4.5 BONE MINERAL MEASUREMENT

4.5.1 Instrument (QDR 4500)

Bone densitometry scans were performed in the Department of Radiography, King Edward the VIII Hospital, using a Hologic QDR® 4500, dual-energy X - ray bone densitometer (Hologic QDR Series Inc.Waltham, MA). This instrument provided the acquisition and analysis of scans of the AP lumbar spine, supine lateral spine, hip, forearm, and whole body. Scans for the study were made of the AP lumbar spine (LS) the proximal femur and whole body.

Dual Energy X - ray Absorptiometry (DEXA) is the preferred method used to measure bone density. DEXA measures the transmission of X - rays of two different photon energies through the body, allowing for measurement of bone and soft tissue. The X - ray photons are produced by an X - ray tube consisting of a cathode (negatively charged) and an anode (positively charged), which is encased in a vacuum tube and uses a high voltage source. Before reaching the patient, the X - ray beam is collimated into a narrow pencil-beam or fan-beam. Collimation is used to keep the scattered electrons from reaching the detector. The beam passes through the patient and is selectively attenuated by the patient's bone and soft tissue (QDR Series user's guide for bone densitometry supplied by Hologic, Inc. (2000).

After the X - ray beam leaves the patient, it passes through the X - ray detector where the intensity of transmitted radiation is recorded. The X - ray tube, collimator and detector are aligned and mechanically linked using a motorized scanner arm.

Using the two different X - ray energies allows a dual X - ray absorptiometry device to record attenuation profiles at two different photon energies allowing two types of tissue to be distinguished: bone (hydroxyapatite) and soft-tissue (everything else). Integral bone mass in the path of an X - ray beam divided by projected area of bone (all pixels recognized as bone by edge-detection algorithm) results in BMD, which is reported in g/cm^2 .

4.5.2 Scan sites

The following sites were scanned: AP lumbar spine, proximal femur and whole body. At all sites, the average BMDs were measured in grams per centimetre squared (g/cm^2) and both T and Z scores reported. The Z score is a measure of the difference between the patient's BMD and that of healthy women of the same age. The T score is a measure of the difference between the patient's BMD and that of a young adult population. The Z and T scores were calculated using the National Health And Nutrition Examination Survey (NHANES) reference data provided by Hologic.

AP Lumbar spine

The lumbar spine, consists of the mid to lower spine, the sacrum and coccyx. Bone loss due to aging, osteopenia or osteoporosis is often observed in the lumbar area. The AP lumbar spine is the most widely used anatomical site for the evaluation of osteoporosis. The vertebral bodies, L1 through L4, contain approximately 40 % cortical and 60 % trabecular bone. The high amount of trabecular bone and the relative ease of reproducibility of positioning of the spine provided an important source for baseline bone density information (Figure 3).

Proximal femur

The femur is the skeletal site where the most serious consequences of osteoporosis (fractures) have occurred. BMD results for the proximal femur (hip) scans, were reported for five different anatomical areas (Figure 4) (QDR Series user's guide for bone densitometry supplied by Hologic, Inc. (2000).

- **Total hip**, which is the most reproducible measurement of the hip and is the clinical measurement of the hip BMD.
- **Femoral Neck** region contains a large portion of trabecular bone.
- **Trochanter** is the triangular region whose boundaries are the lateral edge of the femur and the inferior edge of the neck box (seen on scan) and the solid line where the edge of the femur changes curvature below the trochanter.
- **Inter-trochanteric** region is below the femoral neck.
- **Ward's Triangle** is located automatically by the system and places a small box approximately 1 cm x 1 cm at the area of minimum density in the femoral neck region.

Whole Body

The Hologic QDR 4500 instrument allows regional and global measurement of the Whole Body. The QDR 4500 densitometer is able to directly measure fat and skeletal mass. The anatomical areas analysed included the head, the left and

right arm, the left and right rib cage, the thoracic spine, the lumbar spine and the left and right leg. The sub-regions when analysed included all of the areas except the head (QDR Series user's guide for bone densitometry supplied by Hologic, Inc. (2000).

4.5.3 Subject preparation for examination

As all subjects and controls entered into the study were subjected to a number of routine questions prior to scanning, to establish any knowledge of known pregnancy or internal objects that could interfere with the scan. Prior to the scan, volunteers were instructed to remove their clothing and change into an examination gown, and to remove all metal objects such as jewellery. The patient's height, and weight and their date of birth were recorded. The weight limit of the scanner table is approximately 120 kilograms.

4.5.4 Positioning for scanning

Lumbar spine position

For the lumbar spine examination, the subjects were placed supine on the table with their head at the head end of the table presuming that the person scanning is facing the table. The subject's legs were elevated onto a 'Knee Positioner' that was supplied by Hologic. This is a large square pillow that allows positioning of the femurs so that they are as close to 90° to the spine as possible in order to flatten the back. The knee positioner helped to reduce the lordotic curve

of the subject's spine, by keeping the femurs vertically aligned. A pillow was placed under the subject's head and their arms were placed on either side of their body or over their heads.



Figure 3: AP Lumbar spine position. The subject's legs elevated onto a “Knee Positioner” supplied by Hologic.

The angle of the patient's legs from femur to knee was approximately 45° (i.e. hips fixed at 45°), which is recommended. The starting point for scanning is the middle of L5 indicated on the monitor. If a small amount of the pelvis was not seen, the starting point was readjusted. The area of concern or region of interest (ROI) was L1 – L4. The average bone mineral densities (g/cm^2) were determined for lumbar vertebrae 1 through 4.

Proximal hip position

For the hip scan, the legs were straightened using the Perspex immobiliser, called the 'Hip Positioner'. This was placed between the feet. The foot and leg of the side to be examined were slightly rotated inwards, with the foot against the positioner. The foot was secured against the positioner using a strap. The greater trochanter could then be felt, and the beam centred approximately 1 cm above the trochanter before scanning. The subject's head was placed on a pillow and the arms folded across the chest.



Figure 4: Proximal hip position using perspex immobiliser, called the 'Hip Positioner'

Whole Body position

For the Whole Body examination the subject's body was placed so that the head was aligned with a centreline on the table and the feet, were placed on either side of the centreline at the other end of the table. The body, including the feet was positioned so that the entire body was within the scan limit borderline. The subject's arms were placed at their sides, with palms down, separated from the thighs, but within the scan limit border. The subjects lay flat on the table without a pillow under the head. The subject's feet were rotated inward until the toes touched, and a tape was placed around the feet to maintain the rotation.

4.5.5 Quality Control

At the commencement of each scan session, a daily Quality Control (QC) was performed using a lumbar spine bone phantom called the 'Spine Phantom' (Figure 5) supplied by Hologic. The phantom was of known mineral content, which ensured that the systems software was performing properly prior to the scanning of subjects. The phantom was designed to produce scan data that resembles patient data. A 'Step Phantom' was used for QC only when Whole Body scans were performed for the calibration of body composition.

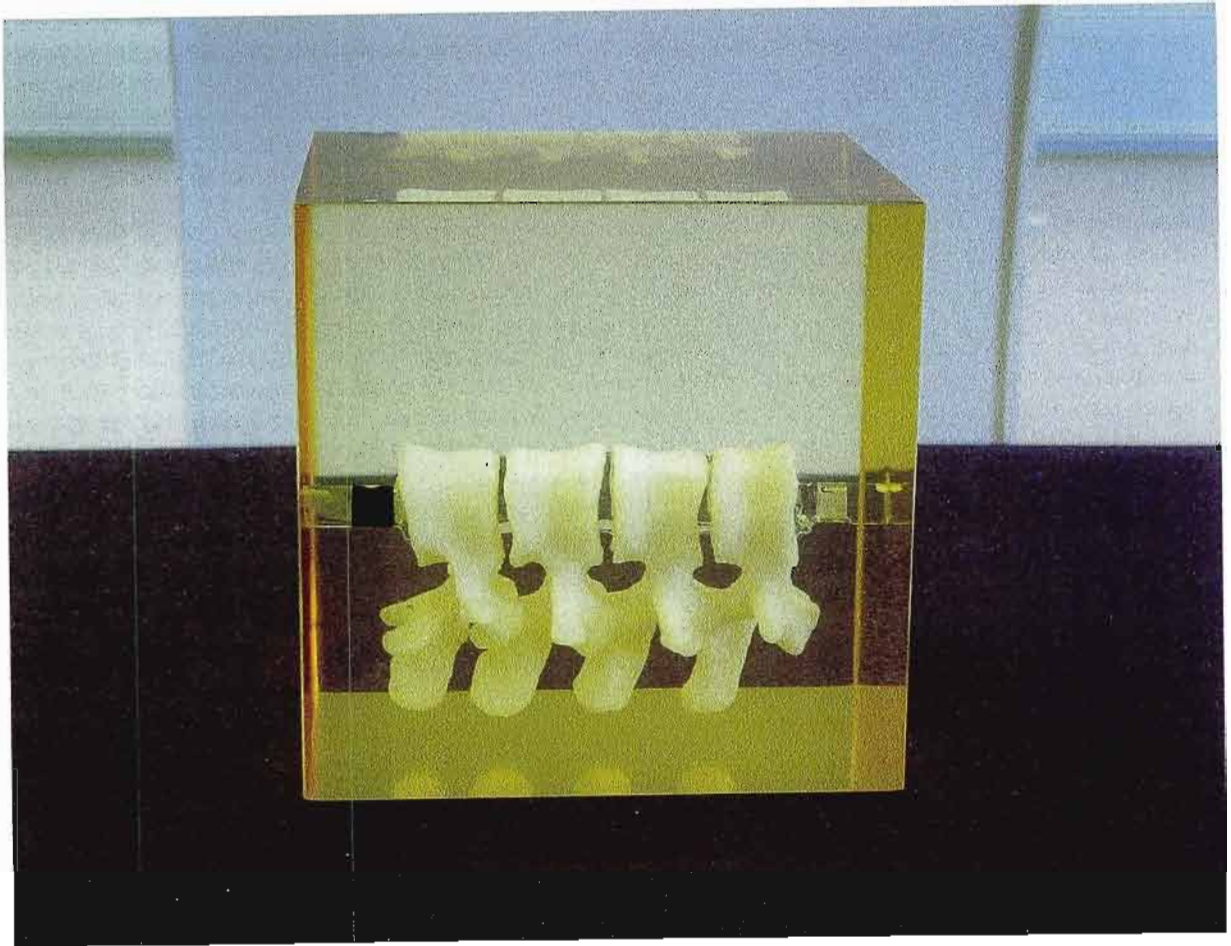


Figure 5: Spine Phantom supplied by Hologic

4.5.6 Reproducibility

A number of subjects were subjected to re-scanning of all sites in order to demonstrate reproducibility of results.

4.5.7 Measurement of percentage fat.

The DEXA, which is based on a three-compartment model, divides the body into total body mineral, fat-free soft tissue mass, and fat tissue mass. The technique is based on the assumption that bone mineral content is directly proportional to the amount of photon energy absorbed by bone being studied.

Analysis of fat mass, lean mass, and percentage fat mass is reported for the entire body and head, arms, trunk, pelvis and legs.

4.6 LABORATORY ASSESSMENT

4.6.1 Blood

A variety of hormone concentrations were measured on the serum samples, namely lutenizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol and progesterone using the enzyme-immunological test for the quantitative determination of the hormones in vitro. Boehringer Mannheim Immunodiagnostics supplied the kits for manual determinations.

Quality control was ensured with the use of Precinorm[®] IM and Precipath[®] IM. The standards provided by each kit were calibrated against the WHO standard 2nd IRP (78/549).

The instrument used was the ES 600/700 a fully automated sample-selective multi-batch analyser installed by Boehringer Mannheim Diagnostics. The systems were designed to use the Boehringer Mannheim ELIZA (enzyme linked immunosorbent assay) coated-tube technology.

4.6.2 Urine

Aliquots of urine, frozen at -20° C, were thawed in a waterbath and spun before use. Routine tests on urine included urinary calcium, phosphorus, and creatinine. Specific bone markers excreted in urine such as deoxypyridinoline cross-links were measured by immunoassay.

4.7 BONE MARKERS

Enzyme – linked immunosorbant assays (ELISA) are used to detect antibody or antigen levels. The antigen-specific antibody is linked to a suitable enzyme, and a substrate for that enzyme is added. Colour formed by cleavage of the substrate is read spectrophotometrically, and the amount of colour measured is directly proportional to the concentration of antigen being measured.

The ELISA may be performed using a direct or competitive method. In the direct ELISA the patient's serum is added to the solid phase containing the specific antibody and incubated for a specific time, at a specific temperature. The direct ELISA produces more colour as the unknown antigen concentration increases in the sample.

Conversely with low concentrations of antigen smaller amounts of conjugate will bind resulting in less substrate cleaved and less colour produced and lower spectrophotometer readings. The antigen is therefore directly proportional to the colour produced.

Competitive ELISA's differ in that the antigen in the sample competes with the conjugate for the active sites on the bound antibody. The sample and conjugate are added at the same time to the solid phase. If the antigen concentration is very high there will be little conjugate bound to the immobilized antibody. Therefore there will be little colour development as there will not be enough enzyme to cleave the substrate. Hence the amount of unknown antigen in the sample is inversely proportional to the colour produced.

In this study, direct ELISA was used for the measurement of BSAP and competitive ELISA's used for deoxypyridinoline crosslinks and osteocalcin.

4.7.1 Bone-specific alkaline phosphatase (BSAP)

A commercial kit, Alkphase – B™ kit (Metra Biosystems, Inc., Mountain View, CA 94043, USA) was used to measure bone-specific alkaline phosphatase.

4.7.1.1 Principle

Alkphase–B is an immunoassay in a microtitre plate system utilizing a monoclonal anti-bone alkaline phosphatase (BAP) antibody coated on a plate to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a para - nitrophenyl phosphatase substrate (pNPP).

4.7.1.2 Equipment

A microtitre plate reader capable of reading optical density (OD) readings > 2.0, and installed with quantitation software with quadratic calibration curve fitting. (Biochromatic Labsystem Multiscan)

4.7.1.3 Materials

Alkphase – B kit - Metra Biosystems Cat. No.8012 (S.A. Scientific)

4.7.1.4 Reagents

A set of five BAP standards was included in the kit with concentrations of: 0, 2, 20, 50, 80 and 140 BAP U/L BAP. The standards were purified from osteosarcoma SAOS - 2 cells in a buffered solution containing magnesium chloride, zinc sulphate, surfactant, carrier protein, blue dye, and sodium azide (0.05 %) as a preservative.

Purified murine monoclonal anti-BAP IgG antibody was adsorbed onto stripwells. The quality controls included low and high values using BAP purified from osteosarcoma SAOS - 2 cells in a buffered solution, the two values were intended to verify the validity of the curve and sample results. Each laboratory is required to establish its own parameters for acceptable assay limits.

The 10 X wash buffer was a non-ionic detergent buffered solution and the assay buffer a solution containing magnesium chloride, zinc sulphate, and the substrate buffer, a 2 – amino – 2 – methyl – 1 propanol solution with ethylenediaminetetraacetic acid (EDTA), magnesium chloride and zinc sulphate. The substrate tablets contained 20 mg each of P – nitrophenyl phosphate. The supplied stop solution was a 1N NaOH solution.

4.7.1.5 Method

1. All reagents and samples were brought to room temperature (20 – 28° C) prior to use. The kit was removed from the fridge at least 2 hours before use (or overnight).
2. The amount of each reagent required was determined for the number of anti - BAP coated strips to be used and reconstituted with appropriate diluents.
3. The required amount of 1 X wash buffer was prepared by diluting 10 X wash buffer 1:10 with de-ionized water and stored at room temperature.
The wash buffer was used within 24 hours of preparation.
4. The desired number of anti- BAP coated strips was placed into the stripwell frame supplied and 125 µL of assay buffer was added to each well.
6. Next, 20 µL of standard, controls and samples were dispensed into the wells of the plate, in duplicate.
7. Samples were incubated at room temperature for 3 hours.
8. Working substrate was prepared 1hour before use by placing one substrate tablet into each bottle required and mixed by vigorous shaking.

9. The plate it was inverted manually, then using a wash bottle with the 1 X wash buffer the plate was washed 3 times. To ensure that the plate was completely dry it was blotted with paper towel after the last wash.
10. 150 μ L of working substrate was added to each well.
11. Further incubation was required for 30 minutes at room temperature.
12. Following incubation, 100 μ L of stop solution was added to each well.
13. The plates were read at an absorption setting of 405 nm within 15 minutes, on a microtitre plate reader, and the absorbance's were quantitated using the quadratic calibration software.

4.7.1.6 Standard Curve and Calibration

The standards mentioned above (BAP purified from osteosarcoma SAOS - 2 cells) were used in serial dilutions to calculate the concentration of the samples. Dilutions were expressed in U/L as follows: 0, 2, 20, 50, 80, 140. A graph was plotted using the absorbance and concentration values of these dilutions. The curve was a linear log fit and the correlation coefficient was calculated for each graph with the values of 0.99 for each plot. The sample results using computer software were reported from the graph of absorbance against concentration.

4.7.2 Osteocalcin

A commercial kit, the NovoCalcin™ kit (Metra Biosystems, Inc., Mountain View, CA 94043, USA) was used for osteocalcin measurement.

