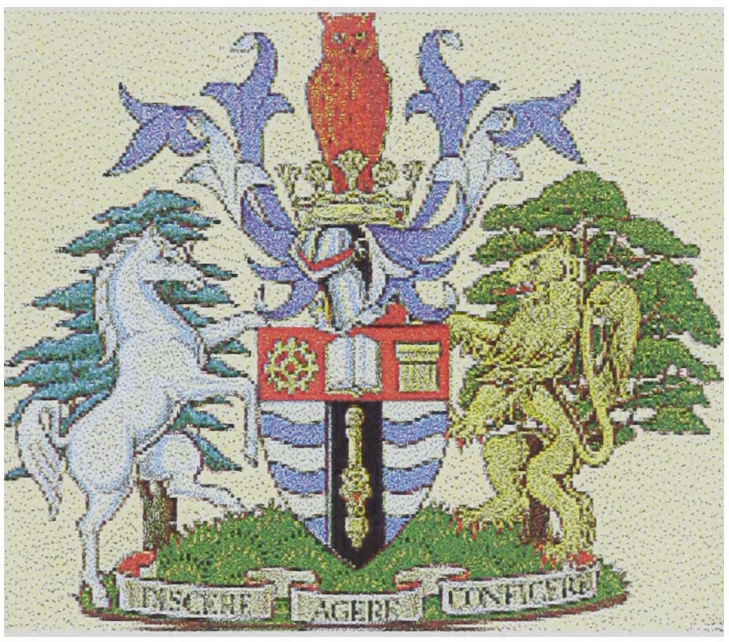
THE RELATIONSHIPS BETWEEN BORON, STRESS HORMONES AND BONE MARKERS IN HUMANS OF DIFFERENT AGES, LIFE STYLE AND HEALTH STATUS.

N.H.CHUMMUN



A PROJECT SUBMITTED IN PART FULFILMENT OF THE REQUIREMENTS FOR

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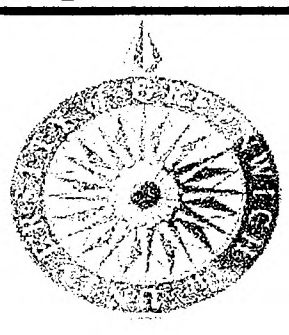

UNIVERSITY OF GREENWICH

ABSTRACT.

Hormones (sex hormones, thyroxine, cortisol and parathyroid), cytokines (interleukins-1 and 2 and tumour necrosis factor), life style (exercise, smoking and reproductive history), nutrition (calcium, meat, vegetables, vitamin D, caffeine and alcohol) and diseases (diabetes mellitus, malabsorption and thyrotoxicosis) affect bone mass. The loss of calcium, phosphate and magnesium from bone makes it weaker which enhances the incidence of bone diseases such as osteoporosis and osteoarthritis and increases the risks of fractures causing pain, sufferings and even death particularly in older females. Treatment of these diseases and the 200,000 related fractures costs the National Health Service over £940 millions annually (National Osteoporosis Society, 1998/1999).

The multifactorial aetiology of osteoporosis and difficulties in deciding the most effective treatment to suit individual sufferer makes preventative measures more suitable in lowering the incidence of this disease and therefore reduces human sufferings, cost and mortality.

Boron, a ubiquitous element in soil, water, ground vegetables and fruits modulates sex hormones in animals. Its role in human is unclear and inconclusive. Boron prolonged the half-life of 17β oestradiol and delayed the loss of bone mass particularly in post-menopausal women with low magnesium (Neilsen, 1990) and increased absorption and retention of calcium, phosphate and magnesium during vitamin D deficiency (Hunt, 1994). Excessively high level of boron suppressed sex steroids and increased the loss of bone minerals (Benderdour et al, 1998). Cortisol reduced bone mass (Delany et al, 1995) but the effects of catecholamines on bone are largely undetermined.

This study aims to assess the relationship between boron, cortisol, catecholamines, serotonin and bone turnover in relation to age, gender,

life style, nutrition, reproductive history and health status in men and women of 3 age groups, mainly nurses and to make recommendations, if appropriate, to improve bone mass or reduce the rate of bone loss.

172 male and female volunteers were placed in respective groups aged 11-20, 20-40, over 40 years old and all the pregnant subjects in one group. Early morning urine was analysed for calcium and magnesium by flame spectrophotometry, phosphate, hydroxyproline and creatinine by spectrophotometry, boron by inductive coupled plasma spectrophotometry and cortisol, adrenaline, dopamine and serotonin by high performance liquid chromatography. Information about each subject's life style, nutrition and health was obtained using a questionnaire. Data were processed and analysed using excel and minitab packages and only significance level at $P<0.05$ or less was accepted using ANOVA, t-test and Pearson's correlation coefficient .

There were positive trends between urinary levels of boron and calcium and significant correlation ($P<0.05$) between boron and phosphate in all the male subjects particularly in M20-40, suggesting that these subjects would be more prone to bone loss. In addition, the levels of boron were significantly higher ($P<0.05$) in post-menopausal compared to pre-menopausal subjects, in F<20 low alcohol drinkers compared to M<20, during stress in M20-40 compared to F20-40, in F>40 with the history of osteoporosis compared to those in M>40 as well as in F20-40 heavy smokers compared to those in M20-40 and in subjects taking the contraceptive pill. Age, exercise, hysterectomy, HRT, vegetables consumption and vitamin supplements did not significantly influence urinary boron levels. Heavy alcohol consumption, greater stress, heavy smoking and family history of osteoporosis increase urinary boron levels and this might enhance bone damage, particularly in post-menopausal subjects.

Urinary cortisol, adrenaline and dopamine levels were raised ($P<0.05$) in F>40 and M>40 and correlated with an increased calcium excretion which suggest that the increased catecholamine levels in these subjects may promote calcium loss and compromise ageing bone. Regular intake of alcohol above 1500 ml of beer, 750 ml of wine and 150 ml of spirits per week, heavy smoking of over 10 cigarettes or 3 ounces of tobacco per day, eating less than 3 vegetables, not taking weight bearing exercises at least 3 times per week and a lack of multivitamin supplements during puberty and middle age, increased bone turnover and may predispose bone to fractures. Male nurses were at a greater risk ($P<0.05$) than the females from increased turnover of phosphate, magnesium, calcium and hydroxyproline as these also positively correlated with either boron, adrenaline or cortisol. The levels of urinary catecholamines were significantly ($P<0.05$) higher in M20-40 compared to F20-40 who were stressed and suggested either male subjects had poor coping mechanism or that female subjects had a different response mechanism. The correlation between boron, cortisol, adrenaline and phosphate in the male subjects as a whole but particularly in M20-40, in whom adrenaline also correlated with hydroxyproline, suggest that these male subjects are at greater risks of bone damage and poor health.

Improved dietary intakes of calcium, vegetables and boron and a healthier life style of reduced alcohol and smoking with more weight-bearing exercises could significantly reduced bone loss and therefore prevent osteoporosis. In addition, male nurses should minimise stress levels not only to protect bone loss but for a better health.

ACKNOWLEDGEMENTS

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THIS WORK IS DEDICATED TO THE

SACRIFICES AND CHERISHED MEMORIES

OF MY LATE

MOTHER AND FATHER.

BORON.

You say you are afraid of the α-rays

Put on sheets of paper to keep it away.

A β-rays needs much more care

Place sheets of metal here and there,

And as for the powerful γ-rays

Pay careful heed to what I say:

Unless you wish to spend weeks in bed

Take cover behind thick slabs of lead.

Fast neutrons pass through everything

Wax slabs remove their nasty thing

These slow them down; even a moron

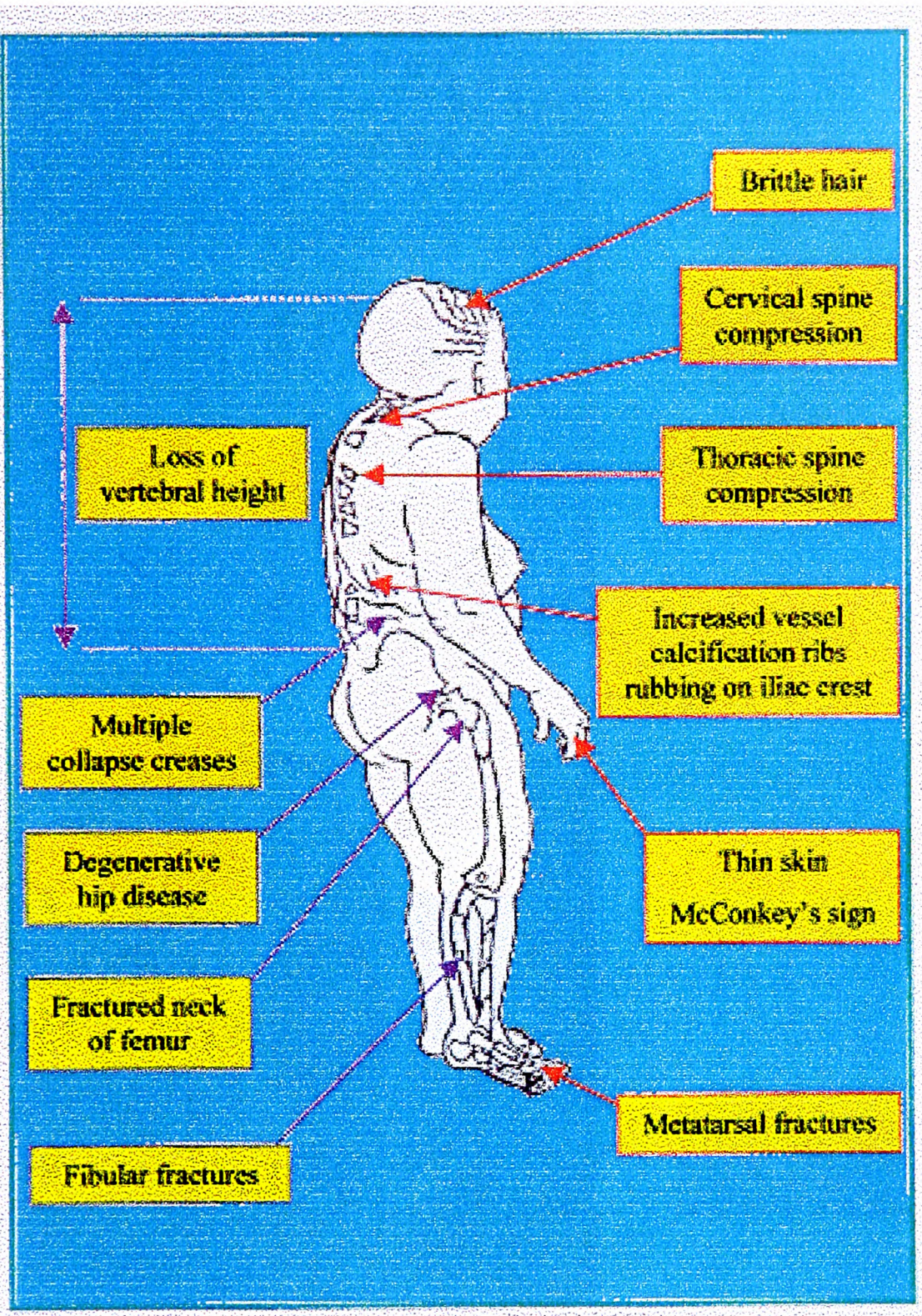
Knows they can be absorbed by boron.