4.7.2.1 Principle

The NovoCalcin assay is a competitive, enzyme linked immunosorbent assay (ELISA). The assay uses osteocalcin coated strip wells, a monoclonal anti-osteocalcin antibody, and an anti-mouse alkaline phosphatase conjugate and a para- nitrophenyl phosphatase substrate (pNPP) to quantify osteocalcin in serum.

4.7.2.2 Equipment

Biochromatic Labysystem Multiscan plate reader with appropriate software for the calibration of a 4 - parameter calibration curve fitting to analyse osteocalcin assay results.

4.7.2.3 Materials

NovoCalcin™ Kit – Metra Biosystems Cat. No. 8002 (SA Scientific).

4.7.2.4 Reagents and preparation

A set of five standards were included in the kit, derived from human osteocalcin with the values: 0, 2, 4, 8, 16, 32 ng/mL.

Within the first hour of the test assay, the standards and controls were reconstituted with 10 x wash buffer and allowed to stand for at least 15 minutes for the pellet to completely dissolve.

The osteocalcin coated strips, was coated (adsorbed) with human osteocalcin, and the standards supplied, were prepared from human osteocalcin extracted from bone. (Reference:NovoCalcin™ packet insert)

The low (0.71 – 1.19 ng/mL) and high (2.62 – 4.43 ng/mL) controls supplied, had values to verify the validity of the curve and sample results.

Using deionized water the wash buffer was prepared by diluting the 10 X wash buffer 1:10. The wash buffer was kept at room temp (20 - 25° C) and was used within 24 hours of preparation. Enzyme conjugate was prepared within 2 hours of use as per table insert. Each required vile of enzyme conjugate was reconstituted with 1 X wash buffer to allow the pellet to completely dissolve.

Substrate buffer supplied, contained 20 mg each of p – nitrophenyl phosphate (p-NPP). This was brought to room temperature prior to the assay.

The monoclonal anti- osteocalcin antibody used was raised against bovine osteocalcin. Because of the high homology of bovine osteocalcin with human osteocalcin, there is a 100 % cross reactivity in the assay between these two species. The antibody is believed to be a “conformationally dependent” antibody and thus will recognize only intact (*de nova*) osteocalcin and not fragments from resorbed bone tissue.

The supplied enzyme conjugate (lyophilised) was goat anti-mouse IgG-alkaline phosphatase. A stop solution of 1 N Na OH was supplied.

4.7.2.5 Method

1. The serum, which had been stored at -20°C was thawed, mixed and used immediately as osteocalcin is sensitive to proteolysis.
2. The kit (NovoCalcinTM) was removed from the refrigerator at least 2 before proceeding with the test.
3. The amount of each reagent was determined for the number of strip-wells as per the table insert.
4. The required amount of 1 X wash buffer was prepared by diluting 10 X wash buffer with deionized water and stored at room temperature and discarded if not used within 24 hours of preparation.
5. The control and standards were reconstituted with 0.5 mL of 1 X wash buffer.
6. To each enzyme conjugate vial required, 10 mL of 1 X wash buffer was added to reconstitute the content.
7. The desired number of osteocalcin strips was placed into the stripwell frame and 25 μL of standards, controls and samples dispensed into the microtitre plate, in duplicate.
8. To each of the above 125 μL of the anti-osteocalcin was added, then incubated for 2 hours at room temperature, in the dark and during this period the substrate buffer was made up one hour before use.
9. Following incubation, the microtitre plate was inverted manually, and then using the wash bottle with 1 X wash buffer the plate was washed 3 times. A paper towel was used to blot the plate dry after last wash.

10. 150 µL of enzyme conjugate was added to each well and incubated at room temperature for 60 minutes in the dark.
11. During incubation, the working substrate reagent was prepared by putting one substrate tablet into each substrate bottle required and allowed between 30 - 60 minutes to dissolve, before mixing thoroughly.
12. After incubation the plate was emptied by manually inverting it, followed by 3 gentle washes using the 1 X wash buffer. For the last wash the plate was blotted dry using paper towel.
13. 150 µL of working substrate was added to each well.
14. This was followed by a further incubation at room temperature for 35 minutes. The incubation was carried out on the bench top with no light precautions.
15. 50 µL of 3N NaOH was added to each well to stop the reaction.
16. Within 30 minutes, the plates were read at an absorption setting of 405 nm on a microtitre plate reader, and the absorbances were quantitated using the quadratic calibration software.

4.7.2.6 Standard curve and Calculation

The standards mentioned above were used in serial dilutions to calculate the concentration of the samples and controls. Following the entry of the tests, standards and control data using quantitation software with a 4 - parameter calibration curve fitting equation osteocalcin levels were analysed.

Equation: $y = (A - D)/(1 + (x/C)^B) + D$

tablet, containing p - nitrophenyl phosphate (20 mg each) and allowing 30 - 60 minutes for the tablets to dissolve, before vigorously shaking to mix completely.

The stop solution, 1N NaOH was supplied.

4.7.3.5 Method

1. Aliquots of the same urines as that for Dpd assessment were sent to the routine biochemistry laboratory for urinary creatinine levels.
2. Urines for assay were diluted 1:10, in duplicate, using 50 μ L urine and 450 μ L assay buffer, which had already been made up.
3. The standards were diluted as for the samples and ready for use after mixing with a vortex.
4. The substrate buffer was allowed to come to room temperature.
5. The amount of each reagent was determined for the number of anti- Dpd coated strips required, as per the table.
6. The required amount of wash buffer was prepared by diluting the 10 X wash buffer concentrate 1:10 with deionized water and stored at room temperature and used within 24 hours.
7. The required number of vials of enzyme conjugate was reconstituted using 7 ml of assay buffer and stored immediately in the refrigerator until use.
8. The desired number of strips was placed in the stripwell frame.
9. 50 μ L of diluted standards, controls and urines were added to each well of the plate in duplicate as per the template and all precautions were taken to avoid prolonged exposure to light.

10. 100 μ L of the cold (refrigerated), reconstituted enzyme conjugate was added to each well, using the multichannel pipette and the samples covered with a plate and incubated in the refrigerator (2 – 8° C) for 2 hours, in the dark for stability.
11. During the incubation period, the working substrate solution was prepared.
12. Following incubation, the stripwells were manually inverted/emptied over the sink and washed 3 times with cold 1 X wash buffer, ensuring that the wash step took no longer than 2 minutes.
13. After the last wash, the plate was dried with a paper towel, and then it was left for a further 5 – 10 minutes to drain on the paper towel.
14. 150 μ L of the substrate solution was added to each well and incubated at room temperature for a further 60 minutes at room temperature but in the dark.
15. 100 μ L of stop solution was added to each well to stop the reaction.
16. Within 15 minutes the absorbance were read at 405 nm on a microtitre plate reader, and the absorbances were quantitated using the quadratic calibration curve software.

4.7.3.6 Standard Curve and Calibration

The set of six standards (Dpd purified from bovine bone) were used in serial dilutions to calculate the concentrations of samples and controls. Dilutions were expressed in nM as follows: 0, 3, 10, 30, 100, and 300 nM Dpd. Quantitation software with a 4-parameter calibration curve fitting equation was used to analyse the Pyrilinks - D assay results.

tablet, containing p - nitrophenyl phosphate (20 mg each) and allowing 30 - 60 minutes for the tablets to dissolve, before vigorously shaking to mix completely.

The stop solution, 1N NaOH was supplied.

4.7.3.5 Method

1. Aliquots of the same urines as that for Dpd assessment were sent to the routine biochemistry laboratory for urinary creatinine levels.
2. Urines for assay were diluted 1:10, in duplicate, using 50 μ L urine and 450 μ L assay buffer, which had already been made up.
3. The standards were diluted as for the samples and ready for use after mixing with a vortex.
4. The substrate buffer was allowed to come to room temperature.
5. The amount of each reagent was determined for the number of anti- Dpd coated strips required, as per the table.
6. The required amount of wash buffer was prepared by diluting the 10 X wash buffer concentrate 1:10 with deionized water and stored at room temperature and used within 24 hours.
7. The required number of vials of enzyme conjugate was reconstituted using 7 ml of assay buffer and stored immediately in the refrigerator until use.
8. The desired number of strips was placed in the stripwell frame.
9. 50 μ L of diluted standards, controls and urines were added to each well of the plate in duplicate as per the template and all precautions were taken to avoid prolonged exposure to light.

10. 100 μ L of the cold (refrigerated), reconstituted enzyme conjugate was added to each well, using the multichannel pipette and the samples covered with a plate and incubated in the refrigerator (2 – 8° C) for 2 hours, in the dark for stability.
11. During the incubation period, the working substrate solution was prepared.
12. Following incubation, the stripwells were manually inverted/emptied over the sink and washed 3 times with cold 1 X wash buffer, ensuring that the wash step took no longer than 2 minutes.
13. After the last wash, the plate was dried with a paper towel, and then it was left for a further 5 – 10 minutes to drain on the paper towel.
14. 150 μ L of the substrate solution was added to each well and incubated at room temperature for a further 60 minutes at room temperature but in the dark.
15. 100 μ L of stop solution was added to each well to stop the reaction.
16. Within 15 minutes the absorbance were read at 405 nm on a microtitre plate reader, and the absorbances were quantitated using the quadratic calibration curve software.

4.7.3.6 Standard Curve and Calibration

The set of six standards (Dpd purified from bovine bone) were used in serial dilutions to calculate the concentrations of samples and controls. Dilutions were expressed in nM as follows: 0, 3, 10, 30, 100, and 300 nM Dpd. Quantitation software with a 4-parameter calibration curve fitting equation was used to analyse the Pyrilinks - D assay results.

Pyrilinks - D is expressed as ratio of Dpd/mmol creatinine, and the results of
Pyrilinks - D/Creatinine mmol/L as Dpd/mM

4.7.4 Cross - linked N - telopeptides of type 1 collagen (NTx)

A commercial kit the Osteomark[®] (Ostex International, Inc. Seattle, WA 98134 USA) was used for cross-linked N-telopeptides of type 1 collagen in human urine.

4.7.4.1 Principle

The assay is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA) that makes use of microwells as the solid phase into which NTx has been absorbed. NTx in the specimen competes with the solid phase NTx for binding sites of monoclonal antibody labelled with horseradish peroxidase. The amount of antibody bound to solid phase is therefore inversely proportional to the amount of NTx in the specimen. Quantitation of the NTx concentration in the specimen was determined spectrophotometrically and calculated from a standard calibration curve. Assay values were corrected for urinary dilution by creatinine analysis and expressed in nanomoles bone collagen equivalents per litre (nM BCE) per millimole creatinine per litre (nM creatinine).

4.7.4.2 Equipment

Single and multichannel pipettors capable of delivering 25 µL, 100 µL and 200 µL volumes and suitable laboratory equipment for liquid measurement.

Automated microwell washer and deionized water.

A microtitre plate reader that must read at 450 nm with 630 nm reference filter and detect absorbances from 0 to 3.000 optical density units with software capable of calculating a 4 - parameter curve fit. (Biochromatic Labysystem Multiscan)

4.7.4.3 Materials

Osteomark® Kit. Ostex International, Inc., 2203 Airport Way South, Seattle, WA 98134 USA.

4.7.4.4 Reagents

A set of assay standards (calibrators) with values of 1, 30, 100, 300, 1000, 3000 nM BCE, 1 vial each, which is a purified NTx antigen in buffered diluent. The antigen coated 96 - well, flat - bottomed plate was supplied with purified human NTx antigen adsorbed onto microwell strips.

The level I and level II urine controls, manufactured with human urine and with known NTx concentration, had values intended to verify the validity of the

curve and sample results. The NTx range of level I urine control was 320 - 480 nM BCE and level II urine control between 1120 - 1680 nM BCE.

The antibody conjugate concentration, purified from murine monoclonal antibody directed against NTx and conjugated horseradish peroxidase was supplied as well as the antibody conjugated diluent.

The chromogen reagent, 1 vial of 3,3', 5,5 - tetramethylbenzidine in dimethylsulfoxide was supplied as a 100 X concentrated reagent.

The 30 X wash concentrate was supplied as an ionic detergent solution and the stopping reagent, 1N sulfuric acid.

4.7.4.5 Storage of reagents

After preparation, the reagents were stored at between 2 - 8° C until used.

4.7.4.6 Method

1. Aliquots of the urines specimens, as that for NTx assessment were sent to the routine biochemistry laboratory for measurement of urinary creatinine levels.
2. At least one hour before use all specimens for NTx assay and reagents were brought to room temperature (20 – 28° C) for

3. The frozen urine samples were thawed at 37° C in the water bath, then brought to room temperature and thoroughly mixed prior to use in the assay. Urine samples containing particulates were centrifuged before use and those that were cloudy or turbid were allowed to settle for 5 to 10 minutes prior to use.
4. The chromogen reagent was brought to room temperature, as it contained dimethylsulfoxide and may have solidified when refrigerated.
5. The working strength wash solution was prepared by diluting 30 X wash concentrate 1:30 with deionized water and mixed for a minimum of five minutes.
6. The desired number of antigen-coated strips to be used was calculated so that each standard, control and urine specimen were run in duplicate and any unused strips were placed back in the pouch and sealed.
7. Using a clean disposable plastic container the antibody conjugate concentrate was diluted 1:101 using the antibody conjugate diluent. For each antigen coated microwell strip that was used 20 µL of antibody conjugate concentrate was diluted into 2 mL of antibody conjugate diluent. The mixture was mixed by gentle inversion to avoid foaming of samples.
8. Next, 25 µL of each standard, control or urine sample was pipetted into the bottom of duplicate wells using a new tip for each standard, control or urine specimen.
9. Using the multichannel pipette, 200 µL of working strength conjugate solution was delivered into each microwell.
10. A plate sealer was applied and swirled for 5 -10 seconds to ensure mixing.
11. The plate was incubated at room temperature (18 – 28° C) for 90 minutes.

12. During the last 10 minutes of incubation, the chromogen/buffered substrate was prepared, by making a 1:101 dilution of the chromogen reagent. The volume of each reagent was determined by the number of samples to be assayed. As a guideline, for each antigen coated microwell strip used, 20 μL of the chromogen was diluted into 2 mL of the buffered substrate. It was important not to vortex or shake the mixture prior to use and to ensure that the chromogen/buffered substrate solution was used within 30 minutes of preparation.
13. At the end of the incubation period, the plate sealer was removed, the liquid aspirated from each well and the plate washed five (5) times with the working strength wash solution using the automated plate washer.
14. Following the final wash, the plate was inverted onto absorbent paper towel, and 200 μL of prepared chromogen/buffered substrate was immediately added to each microwell, using a multichannel pipette and then sealed with a new plate sealer.
15. Further incubation at room temperature for 15 minutes followed. A blue colour developed in the wells containing bound antibody-horseradish peroxidase conjugate.
16. Following incubation, the plate sealer was carefully removed and then using the multichannel pipettor, a 100 μL of stop solution was added to each well in the same order as addition of the chromogen/buffered substrate reagent. It was noted that the wells, which developed a blue colour, turned a yellow colour.

17. To ensure mixing, the plate was swirled gently on a flat surface for 5 -10 minutes and then allowed to stand for 5 minutes before reading absorbance values
18. Within 30 minutes of adding the stop solution, the absorbance of the calibrators, controls and urine samples were read with the use of a microwell plate reader at 450 nm. The reference filter used was 630 nm. It was ensured that the reader had a maximum optical density reading of ≥ 3.000 .

4.7.4.7 Standard curve and calculation

The calibrators/ standards mentioned above (purified NTX antigen in buffered diluent) were used in serial dilutions to calculate the concentration of the samples. Dilutions were expressed in nM BCE as follows: 1, 30, 100, 300, 1000 and 3000. Computer software using a 4 - parameter curve fitting equation was used to analyse the data entered of Controls and urine specimens to determine their values (nM BCE).

The concentration values for urine specimens were reported as nM BCE/mM creatinine.

An example of the calculation as per the packet insert was as follows:

Assay value	=	360 nM BCE
Urine creatinine	=	<u>60 mg/dL creatinine</u>
		11.3*

$$\begin{array}{rcl}
 & = & 5.3 \text{ nM creatinine} \\
 \underline{360 \text{ nM BCE}} & = & 68 \text{ nM BCE/mM creatinine} \\
 5.3 \text{ mM creatinine} & &
 \end{array}$$

* The conversion factor (11.3) used to convert mg creatinine per dL to millimole creatinine per litre.

4.8 STATISTICAL ANALYSIS

Results for this study are presented as the mean and one standard deviation of the mean. The two – tailed unpaired Student t-test was used to compare the means of two groups with normal distribution and equal variance. For comparing more than two groups, the non-parametric tests, analysis of variance (ANOVA) and Kruskal - Wallis tests were used. The Pearson's test was used to determine the correlation between the parameters. Contingency tables were analysed using Fisher exact test. Alpha was set at 5 %.

CHAPTER FIVE

5.0 RESULTS

5.1 DEMOGRAPHICS OF STUDY POPULATION

Runners

Fifty-five (55) premenopausal ultra-distance marathon runners were enrolled in the study. Of the 55 subjects 30 were Whites, 12 Indians 10 Coloureds and 3 Blacks.

The mean age was 39 ± 5 years (range: 31 - 51 yrs). The mean weight was 55.1 ± 6.3 kilograms (range: 42 - 68 kg), mean height 1.61 ± 0.1 metres (range: 1.48 - 1.71 m) and the mean BMI was 21.35 ± 1.8 kg/m² (range: 17.23 - 26.37 kg/m²). The total distance run during the six months prior to the study was 1431 ± 432 kilometres (range: 490 - 2511 km).

Controls

Forty-seven (47) premenopausal sedentary healthy women were included in the study.

The mean age was 34 ± 6 years (range: 31 - 51 years) the mean height was 1.61 ± 0.1 metres (range: 1.49 - 1.6 metres), the mean weight was 60.27 ± 11.7 kilograms (range: 38.5 - 115 kg) and the BMI was 23.38 ± 4.2 (range: 16.51 - 41.23).

The runners (n = 55) were significantly older than the controls and had a significantly lower weight and BMI value (p < 0.01) (Table 3).

Table 3: Demographic data of runners and sedentary controls, expressed as the mean and 1 SD, and the range. Statistical analysis by unpaired t – test.

	Controls (n = 47)	Runners (n = 55)	P Value
Age (years)	33.8 ± 6.3 (20 - 48)	38.8 ± 4.7 (31 - 51)	< 0.0001
Weight (kg)	60.3 ± 11.7 (38.5 - 115)	55.1 ± 6.3 (41.5 - 67.5)	< 0.01
Height (cm)	1.61 ± 0.1 (1.49 - 1.76)	1.61 ± 0.1 (1.48 - 1.71)	ns
BMI (kg/m ²)	23.38 ± 4.2 (16.51 41.23)	21.35 ± 1.8 (17.28 - 26.37)	< 0.01
Ethnic Distribution			
No. Whites	15	30	
No. Indians	29	12	
No. Coloureds	3	10	
No. Blacks	0	3	

5.2 COMPARISON OF TOTAL COHORT OF RUNNERS WITH SEDENTARY CONTROLS

5.2.1 BMD measurements and body composition

Bone mineral density (BMD) at the lumbar spine and hip was measured in all the runners and controls. Measurements of the BMD at the whole body, trunk, arms and legs and percentage body fat were taken in all runners and 18 controls.

The mean BMD measurements at the each of the sites and the percentage body fat in controls and runners are shown in Table 4.

There was no significant difference in the BMD at the lumbar spine, total body and trunk in the controls and runners. The BMD at all the sites in the hip region were higher in the runners compared to controls, however, this was only significant at the total hip ($p < 0.05$), neck ($p < 0.05$) and intertrochanteric area ($p < 0.05$). The BMD at the total legs was also significantly higher in the runners compared to controls ($p < 0.01$). In contrast the BMD at the total arms was lower in the runners compared to controls ($p < 0.001$).

Comparing the body composition in the runners and controls, the percentage body fat was significantly lower in the runners ($p < 0.001$).

There was a significant negative correlation between the percentage body fat

and BMD at the total legs in the runners. In contrast there was a significant positive correlation between percentage body fat and the BMD at the total trunks, total legs, total hip, femoral neck, trochanter and inter-trochanteric sites (Table 5)

5.2.1.1 BMD T score runners and controls

Using the T scores, runners and controls were categorized into those with normal BMD i.e. T score > -1.0 SD; osteopaenia T score > -1.0 to -2.5 SD and osteoporosis T score < -2.5 SD. There was no significant difference in T scores between the runners and controls (Table 6).

Table 4: Bone mineral densities at various sites and percentage body fat in runners and controls, expressed as the mean and 1 SD. Statistical analysis was by unpaired t – test. (* = data available for 18 subjects)

BMD g/cm ²	Controls (n = 47)	Runners (n = 55)	P Value
Total lumbar	0.981 ± 0.131	0.990 ± 0.110	ns
L1	0.872 ± 0.126	0.889 ± 0.109	ns
L2	0.979 ± 0.144	1.165 ± 1.127	ns
L3	1.026 ± 0.145	1.028 ± 0.110	ns
L4	1.023 ± 0.127	1.037 ± 0.126	ns
Total hip	0.881 ± 0.125	0.935 ± 0.132	<0.05
Neck	0.785 ± 0.106	0.831 ± 0.127	<0.05
Trochanter	0.680 ± 0.102	0.722 ± 0.120	(0.06)
Inter- trochanter	1.036 ± 0.153	1.111 ± 0.172	<0.05
Total body	1.088 ± 0.774*	1.107 ± 0.069	ns
Total arms	0.923 ± 0.154*	0.722 ± 0.050	<0.0001
Total legs	1.014 ± 0.080*	1.126 ± 0.119	<0.001
Trunk	0.627 ± 0.1051*	0.635 ± 0.060	ns
Total body fat (%)	38.2 ± 7.0*	21.6 ± 5.7	<0.0001

Table 5: Correlation between percentage body fat and BMD in runners and controls at various sites using Pearson’s Correlation, (r).

	Runners		Controls	
	Pearsons Correlation r	P Value	Pearsons Correlation r	P Value
Total body	-0.06	ns	0.26	ns
Total arms	-0.04	ns	-0.29	ns
Total trunk	-0.03	ns	0.61	< 0.01
Total spine	-0.00	ns	0.27	ns
Total legs	-0.38	0.005	0.47	0.0506
Total hip	-0.10	ns	0.81	< 0.001
Femoral neck	-0.14	ns	0.63	< 0.005
Trochanter	-0.08	ns	0.71	< 0.001
Inter-trochanter	-0.16	ns	0.76	< 0.001**
Lumbar spine	-0.01	ns	0.34	ns

**Table 6: The number of runners and controls, with BMD T scores of
> - 1.0, > - 1.0 to – 2.5 and < - 2.5 measured at different sites.**

	RUNNERS	CONTROLS
T- score (Total Body)		
Number	55	18
> -1.0	50	14
> -1.0 to – 2.5	5	4
< - 2.5	0	0
T- score (Total Hip)		
Number	54	48
> -1.0	40	31
> -1.0 to – 2.5	10	15
< -2.5	4	2
T- score (Total Lumbar)		
Number	55	48
> -1.0	40	30
> -1.0 to –2.5	15	17
< -2.5	0	1

5.2.2 Biochemical parameters

5.2.2.1 Gonadal hormones and reproductive status

The results for the FSH, LH, progesterone and oestradiol concentrations measured in 55 runners and 18 controls are presented in Table 7. The LH concentration was significantly higher in the runners compared to controls, as was the oestradiol concentration. The mean progesterone concentrations were significantly lower in runners

The calculated MHI values were higher in runners compared to controls (11.2 ± 1 menses/year vs. 10.3 ± 2 menses/year), but did not reach statistical significance. (Table 7).

Table 7: Gonadal hormone concentrations in runners and controls, expressed as the mean and 1 SD. Statistical analysis by unpaired t – test.

	Controls (n = 18)	Runners (n = 55)	P Value
FSH (mIU/ml)	8.1 ± 8.9	9.21 ± 10.2	ns
LH (mIU/ml)	5.54 ± 4.0	9.67 ± 13.9	< 0.0001
Progesterone (ng/ml)	10.26 ± 19.4	7.82 ± 13.6	< 0.05
Oestradiol (pg/ml)	172.81 ± 161.8	357.87 ± 362.2	< 0.05
MHI (menses/year)	11.2 ± 1	10.3 ± 2	ns

5.2.2.2 Markers of bone turnover

Biochemical markers of bone turnover were measured and compared in 55 runners and 18 controls. Due to insufficient serum sample, osteocalcin concentrations were available in 26 runners. Significantly lower mean deoxypyridinoline (Dpd) and mean osteocalcin concentration were found in the runners compared to the controls. There was no difference in the concentration of urinary NTx or bone specific alkaline phosphatase (Table 8).

Table 8: Markers of bone turnover in runners and controls, expressed as the mean and 1 SD. Statistical analysis by unpaired t – test.

	Controls (n = 18)	Runners (n = 55)	P Value
NTx (nM BCE/mM creatinine)	42.5 ± 25.6	55.8 ± 76.3	ns
DpD (nM DpD/mM creatinine)	6.3 ± 3.5	4.9 ± 1.9	< 0.05
BSAP (U/L)	14.5 ± 6.2	13.9 ± 3.9	ns
Osteocalcin (nmol/L)	2.0 ± 1.9	1.1 ± 0.5 (n = 26)	< 0.05

5.3 COMPARISON BETWEEN RUNNERS WITH A HISTORY OF STRESS FRACTURES AND TOTAL COHORT OF RUNNERS

Of the 55 runners, 10 had a history of stress fractures. These 10 runners were then compared to the 45 runners without stress fractures for the demographic features including the number of years run and the distance run in the preceding six months, BMD and body composition and gonadal and reproductive status.

5.3.1 Site of stress fractures

Ten runners all in the white population group had a history of stress fractures. Sites included right and left tibia, clavicle and ribs right big toe, left ankle, and inferior pubic ramus.

5.3.2 Demographics

There were no significant differences in the age and BMI between the runners with stress fractures and those without. Although the runners with stress fractures had a longer history of running and distance covered in the preceding six months this did not reach statistical significance (Table 9).

Table 9: Demographics of runners with a history of stress fractures and those without. Results expressed as the mean and 1 SD. Statistical analysis by unpaired t = test.

	Runners no stress fractures (n = 45)	Runners with stress fractures (n = 10)	P Value
Age (years))	38.5 ± 4.9	39.8 ± 4.1	ns
Weight (kg)	55.0 ± 6.3	55.5 ± 6.8	ns
Height (m)	1.60 ± 0.1	1.62 ± 0.1	ns
BMI (kg/m ²)	21.4 ± 1.9	21.0 ± 2.7	ns
Number of years running	7.0 ± 3.8	9.1 ± 4.4	ns
Total km run/week in previous six months	57.7 ± 15.5	68.4 ± 26.7	ns

5.3.3 Comparison of BMD measurements and body composition in runners with and without a history of stress fractures

The BMD measurements and percentage body fat were compared in runners with and without stress fractures (Table 9). There was no difference in the BMD measurements at all sites between runners with stress fractures and those without. Since all the stress fractures occurred in the White runners, their BMD measurements were compared to an ethnically matched group without stress fractures. Once again there were no differences.

The only significant difference was that the runners with stress fractures had a lower percentage body fat compared to the runners without stress fractures ($p < 0.05$). There was also no difference in the mean MHI between the groups (Table 10).

Table 10: BMD and body composition of runners with stress fractures and without a history of stress fractures. Results expressed as the mean and 1 SD. Statistical analysis by unpaired t – test.

BMD g/cm ²	Runners no stress fractures (n = 45)	Runners with stress fractures (n = 10)	P Value
Total Body	1.104 ± 0.069	1.116 ± 0.774	ns
Total arms	0.722 ± 0.051	0.723 ± 0.043	ns
Total trunk	0.632 ± 0.058	0.650 ± 0.969	ns
Total spine	0.934 ± 0.084	0.953 ± 0.163	ns
Total legs	1.121 ± 0.126	1.151 ± 0.080	ns
Total hip	0.926 ± 0.124	0.979 ± 0.164	ns
Femoral neck	0.826 ± 0.123	0.855 ± 0.148	ns
Trochanter	0.718 ± 0.111	0.743 ± 0.153	ns
Intertrochanter	1.097 ± 0.162	1.175 ± 0.208	ns
Total lumbar	0.990 ± 0.110	1.993 ± 0.117	ns
Total body fat (%)	22.3 ± 5.9	18.6 ± 3.5	<0.05
MHI (menses/year)	10.49 ± 1.9	9.51 ± 2.3	ns

5.3.3.1 Gonadal hormones and reproductive status in runners with and without stress fractures

There was no difference in the mean concentrations of FSH, LH, progesterone and oestradiol in the runners with stress fractures (n = 10) and those without stress fractures (n = 45). There was also no difference in the mean MHI between the groups (Table 11).

Table 11 Gonadal status in runners with or without stress fractures.

The results are expressed as mean and 1 SD. Statistical analysis: unpaired t – test.

	No stress fractures (n = 45)	Stress fractures (n = 10)	P Value
FHS (mIU/ml) (Ref. range: 0-150)	9.5 ± 10.3	7.9 ± 10.5	ns
LH (mIU/ml) (Ref. range: 0-150)	9.8 ± 14.3	9.3 ± 13.1	ns
Progesterone (ng/ml) (Ref. range: 0-30)	8.0 ± 13.8	6.9 ± 13.0	ns
Oestrogen (pg/ml) (Ref. range: 0 – 180)	332.2 ± 340.8	473.2 ± 448.6	ns
MHI (menses/year)	10.49 ± 1.9	9.51 ± 2.3	ns

5.3.3.2 Bone markers in runner with and without stress fractures

Biochemical parameters of bone turnover were measured and compared in 45 runners without stress fractures and 10 with stress fractures. A significantly lower mean urinary NTx was found in the stress fracture runners compared to those without ($p < 0.0001$). There was no difference in the concentrations of bone specific alkaline phosphatase or deoxypyridinoline (Table 12).

Table 12 Bone markers in runners with and without stress fractures.

**Results are expressed as mean and 1 SD. Statistical analysis:
unpaired t - test .**

	Runners no stress fractures (n = 45)	Runners with stress fractures (n = 10)	P Value
Ur. Creatinine (mmol/L)	5.90 ± 4.10	6.31 ± 4.20	ns
NTx (nM BCE/mM creatinine)	59.63 ± 83.90	38.20 ± 9.72	0.0001
DpD (nM DpD/mM creatinine)	4.82 ± 1.90	5.95 ± 2.03	ns
BASP (U/L)	13.76 ± 3.84	14.68 ± 4.21	ns

5.4 COMPARISON OF ETHNIC GROUPS

5.4.1 BMD and body composition

The BMD, body composition and training regime parameters were compared in 30 White, 12 Indian and 10 Coloured runners. The sample size for African runners ($n = 3$) was too small to include in the analysis. The White runners were significantly older than the Indian runners ($p < 0.01$). There was no significant difference in the BMD at all sites in the different ethnic groups. The Indian runners had a significantly higher percentage body fat compared to both the White ($p < 0.01$) and Coloured ($p < 0.001$) runners. There was no significant difference between the percentage body fat in the Coloured and White runners and the calculated menstrual history index (MHI) in 30 White, 12 Indian and 10 Coloured runners and showed no significant difference (Table 13).

Training regime

The distance run in the preceding six months, reported as km/week was significantly higher in the White runners compared to Indians ($p < 0.05$), while the Coloured runners also appeared to have run a longer distance this was not significant. However, the Coloured runners had run for a significantly longer duration than the Indian runners ($p < 0.05$) (Table 13).

There was no significant difference in the MHI between the ethnic groups (Table 13)

Table 13 BMD, Body composition, duration of running and kilometres run and MHI in the different ethnic groups, given as the mean and 1 SD. Statistical analysis by ANOVA and post hoc testing (* P < 0.01, ** P < 0.05 *** P < 0.001)

	Whites (n = 30)	Indians (n = 12)	Coloureds (n = 10)
Age (years)	41 ± 4	35 ± 3 *	38 ± 6
Weight (kg)	56 ± 6.1	50.42 ± 6.8	57.7 ± 5.1
Height (cm)	1.62 ± 0.05	1.56 ± 0.07	1.62 ± 0.06
BMI (kg/cm²)	21.31 ± 1.79	20.67 ± 2.05	22.15 ± 1.76
BMDTotal body (g/cm²)	1.113 ± 0.075	1.082 ± 0.522	1.112 ± 0.627
BMDTotal arms (g/cm²)	0.733 ± 0.048	0.707 ± 0.058	0.729 ± 0.046
BMDTotal trunk (g/cm²)	0.645 ± 0.068	0.620 ± 0.043	0.650 ± 0.042
BMDTotal spine (g/cm²)	0.936 ± 0.118	0.927 ± 0.050	0.959 ± 0.099
BMDTotal hip (g/cm²)	0.933 ± 0.142	0.912 ± 0.123	0.988 ± 0.098
BMDTotal legs (g/cm²)	1.148 ± 0.144	1.068 ± 0.064	1.128 ± 0.050
Total body fat (%)	19.82 ± 5.04	29.78 ± 5.04 *	20.1 ± 5.67***
Duration of running (years)	7.6 ± 3.8	5.6 ± 2.19	10.0 ± 5.1
Total kilometres/week r	62.7 ± 18.6	46.5 ± 17.2**	56.7 ± 18.7
MHI (menses/year)	9.86 ± 2.17	10.77 ± 2.12	10.74 ± 1.35

5.4.2 Comparison of markers of bone turnover in the different ethnic groups

Biochemical markers of bone turnover were measured and compared in 30 White, 12 Indian and 10 Coloured runners. The NTx concentration in the Indian runners was significantly higher compared to the Coloured runners ($p < 0.05$) and ($p < 0.01$) respectively. Additionally, there was no difference in the concentrations of urinary DpD, osteocalcin and bone specific alkaline phosphatase in all race groups. Due to insufficient samples, osteocalcin concentration was available in a total of 26 runners (Table 14).