Remember, remember all that I have said

Because it's no use remembering when you are dead.

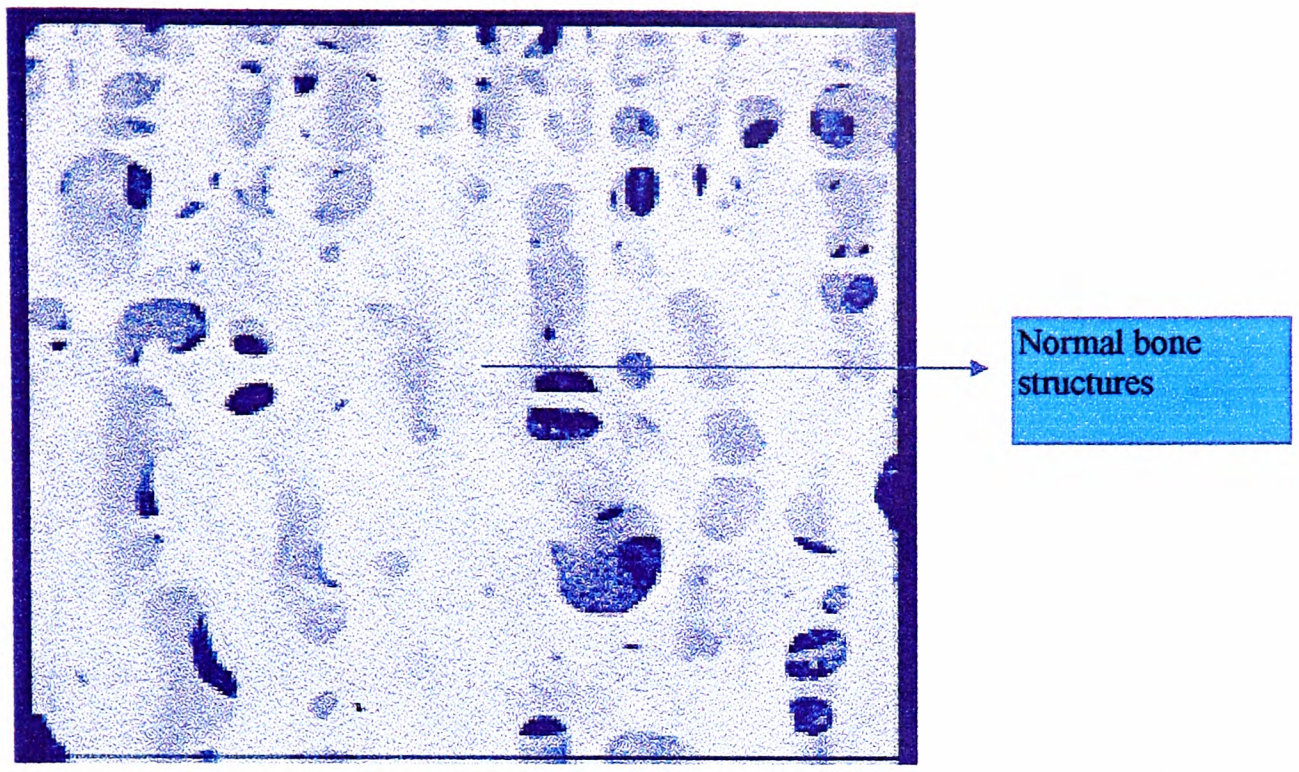
Harry Chummun.

1999.

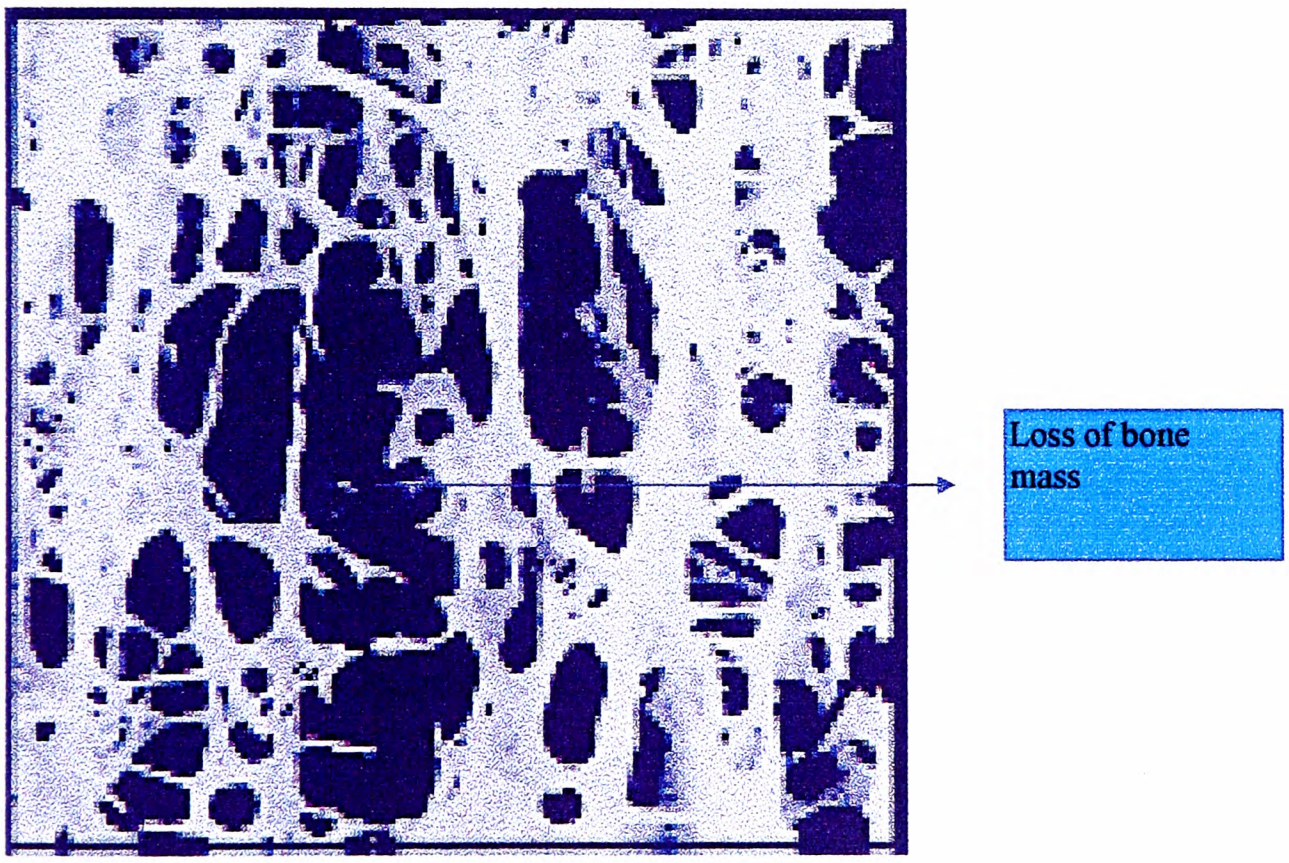


Bone changes due to osteoporosis

Source: Birdwood G. (1996)



Normal bone.



Osteoporotic bone

OSTEOPOROSIS CAUSES BONE TO BECOME POROUS WHICH BREAKS VERY EASILY. 1 IN 3/4 WOMEN AND 1 IN 12 MEN WILL DEVELOP OSTEOPOROSIS. IT AFFECTS PEOPLE OF ALL AGES BUT PARTICULARLY AFTER THE AGE OF 50, CAUSING FRACTURES, INCREASING MORBIDITY AND MORTALITY. (NATIONAL OSTEOPOROTIC SOCIETY 1999).

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ABBREVIATIONS

5 HIAA	hydroxyindoleacetic acid
5 HT	5-hydroxytryptamine (serotonin)
AAS	atomic absorptiometry
ACTH	adrenocorticotropic hormone
AD	adrenaline
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CATS	catecholamines
COMT	catechol-O-methyltransferase
DA	dopamine
DHBA	dihydroxybenylamine
Dpd	deoxypyridinoline
EGF	epidermal growth factor
EDTA	ethylenediaminetetoacetic acid
F<20	females under the age of 20 years
F>40	female over the age of 40 years
F20-40	females between 20 and 40 years
FGF	fibroblasts growth factor
HPLC	high performance liquid chromotogarphy
HRT	hormone replacement therapy
ICP-MS	inductive-coupled plasma spectrophotometry
IGFs	insulin-like growth factors
IL	interleukin
M<20	males under the age of 20 years
M>40	females over the age of 40 years
M20-40	males between 20 and 40 years
MAO	monoamine oxidase

mRNA	messenger ribonucleic acid
NA	noradrenaline
NAD	nicotinamide adenine dinucleotide.
O/A	osteoarthritis
O/P	osteoporosis
P	pregnant women
PDGF	platelets-derived growth factor
PGs	prostaglandins
PTH	parathyroid hormone
Pyd	pyridinoline
TNF	tumour necrotic factor

CHAPTER 1

STRUCTURES AND FUNCTIONS OF BONE.

1.0.0. Introduction.

Bone, a specialised form of connective tissue, made durable by the deposition of minerals within its infrastructure, can be long, short, flat and irregular. It gives shape and form to the body, protects internal organs, stores minerals and provides the site for blood cell production. It is made up of cortical and trabecular bone tissues which are living tissues and undergo continuous growth, development and repair. These tissues originate from mesenchymal stem cells and eventually differentiate into osteoblasts, osteoclasts, osteocytes and osteogenic cells. In addition, bone is also made up of organic and inorganic substances. The organic component consists of the bone cells and collagenous and non-collagenous proteins. The inorganic substances are mainly calcium hydroxyapatites, made up principally of calcium and phosphate crystals with sodium and magnesium. The intercellular bone matrix is impregnated with inorganic calcium salts which provide tensile and compressible strength. Several cytokines, such as interleukins 1 and 2, hormones, such as cortisol, insulin, oestrogen and testosterone, life style such as alcohol consumption, exercise and dietary minerals, such as calcium and vitamins, influence bone cells activities and either promote growth or resorption of bone.

Long bone has a central diaphysis of dense and compact bone and the region at each end are the epiphysis, growth plates and metaphysis which become calcified following completion of pubertal growth. Growth in length occurs by the deposition of new bone tissue at the metaphysis which then has to undergo subsequent mineralisation. The diaphysis consists of compact bone and

the periosteum, which is the outer surface layer of the diaphysis, consists of fibrous tissues and nerve endings and is richly supplied with blood vessels. Its innermost layer is secured to the underlying bone by tufts of collagen fibres which extend from the fibrous layer into the bone matrix. The endosteum layer separates the medullary cavity from the surrounding compact bone and contains osteoblasts as well as osteoclasts.

Bone is made up of two main types of tissues; trabecular and cortical. Trabecular bone found internal to cortical bone and at the end of long bones, comprises of trabeculae seperated by marrow spaces (Marieb, 1997). This bone has a high surface-to-volume ratio and provides the environment for calcium and phosphates metabolism. Cortical bone makes up the outer layer of long bones and vertebrae and has a low surface-to-volume ratio (Reid, 1997). About 80% of the skeleton is cortical bone providing ridigity and density as well as weight bearing properties.

Bone contains collagen fibres arranged in parallel or concentric sheets or lamellar bone (Weinstein & Buckwater, 1994). Lamellar bone is found around blood vessels forming Haversian systems in long bone. Collagen fibres, found in woven bone, are formed when bone is laid down rapidly, as in foetal growth or during healing of fractures, and are interlaced and randomly dispersed with hydroxyapatite crystals in a disorganised fashion unlike in lamellar bone where they are deposited parallel to the collagen fibres (Odell & Heath III, 1993). Bone tissues are dynamic and continually being remodelled. In the foetus, bone develops from previously laid down cartilage, which is eventually resorbed and replaced with bone tissues through endochondrial ossification (Suta & Yasin, 1998). However, bones of the calvaria, such as mandible and maxilla, developed from mesenchymal cells, and are formed through intra-mesenchymal ossification. In bone modelling, a process associated with growth and shaping of bone during childhood and adolescence, longitudinal growth of long bones depend on proliferation and differentiation of cartilage cells at the growth plates

while growth in width and thickness is accompanied by formation of bone at the periosteal surface (Ng & Roma, 1997). In adults, after the closure of the epiphysises, growth in length ceases, but remodelling of bone, a life-long renewal activitiy continues through which old bone is removed by osteoclasts and replaced with new uncalcified bone tissues followed by mineralisation with hydroxyapatite crystals by osteoblasts (Eriksen et al, 1993). Parfitt, (1994) estimated that in humans, as much as 25% of trabecular bone and 3% of cortical bone is resorbed and replaced each year. This coupling of bone resorption and formation is influenced by hormones, local growth factors, cytokines and nutrients (Molgaard & Thompson, 1997).

1.1.0. Functions of bones.

Bone provides a supportive framework for soft tissues and protects delicate internal organs such as the bones of the skull, which form a bony cage for the brain tissues. Tendons are attached to the ends of long bone to facilitate movements, for example, bone of the legs during walking. The matrix of long bone stores minerals such as calcium, phosphate and magnesium and releases them into blood when their levels fall below normal ranges. Bone marrow produces most of the blood cells.

1.1.1. Bone matrix.

Bone matrix, produced by osteoblasts, consists of collagen and mucopolysaccharides. The mineralised organic matrix makes bone resistant to tractional and torsional forces. Intrinsic bone matrix disorders are rare and usually involves defective mineralisation for example hypophosphatasia, a rare autosomal recessive disorder in children and adults with associated rickets. Extrinsic factors which inhibit bone matrix mineralisation are even rarer for example, fibrogenesis imperfecta, in which serum alkaline phosphatase is increased with disorganised matrix and delayed bone mineralisation.

1.1.2. Bone collagen.

Collagen, a major component of connective tissues, is synthesised by osteoblasts and consists of a helix of three long alpha polypeptide chains. It has a triple helical structure made up of three collagen polypeptide sub-units, called α- chain, which are twisted together to form the helix. The innermost polypeptide consists of a repeating sequence of glycine residue (GLY-X-Y)n in which the X and Y positions can be any amino acid. About 100 of the X positions are occupied by proline and 100 Y by hydroxyproline. Proline and hydroxyproline residues impart considerable rigidity to the structure. Collagen fibres are further stabilised by the formation of covalent cross-links both within and between the triple helical units. These crosslinks are formed through the action of lysyl oxidase, a copper-dependant enzyme that oxidatively deaminates the ε-amino group of lysine and hydroxylysine residues, yielding reactive aldehydes which stabilise the covalent cross-links to provide tensile strength to the fibres (Bonde et al, 1994). The deposition of calcium phosphate crystals during bone mineralisation, imparts further hardness and strength to the collagen.

Pyridinoline and deoxypyridinoline are non-reducible cross-links of collagen and while pyridinoline and hydroxyproline are also found in other tissues such as ligament and cartilage, deoxypyridinoline is relatively bone specific. The ratio of pyridinoline : deoxypyridinoline, in urine, is 3.5:1 (Eyre & Kobb, 1984). Total urinary pyridinoline and deoxypyridinoline can be measured by reverse phase HPLC but they do not always reflect changes in plasma pyridinoline or deoxypyridinoline as little is known of the renal handling or metabolism of these substances (Colwell et al, 1993).

Collagen is formed from protocollagen molecules synthesised and secreted by the osteoblasts which then undergo a number of post-translational modifications **(Fig.1.0)** including the hydroxylation of proline and lysine to form hydroxyproline and hydroxylysine respectively. The terminal propeptides

are cleaved following secretion and in the final conversion of procollagen to collagen, one third of the mass is cleaved from the protein (Devlin, 1997). Collagen interacts with other bone tissue components such as proteoglycan and minerals to determine the structures of the bones. Bone collagen is unique as it is only mineralised in bone (St. John-Dixon & Woolf, 1988).

Breakdown of bone collagen releases hydroxyproline, deoxypyridinoline, pyridinoline and stored minerals such as calcium,

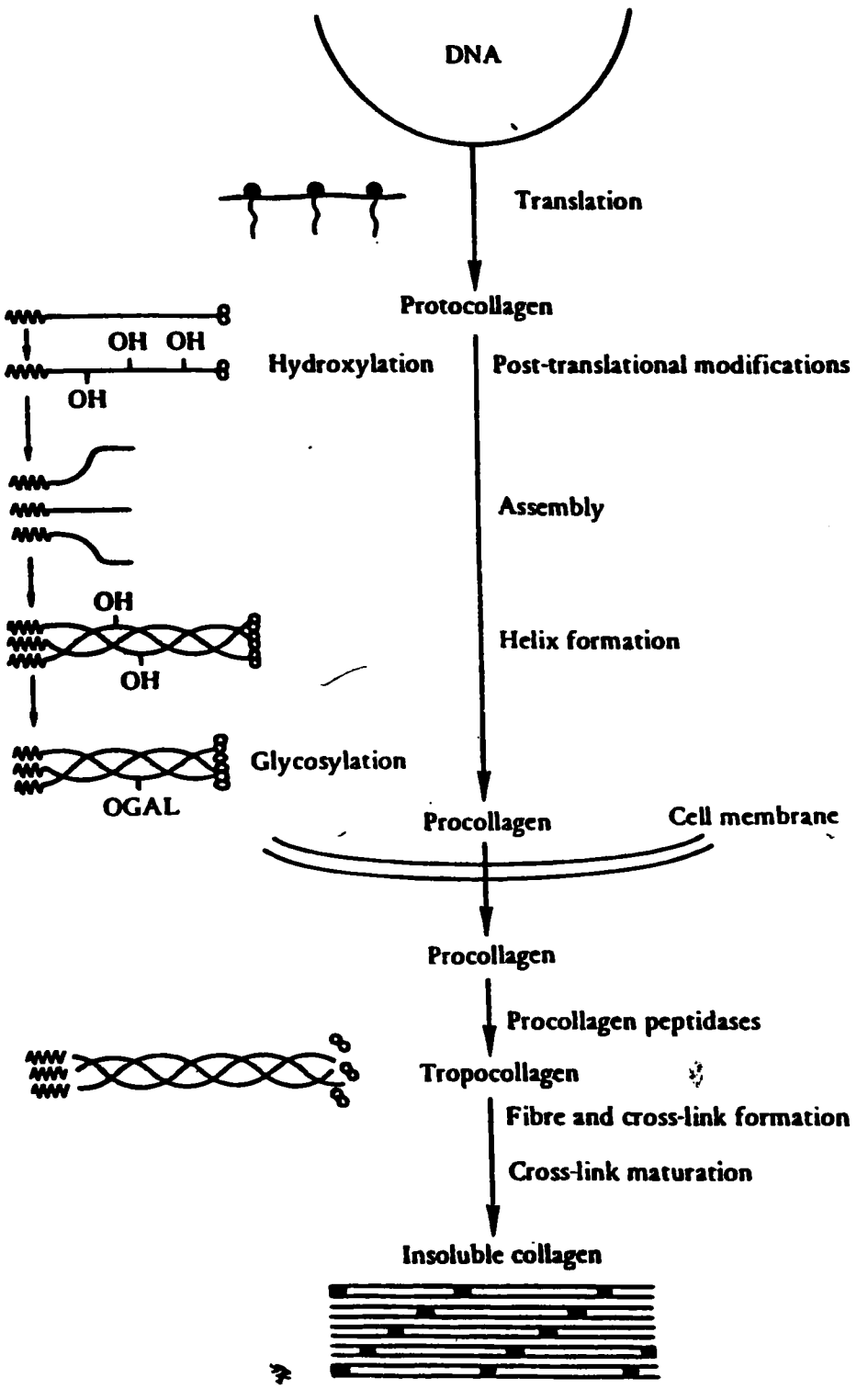

Fig. 1.0. The biosynthesis of collagen.(St.John-Dixon & Woolf D.A.1988).

magnesium and phosphate (Skodjt & Russell, 1992) into blood and excess is excreted mainly in urine. The urine level of these substances reflects the rate of collagen and bone turnover (Sproot & Muller, 1997).