Table 14 Markers of bone turnover in the different ethnic groups, expressed as mean and 1 SD. Statistical analysis by ANOVA with post hoc testing, (* P < 0.05 and ** P < 0.01)

	Whites (n = 30)	Indians (n = 12)	Coloureds (n = 10)
NTx (nM BCE/mM creatinine)	61.6 ± 100.5	47.42 ± 22.6*	36.04 ± 15.01**
DpD (nM DpD/mM creatinine)	5.40 ± 1.87	5.35 ± 2.32	3.25 ± 0.9
BSAP U/L	12.82 ± 2.83	10.93 ± 2.35	15.0 ± 5.1
Osteocalcin (nmol/L)	1.108 ± 0.5 (n = 13)	0.75 ± 0.31 (n =6)	0.96 ± 0.26 (n = 7)

CHAPTER SIX

6.0 DISCUSSION

6.1 EXERCISE INDUCED OSTEOPOROSIS

Exercise is accepted as beneficial and is recommended to all ages. Benefits include improved cardiovascular fitness (Powell *et al*, 1987), emotional health (McCann and Holmes, 1984) and an increase in bone mineral density or a reduction in bone loss in young, premenopausal, and postmenopausal women. Low to moderate intensity, weight bearing exercise is the key approach for the prevention and treatment of osteoporosis (Dalsky *et al*, 1988; Wolman, 1990; Drinkwater, 1996).

However, exercise may be detrimental to bone health. Women athletes who participate in vigorous physical activity are predisposed to the development of the "Female Athlete triad". This comprises an eating disorder, amenorrhoea and osteoporosis. The eating disorder is thought to be due to a desire to lose weight and to improve performance (Nattiv *et al*, 1994; Wiggins *et al*, 1997).

Amenorrhoea in female athletes is multifactorial and may be secondary to the eating disorder and the intensity of physical activity. Often the main cause of amenorrhoea is a deficiency of nutrients, insufficient calorie intake, low percentage body fat and low body mass leading to an interruption of the hypothalamic-pituitary axis. The psychological and physical stresses of exercise are also thought to disrupt the hypothalamic-pituitary axis (Wittert, 2000). Low oestrogen

concentrations seen in athletic amenorrhoea are associated with increased bone resorption which results in a reduction in BMD (Wiggins *et al*, 1997) and the longer the menstrual irregularity persists, the greater the deficits in the bone (Fisher *et al*, 1986; Nelson *et al*, 1986; Myburgh *et al*, 1993; Rencken *et al*, 1996).

The effects of exercise on BMD separate from menstrual irregularities depend on whether or not the activity is weight bearing. For example swimmers have been shown to have equal to or lower bone mineral densities than sedentary women or women participating in weight bearing activities (Sanborn, 1982; Wolman, 1990; Madsen *et al*, 1997). The differences may occur because swimming is not a weight bearing activity and is associated with extended periods of weightlessness, and therefore insufficient stress for osteogenic stimulation (Risser *et al*, 1990). Exercise on its own can also be detrimental to bone mass. This depends on the intensity of athletic training, which includes distance, speed, frequency of exercise, duration of training and level of performance (Drinkwater, 1995; Drinkwater, 1996; Micklesfield *et al*, 1995).

Several South African studies have examined the effect of distance running on women athletes (Watkin *et al*, 1991; Micklesfield *et al*, 1995; Lindeque, 1996; Micklesfield *et al*, 1998; Vorster *et al*, 2001). The steady increase in women participating in ultra-distance running, often culminating in the ultimate challenge, of competing in the Comrades Marathon, gave us the opportunity to further investigate the effects of ultra marathon training on South African women athletes. The aim of the study therefore, was to investigate the effect of ultra-distance running on bone mineral density in premenopausal women from a variety of ethnic

backgrounds and to investigate the impact of the training regime, menstrual status and body composition on BMD.

6.2 BMD IN ULTRADISTANCE RUNNERS AND CONTROLS

Bone mineral density is determined by peak bone mass (PBM) and bone loss. Exercise is an important factor in determining PBM. During childhood and puberty, between the ages of 12 – 16 years, bone mass increases in a linear fashion until about the age of 20 – 30 years when the lifetime maximum in bone mass (peak bone mass) is achieved. Thereafter bone remodelling occurs throughout life. At about the fourth decade age-related decline in bone mass occurs in both men and women. In men there is a 0.7 % - 1 % bone loss per annum. Whereas in post menopausal women there is a period of accelerated bone loss of about 3 % - 5 % per annum with the onset of menopause. By age 80 years women will have lost 40 % of their PBM and men 25 %. Thus the bone mass at a later age depends on the PBM achieved at skeletal maturity and subsequent bone loss or both (Riggs and Melton III, 1986; Lindsay, 1993; Bilezikian, 1999).

The effect of exercise on BMD is dependent on numerous factors, which include a genetic contribution, diet, hormonal status, percentage body fat, intensity of exercise, anatomical site where stress is applied and muscle mass. In female runners, the age of menarche, intensity of training, eating habits (i.e. energy intake), menstrual function, body mass and body mass index (BMI) influence the loss or gain of bone mass.

Previous studies have shown the positive role of exercise on BMD (Dalsky *et al*, 1988; Micklesfield *et al*, 1995; Micklesfield *et al*, 1998; Vorster *et al*, 2001) and numerous others have illustrated the detrimental effects of exercise on bone mass (Myburgh *et al*, 1990; Myburgh *et al*, 1993; Micklesfield 1995; Drinkwater, 1996).

In the study comparing the BMD and menstrual status of 25 athletes with stress fractures and 25 athletes who did not have stress fractures (Myburg *et el* 1990), the athletes with stress fractures had a lower BMD at the spine, femoral neck and greater trochanter. In addition more athletes with stress fractures had menstrual irregularities than control athletes. In a further study BMD was measured by DEXA and SPA in 12 amenorrhoeic and 9 eumenorrhoeic young white athletes (Myburgh *et al*; 1993). Lower BMD values were reported at the spine, whole body, proximal femur and mid-femoral shaft in the amenorrhoeic athletes. However, this study had a small number of subjects and did not include sedentary controls. Further this study did not show an effect of body mass on BMD. This may be due to the homogeneity of the subjects with respect to body mass.

The association between menstrual irregularity and low BMD in athletes was further studied in 15 mature premenopausal runners without menstrual irregularity and 10 runners with no menstrual irregularity (Micklesfield *et al* (1995). Lower BMD was reported in runners who had menstrual irregularity. The BMD also correlated with the MHI and years of oligoamenorrhoea. In a longitudinal

study, follow up BMD in the lumbar spine was significantly lower in the runners with a history menstrual irregularity (Micklesfield *et al* 1998).

In this study, the BMD at total hip, femoral neck, trochanter, intertrochanteric area, total legs, in ultra-distance runners was compared to normal sedentary volunteers. The runners and controls from the different ethnic groups were pooled based on the findings of previous South African studies that showed no differences in the BMD in the different ethnic groups. There was a significant increase in BMD at the hip area and legs, in the runners compared to controls, which appears to support other studies (Wolman, 1990; Heinonen *et al*, 1993; Wolman, 1994; Madsen *et al*, 1997) that illustrate the benefit of repetitive weight-bearing exercise on BMD. However, the BMD for total arms was significantly reduced. Similar findings have been reported by Vorster *et al* (2001) in a study on nine White female athletes between the ages of 20 – 29 years. This is explained by the fact that the radius is not a weight bearing bone and is therefore not subjected to repeated mechanical stresses in long distance runners. This concept is further substantiated by the increase in forearm BMD seen only in competitive tennis/squash players. (Madsen *et al*, 1997).

6.3 BODY COMPOSITION AND BONE MASS

Body weight is an important determinant of bone mass. Primary osteoporosis commonly occurs in thin, slightly built women. In contrast obesity is a negative risk factor of osteoporosis, and osteoporosis is one of the few conditions in which excess body weight is protective (Felson *et al*, 1993). Body

composition is also an important determinant of bone mass and menstrual status. Ducey *et al* (2000) in a recent study proposed that a specific molecular mechanism might link bone mass with body weight. Their findings suggested that the hormone leptin produced by adipocytes might have a specific role in the control of body weight. Leptin has also been implicated in the pathogenesis of hypothalamic amenorrhoea (Laughlin, 1997).

Women participating in competitive sports such as ultradistance running generally have a low body mass and low percentage body fat in an effort to maximise performance. The low percentage body fat and mass in athletes, particularly females, may be as a result of poor energy intake, eating disorders and the perception of body size. The low percentage body fat in turn may lead to menstrual dysfunction (Carlberg *et al*, 1982; Loucks and Horvath, 1985; Drinkwater *et al*, 1990) and osteoporosis. Reports on the relationship between body composition and menstrual function in athletes have shown conflicting results. While numerous studies have found that amenorrhoea and oligomenorrhoea in athletes is associated with a lower percentage body fat and low body weight (Frisch and McArthur, 1974; Carlberg *et al*, 1982; Drinkwater *et al*, 1990; Watkin *et al*, 1991; Gremion *et al*, 2001), other studies have not confirmed this association (Loucks and Horvath, 1985; Feicht *et al*, 1978).

In this study we measured the body weight, BMI and percentage body fat in the runners and controls. The percentage body fat was measured using DEXA. The runners had a significantly lower body weight compared to the controls (55.1 ± 6.3 kg vs. 60.23 ± 11.7 kg; $p < 0.01$). In addition there was also a significantly

lower percentage body fat ($21.6 \pm 5.7\%$ vs. $38.2 \pm 7\%$, $p < 0.0001$). This is similar to the findings of Madsen *et al* (1997).

The beneficial effect of increased percentage fat on BMD was confirmed in the control group, where a significant positive correlation between percentage body fat and BMD at the total trunk and all the hip areas was found. The lack of a correlation between BMD at the lumbar spine and percentage body fat is surprising. This may be related to the small numbers of controls. In contrast there was a significant negative correlation between the percentage body fat and BMD at the total legs in the runners. It is accepted that people who exercise regularly tend to have a lower percentage body fat. This is related to both intensity and duration of exercise and more judicious diet. Thus the lower percentage body fat in the runners is a possible surrogate marker for the fitness level of an athlete. We have also shown that the BMD at the legs was significantly higher in the runners compared to the controls.

In addition when the percentage body fat was compared in the three different ethnic groups there was a significantly lower percentage body fat in the white runners compared to the Indian runners ($19.8 \pm 5.0\%$ vs. $29.8 \pm 5.4\%$; $p < 0.001$). This may be explained by the higher total distance run by the whites compared to the Indians (1504 ± 445 km vs. 1116 ± 431 km; $p < 0.05$). The percentage body fat was also lower in the Coloured runners compared to the Indians ($20.1 \pm 5.7\%$ vs. $29.8 \pm 5.4\%$; $p < 0.001$). Although there was no significant difference in the total distance run in the two groups, the runners of mixed race had been running for a significantly longer duration (9.8 ± 5 yrs vs. 5.6

± 2.2 yrs; $p < 0.05$). Despite these differences in the percentage body fat there were no differences in the BMD at all the regions measured in the three groups.

Despite the lower percentage body fat none of the runners were found to be amenorrhoeic and only 25 % had a history of oligomenorrhoea. There was also no significant difference in the menstrual history in the three ethnic groups. The variance in the studies on the relationship between body composition and amenorrhoea may be related in part to the variations in the methods used to determine body fat. In addition the relationship between percentage body fat and amenorrhoea does not appear to be absolute. Marcus *et al*, (1995) found although the amenorrhoeic women in their cohort of elite women distance runners had very low values for percentage body fat, there was no difference between the amenorrhoeic and cyclic women. In fact the lowest percentage body fat was found in a woman who had regular menses. This suggests that although weight loss may be one of the most powerful physiological hypothalamic stressors (Petit and Prior, 2000) other factors such as rate of weight loss, training regimen, reproductive maturity at the time of initiation of the exercise program and specific sporting activity may have an important role in inducing amenorrhoea associated with exercise (Loucks and Horvath, 1985).

These findings suggest that exercise in sub-elite athletes has a significant beneficial effect on BMD, a low percentage body fat is not an independent risk factor for low bone mass in these athletes and the effect of low percentage body fat is mediated through its effect on the menstrual cycle.

6.4 MENSTRUAL FUNCTION AND BONE MASS IN ATHLETES

Amenorrhoea or menstrual irregularity is an important component of the "Female Athlete Triad". Women participating in strenuous exercise have a higher prevalence of amenorrhoea compared to the normal population (Dale *et al*, 1962) and the lower bone mass associated with exercise-induced amenorrhoea is thought to be due to oestrogen deprivation resulting in increased resorption of bone and bone loss.

6.4.1 Menstrual history

The association between amenorrhoea and bone mass has been extensively studied. A lower BMD has been reported in amenorrhoeic distance runners compared to distance runners with normal menstrual cycles at the lumbar spine (Marcus *et al*, 1985) and at other regions including the weight bearing bones of the appendicular skeleton (Myburgh *et al*, 1995). However it has also been shown that a history of amenorrhoea or oligoamenorrhoea regardless of the resumption of normal menses was more important than the current menstrual status for the prediction of bone mass (Micklesfield *et al*, 1995). Ultramarathon runners with a history of oligoamenorrhoea or amenorrhoea had a lower BMD at the lumbar spine compared to runners with normal menstrual cycles. In addition the MHI was significantly higher in the runners who had never had menstrual abnormalities and there was a significant correlation between the MHI and BMD at the lumbar spine.

In this study the MHI was recorded in sedentary controls and runners. Although 25 % of the total cohort of runners had a history of oligomenorrhoea compared to 6 % of the controls, none of the runners had a history of amenorrhoea and there was no significant difference in the MHI between the runners and controls. This may explain why this cohort of runners did not demonstrate a lower BMD at the lumbar spine as previously reported by Micklesfield *et al* (1998). When comparing the MHI of runners in the runners of the three different ethnic groups (Whites, Indians and Coloureds), a lower MHI was recorded in the White runners compared to the Indian runners (9.9 vs. 10.8), however this did not reach statistical significance ($p = 0.06$). Although there were no significant differences in the BMD at the various sites between the different ethnic groups, a history of stress fractures was only present in the white runners and the lower MHI may indicate an independent increased fracture risk in the White runners.

6.4.2 Gonadal hormones

Since menstrual irregularities contribute to bone mass, several studies have investigated the menstrual history in ultra distance runners (Marcus *et al*, 1985; Micklesfield *et al*, 1995). Low oestrogen concentrations associated with a lower bone mass have been reported in amenorrhoeic runners (Fisher *et al*, 1986). A lower BMD at the hip and spine has been reported in amenorrhoeic ultra distance runners in several studies (Drinkwater *et al*, 1984; Marcus *et al*, 1985; Drinkwater *et al*, 1990; Myburgh *et al*, 1993; Hetland *et al*, 1993). However, disturbances in

progesterone production related to exercise is not associated with decreased bone mass (Hetland *et al*, 1993; Winters *et al*, 1996; De Souza *et al*, 1997).

In the present study oestradiol and progesterone concentrations were measured in the runners and controls. In view of the fact that none of the runners and controls was amenorrhoeic we did not expect any hormonal differences. However, the oestradiol concentration was significantly increased in the runners ($p < 0.05$) compared to the control group. The unexpected finding of higher levels of oestrogen in the runners could be due to a number of reasons. Firstly, none of the runners were amenorrhoeic at the time blood was drawn. Secondly, blood was not drawn at mid-cycle and thirdly 15 % of the runners were on oral contraception, which may be responsible for elevated levels. Further information on the use of oral contraceptives was not available in 47 %. Since this group of runners were in the childbearing age group, and increased awareness of the risk of exercise induced amenorrhoea, it is possible that more of the runners were in fact on hormonal therapy.

In contrast the progesterone concentrations were significantly lower in the runners compared to controls ($p < 0.05$). The lower progesterone levels could be explained on subtle changes in menstruation such as short and inadequate luteal phase, which usually do not manifest as amenorrhoea. This is supported by the findings of a study examining menstrual phase dynamics, progesterone levels and the relationship to bone mass in eumenorrhoeic sedentary women and runners (De Souza *et al*, 1997). The women were categorized into three groups; sedentary ovulatory, exercising ovulatory and those exercising with luteal phase

defects. A lower total production of progesterone was found in the exercising and sedentary ovulatory groups compared to the runners with luteal phase defects. The production of progesterone during the luteal phase was also lower in the runners with luteal phase defects compared to runners who were ovulating. However there was no association between the lower progesterone levels and bone mass. These findings suggest that runners may have subtle alterations in the menstrual cycle resulting in lower progesterone levels and that these do not negatively impact on bone mass.

There was no difference in the FSH concentration in either group. However, the LH concentrations were significantly higher in the runners ($p < 0.0001$) compared to the controls. These findings compare to studies of athletes engaged in sports, which emphasize strength over leanness such as swimming and rowing, and not associated with restrictive diets. Their endocrine profile is characterised by elevated LH levels, elevated LH/FSH ratios and mild hyperandrogenism rather than reduced oestrogen concentration observed in athletes engaged in sports requiring leanness such as running (Frisch *et al*, 1984; Cummings *et al*, 1987; Constantini and Warren, 1995).

6.5 BONE TURNOVER IN ULTRADISTANCE RUNNERS AND HEALTHY SEDENTARY VOLUNTEERS

The pathogenesis of exercise-induced osteoporosis is complex with the interplay of several factors including oestrogen deprivation, changes in body composition and type of sport and training regimen. Essentially there is an

uncoupling of bone formation and resorption. The examination of biochemical markers in serum and urine are utilised as indicators of the overall bone formation and resorption and are an addition to the use of BMD in examining the effect of exercise on the skeleton in runners (Malm *et al*, 1993; Brahm *et al*, 1996). There are several limitations in the use of biochemical markers of bone formation and resorption. There is a significant intra-individual variation in the values, a diurnal circadian rhythm and variation in reproducibility. In addition they represent an average of turnover from all skeletal sites in the body and therefore are not site specific (Creighton *et al*, 2001).