1.1.3. Bone cells.

Bone cells **(fig. 1.1)** are intricately involved in bone growth and development and are sensitive to local and systemic factors. The influence of these factors on the coupling process is usually balanced to determine rate of bone growth, development and mass and any imbalance, may lead to reduced bone mass and increase the risk of fractures. The 4 bone cells are osteoblasts, osteoclasts, osteocytes and osteogenic cells (Mundy & Martin, 1993).

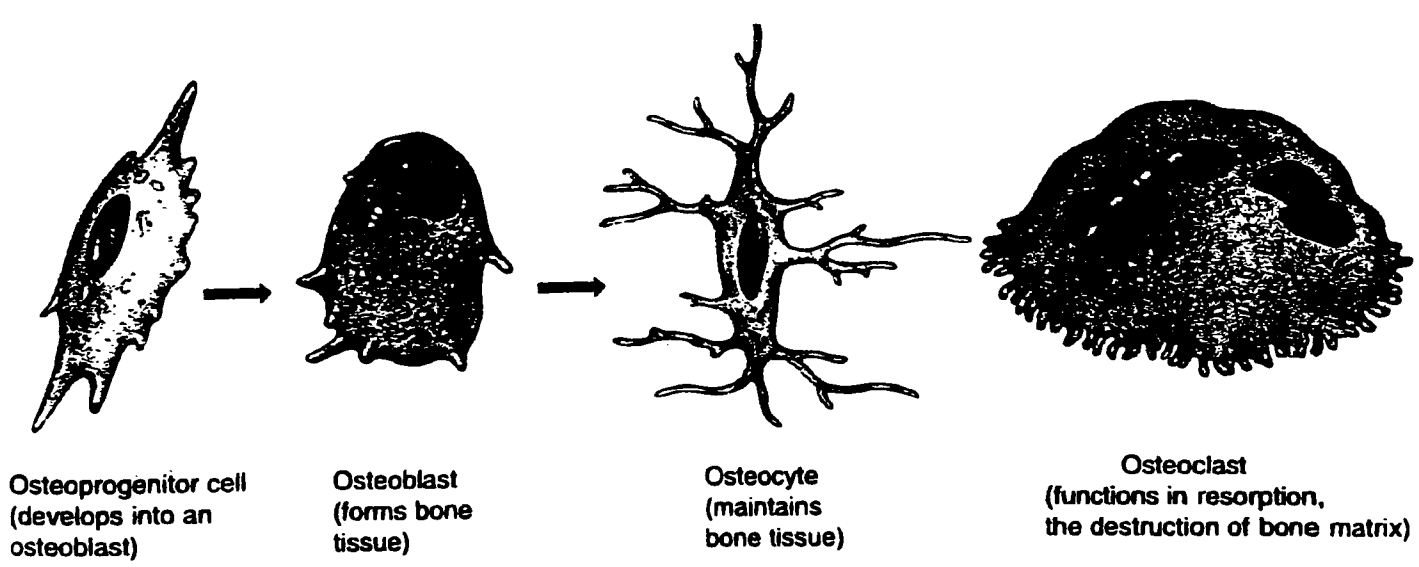

Fig.1.1. The four different bone cells (Marieb E. 1997).

1.1.3(a) Osteoblasts.

Osteoblasts, bone forming cells, are derived from stromal fibroblast-like cells within the bone marrow. They are uniform in size, communicate with each other by fine cytoplasmic processes, synthesise and secrete bone matrix proteins including collagen, glycoproteins and proteoglycans and calcium binding proteins (Rey & Kim, 1996) and produce large amount of alkaline phosphate, an enzyme which initiates and promotes mineralisation of the newly deposited matrix (Wlodarski, 1990). The activities of these cells are influenced by various hormones such as parathyroid hormone (Mansen &

Palmer, 1998), vitamin D, sex hormones, cytokines and growth factors (Buckwater et al, 1996).

1.1.3(b) Osteoclasts.

These uni- and multi-nucleated bone cells are responsible for resorption of bone and initiation of skeletal remodelling. They evolved from the macrophage-monocyte lineage, vary in size and are highly mobile (Suda et al, 1992). They are rich in several enzymes such as collagenase, lysosomal enzymes and acid phosphatase, which are involved in bone resorption to solubilise the bony matrix resulting in the release of calcium, phosphate and other minerals (Kean et al, 1994). Osteoclasts respond to several hormones, such as parathyroid, calcitonin and sex hormones, metabolites of vitamins A and D, and dietary factors such as alcohol (Odell & Heath III, 1993).

1.1.3(c) Osteocytes.

Osteocytes are osteoblasts trapped in the lacunae by the calcifying matrix they have secreted (Johansen & Stone, 1996). Lacunae connect the osteocytes to one another to facilitate movements of nutrients and metabolites. They act as a 'bone pump' releasing calcium from perilacunar bone when serum calcium is low and is also associated with matrix mineralisation (Kingsmil & Boyde, 1998).

1.1.3(d) Osteogenic cells.

Osteogenic cells are undifferentiated bone cells found in the periosteum and epiphyseal plates of growing bones. Eventually, these cells differentiate into osteoblasts and are active during growth and healing of fractures and may contribute to the continual replacement of worn out tissues (Takwshima & Kitano, 1998).

1.1.4. Bone development.

The mature skeleton develops by the transformation of embryonic fibrous connective and hyaline tissues into bone by the processes of intramembraneous and endochondral ossification respectively.

1.1.4(a) Intramembraneous ossification.

Foetal fibrous connective tissues, formed by mesenchymal cells, serve as the initial supporting structures on which ossification begins. Mesenchymal cells, in the fibrous membrane, differentiate into osteoblasts which then secrete osteon (bone unit) around the collagen fibres and, subsequently, produce alkaline phosphatase to initiate mineralisation of the osteon into mature bone matrix. However, mesenchymal cells, located just innermost to the periosteum, differentiate into osteoblast and produce osteon which is subsequently mineralised to produce compact bone **(fig.1.2).** Mature osteoblasts, trapped within concavities inside the newly secreted matrix, differentiate into osteocytes and vascular tissue, within the trabecular bone which form the red bone marrow (diploe). The vascular mesenchymal, on the outside of the fibrous membrane condenses to become the periosteum.

1.1.4(b) Endochondral ossification.

The process of ossification of long bone, in which hyaline cartilage is converted into mature bone tissues, begins around week 3 of gestation, with the formation of a bone collar around the hyaline cartilage and the appearance of a primary ossification in the centre of the cartilage shaft **(fig. 1.4.).** The chondroblasts, active mitotic cartilage cells, differentiate into osteoblasts which then infiltrate the primary ossification centre followed by cavitation of the hyaline cartilage shaft. The periosteal bud subsequently invades the internal cavities to form the spongy bone. As the primary ossification centre expands, osteoclasts form the medullary cavity (Marieb, 1997).

Fig. 1.2(a)

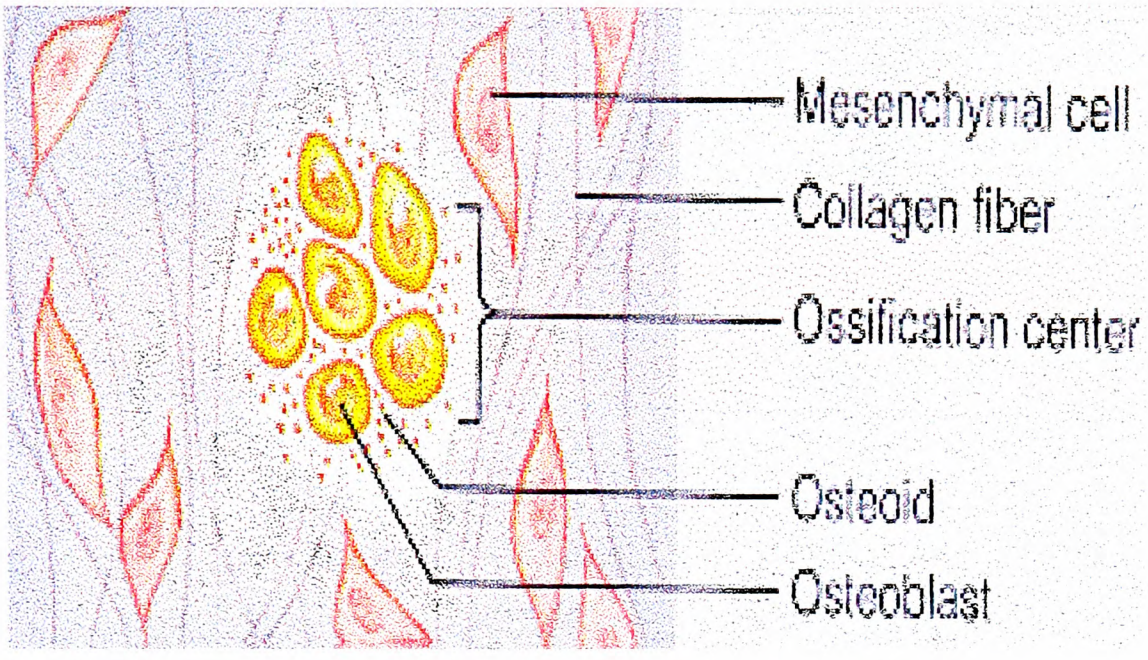

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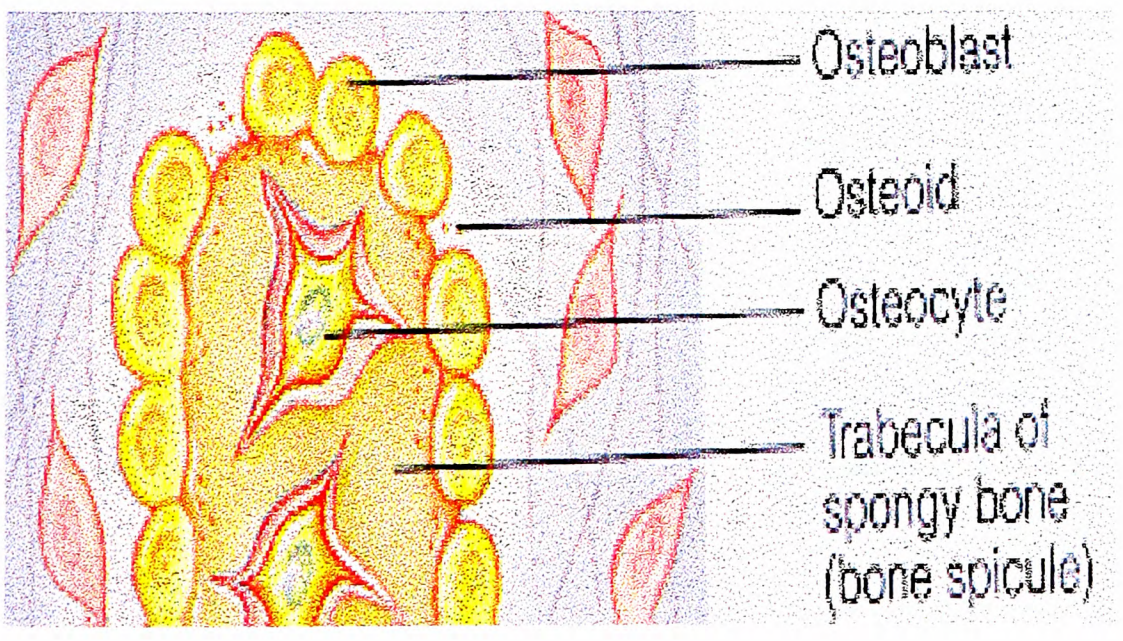
Fig. 1.2(b)