Several studies have investigated the effect of exercise on markers of bone turnover and have reported conflicting results (Table 15).

In marathon/long-distance runners significantly reduced serum osteocalcin concentrations and bone alkaline phosphatase activity at the end of the marathon have been reported (Malm *et al*, 1993). These findings suggest a temporary inhibition of osteoblastic function during the marathon. Brahm *et al* (1996), reported a similar effect on bone formation. However in this cohort of subjects there was also an increase in bone resorption as indicated by an increase in carboxyterminal cross-linked telopeptide of type 1 collagen (ICTP) concentrations.

In a later study Brahm *et al*, (1997) compared PICP, ITCP, osteocalcin and bone specific alkaline phosphatase concentrations in 30 young runners (mean age 32 years) and 30 age and sex matched controls. The concentrations of PICP and ITCP were significantly lower in the runners compared to controls. There was no

Table 15: Studies reporting effect of exercise on markers of bone turnover. ↓ = Reduced concentration of bone marker; ↑ = Increased concentration of bone marker. OC = Osteocalcin; BSAP = Bone specific alkaline phosphatase; PICP = Procollagen 1 carboxyterminal peptide; ICTP = carboxyterminal cross-linked telopeptide of type 1 collagen.

	Study population	Bone markers measured	Results
Malm <i>et al</i> 1993	23 athletes (15 women 8 men)	Hydroxyproline OC BSAP	↓OC (20 % men 10 % women) ↓BSAP (women)
Brahm <i>et al</i> 1996	20 runners (10 men 10 women)	PICP ICTP OC BSAP	↑ ICTP (runners) ↓OC (men) BSAP (no difference)
Brahm <i>et al</i> 1997	30 runners (23 men 7 women) 30 controls	PICP ICTP OC BSAP	↓ PICP (runners) ↓ ITCP (runners) OC and BSAP (no difference)
Matsumoto <i>et al</i> 1997	103 male and female athletes	PICP BSAP Dpd Pyd	PICP and BSAP (no differences) ↑Dpd and ↑Pyd (male and female judoists)
Zanker and Swaine 1998	33 women distance runners (18 eumenorrhoeic 6 oligo menorrhoeic 9 amenorrhoeic)	OC BSAP Dpd	↓OC and ↓BSAP (runners) ↓Dpd (in oligo and amenorrhoeic subjects)
Creighton <i>et al</i> 2001	41 female athletes 7 controls. (14 volley ball and basket ball players, 13 soccer, track athletes and 7 swimmers)	OC NTx	↑ OC (high and medium impact groups) NTx (no differences in all groups and controls)

significant difference in osteocalcin and bone specific alkaline phosphatase concentrations in the two groups. The authors concluded that the reduction in both formation (PICP) and resorption (ITCP) markers, signified a low bone turnover state in runners. In another study serum osteocalcin and bone specific alkaline phosphatase and urinary deoxypyridinoline/creatinine were measured in 18 eumenorrhoeic, 9 amenorrhoeic and 6 oligomenorrhoeic women distance runners (Zanker and Swaine, 1998). Concentrations of all the bone markers were lower in the amenorrhoeic and oligomenorrhoeic women compared to the eumenorrhoeic women distance runners.

Different sporting activities may also have different effects on bone metabolism. In a study by Creighton et al (2001) osteocalcin and urinary cross-linked N-telopeptide concentrations were compared in 41 female athletes participating in high, medium and non-impact sports (14 volley and basket ball players, 13 soccer and track athletes and 7 swimmers) to a sedentary age-matched control subjects (n = 7). The OC concentration was greater in the high and medium impact groups compared to the non-impact and control group. No difference noted in the bone formation marker (NTx) concentrations in all groups (Creighton *et al*, 2001).

On the other hand in a study on 103 male and female collegiate athletes participating in long-distance running, judo and swimming, higher values for bone resorption markers (Pyd and Dpd) was reported in judoists compared to long distance runners and swimmers (Matsumumoto *et al*, 1997). However, there was

no difference in the bone formation markers (BSAP and PICP) between these groups.

In the present study both osteocalcin, a biochemical marker of bone formation, and deoxypyridinoline, a marker of bone resorption, were significantly reduced in the runners compared to the control group. There was no difference in either the urinary NTx concentration or the serum bone specific alkaline phosphatase. These results are similar to others who have previously found low osteocalcin concentrations (men) and PICP concentrations (women) during the days following marathon or distance running (Malm *et al*, 1993; Brahm *et al*, 1996; Brahm *et al*, 1997) and support the concept of low bone turnover in ultradistance runners. However, these results were in contrast with the report of no significant relationship between training and biochemical markers of bone turnover (Hetland *et al*, 1993). The discordance between osteocalcin and bone specific alkaline phosphatase may be explained by the fact that BSAP only reflects osteoblastic activity, while osteocalcin is released by active osteoblasts and from bone matrix, and by the differences in molecular size and thus clearance. Similarly the discordance between the bone resorption markers may be due to intra-subject variability and reproducibility.

This finding of a low bone turnover explains the lack of a detrimental effect on bone mass in our subjects. The fact that none of the runners were amenorrheic would also explain why bone turnover and particularly bone resorption was not increased.

6.6 STRESS FRACTURES

Stress fractures are common in participants in many types of sports and have been reported to account for 0.7 % - 20 % of all sports related injuries with the highest incidence in track and field athletes (Wilder and Sethi, 2004).

Numerous risk factors have been identified and include a rapid increase in training program, poor prior physical condition, menstrual disturbance, low bone turnover, decreased BMD (Lloyd *et al*, 1986; Myburgh *et al*, 1990, nutritional deficiencies, leg length inequality, running on irregular surfaces and inappropriate footwear (Sanderlin and Raspa, 2003).

An increased prevalence of stress fractures has been reported in female long distance runners in several studies (Barrow, 1988; Myburgh, 1990; Bennell *et al*, 1996; Johnson *et al*, 1994). In a study of 25 athletes, nineteen of whom were women, lower BMD, lower dietary calcium intake, current menstrual irregularity and lower contraceptive use were identified as risk factors for stress fractures (Myburgh *et al*, 1990). Although the association between menstrual disturbance and the prevalence of stress fracture has been confirmed in many studies, stress fractures have also been reported in eumenorrhoeic women. In a study investigating the prevalence of stress fractures and its relationship menstrual history although a higher prevalence was noted in women with menstrual abnormalities, (49 % in women with 0 – 5 menses/year and 39 % in women with 6 – 9 menses/year) women with normal menses also experienced fractures (29 %) (Barrow, 1988). In addition there was an increased prevalence of an eating

behaviour disorder in the amenorrhoeic women. In a prospective study of male and female track and field athletes 21.1 % of the subjects experienced a fracture in the 12 - month follow up period (Bennell *et al*, 1996). In the female athletes lower BMD, a history of menstrual disturbance, less lean mass in the lower limb, a discrepancy in leg length and a lower fat diet were identified as risk factors. No predictive risk factors were identified in the males.

In this study a history of stress fractures was obtained in 10 runners (18 %). On comparing the runners with stress fractures with those without stress fractures, there was no difference in the age, weight, menstrual history, hormonal markers, duration of running and the total distance run in the preceding six months. Of note is that there was no significant decrease in BMD. Rather, the BMD at the spine was significantly higher. However there was a significantly lower percentage total body fat in the runners with stress fractures. In addition the mean NTx concentrations were significantly lower in the runners with stress fractures. This finding suggests that the runners with stress fractures had a lower bone turnover, which may account for the stress fractures. The normal response to an increased mechanical load is an increase in remodelling with repair of the microdamage. With a decreased bone turnover rate there may be insufficient time for repair to occur. Alternatively, it is possible that the stress fractures and evidence for a low bone turnover are unrelated as all the stress fractures had occurred in the past and were not current.

The presence of stress fractures in this cohort of eumenorrhoeic women with normal or increased bone mineral density compared to controls emphasizes

the importance of additional risk factors for stress fractures such as biomechanical factors, excessive repetitive mechanical stress and nutrition.

6.7 ETHNIC DIFFERENCES IN ULTRADISTANCE RUNNERS

Ethnic differences in BMD have been reported in several different populations. Highest BMD measurements have been reported in African-Americans, lowest in Asians and intermediate in Caucasians. These differences may be due to genetic and environmental factors. Studies reporting a higher BMD in African – Americans have implicated a higher rate of obesity, greater frame size and greater muscle mass as contributing factors towards the greater bone mass (Ettinger *et al*, 1997). In a recent study a lower BMD was reported in immigrants from the Indian subcontinent to the United Kingdom compared to Caucasians (Mehta *et al*, 2004). However the difference disappeared on correction for bone size by estimating the volumetric bone mineral apparent density (BMAD).

Early South African studies reported a significantly lower prevalence of osteoporotic fractures in Black Africans compared to Whites (Solomon, 1968), however this was not reflected by a lower BMD as measured by metacarpal index (Solomon, 1979).

In a study comparing the bone mass in White, Coloured and Indian children, there was no difference in the weight and height adjusted bone mass in boys of the three ethnic groups (Patel *et al*., 1993). However, Coloured girls had a significantly greater bone mass than did the White and Indian girls. This is in

contrast to the studies that have shown no differences in spinal and femoral bone mass in White and Coloured adult women (Wagener and Hough, 1987; Kalla *et al*, 1994). The spinal BMD has also been shown to be similar in Black African and White females (Daniels *et al*, 1997), while a higher femoral BMD was seen in the Black African females, and particularly in those who were overweight.

To our knowledge there are no previous studies that have examined the effects of exercise on BMD in different ethnic groups.

One of the aims of this study was to determine whether there are any ethnic differences in the effect of exercise on BMD and bone markers. In this study we enrolled 30 white, 12 Indian and 10 Coloured female long distance runners. Unfortunately we were only able to recruit 3 Black African runners. Thus comparisons were only possible between the White, Coloured and Indian runners.

The white runners were significantly older than Indians and this may be an effect of the small sample size for the Indians. There was no difference in the weight, height and BMI in the three groups.

There was no significant difference in the BMD at all sites in the three ethnic groups. This finding is in contrast to the report of a lower BMD in Indian compared to White and Black medical students (Mohamed, 2001). This could be explained by firstly the effect of exercise on BMD and secondly by the effect of body composition. In the study of medical students the Indian female students had a significantly lower weight and total body fat compared to the Whites. In our

study while there was no difference in weight but the Indian runners had a significantly higher percentage body fat ($p < 0.001$).

Perhaps the most striking difference was the prevalence of stress fractures. While one third of the White runners had experienced a stress fracture, none of the Indian or Coloured runners gave a history of stress fracture. The absence of a difference in BMD in the three groups suggests that other factors beside BMD are important predictors for stress fractures. The only significant differences between the groups were that the White runners had a significantly lower percentage body fat than the Indian runners ($p < 0.001$) and that they also had run a longer total distance than the Indian runners in the preceding 6 months ($p < 0.05$). In addition the white runners tended to have lower MHI than the Indian runners however this did not reach statistical significance. However, although the Coloured runners also had a significantly lower percentage body fat ($p < 0.001$) and had a history of running for a longer duration ($p < 0.05$) than the Indian runners, none of the coloured runners had a history of stress fractures. There were no significant differences in the hormonal profile and biochemical markers, which would account for the increased prevalence of stress fractures in the White runners. Of importance is that the stress fractures had occurred in the past and any precipitating factor may have been corrected or resolved at the time of the study. It is more likely that the stress fractures would have occurred at the time of a rapid increase in training regimen. These runners were ultra-distance runners who had been training regularly. This study was not designed to include other biomechanical parameters such as leg length inequality or inappropriate footwear.

6.8 CONCLUSIONS

In this study the effects of exercise on body composition, BMD, biochemical markers of bone turnover and menstrual status were compared in ultra-distance runners and sedentary controls. To our knowledge this is the first study to compare three different ethnic groups.

1. The beneficial effect of ultra-distance running on BMD at the total legs and hip was demonstrated.
2. The ultradistance runners had a lower percentage body fat compared to controls.
3. This cohort of runners did not demonstrate any menstrual irregularity or oestrogen deficiency. The lower progesterone levels suggest the presence of subtle menstrual disturbance and had no effect on BMD.
4. A reduced bone turnover rate was demonstrated in the ultra-distance runners.
5. Stress fractures were recorded in 18 % of the runners. There was a lower percentage body fat in the runners with stress fractures compared to those without, however there was no difference in the BMD and hormonal status. The stress fractures only occurred in the White runners and may be due to other biomechanical factors.

6.9 LIMITATIONS AND FUTURE STUDIES

1. This was a cross-sectional study and it is possible that the runners had a higher BMD prior to their participation in the training, however our results are consistent with previous studies showing the site-specific increase in BMD with exercise. A longitudinal study will conclusively determine the effect of exercise on BMD.
2. The sample size particularly for the biochemical markers for bone turnover and for the ethnic groups may have not been sufficiently large to pick up differences and we were unable to recruit sufficient Black runners. Larger studies are needed to determine whether ethnic differences exist in BMD, biochemical markers of bone formation, body composition and risk factors for stress fractures.
3. The use of contraceptives was determined from a questionnaire and incomplete data were available. This would explain the surprising results of the higher oestrogen concentrations in the runners. The contraceptive use may have also influenced the BMD and future studies controlling for this variable in pre-menopausal women athletes would be of value.
4. The history of stress fractures was obtained from the questionnaire and as these had occurred in the past we were unable to determine the severity or risk factors. Future studies assessing the BMD, biochemical markers of bone turnover and biomechanical factors at the time of the stress fracture

are indicated to determine the incidence and risk factors for stress fractures in sub-elite endurance runners with normal menstrual function.

5. The relationship between the percentage body fat on BMD and menstrual function remains controversial. Future studies assessing the role of leptin on body composition in normally menstruating and amenorrhoeic athletes may provide valuable information.

BIBLIOGRAPHY

Allen SR. (2001) Exercise: Her options. *South Med J*, 94(12): 1143 - 1144.

Alexander M. (1976) The Comrades Marathon Story. 2nd ed. Juta & Company Limited Cape Town.

American College of Sports Medicine. (1997) Position Stand: The female athlete triad. *Med Sci Sports Exerc*, 29(5) pp. i – ix.

Arden NK, Spector TD, Eds. (1997) Osteoporosis Illustrated. Published by Current Medical Literature Ltd., London NW1 3ND, UK.

Ascott-Evans B H. (1999) The role of quantitative ultrasound in the management of osteoporosis. 35th SEMDSA, 9th Bone & Mineral Metabolism DESSA Workshop. April: (Abstract) 45.

Baron RE. (1996) Chapter 1. Anatomy and ultrastructure of bone. In: Primer on the bone diseases and disorders of mineral metabolism. 3rd ed. Favus MJ Ed. Philadelphia, Lippincott - Raven. 3 - 10.

Barrow GW, Dahan S. (1988) Menstrual irregularity and stress fractures in collegiate female distance runners. *Am J Sports Med*, 16(3): 209 - 216.

Bass SL, Myburgh KH. (2000) Chapter 15: The role of exercise in the attainment of peak bone mass and bone strength. In: Sports Endocrinology. Michelle P Warren, Constantini Naama W Eds. Human Press Inc. Totowa, New Jersey. 253 - 280.

Bennel KL, Malcolm SA, Thomas SA, Wark JD, Brukner PD. (1996) The incidence and distribution of stress fractures in competitive track and field athletes. *Am J Sports Med*, 24(2): 211 - 217.

Bennell KL, Malcolm SA, Thomas SA, Reid SJ, Brukner PD, Ebeling PR, Wark JD. (1996) Risk factors for stress fractures in track and field athletes: A twelve-month prospective study. *Am J Sports Med*, 24(6): 810 - 818.

Bilezikian JP. (1999) Osteoporosis in men. *J Clin Endocrinol Metab*, 84(10): 3431 – 3434.

Bishop ML, Duben-Engelkirk JL, Fody EP Eds. (2000) Clinical Chemistry: Principles, procedures, correlations 4th ed. J.B. Lippincott Company. Philadelphia.

Blain H, Vuillemin A, Guillemin F, Durnat R, Hanesse B, de Talance N, Doucet B, Jeandel C. (2002) Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J Clin Endocrinol Metab*, 87(3): 1030 - 1035.

Boling EP. (2001) Gender and Osteoporosis: Similarities and Sex-Specific Differences. *J Gend Specif Med*, 4(2): 36 - 43.

Bollen AM, Eyre DR. (1994) Bone resorption rates in children monitored by the urinary assay of collagen type 1 cross-linked peptides. *Bone*, 15(1): 31 - 34.

Bonjour J-P, Theintz G, Buchs B, Slosman, Rizzoli R. (1991) Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. *J Clin Endocrinol Metab*, 73: 555 - 563.

Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E. (1998) Moderate exercise during growth in pubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. *J Bone Miner Res*, 13(12): 1814 - 21.

Brahm H, Piehl-Aulin, Ljunghall S. (1996) Biochemical markers of bone metabolism during distance running in healthy, regularly exercising men and women. *Scand J Med Sci Sports*, 6: 26 - 30.