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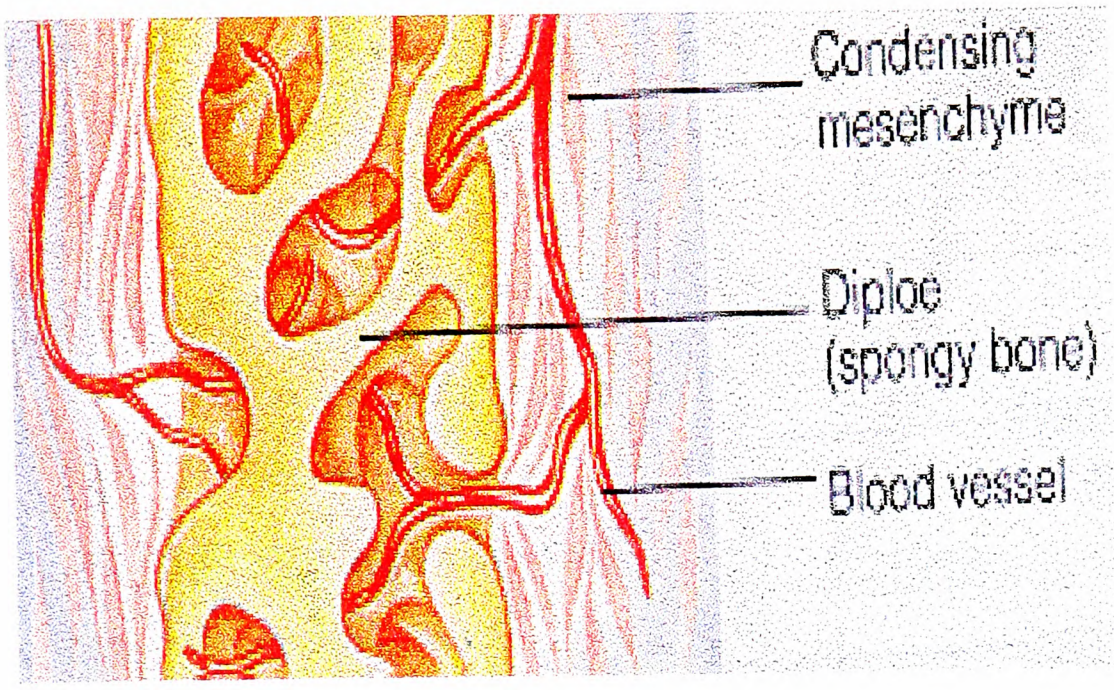

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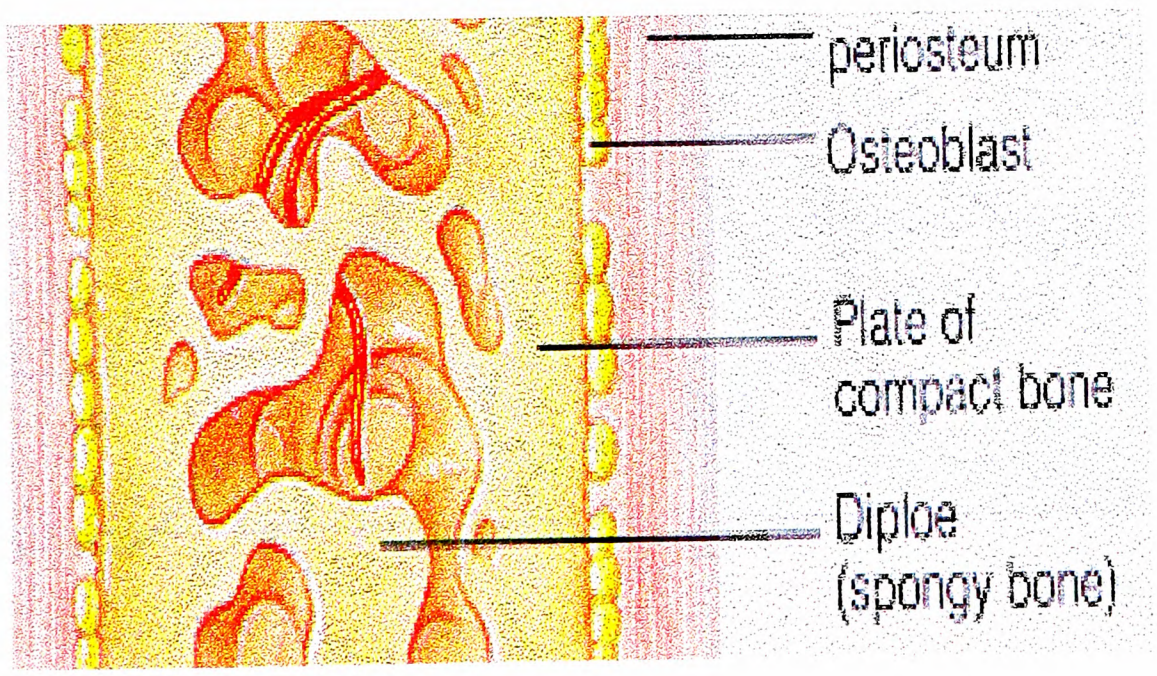


Fig. 1.2. The four stages (a, b, c & d) of intramembraneous ossification. (Source: Marieb E. 1997).

Finally, secondary ossification centres appear at the two epiphysises to produce similar events as in primary ossification. When secondary ossification is completed, only the hyaline cartilage and the growth plate between the diaphysis and epiphysis, remain.

1.1.5. Types of bone tissues.

Cortical and trabecular bones consist of similar minerals and proteins. Cortical bone differs in some respect from trabecular bone in that it appears almost ivory-like in its solidity but is far from inert, though it does have a low metabolism, with fewer remodelling sites and a lower turnover than the more open-structured trabecular bone (Birdwood, 1996).

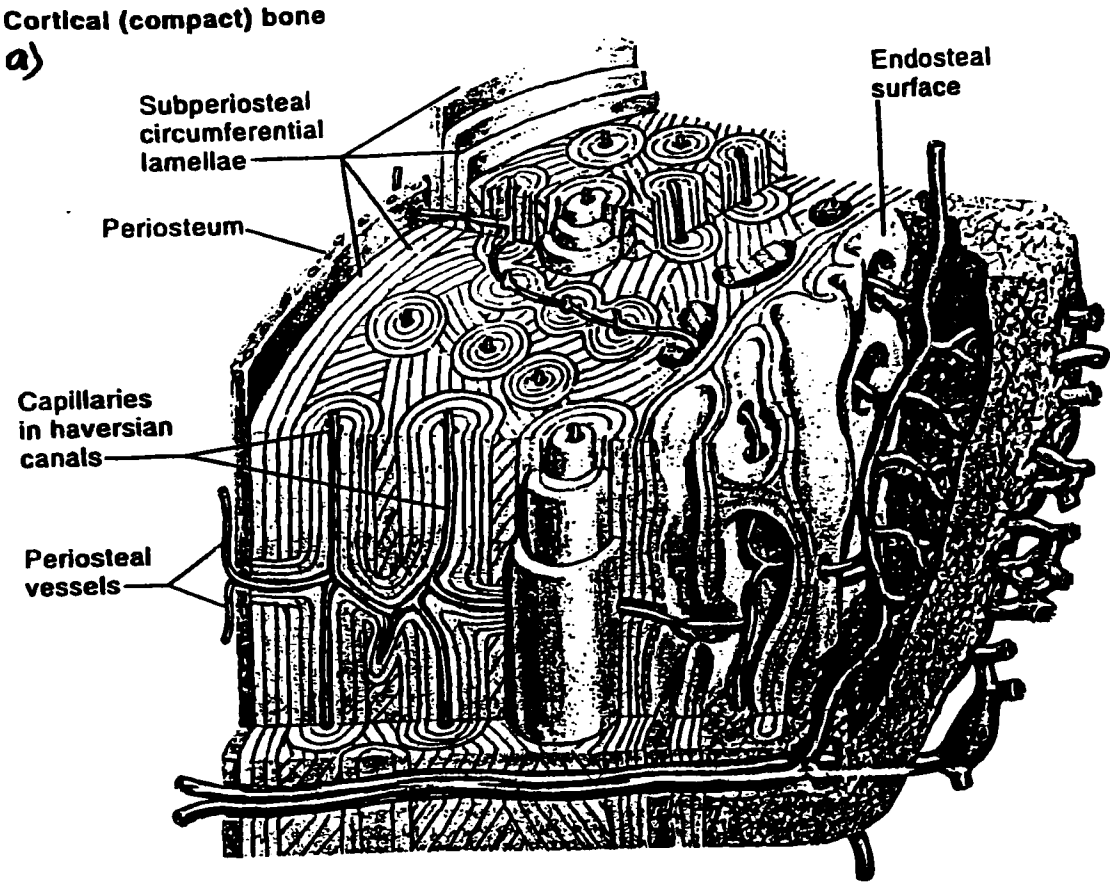
1.1.5(a) Cortical bone.

Cortical or lamellar bone, has many openings or passageways for nerves, blood vessels, and lymphatic vessels, serving the osteons of the Haversian system. Each osteon is made up of bone matrix arranged in concentric rings, lamellae, around the central Haversian canal, which is orientated along the long axis of the bones (Pfeiffer, 1998; **fig.1.3**). In contrast, the Volkmann's canals are found at right angle to the long bone axis and connect the blood vessels and nerves of the periosteum to those of the Haversian canals and the medullary cavity.

Osteocytes lie between the lamellae in small concavities known as lacunae. All lacunae are connected to each other and to the Haversian canals by canalliculi to facilitate the exchange of nutrients and waste products. The closely packed osteons make the cortical bone very solid and allows this type of bone to generate more powerful muscular actions.