Brahm H, Ström H, Piehl-Aulin K, Mallmin H, Ljunghall S. (1997) Bone metabolism in endurance trained athletes: A comparison to population-based controls based on DXA, SXA, quantitative ultrasound, and biochemical markers. *Calcif Tissue Int*, 61: 448 - 454.

Brahm H, Mallmin H, Michaëlsson K, Ström H, Ljunghall S. (1998) Relationships between bone mass measurements and lifetime physical activity in a Swedish population. *Calcif Tissue Int*, 62: 400 - 412.

Burger EH, Klein-Nulend J. (1999) Mecanotransduction in bone – role of lacuno-canicular network. *FASEB J*, 13: S101 - S112.

Caillot-Augusseau A, Lafage-Proust M-H, Soler C, Pernod J, Buboïs F, Alexandre C. (1998) Bone formation and resorption biological markers in cosmonauts during and after a 180-day space flight (Euromir 95). *Clin Chem*, 44(3): 578 - 585.

Carbon R. (1994) Female Athletes. *Br Med J*, 309: 254 - 258.

Carlberg KA, Buckman MT, Peake GT, Riedesel ML. (1983) Body composition of oligo / amenorrheic athletes. *Med Sci Sports Exerc*, 15(3): 215 - 217.

Carter DR. (2000) Pilot study to predict bone strength during osteoporosis.
<http://wwwguide.stanford.edu/Projects/2kprojects/osteo1.html>

Cassim B. (2001) Epidemiology and causes of osteoporosis. *Continuing Med Edu*, 19: 72 - 85.

Chestnut III CH. (1993). Bone mass and exercise. *Am J Med*, 95 (Supp 5A): 5A-34S - 5A - 36S.

Chestnut III CH, Bell NH, Clark GS, Drinkwater BL, English SC, Johnston Jr. C, Notelovitz M, Rosen C, Cain DF, Flessland KS, Mallinak NJS. (1997) Hormone replacement therapy in postmenopausal women: Urinary n-telopeptide of type 1

collagen monitors therapeutic effect and predicts response of bone mineral density. *Am J Med*, 102: 29 - 37.

Christenson RH. (1997) Biochemical markers of bone metabolism: An Overview. *Clin Biochem*, 30: 573 - 578.

Comrades Marathon Association. (2004) <http://www.comrades.com>

Consensus Development Conference. (1993) Diagnosis, prophylaxis and treatment of osteoporosis. *Am J Med*, 94: 646 - 50.

Constantini NW & Warren MP. (1995) Menstrual dysfunction in swimmers: a distinct entity. *J Clin Endocrinol Metab*, 80: 2740 - 2744.

Cooper C. (1997) The crippling consequences of fractures and their impact on quality of life. *Am J Med.*, 103(Suppl): 12 - 19.

Cormier C, Souberbielle J-C, Kindermans C. (1997) Diagnostic and therapeutic aspects of calcitropic hormones. *Curr Opin Rheumatol*, 9: 370 - 376.

Council of the National Osteoporosis Foundation. (1996) Guidelines for the early detection of osteoporosis and prediction of fracture risk. *S Afr Med J*, 86(9): 1113 - 1116.

Creighton DL, Morgan AL, Boardley D, Brolinson PG. (2001) Weight-bearing exercise and markers of bone turnover in female athletes. *J Appl Physiol*, 90(2): 565 - 570.

Cummings DC, Wall SR, Galbraith MA, Belcastro AN. (1987) Reproductive hormone responses to resistance exercise. *Med Sci Sports Exerc*, 19: 234 - 238.

Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, & Vogt. (1995) Risk factors for hip fracture in white women. Study of osteoporotic fractures research group. *N Eng J Med*, 332: 767 - 773.

Dale E, Gerlach DH, Wilhite AL. (1979) Menstrual dysfunction in distance runners. *Obstet Gynecol*, 54: 47 - 53.

Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ. (1988) Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. *Ann Int Med*, 108: 824 - 828.

Daniels ED, Pettifor JM, Schnitzler CM, Russell SW, Patel DN. (1995) Ethnic differences in bone density in female South African nurses. *J Bone Miner Res*, 10(3): 359 - 367.

Daniels ED, Pettifor JM, Schnitzler CM, Moodley GP, Zachen. (1997) Differences in mineral homeostasis, volumetric bone mass and femoral neck axis length in black and white South African women. *Osteoporos Int*, 7(2): 105 - 112.

Davidson M, DeSimone ME. (2002) Osteoporosis update. *Clin Reviews* 12(4): 75 - 82.

Dempster DW. (1995) Chapter 3. Bone remodeling. In: Riggs BL, Melton III LJ Eds. *Osteoporosis. Etiology, diagnosis, and management*. Lippincott-Raven, Philadelphia. Pg. 67 - 91.

De Souza MJ, Miller BE, Sequenzia LC, Luciano AA, Stier S, Prestwood K, Lasley BL. (1997) Bone health is not affected by luteal phase abnormalities and decreased ovarian progesterone production in female runners. *J Clin Endocrinol Metab*, 82(9): 2867 - 2876.

Drinkwater BL, Nilson K, Chestnut III CH, Bremner WJ, Shainholtz S, Southworth MB. (1984) Bone mineral content of amenorrheic and eumenorrheic athletes. *N Engl J Med*, 311(5): 277 - 281.

Drinkwater BL Nilson K, Ott S, Chestnut III CH. (1986) Bone mineral density after resumption of menses in amenorrheic athletes. *J Am Med Assoc*, 256(3): 380 - 382.

Drinkwater BL, Breumner B, Chestnut III CH. (1990) Menstrual history as a determinant of current bone density in young athletes. *J Am Med Assoc*, 263(4): 545 - 548.

Drinkwater BL. (1995) Weight-bearing exercise and bone mass. *Phys Med Rehab Clinics of N Am*, 6(3): 567 - 577.

Drinkwater BL. (1996) Exercise and bones. *Am J Sports Med*, 24(6): S33 - S35.

Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell*, 100: 197 - 207.

Dunitz M. (1995). Chapter I: Bone Remodeling. In: Mundy GR, Bone remodeling and its disorders. London. Pg 1 - 11.

Durnin JVGA, Womersley J. (1974) Body fat assessed from total body density and its estimation from skin fold thickness: measurements on 481 men and women aged from 16-72 years. *Br J Nutr*, 32: 77 - 97.

Ebling PR. (2000) Pharmacological Management of Osteoporosis in 2000. *Aust J Hosp Pharm*, 30: 111 - 15.

Elliot KJ, Sale C, Cable NT. (2002) Effects of resistance training and detraining on muscle strength and lipid profiles in postmenopausal women. *Br J Sports Med*, 36: 340 - 344.

Erickson SM, Servier TL. (1997) Osteoporosis in active women: Prevention, diagnosis, and treatment. *Physic Sportsmed*, 25(11): 1 - 9.

Ettinger B, Sidney S, Cummings SR. (1997) Racial Differences in Bone Density between Young Adult Black and White Subjects Persist after Adjustment for Anthropometric, Lifestyle, and biochemical Differences. *J Clin Endocrinol Metab*, 82: 429 - 434.

Faulkner KG. (2001) Update on bone density measurements. In: Rheumatology disease clinics of North America. Osteoporosis. Nancy E Lane, Guest Ed Vol 27 No.1 Feb., WB Saunders Company USA. 81 - 99.

Feicht CB, Johnson TS, Martin BJ, Sparkes KE, Wagner WW. (1978) Secondary amenorrhoea in athletes. *Lancet*, 2: 1145 - 1146.

Felson DT, Zhang Y, Hannan MT, Anderson JJ. (1993) Effects of weight and body mass index on bone mineral density in men and women: Framingham study. *J Bone Miner Res*, 8: 567 - 73.

Fenton AJ. (2000) Update on athletic amenorrhoea and bone loss. (Roche Pharmaceuticals weekend work-shop proceedings bulletin, Christchurch Feb. 2000) Advances in diagnosis, prevention and treatment of osteoporosis. 22 - 24.

Ferrari SL, Garnero P, Ahn-Luong LE, Montgomery H, Humphries S, Greenspan S. (2000) A functional polymorphic variant in the IL-6 gene promoter associated with low bone resorption in postmenopausal women. *Osteoporos Int*, 11(Suppl 2): S147.

Finkelstein JS, Sowers M, Lee MT, Ettinger B, Neer RM, Kelsey JL, Cauley JA, Huang M-H, Greendale GA. (2002) Ethnic variation in bone density in premenopausal and early perimenopausal women: Effects of anthropometric and lifestyle factors. *J Clin Endocrinol Metab*, 87(7): 3051 - 3056.

Fisher EC, Nelson ME, Frontera WR, Turksoy RN, Evans WJ. (1986) Bone mineral content and levels of gonadotropins and estrogens in amenorrheic running women. *J Clin Endocrinol Metab*, 62: 1232 - 1236.

Forwood MR. (2000) Exercise recommendations for osteoporosis. *Aust Fam Physician*, 29(8): 761 - 764.

Friedman JM. (2000) Obesity in the new millennium. *Nature*, 404(6778): 632 - 634.

Frisch RE & McArthur JW. (1974) Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*, 185: 949 - 951.

Frisch RE, Wyshak G, Vincent L. (1980) Delayed menarche and amenorrhea in ballet dancers. *N J Med*, 303(1): 17 - 19.

Frisch RE, Hall GM, Aoki TT, Birnholz J, Jacob R, Landsberg L, Munro H, Parker-Jones K, Tulchinsky D, Young J. (1984) Metabolic, endocrine, and reproductive changes of a woman channel swimmer. *Metabolism: Clin Experiment*, 33: 1106 - 1111.

Frost HM. (2000) Muscle, bone, and the Utah paradigm: a 1999 overview. *Med Sci Sports Exerc*, 32(5): 911 - 917.

Frost ML, Blake GM, Fogelman I. (2001) Quantitative ultrasound and bone mineral density are equally strongly associated with risk factors for osteoporosis. *J Bone Miner Res*, 16: 406 - 416.

Galle PC, Freeman EW, Galle MG, Huggins GR, and Sondheimer ST. (1983) Physiological and psychologic profiles in a survey of women runners. *Fertil Steril*, 39: 633 - 639.

Gambacciani M, Ciaponi M, Cappagli B, Piaggese L, De Simone L, Orlandi R, Genazzani AR. (1997) Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. *J Clin Endocrinol Metab*, 82: 414 - 417.

Ganant HK, Vogler JB, Block JE. (1988) Radiology of osteoporosis. In: RiggsBL, Melton LJ III, Eds. Osteoporosis, Etiology, diagnosis and management. New York Raven Press. 181 - 822.

Garnero P, Shih WJ, Gineyts E, Karpf, DB, Delmas PD. (1994) Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to Alendronate treatment. *J Clin Endocrinol Metab*, 79: 1693 - 1700.

Garnero P, Arden NK, Griffiths G, Delmas PD, Spector TD. (1996) Genetic influence on bone turnover in postmenopausal twins. *J Clin Endocrinol Metab*, 81(1): 140-6.

Gidwani GP. (1999) Amenorrhea in the athlete. *Adolesc Med*, 10: 273 - 290.

Goltzman D. (2000) The contribution of basic science to the field of osteoporosis. *Osteoporos Int*, (Suppl 2): S43 - S46.

Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. (1996) Reduced bone density and osteoporosis associated with polymorphic Sp 1 binding site in the collagen type1 α 1 gene. *Nat Genet*, 14: 203 - 205.

Gremion G, Rizzoli R, Slosman D, Theintz G, Bonjour J-P. (2001) Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. *Med Sci Sports Exerc*, 33(1): 5 - 21.

Grinspoon S, Thomas E, Pitts S, Gross E, Mickley D, Miller K, Herzog D, Klibanski A. (2001) Prevalence and predictive factors for regional osteopenia in women with anorexia nervosa. *Ann Int Med*, 133(10): 790 - 794.

Gutin B, Kasper MJ. (1992) Can vigorous exercise play a role in osteoporosis prevention? A review. *Osteoporos Int*, 2(2): 55 - 69.

Harmon KG. (2002) Evaluating and treating exercise-related menstrual irregularities. *Physician Sports Med*, 30(3): 1 - 8.

Heaney RP. (1993) Bone mass, nutrition, and other lifestyle factors. *Am J Med*, 95(Suppl 5A) 5A - 29S - 5A - 32S.

Heaney RP, Matkovic V. (1995) Chapter 5. Inadequate peak bone mass. In: Riggs BL, Melton LJ III, Eds. *Osteoporosis: Etiology, diagnosis and management*. 2nd ed. Philadelphia, Lippincott-Raven. 115 - 131.

Heaney RP. (1988) Chapter 13. Nutritional factors in bone health. In: *Osteoporosis, etiology, diagnosis and management*. Riggs BL, Melton III LJ Eds. Lippincott – Raven Philadelphia. 359 - 372.

Heaney RP. (2000) Sources of bone fragility. *Osteoporos Int*, (Suppl 2): S43 - S46.

Heath H. (1985) Athletic women, amenorrhea, and skeletal integrity. *Ann Int Med*, 102(2): 258 - 260.

Heinonen A, Oja P, Kannus P, Sievänen H, Mänttari A and Vouri I. (1993) Bone mineral density of female athletes in different sports. *Bone Miner*, 23: 1 - 14.

Heinonen A, Kannus P, Sievänen H, Oja P, Pasanen M, Rinne M, Uusi-Rasi K, Vuori I. (1996) Randomized controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. *Lancet*, 348: 1343 - 1347.

Heinonen A, Oja P, Sievänen. Pasanen M, Vuoril. (1998) Effect of two training regimens on bone mineral density in healthy perimenopausal women: A randomized controlled trail. *J Bone Miner Res*, 3: 483 - 490.

Hendrix MK and Lohman TG. (1981) Incidence of menstrual disorders in female collegiate athletes and its relation to body fat content. *Med Sci Sports Exerc*, 13: 104.

Hetland ML, Haarbo J, Christiansen C. (1993) Running induces menstrual disturbances but bone mass is unaffected, except in amenorrheic women. *Am J Med*, 95: 53 - 59.

Hindsø K, Lauritzen JB. (2000) Intervention study with hip protectors in orthopaedic patients with hip fractures. *Osteoporos Int*, 11 (Suppl 2): S150.

Hologic® QDR Series User's Manual. (2000) Hologic, Inc. 35 Crosby Drive Bedford, MA 01730 USA.

Holloway WR, Collier FM, Aitken CJ, Hodge JM, Malakellis M, Gough TJ, Collier GR, Nicholson GC. (2002) Leptin inhibits osteoclast generation. *J Bone Miner Res*, 17(2): 200 - 209.

Holmes-Walker J, Prelevic GM, Jacobs HS. (1995) Effects of calcium and exercise on bone mineral density in premenopausal women with osteoporosis. *Curr Opin Obstet Gynecol*, 7: 323 - 326.

Horribin DF. (1996) Osteoporosis (Congress)
Session IV: Nutrition and bone, calcium metabolism, osteoporosis and essential fatty acids: A Review. Pg. 41 - 44.

Hough S. (2004) Population differences in parameters of bone and mineral metabolism – the African fracture paradox. *JEMDSA*, 9(1): 5 - 6.

Hughes VA, Frontera WR, Dallal GE, Lutz KJ, Fisher EC, Evans WJ. (1994) Muscle strength and body composition: associations with bone density in older subjects. *Med Sci Sports Exerc*, 967 - 974.

Ingber D. (1999) How cell (might) sense microgravity. *FASEB J*, 13: S3 - S15.

Internet (2001) What is osteoporosis? *National Institutes of Health, Sponsored by GE Medical Systems, Last modified: Jan 25, 2001*
http://www.hersource.com/osteo/c1/whatis_osteo.cfm.

Iqbal MM. (2000) Osteoporosis: Epidemiology, Diagnosis, and treatment. *South Med J*, 92(1): 2 - 18.

Ivers RQ. (2000) Visual Impairment and risk of hip fracture. *Osteoporos Int*, (Suppl 2): S43 - S46.

Ivers RQ, Cummings RG, Mitchell P, Peduto A, (2000) Visual impairment and risk of hip fracture. *Osteoporos Int*, 11 (Suppl 2): S113.

Jilka RL, Weinstein RS, Bellido T, Parfit AM, and Manolagas SC. (1998) Osteoblast programmed cell death (apoptosis): Modulation by growth factors and cytokines. *J Bone Miner Res*, 13: 793 - 802.

Jilka RL. (1998) Cytokines, bone remodeling, and estrogen deficiency: A 1999 update. *Bone*, 23: 75 - 81.

Joakimsen M, Magnus JH, Fønnebø. (1997) Physical activity and predisposition for hip fractures: A review. *Osteoporos Int*, 7: 503 - 513.

Johnson BD. (2003) Bone mineral health in children. http://www.pens.org/articles/johnson-barbara-d_bone-mineral-health-in-children.htm

Johnston Jr. CC and Melton III LJ. (1995) Chapter 12. Bone densitometry. In: Riggs BL, Melton III LJ, Eds Osteoporosis: Etiology, diagnosis, and management. 2nd ed. Lippincott - Raven Philadelphia. 275 - 297.

Kalla AA, Fataar AB, Bewerunge. (1994) Assessment of age-related bone loss in normal South African women by means of the Hologic 1000 system. *S Afr Med J*, 84: 398 - 404.