1.1.5(b). Trabecular bone.

Trabecular bone is spongy in appearance and enclosed in a hard outer



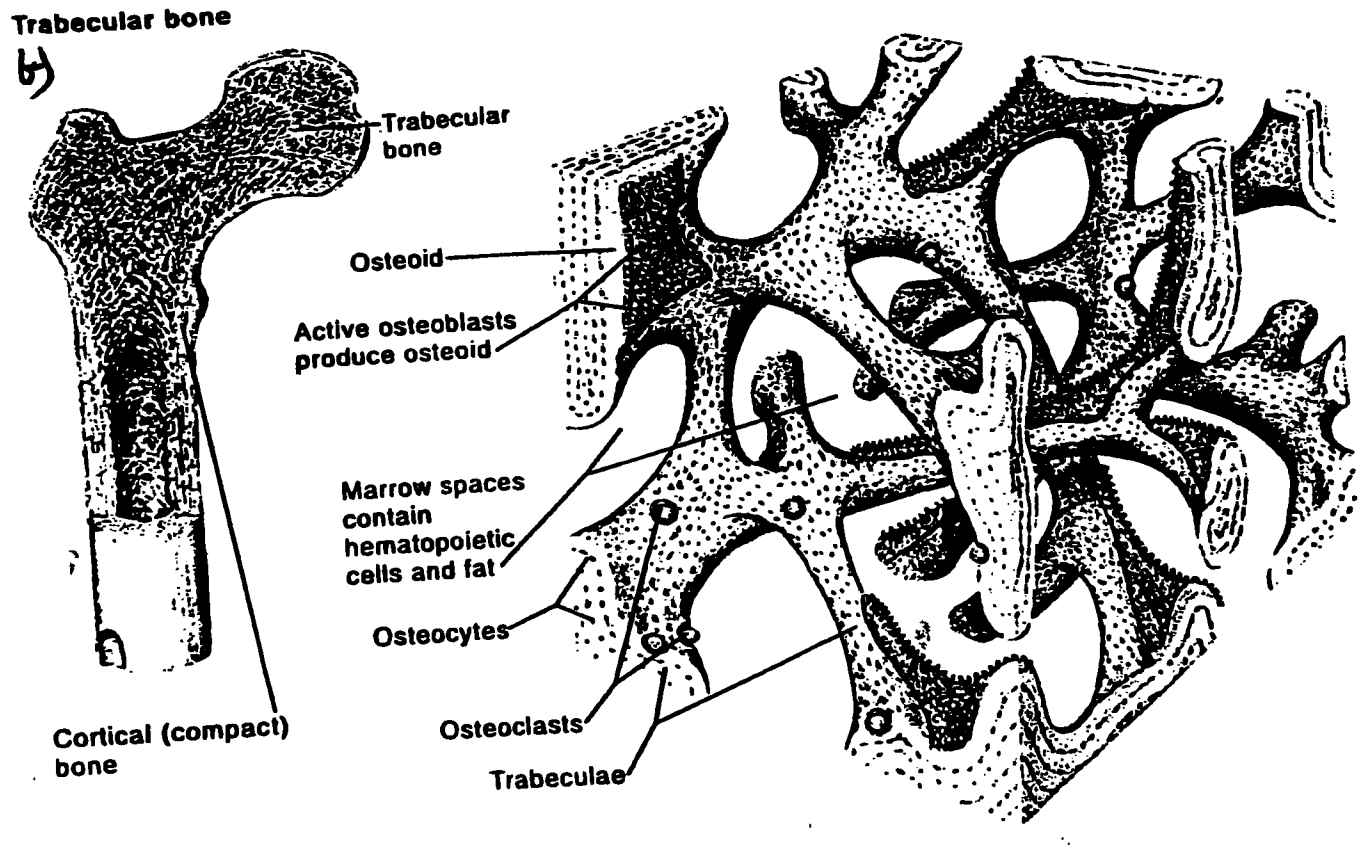


Fig. 1.3a & 13b. Structures of cortical and trabecular bone tissues. (Birdwood G, 1996).

crust of cortical bone **(fig. 1.3a).** Trabecular bone is made up of trabecullae (Nakamura & Kurokawa, 1998) containing irregularly arranged lamellae and osteocytes, interconnected by canalliculi, but without osteon (Weinstein & Buckwater, 1994). Trabecular bone obtains nutrients by diffusion from the bone marrow spaces. They are relatively light but strong to protect against physical stress. During growth, it adapts its responses in accordance with Wolff's law which states that a bone, normal or abnormal, develops the structure most suited to resist the forces acting upon it (Birdwood, 1996). Mechanical stress stimulates local trabecular bone formation, seen in athletes, as a result of physical training (Kanders & Demster, 1988). Although lighter than cortical bone, it provides strong bone surfaces (abundant in the ends of long bones) to minimise wear and tear. Loss of trabecular bone tissues is very common with ageing and, if not arrested, can lead to osteoporosis.

Cortical and trabecular bones respond to a vast array of extrinsic influences such as nutritional factors as well as intrinsic factors such as cytokines and hormones (Anderson et al, 1996). While these factors are necessary for healthy bone, they can under certain conditions, also contribute to adverse bone changes which can weaken bone and increase the risk of damage.

1.1.6. Mineralisation of bone.

Mineralisation of bone matrix starts with the laying down of amorphous calcium phosphate crystals within the osteon which is then converted into hydroxyapatite following several reactions. The ratio of calcium to phosphorus, required, can vary markedly under different nutritional conditions but under normal condition is 1.3: 2.(Bruni et al, 1997). The process begins when osteoblasts secrete procollagen fibrils which assemble, extracellularly along the linear collagen fibres for crystal nucleation (St. John -Dixon & Woolf, 1988). Hydroxyapatite crystals are deposited within the cavity in the collagen fibrils, where mineralisation is initiated, and as the crystal deposition increases, it

spreads to form mature and fully calcified bone tissue. Following formation of the osteoid by osteoblasts, there is about 10-15 days lag after which mineralisation begins. This is completed in about two third of the duration for the whole process with the ossification of the final third taking several months. Microcrystals, not bound to collagen, can also be found in other parts of the body such as walls of blood vessels, and occasionally, in synovial fluid of people with osteoarthritis (Masuda & Salu, 1997).

Bone matrix mineralisation is facilitated by collagen and non-collageneous proteins acting as nucleators and by alkaline phosphatase. Calcium and phosphate levels in the extracellular fluid are critical for effective mineralisation. Inhibitors to mineralisation, such as proteoglycan can prevent calcium crystalisation and must be reduced or eliminated.

1.1.7. Types of bone growth.

Foetal fibrous membraneous bone tissues undergo maturation process by intramembraneous ossification to become flat bone whilst cartilagenous foetal bone tissues become mature long bone through the process of endochondral ossification. During youth, long bone continues to grow in length as the epiphyseal plates move further apart for new bone deposition. Growth in the width of bones occurs as new bone tissues are deposited on the periosteal surfaces.

Bone growth occurs throughout childhood and ends in early adulthood. Growth hormone effects, mediated by insulin-like growth factors, is the single important stimulus for bone growth during infancy and childhood.Thyroid hormone modulates growth hormone to ensure skeleton has proper proportion as growth occurs. Sex hormones, in both genders, initiate the growth spurt seen at puberty (fig. 1.6). After menopause, bone growth is reduced with loss of bone mass. Life style such as smoking and alcohol consumption, and nutrition, such as calcium and phosphate, can also influence these processes in all ages, but can

be critical during puberty and immediately after the menopause.

1.1.7(a) Longitudinal bone growth.

Growth in length of long bone occurs through the process of longitudinal bone growth, mimicking endochondral ossification, in which the epiphyseal plate grows by mitosis of the cells onto their distal faces **(fig. 1.4).** The chondrocytes on the proximal face of the plates hypertrophy before the matrix is calcified. The osteoblasts, in the medullary cavity, then ossify the cartilage spicules forming spongy bones (Tortora & Anagnostakos, 1996). This is followed by continuous bone remodelling, removal of old and damaged tissues, and replacing them with new tissues. Long bone growth is reduced towards the end of adolescence (18-21years) when epiphysis and diaphysis fuse. However, bone growth mechanisms are still present and can be influenced by factors such as exercises (Coralli & Raisz, 1986).

1.1.7(b) Appositional bone growth.

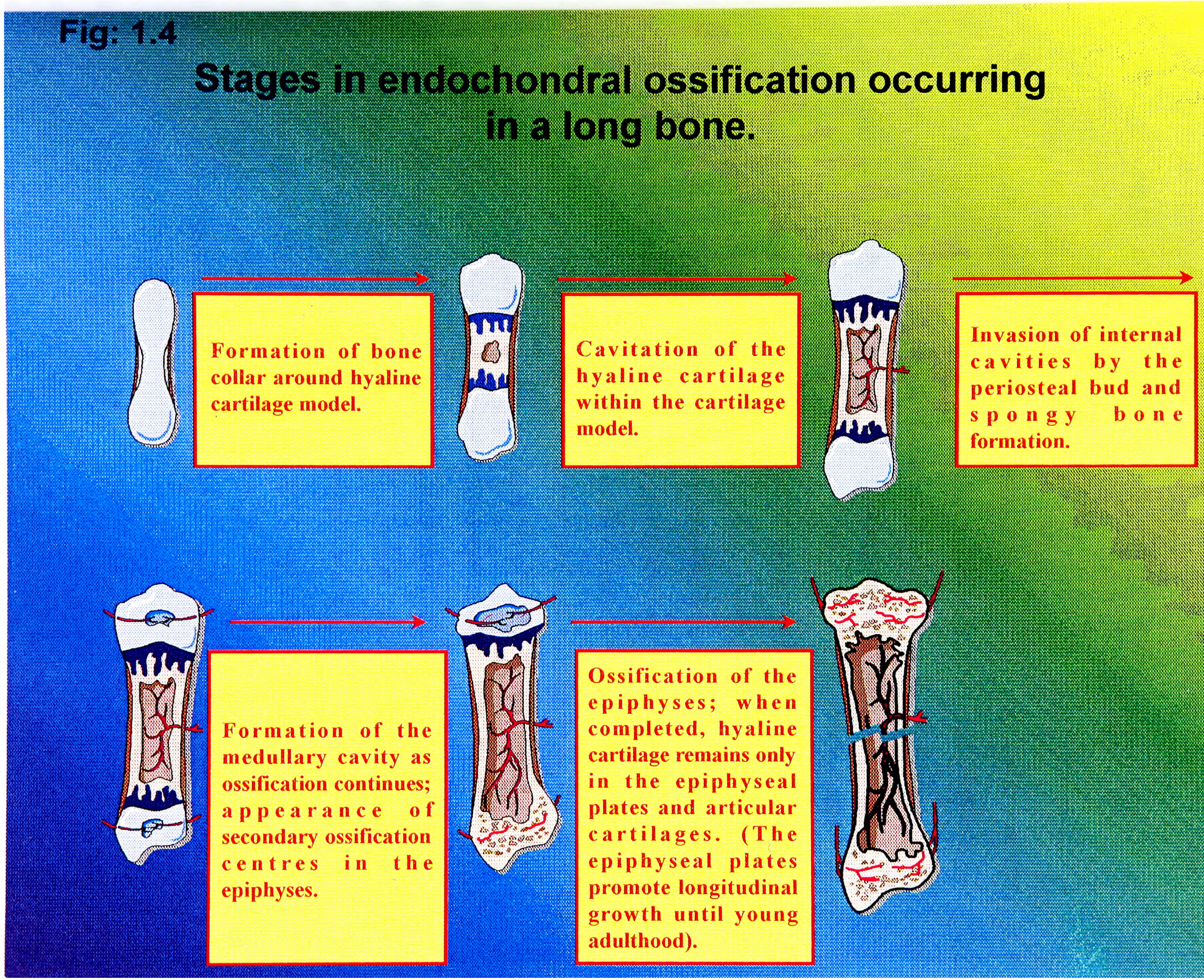
Appositional growth increases bone thickness (diameter) and is due to osteoblasts, beneath the periosteum, secreting bony matrix and depositing it on the external surface of the bone (Decker & Marshall, 1996). This increase in compact bone is usually followed by osteoclast activities on the endosteal surface but these are less compared to those of the osteoblast so that the coupling process produces a thicker and stronger bone.

1.1.7(c) Bone modelling.

Bone growth occurs, evenly, during the first two decades of life, with an additional spurt during adolescence, followed by a period of consolidation during adulthood in which the bone reaches its peak mass, usually around the third decade of life for cortical bone and a few years earlier

Fig: 1.4

Stages in endochondral ossification occurring in a long bone.



Source: School of Health, University of Greenwich, (1999),

for trabecular bone. After menopause, bone growth slows down and is superseded by bone resorption. The modelling of bone ensures that existing bones meet the mechanical demands placed upon them.

1.17(d) Bone remodelling.

In adult, there is continuous turnover of bone to ensure adaptation and enable the repair of damaged tissues. Remodelling involves the coupled cycle between osteoclast and osteoblast and lasts approximately 13 weeks. The turnover of trabecular bone is much greater, and therefore is under increased risks of damage by hormonal and local factors than cortical bone. Remodelling of compact bone starts with osteoclasts forming a cutting cone and moving progressively through older mineralised bone to create a cavity for a new Haversion system. This cavity is then colonised by a vascular stroma and poorly differentiated cells followed by deposit of lamellae by osteoblasts. In trabecular bone, osteoclastic resorption results in the formation of Howship's lacunae which are then filled by new bone tissue from active osteoblasts. The coupling of the functional units of osteoclast and osteoblast is synchronous, so that resorption at one site is balanced by formation at another, with no net weakening of the skeleton **(fig.1.5).** While the coupling osteoblasts and osteclasts maintains bone mass fairly constant, general and local factors can override these mechanisms and therefore influence the final outcome (Cummings et al, 1985). Increased resorption in cortical bone results in wider Haversian canals with increased cortical porosity while in trabecular bone, there is a loss of trabeculae with an increased number of spicules.

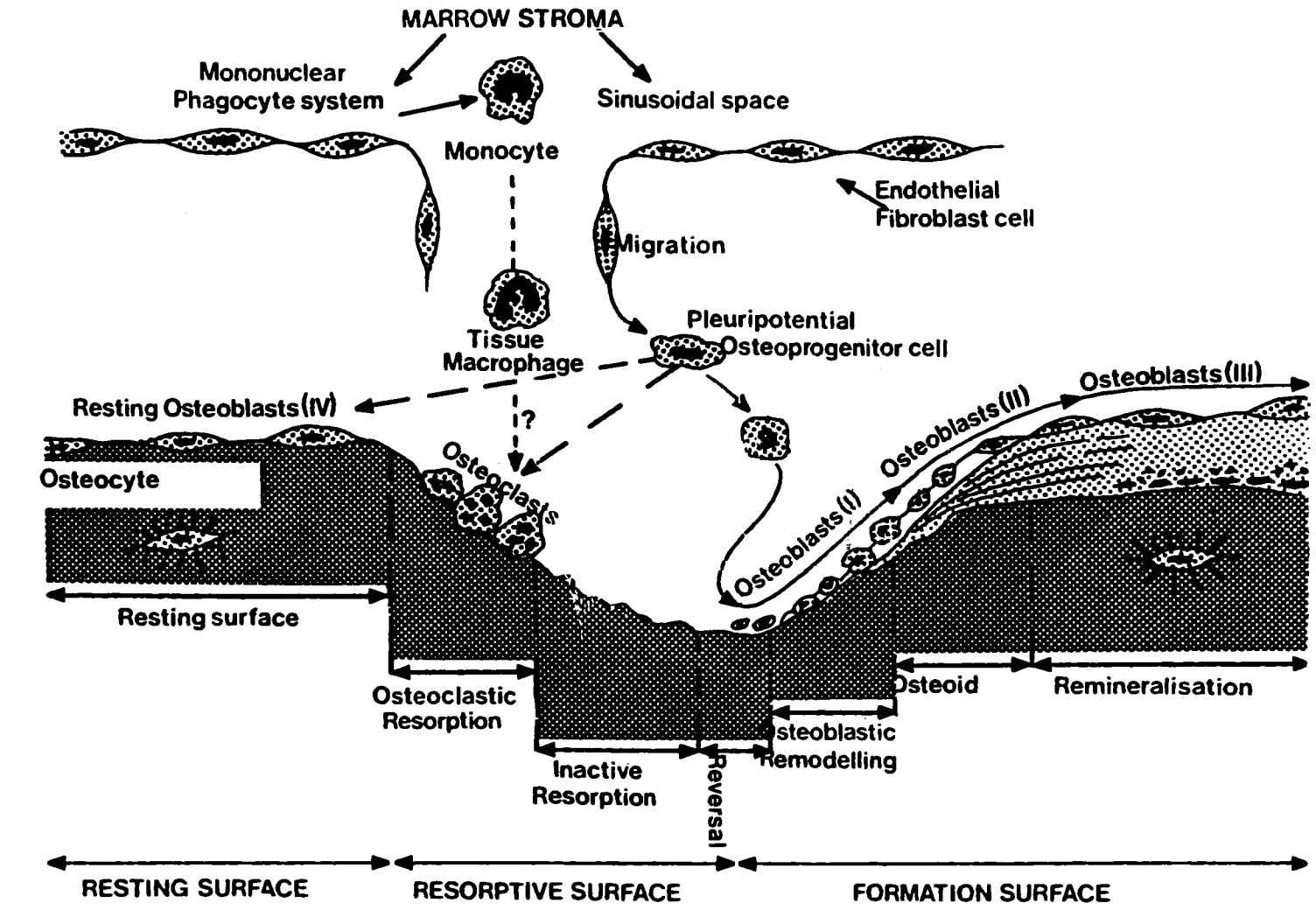


Fig.1.5. Remodelling of bone (Compston E, 1996).

1.1.8. Factors affecting bone growth, composition and mass.

Peak bone mass refers to the highest volume of bone tissues present during the life-time and depends on the balance between bone formation and resorption (Nieves et al, 1998). Bone mass increases progressively during foetal life, adolescence and early adulthood to reach a peak around the age of 30 years and thereafter falls slowly in both sexes until menopause (Birdwood, 1996; **fig.1.6).** Immediately after menopause, bone mass is further reduced in women compared to men due to the reduction in oestrogen level (Reid, 1997). Genetic, environmental factors and life style influence the activity of bone cells and, therefore, will dictate the final peak bone mass (Barr & McKay, 1998).

1.1.8(a) Genetic factors.

Genetic factors account for between 75-80 % of peak bone mass (Eisman et al, 1995). Women who develop osteoporosis early in life are apt to have daughters at risks of getting the disease as bone mass of sisters, mothers and daughters are closely related (Krall & Dawson, 1993). Males, generally, have a greater bone and muscle mass and bone density than females (O'Neill et al, 1996). During pubertal growth spurt, bone of negros shows a greater acceleration of growth than their caucasian counterparts so that by adulthood, they have a greater bone mass (Gilsanz et al, 1991). Peak bone mass is also influenced by the presence of vitamin D (Cooper & Umbach, 1996) and oestrogen receptors in bone and by transforming growth factor β (TGFβ). Body size, lean mass and fat mass are important in bone mass determinations and are partly influenced by genes (Schwartz, 1993).

1.1.8(b) Age.

Bone mass and bone minerals are progressively lost with age, reducing bone strength and predisposing them to fractures under minimal stress (Wang & Kaisak, 1998). From childhood to puberty, there is an increase in new bone deposition through the process of osteogenesis (Tortora & Anagnostakos, 1996). At puberty, this linear growth is superimposed by an additional increase through the influence of sex hormones which lasts until the 3rd decade of life when the rate of increase slows down (Beck et al, 1993; **fig.1.6**).

After menopause, overall bone mass decreases as the levels of sex hormones fall (Odell & Heath III, 1993) and there is also widening of the Haversian canals on the endosteal surface of cortical bone, with a reduction in new bone deposition and which leads to increased intra-cortical porosity. In trabecular bone, there is a thinning and loss of trabeculae and an increase in microfractures (Bohannon & Kinnett, 1994). In both, men and women, age-related decline in bone mass continues well into extreme old age (Jones et al,

1994) but in female, there is an additional period of rapid bone loss predominantly due to oestrogen withdrawal (Rigg & Melton, 1986). However, there is a large variations in normal values of peak bone density within any given age group, gender or culture (Seeman & Young, 1993).