Kanis JA. (1994) Osteoporosis. *Blackwell Science Ltd.*, UK.

Kannus P, Palvanen M, Kaprio J, Parkkari J, Koskenvuo M. (1999) Genetic factors and osteoporotic fractures in elderly people: prospective 25 year follow up of a nationwide cohort of elderly Finnish twins. *Br Med J*, Nov 20; 319 (7221): 1334 - 7.

Katz WA, Sherman C. (1998) Exercise for osteoporosis. *Physician Sports Med*, 26(2): 10 - 19.

Kaufman JD. (2000) Osteoporosis tests.

http://orthoinfo.aaos.org/fact/thr_report.cfm?Thresd_ID=176

Kelly PJ, Eisman JA, Stuart MC, Pocock NA, Sambrook PN, Gwinn TH. (1990) Somatomedin - C, physical fitness and bone density. *J.Clin Endocrinol Metab.*, 70:718 - 723.

Khan KM, Liu-Ambrose T, Sran MM, Ashe MC, Donaldson MG. (2002) Because 'osteoporosis' is rare, should the ACSM include 'osteopenia' among the criteria for defining the 'female athlete triad' syndrome? UBC Bone and Health Research Group, Departments of Family practice & Human Kinetics; BC Women's Hospital and Health Centre, Osteoporosis Programme, UBC, Vancouver, Canada. <http://www.bonebook.net/articles/LeaderV6post.doc>.

Khosla S and Melton LJ III (1995) Chapter 8. Secondary osteoporosis. In: Riggs BL, Melton III LJ Eds. Osteoporosis. Etiology, diagnosis, and management. Lippincott-Raven, Philadelphia. Pg. 183 -204.

Kirchner EM, Lewis RD, O'Connor PJ. (1995) Bone mineral density and dietary intake of female college gymnasts. *Med Sci Sports Exerc*, 543 - 549.

Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. (1996) Association of bone mineral density with polymorphism of estrogen receptor gene. *J Bone Miner Res*, 11(3): 306 - 311.

Korpelainen R, Orava S, Karpakka J, Hulkko A. (2001) Risk factors for recurrent stress fractures in athletes. *Am J Sports Med*, 29(3): 304 - 310.

Kröger H, Heikkinen J, Laitinen K, Kotaniemi A. (1992) Dual-energy X-ray absorptiometry in normal women: A cross-sectional study of 717 Finnish volunteers. *Osteoporosis Int*, 2: 135 - 140.

Kruger MC, de Winter RM, Becker PJ, Vorster HH. (2004) Changes in markers of bone turnover following urbanization of black South African women. *JEMDSA*, 9(1): 8 - 14.

Kumar V, Cotran RS, Robbins SL. (1997) Basic Pathology 6th ed. WB Saunders Company, Philadelphia, USA.

Lane NE, Oehlert JW, Bloch DA, Fries JF. (1998) The relationship of running to osteoarthritis of the knee and hip and bone mineral density of the lumbar spine: A 9-year longitudinal study, *J Rheumatol*, 25: 334 - 341.

Lane NE. (Guest editor) (2001) Rheumatic Disease Clinics of North America. (February) Osteoporosis. Vol 27; No 1 WB Saunders Company USA.

Lau EM, Suriwongpaisal P, Lee JK. (2001) Risk factors for hip fracture in Asian men and women: the Asian osteoporosis study. *J Bone Miner Res*, 16: 572 - 580.

Laughlin GA, Yen SS. (1997) Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. *J Clin Endocrinol Metab*, Jan; 82(1): 318 - 21.

Lee EJ, Long KA, Risser WL, Poindexter HBW, Gibbons WE, Goldzieher J. (1995) Variations in bone status of contralateral and regional sites in young athletic women. *Med Sci Sports Exerc*, 27(10): 1354 - 1361.

Lindeque BG. (1996) Amenorrhoea – or exercise induced osteoporosis – what is new? Congress on Osteoporosis, proceedings of Bakubung meeting, Session III: So What's New? Pg. 37 - 39.

Lindsay R. (1993) Pathogenesis of postmenopausal osteoporosis. *Bailliere's Clin Rheumatol*, 7(3): 499 - 513.

Lindsay R. (1995) Chapter 6; Estrogen deficiency. In: Osteoporosis: Etiology, diagnosis, and management, 2nd ed. B Lawrence Riggs, L. Joseph Melton III Eds. Mayo Foundation. Published by Lippincott - Raven, Philadelphia. 133 – 160.

Lloyd T, Triantafyllou SJ, Baker ER, Houts PS, Whiteside JA, Kalenak A, Stumpf PG. (1986) Women athletes with menstrual irregularity have increased musculoskeletal injuries. *Med Sci Sports Exerc*, 18(4): 374 - 379.

Lloyd T, Chinchilli VM, Johnson-Rollings N, Kieselhorst K, Egli DF, Marcus R. (2000) Adult female hipbone density reflects teenage sports-exercise patterns but not teenage calcium intake. *Pediatr*, 106: 40 - 44.

Lloyd T. (2000) Adult female hipbone density reflects teenage sports-exercise patterns but not calcium intake.

http://www.findarticles.com/m0950/1_106/63602488/p1/article.html

Long-term bed rest 07/08/2002 (Internet)

<http://www.medes.fr/Clinic/Experiments/LTBR/PublicSection/Anglais/PressKit.html>

Loucks AB, Horvath SM. (1985) Athletic amenorrhea: a review. *Med Sci Sports Exerc*, 17(1): 56 - 72.

Loucks AB. (1990) Effects of exercise training on the menstrual cycle: existence and mechanisms. *Med Sci Sports Exerc*, 22: 275 - 280.

Loucks AB. (2000) Chapter 9: Exercise training in the normal female. In: Sports Endocrinology. Michelle P. Warren, Naama W. Constantini Eds. Human Press Inc. Totowa, New Jersey. 165 - 180.

Lukert BP, Raisz LG. (1990) Glucocorticoid – induced osteoporosis: Pathogenesis and management. *Ann Int Med*, 112: 352 – 364.

Lunar News. (1998) Bone Ultrasonometer: Stiffness predicts bone strength. Lunar Corporation June: 13 - 14.

Lutter JM, Cushman S. (1982) Menstrual patterns in female runners. *Physician Sports Med*, 10(9): 60 - 72.

Lytle LD, Messing RB, Fisher L, Phebus L. (1975) Effects of long-term corn consumption on brain serotonin and response to electric shock. *Science*, 190: 692 – 694.

Macut D, Micic D, Pralong FP et al (1998) Is there a role for leptin in human reproduction? *Gynecol Endocrinol*, 12(5): 321 - 326.

Maddalozzo GF, Snow CM. (2000) High intensity resistance training: Effects on bone in older men and women. *Calcif Tissue Int*, 66: 399 - 404.

Madsen KL, Adams WC, Van Loan MD. (1997) Effects of physical activity, body weight and composition, and muscular strength on bone density in young women. *Med Sci Sports Exerc*, 114 - 120.

Maeta M, Oka S, Saito H, Ikeguchi M, Tsujitani S, Kaibara N. (2001) Plasma leptin level, the adipocyte-specific product of the *obese* gene, is associated with tumor progression and is a marker of the nutritional status of patients with gastric cancer. *Yonago Acta Medica*, 44: 143 - 149.

Malm HT, Ronni-Sivula HM, Viinikka LU, Ylikorkala OR. (1993) Marathon running accompanied by transient decreases in urinary calcium and serum osteocalcin levels. *Calcif Tissue Int*, Mar; 52(3): 209 - 11.

Manolagas SC and Weinstein RS. (1999) Perspective new developments in the pathogenesis and treatment of steroid-Induced osteoporosis. *J Bone Miner Res*, 14(7): 1061 - 1066.

Mantzoros CS. (1999) The role of leptin in human obesity and disease: A review of current evidence. *Ann Intern Med*, 130: 671 - 680.

Marcus R, Kosek J, Pfefferbaum A, Horning S. (1983) Age-related loss of trabecular bone in premenopausal women: A biopsy study. *Calcif Tissue Int*, 35: 406 - 409.

Marcus R, Cann C, Madvig P, Minkoff J, Goddard M, Bayer M, Martin M, Gaudiani L, Haskell W, Genant H. (1985) Menstrual function and bone mass in elite women distance runners. *Ann Int Med*, 102: 158 - 163.

Marieb EN. (1989) Chapter 6. Bones and bone tissue. In : Human anatomy and physiology. The Benjamin/Cummings Publishing Company, Inc. Redwood City, California USA. 152 - 171.

Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BEC. (1979) Bone status and fracture rates in two regions of Yugoslavia. *Am J Clin Nutr*, 31: 540 - 549.

Matkovic V, Jelic T Wardlaw GM, Ilich JZ, Goel PK, Wright JK *et al.* (1994) Timing of peak bone mass in Caucasian females and its implications for the prevention of osteoporosis. *J Clin Invest*, 93: 799 - 808.

Matsumoto T, Nakagawa S, Nishida S, Hirota R. (1997) Bone density and metabolic markers in active collegiate athletes: findings in long distance runners, judoists, and swimmers. *Int J Sports Med*, 18(6): 408 - 412.

Mazess RB, Barden HS. (1991) Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. *Am J Clin Nutr*, 53: 132 - 142.

Mazess RB, (1982) On ageing bone loss. *Clin Orthop*, 165: 239 - 252.

McCann L, Holmes DS. (1984) Influence of aerobic exercise on depression. *J Personal Soc Psych*, 46: 1142 - 1147.

McGuigan FEA, Murray L, Gallagher A, Davey-Smith G, Neville CE, Van'T Hof R, Boreham C, Ralston SH. (2002) Genetic and environmental determinants of peak bone mass in young men and women. *J Bone Miner Res*, 17: 1273 - 1279.

Mehta G, Taylor P, Petley G, Dennison E, Cooper C, Walker-bone K. (2004) Bone mineral status in immigrant Indo-Asian women. *Q J Med*, 97: 95 - 99.

Melton III LJ, Chrischilles EA, Cooper C. (1992) How many women have osteoporosis? *J Bone Miner Res*, 7: 1005 - 1010.

Melton III LJ, Khosla S, Atkinson EJ, O'Fallon WM, Riggs BL. (1997) Relationship of bone turnover to bone density and fractures. *J Bone Miner Res*, 12(7): 1083 - 1091.

Meyerson M, Gutin B, Warren MP, May MT, Contento I, Lee M, Pi-Sunyer X, Pierson Jr. RN, Brooks-Gunn J. (1991) Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. *Med Sci Sports Exerc*, 23(1): 15 - 22.

Micklesfield LK, Lambert EV, Fataar AB, Noakes TD, Myburgh KH. (1995) Bone mineral density in mature, premenopausal, ultramarathon runners. *Med Sci Sports Exerc*, 27(5): 688 - 696.

Micklesfield LK, Reyneke L, Fataar AB, Myburgh KH. (1998) Long-term restoration of deficits in bone mineral density is inadequate in premenopausal women with prior menstrual irregularity. *Clin J Sport Med*, 8(3): 155 - 163.

Mohamed S, Norris SA, Daniels ED and Pettifor JM. (2001) Peak bone mass and body composition differences between second year White, Black and Asian medical students in Johannesburg. 37th SEMDSA, 10th Bone and Mineral, 4th SASSO, 5th DESSA Workshop. Pg. 31.

Morris FL, Naughton GA, Gibbs JI, Carlson JS, Wark JD. (1997) Prospective ten-month exercise intervention in premenarcheal girls: positive effects on bone mass. *J Bone Miner Res*, 12(9): 1453 - 62.

Morrison NA, Oi JC, Tokita A, Kelly PJ, Crofts L, Ngugen TV, Sambrook PN and Eisman JA, (1994) Prediction of bone density from vitamin D receptor alleles. *Nature*, 367: 284 - 287.

Mundy GR, (1995) Chapter 1. Bone remodeling. In: Mundy GR Bone remodeling and disorders. Martin Dunitz: (Chapter 1). London.

Mundy GR. (2000) Secondary osteoporosis / Editorial: The potential relevance of leptin and low body weight. *Ann Intern Med*, 133(10): 828 – 830.

Myburgh KH, Hutchins J, Fataar AB, Hough SF, Noakes TD. (1990) Low bone density is an etiologic factor for stress fractures in athletes. *Ann Intern Med*, 113(10): 754 - 759.

Myburgh KH, Watkin VA, Noakes TD, (1992) Are risk factors for menstrual dysfunction cumulative? *Physician Sports Med*, 20(4): 114 - 122.

Myburgh KH, Bachrach LK, Lewis BL, Kent K, Marcus R. (1993). Low bone mineral density at axial and appendicular sites in amenorrheic athletes. *Med Sci Sports Exerc*, 25: 1197 - 1202.

National Institute Of Health Consensus Development Conference Statement. (2000) Osteoporosis prevention, diagnosis, and therapy. March 27 - 29, 2000. 17(1): 1 - 13.

Nattiv A, Agostini R, Drinkwater B, Yeager KK. (1994) The female athlete triad: The inter-relatedness of disorder eating, amenorrhea, and osteoporosis. *Clin Sports Med*, 13(2): 405 - 418.

Neer R. (1995) Chapter 73. Osteoporosis. In: Endocrinology Vol 2, 3rd ed. Leslie J De Groot Eds. Philadelphia: WB Saunders. 1228 - 1258.

Nelson ME, Fisher EC, Castos PD, Meredith CN, Turskoy RN, Evans WJ. (1986) Diet and bone status in amenorrheic runners. *Am J Clin Nutr*, 43: 910 - 916.

New SA. (2001) The role of physical activity in development and maintenance of bone health throughout the lifecycle. The 1st Joint Meeting of the International

Bone and Mineral Society and the European Calcified Tissue Society. Madrid, Spain, June 5 / 10 / 2001.

Nichols DL, Sanborn CF, Bonnick SL, Gench B, DiMarco N. (1994) Relationship of regional body composition to bone mineral density in college females. *Med Sci Sports Exerc*, 178 - 182.

Niesler HM. (1991) Effects of exercise on Laboratory Tests: An overview. *Clin Lab Sci*, 4(3): 164 - 167.

NIH Consensus Development Panel. (2001) Osteoporosis prevention, diagnosis and therapy. *J Am Med Assoc*, 285: 785 - 355.

Noakes TD, van Gend M. (1988) Menstrual dysfunction in female athletes. A review for clinicians. *S Afr Med J*, 73: 350 - 355.

Nordin BEC, Morris HA. (1989) The calcium deficiency model for osteoporosis. *Nutr Rev*, 47: 65 - 72.

Parfitt AM, Mundy GR, Roodman GD, Hughes DE, Boyce BF. (1996) A new model for the regulation of bone resorption, with particular reference to the effects of biphosphonates. *J Bone Miner Res*, 11(2): 150 - 159.

Patel DN, Pettifor JM, Becker PJ. (1993) The effect of ethnicity on appendicular bone mass in white, Coloured and Indian schoolchildren. *S Afr Med J*, 83: 847 - 853.

Pead MJ, Skerry TM, Lanyon LE. (1988) Direct transformation from quiescence to bone formation in adult periosteum following a single brief period of bone loading *in vivo*. *J Bone Miner Res*, 3: 647 - 656.

Pearce G, Bass S, Young N, Formica C, Seeman E. (1996) Does weight-bearing exercise protect against the effects of exercise-induced oligomenorrhea on bone density? *Osteoporosis Int*, 6: 448 - 452.

Peters EM, Goetzsche JM. (1997) Dietary practices of South African ultradistance runners. *Int J Sports Nutr*, 7: 80 - 103.

Petit MA, Prior JC. (2000) Chapter 8: Exercise and the hypothalamus :*Ovulatory adaptations*. In: Sports Endocrinology, edited by Michelle P. Warren, Naama W. Constantini, Published by Humana Press Inc. Pg 133 - 163.

Powell KE, Thompson PD, Casperson CJ, Kendrick JS. (1987) Physical activity and the incidence of coronary heart disease. *Ann Rev Public Health*, 8: 253 - 287.
Prior JC, Vigna YM, Schechter MT, Burgess AE. (1990) Spinal bone loss and ovulatory disturbances. *N Engl J Med*, 323: 1221 - 1227.

Pruitt LA, Jackson RD, Bartels RL, Lehnhard HJ. (1992) Weight-training effects on bone mineral density in early postmenopausal women. *J Bone Miner Res*, 7(2): 179 - 85.

Radiographic Absorptiometry (2002)

http://www.compumed.net/osteogram/RA_b.html

Ralston SH. (1999) The Genetics of Osteoporosis. *Bone*, 25(1): 85 - 86.

Ramsay R, Wolman R. (2001) Are synchronized swimmers at risk of amenorrhoea? *Br J Sports Med*, 35: 242 - 244.

Ramsdale SJ, Bassey EJ. (1994) Change in bone mineral density associated with dietary-induced loss of body mass in young women. *Clin Sci*, 87: 343 - 348.

Ravn P, Cizza G, Bjarnason NH, Thompson D, Daley M, Wasnich RD et al (1999) Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. Early postmenopausal intervention cohort (EPIC) study group. *J Bone Miner Res*, 14: 1622 - 7.

Rencken ML, Charles MS, Chestnut III CH, Drinkwater BL. (1996) Bone density at multiple skeletal sites in amenorrheic athletes. *J Am Med Ass*, 276(3): 238 - 240.

Reid DM, Harvie J. (1997) Secondary osteoporosis. *Bailliere's Clin Endocrinol Metab*, 11(1): 83 - 99.

Riggs BL, Melton LJ III. (1986) Involution osteoporosis. *N Engl J Med*, 314: 1676 - 84.

Riggs BL. (2000) Estrogens/Progestins and bone. *Osteoporos Int*, (Supp 2): S43 - S46.

Rigotti NA, Neer RM, Jameson L. (1986) Osteopenia and bone fractures in a man with anorexia nervosa and hypogonadism. *J Am Med Ass*, 256(3): 385 - 388.

Risser WL, Lee EJ, Leblanc A, Poindexter HBW, Risser JMH, Schneider V. (1990) Bone density in eumenorrheic female college athletes. *Med Sci Sport Exerc*, 22(5): 570 - 574.

Risteli L and Risteli J. (1993) Biochemical markers of bone metabolism. *Ann Med*, 25: 385 - 393.

Robbins JA, Hirsch CH, Whitmer R, Cauley J, Harris TB, Davis UC. (2000) Association of BMD and depression in an elderly population. *Osteoporos Int*, 11(Suppl 2): S112.

Rosen CJ (2002) Chapter 11. The epidemiology and pathogenesis of osteoporosis. *Endotext.com* Pg. 1 - 13.

Rudman D, Feller AG, Nagraj HS, et al. (1990) Effects of growth hormone in men over 60 years old. *N Engl J Med*, 323: 1 - 6.

Sanborn CF, Martin BJ, Wagner WW. (1982) Is athletic amenorrhea specific to runners. *Am J. Obstet Gynecol*, 143: 859 - 861.

Sanborn CF, Albrecht BH, Wagner WN. (1987) Athletic amenorrhea: Lack of association with body fat. *Med Sci Sports Exerc*, 19: 207 - 211.

Sanderlin BW, Raspa RF. (2003) Common stress fractures. *Am Fam Physician*, 68(8): 1 - 8.

Schnitzler CM, Macphail P, Shires R, Schnaid E, Mesquita JM, Robson HJ. (1994) Osteoporosis in African Haemosiderosis: Role of alcohol and iron. *J Bone Miner Res*, 9(12): 1865 - 1873.

Schnitzler CM (1996) The interpretation of bone density. Congress: Osteoporosis, proceedings of Bakubung meeting, Plenary Lecture, PG 8 - 12.

Schnitzler CM. (1996) Osteoporosis: A Clinical Approach 2nd Edition. Published by Sandoz: Preventing Osteoporosis, PO Box 371, Randburg 2125, South Africa.

Schnitzler CM. (2001) Microarchitecture of normal and osteoporotic bone. *S Afr Bone & Joint Decade*, 1(1): 18 - 27.

Schultheis L. (1991) The mechanical control system of bone in weightless space flight and in ageing. *Exp Gerontol*, 26(2-3): 203 - 214.

Shangold MM, and Levine HS. (1982) The effect of marathon training upon menstrual function. *Am J Obstet Gynecol*, 143: 862 - 869.

Shangold MM, Rebar RW, Wentz AC, Schiff I. (1990) Evaluation and management of menstrual dysfunction in athletes. *J Am Med Ass*, 263: 1665 - 1669.

Siddle N, Sarrel P, Whitehead M. (1987) The effect of hysterectomy on the age of ovarian failure: identification of a subgroup of women with premature loss of ovarian function and literature review. *Fertile Sterol*, 47: 94 - 100.

Simkin A, Ayalon J, Leichter I. (1987) Increased trabecular bone density due to bone-loading exercise in postmenopausal osteoporotic women. *Calcif Tissue Int*, 40(2): 59 - 63.

Singh M, Nagrath AR, Maini PS. (1970) Changes in trabecular pattern of upper end of the femur as an index of osteoporosis. *J Bone Joint Surg*, 52 - A (3): 457 - 467.

Sinha P. (2002) Stress fracture. *eMedicine Journal*, March 3(3): 1 - 11.

Sirota LH. (1994) Vitamin and mineral toxicities: Issues related to supplementation practices of athletes. *J Health Edu*, March-April, 25(2): 82 - 87.

Smit TH, Burger EH. (2000) Is BMU-coupling a strain-regulated phenomenon? A finite element analysis. *J Bone Miner Res*, 15: 301 - 307.

Smith AD. (1996) The female athlete triad: Causes, diagnosis, and treatment. *Physician Sports Med*, 24(7): 1 - 8.

Snider CA. (1996) Chapter 76. The pharmacology of estrogens in osteoporosis. In: Principles of bone biology. John P Bilesikian, Lawrence G. Raisz and Gideon A Rodan eds. Academic Press. 1065 - 1066.

Soicher J W. (1991) Modern bone densitometry and the radiographer. *S Afr Radiographer*, Vol. XXIX (2): 5 - 10.

Solomon L. (1968) Osteoporosis and fracture of the femoral neck in the South African Bantu. *J Bone Joint Surg*, 50B: 2 - 13.

Solomon L. (1979) Bone density in ageing Caucasian and African populations. *Lancet*, Dec. 22/29: 1326 - 1330.

Stewart TL and Ralston SH. (2000) Role of genetics in the pathogenesis of osteoporosis. *J Endocrinol*, 166: 235 - 245.

Stracke H and Kuhnel W. (1985) Biochemical parameters in the diagnosis of bone disease. (Translation from German) Polyglot language service. 340 Brannan Street, Suite 305 San Francisco, CA 94107. USA.

Sundaresan SM, Roemmich JN, Rogol AD. (2000) Chapter 17: Exercise and the developing child. In: Sports Endocrinology. Michelle P. Warren, Naama W. Constantini Eds. Humana Press Inc. Pg. 303 - 318.

Taffe DR, Robinson TL, Snow CM, Marcus R. (1997) High-impact exercise promotes bone gain in well-trained female athletes. *J Bone Miner Res*, 12(2): 255 - 260.

Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. (2002) Leptin regulates bone formation via sympathetic nervous system. *Cell*, 111: 305 - 317 (November 1).

Thachuk TL. (1996). Effects of exercise on bone mineral density.
http://otpt.ups.edu/Gerontological_Resources/Gerontology_Manual/Thack.html

Turner RT, Riggs BL, Spelsberg TC. (1994) Skeletal effects of estrogen. *Endocr Reviews*, 15(3): 275 - 308.

Van den Graaff KM, Fox SI, LaFleur KM. (1997) Synopsis of human anatomy and physiology, Wm. C. Brown Publishers USA.

Van Duuren EM, (1996) Osteoporosis in men. Congress on osteoporosis, proceedings of Bakubung meeting, Session III: So what's new? Pg. 35 - 37.

Vidyashankar C (2002). Bone mineral density low in Asian Indians. *J Indian Med Assoc*, 100: 598 - 602.

Vorster H, Venter CS, Beetge GJA, Steyn HS. (2001) The effect of exercise on bone mineral density in premenopausal female athletes compared with non-athletes. *S A J Clin Nutr*, 14(1): 20 - 25.

Wagener GW, Hough FS. (1987) Metacarpal bone mass in the white and Coloured populations of the Cape. *S Afr Med J*, 72: 205 - 208.

Wallace BA, Cumming RG. (2000) Systematic review of randomized trials of the effect of exercise on bone mass in pre and postmenopausal women. *Calcif Tissue Int*, 67(1): 10 - 18.

Warren AK, Sherman C. (1998) Exercise for osteoporosis. *Phys Sportsmed*, 26(2): 10 - 19.

Warren MP. (1980) The effects of exercise on pubertal progression and reproductive function in girls. *J Clin Endocrinology Metab*, 51:1150 - 1157.

Warren MP. (1983) The effects of undernutrition on reproductive function in the human. *Endocrinol Rev*, 4: 363 - 377.

Warren MP, Brooks-Gunn J, Hamilton LH, Warren LF, Hamilton WG. (1986) Scoliosis and fractures in young ballet dancers: relation to delayed menarche and secondary amenorrhea. *N Engl J Med*, 314: 1348 - 1352.

Warren MP, Voussoughian F, Geer EB. (1999) Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. *J Clin Endocrinol Metab*, 84(3): 873 - 877.

Warren MP, Shantha S. (2000) The female athlete. *Ballière's Clin Endo Metab*, 14(1):37 - 53.

Warren MP, Perlroth NE. (2001) The effects of intense exercise on the female reproductive system. *J Endocrinol*, 170(1): 3 - 11.

Warren MP, Russalind HR, Bronson EM. (2002) Exercise-associated amenorrhea: Are altered leptin levels and early warning sign? *Phys Sportsmed*, 30(10): 1 - 10.

Watkin VA, Myburgh KH, Noakes TD. (1991) Low nutrient intake does not cause the menstrual cycle interval disturbances seen in some ultramarathon runners. *Clin J Sports Med*, 1: 154 - 161.

Whitfield JF. (2002) Leptin – A new member of the bone builders' club? *Medscape Women Health eJournal*, 7(4): 1 - 5.

Wiggins DL, Wiggins ME. (1997) The female athlete. *Clin Sports Med*, 16(4): 593 - 611.

Wilder RP, Sethi S. (2004) Overuse injuries: Tendinopathies, stress fractures, compartment syndrome, and shin splints. *Clin Sports Med*, 23(1): 1 - 21.

Winters KM, Adams WC, Meredith CN, Van Loan MD, Lasley BL. (1996) Bone density and cyclic ovarian function in trained runners and active controls. *Med Sci Sports Exerc*, 28: 776 - 785.

Wittert G. (2000) Chapter 3. The Effects of exercise on the hypothalamo-pituitary-adrenal axis. In: Sports Endocrinology. Michelle P. Warren, Naama W. Constantini Eds. Humana Press Inc. Totowa, New Jersey Pg. 43 - 55.

Woitge HW. (2001) Biochemical markers to survey bone turnover. In: Rheumatology disease clinics Of North America. Osteoporosis. Nancy E Lane, Guest ed. Vol 27 No.1 Feb; WB Saunders Company. 49 - 79.

Wolman RL, Harris MG. (1989) Menstrual abnormalities in elite athletes. *Clin Sports Med*, 1: 95 - 100.

Wolman RL. (1990) Bone mineral density levels in elite female athletes. *Ann Rheum Dis*, 49: 1013 - 1016.

Wolman RL. (1994) Osteoporosis and exercise. *Br Med J*, 309: 400 - 403.

Wüster C, Soballa T, Heilmann P, Ziegler R. (1996) Quantitative ultrasound densitometry (QUS) for the evaluation of osteoporosis risk: limitations and application possibilities. Congress on osteoporosis, proceedings of Bakubung meeting. Plenary Lecture: 12 - 13.

Zanker CL, Swaine IL. (1998) Relationship between bone turnover, oestradiol, and energy balance in women distance runners. *Br J Sports Med*, 32:167 - 171.

Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC. (1998) The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. *J Bone Miner Res*, 13(10): 1594 - 1601.

Zumda J, CauleyJ, Stone K *et al.* (2000) An interleukin 6 promoter polymorphism is associated with hip bone loss in older women. *Osteoporos Int*, 11(Supp 2): S58.

APPENDIX 1

INFORMED CONCENT
BONE MINERAL DENSITY IN ULTRADISTANCE FEMALE RUNNERS
RESEARCH TRIAL

I, _____ hereby consent to take part in the trial entitled:

***“BONE MINERAL DENSITY IN SOUTH AFRICAN ULTRA DISTANCE
PREMENOPAUSAL RUNNERS OF ALL ETHNIC BACKGROUNDS”***

that has been explained to me by **Avril McGregor**. I am familiar with all the procedures that will be undertaken and the risks and discomforts that I may experience.

This research trial will investigate the effect of ultra distance running on BMD and menstrual cycle. The results obtained will help us to determine risk factors that may result in osteoporosis.

You are asked to accurately record your dietary intake (with special reference to calcium-containing foods (e.g. milk, yoghurt, and cheese), menstrual cycle and the distance run during training and races. The diary must be filled in every day. A simple questionnaire will also be filled in and returned to the research coordinator.

You will be required to come to the laboratory on one occasion. Your height and weight will be measured. You will have a bone scan of your spine, hip and whole body. The X-ray dosage is approximately equivalent to a chest X-ray. If you are pregnant or suspect you may be pregnant, you must not take part in the trial. A venous blood sample will be taken by a registered medical technologist in order to measure hormone concentrations and routine biochemistry such as haemoglobin, calcium concentrations as well as specific markers on bone metabolism. You will feel the normal discomfort of blood sample. Your visit to the laboratory and X-ray department will take approximately one hour.

If you are travelling to KwaZulu Natal for the Comrades marathon from some distance, you will be scanned during your visit (irrespective of the phase of your menstrual cycle) but your blood test may be taken at a later time.

All results and information gathered for the trial will be treated in the strictest confidence. The results of the bone scan will be forwarded to your GP.

You are free to withdraw from the study at any time, without consequence or prejudice towards you.

I confirm that I have read and understood the “Information to Subjects” sheet concerning this trial. I agree that the procedures detailed on that information sheet will be carried out and supervised by

_____ Researcher
Researcher's full name

I acknowledge that I understand the contents of this form and the “Information to Subjects” sheet and as the Subject freely consent to these procedures being conducted. I am aware that I may withdraw my consent at any time without prejudice.

Signed _____ Date _____
Subject

Signed _____ Date _____
Witness

Signed _____ Date _____
Researcher

APPENDIX 2.

BONE DENSITY RESEARCH TRIAL FOR PRE-MENOPAUSAL ULTRADISITANCE WOMEN RUNNERS

(Dept. Physiology, Nelson Mandela School of Medicine, Umbilo Rd. Durban)

QUESTIONANAIRE TO BE COMPLETED BY ALL PARTICIPANTS

Name _____
Postal Address _____
_____ Code: _____

Phone Numbers (H) _____ (W) _____ (C) _____
Date of Birth ____/____/____
Name and address of General Practitioner (Doctor)

How many years have you been running? _____

How many years have you been racing? _____

What is your average monthly training distance?

1998 Jan _____ Feb _____ March _____ April _____ May _____ June _____
1999 July _____ Aug _____ Sept _____ Oct _____ Nov _____ Dec _____

Do you Smoke? _____ If so, how many/day _____

Have you had shin splints, or shin pain that has caused you to take off time and stop running? _____

If yes, approximate date and time period _____

Have you had a medically diagnosed stress fracture? _____

If yes, where and when (year) _____

Have you ever broken a bone? _____

If yes, which bone and when (year) _____

Do you have any family history of osteoporosis? _____

If yes, which member(s) of the family _____

How many children have you had? Number _____ Ages 1 ____ 2 ____ 3 ____ 4 ____

How many months did you breast feed? (total in months for all children) _____

At what age did you start menstruating? _____

Are you on a contraceptive? _____

If yes, state whether injection or pill _____

How many years in total have you been taking the pill/injection?

Have you had a hysterectomy? _____ If yes, when (year) _____

Has your period ever stopped? (Excluding pregnancy) _____

If yes, when and for how long?

Have you ever experienced menstrual irregularities? _____

If yes, when? (State your age and year e.g. 16 yrs for 2 months)

Are you pregnant? _____ If yes, how many months? _____

Are you on calcium supplement? ____ If yes, state brand and dosage /day

Are you on magnesium supplement? ____ If yes, state brand and dosage /day

Are you on any other mineral supplement? ____ If yes, state brand and dosage /day _____

Have you ever been diagnosed with any medical disorder? (e.g. Diabetes, Porphyria, Anaemia, Asthma, Osteoporosis etc.) _____

If yes, state the name of the disorder _____

Are you on any medication? (prescribed or self-medicated) _____

If yes, state name of the drug and daily dosage _____

Do you suffer from any medically diagnosed allergies? _____

If on treatment for any allergy, state the treatment and daily dosage _____

Do you donate blood? _____

If yes, when did you last donate blood?

What is the total number of units donated, since you began donating blood (approx) _____

Trial Co-ordinator: Avril McGregor

Tel (H) ____ (W) ____ (C) ____

(All information obtained and results of tests will be treated with strictest confidentiality).

APPENDIX 2 (cont.)
ULTRA DISTANCE RUNNERS – RECORD KEEPING

NAME: _____ MONTH _____ YEAR _____

	S	M	T	W	T	F	S	S	M	T	W	T	F	S	A	M
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Training																
km																
Time																
Walking (km)																
Other exercise (min)																
DIET:																
Milk (cups)																
Yoghurt (cups)																
Cottage cheese (T)																
Cheese (T)																
MENSTRUAL:																
Menstruation (T)																
O/C pill (T)																
O/C injection (T)																
RACES:																
Distance (km)																
Time (hrs/mins)																

	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Training															
km															
Time															
Walking (km)															
Other exercise (min)															
DIET:															
Milk (cups)															
Yoghurt (cups)															
Cottage cheese (T)															
Cheese (T)															
MENSTRUAL:															
Menstruation (T)															
O/C pill (T)															
O/C injection (T)															
RACES:															
Distance (km)															
Time (hrs/mins)															

NOTE: DIET/ MENSTRUAL CYLCLES/KMS RUN – RECORD KEEPING
Bone Density Trail, Dept. of Physiology, Nelson Mandela School of Medicine