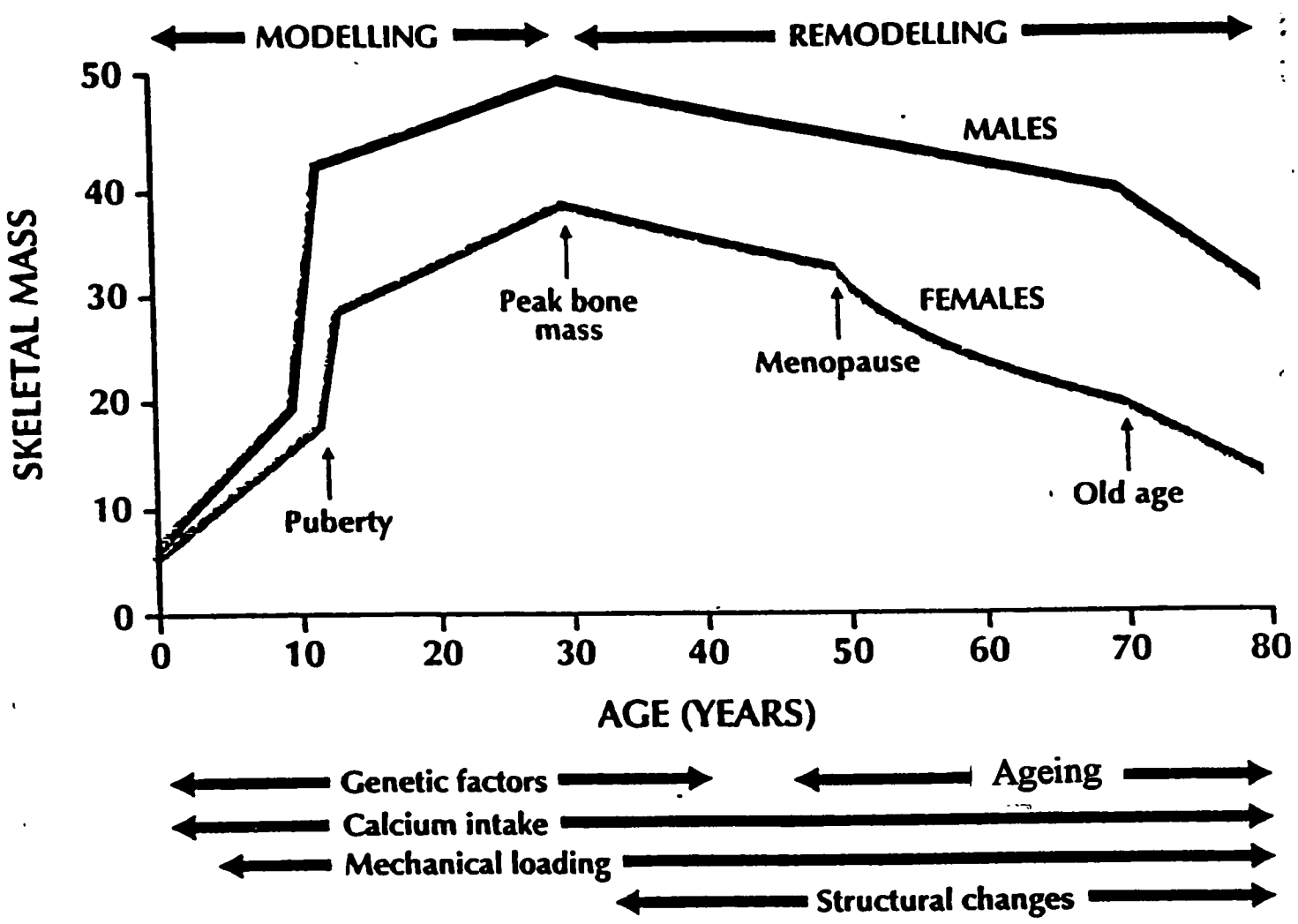


Fig.1.6. The relationship between age and bone mass.

(Birdwood G. 1996).

(The peak bone mass attained in early adulthood, is normally 30-50% higher in men. Women, therefore, only need to lose about half as much bone as men to become osteoporotic. Factors influencing modelling and remodelling are given at the bottom of graph).

1.1.8(c) Gender.

Males generally have a larger bone mass and density and greater muscle mass than females (O'Neil et al, 1996). Females have an increased osteoclastic

activity immediately after menopause than males and as a result the incidence of fractures is higher in females than in males over the age of 65 years (Lashas et al, 1996).

1.1.8(d) Culture and race.

During pubertal growth spurt, black girls show a greater acceleration of bone growth than white girls (Gilsanz et al, 1991; **fig.1.7),** so that by maturity, black females have, on average, about 10% greater bone mass than caucasians females. Fracture rates are more common in caucasians than Asians with the least in blacks. These differences also apply to males (Barron et al, 1994).

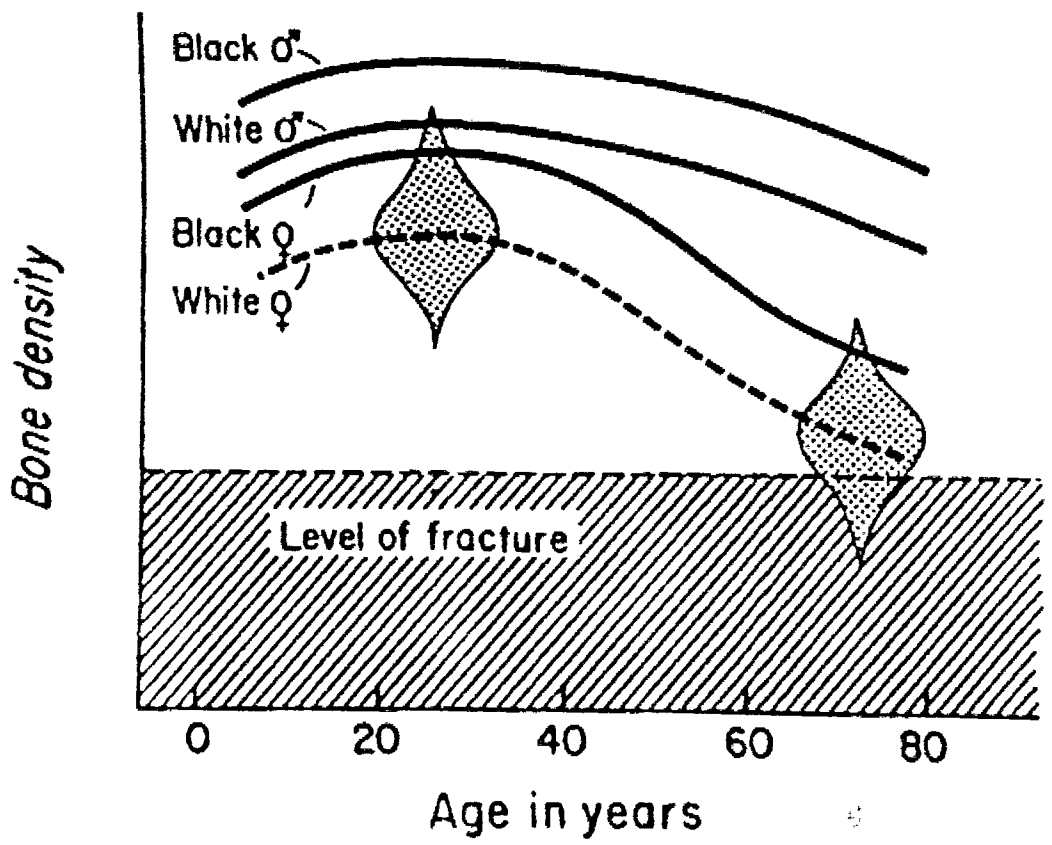


Fig.1.7. Cultural / racial differences in bone mass.(Reid IR, 1997).

1.1.8(e) Sex hormones.

Human growth hormone is responsible for 50% of bone mass production in pre-pubertal females and only 10% in males (Riggs et al, 1982). At puberty,

the bone growth spurt is due to increases in sex hormones in both genders (Marieb, 1997). Hypogonadism, due to surgery or chemicals, is associated with a reduction in peak bone mass in young males and females (Seeman & Young, 1993). In post-menopausal women, bone mass decreases due to reduction in oestrogen level (Marcus, 1996). Oestrogen binds to oestrogen receptors within osteoblast to mediate anti-resorptive effects on bone, but with advancing age, there is an increased loss of oestrogen receptor regulation (Ng & Roma, 1997). The reduction in the sex hormone levels also induces a transient increase in osteoclastogenesis in both genders and is associated with the early phase of rapid bone loss (Most et al, 1997). There is an increased production of cytokines such as IL-1, IL-6 and TNF by osteoblast, as a result of falling level of oestrogen, so that bone resorption is further increased (Lader & Flanagan, 1998).

Nguyen et al, (1995) reported a positive correlation between bone mass and reproductive history, whilst Bauer et al, (1993) found a negative effect of parity on bone mass as well as fracture risks. Breast feeding may cause a decline in bone mass density in the nursing mother as calcium, phosphate and magnesium are diverted into the child's feed but any osseous damage is reversed when breast feeding is stopped (Bauer et al, 1993). Testosterone deficiency has been associated with osteoporosis in men as testosterone increases differentiation and proliferation of osteoblasts (Anderson, 1998).

1.1.8(f) Parathyroid hormone (PTH).

This hormone, produced by the parathyroid glands, stimulates osteoclastic resorption of bones (Kochersberger et al, 1987). It increases osteoclast's production of adenylate cyclase, intracellular calcium, cyclic adenosine monophosphate (cAMP) and activates a protein kinase which inhibits collagen synthesis at the mRNA (messenger ribonucleic acid) level. PTH increases calcium and decreases phosphate resorption from the kidney

tubules, but while serum calcium level increases, there will be a decrease in the calcium phosphate product required for bone mineralisation because the ratio of calcium to phosphate will be subsequently disturbed (Sharon, 1989). It also increases 1,25-dihydroxy-vitamin D synthesis by the proximal convoluted tubules with a subsequent increase in intestinal calcium absorption (Thorsen et al, 1996). However, Arnaud (1993) suggested that PTH does not play any significant role in bone resorption, particularly during early menopause, as the reduced oestrogen is most probably responsible for the bone resorption.

1.1.8(g) Calcitonin.

Calcitonin is produced by the thyroid glands and inhibits bone resorption by binding to high affinity receptors on osteoclasts (Hillard & Stevenson, 1991). It rapidly impairs osteoclast functions as there is a decreased urinary excretion of pyridinoline cross-links (Itabashi, 1998). It also responds to short-lived changes in serum calcium due to dietary loading in order to prevent hypercalcaemia. Calcitonin increases intracellular cyclic adenosine monophosphate which produces an inhibitory effect on osteoclast (Overgaar et al, 1992). Calcitonin levels in normal women are lower than in men of corresponding age and the levels consistently decline with age (Itabashi, 1998).

1.1.8(h) Insulin and insulin-like growth factors(somatomedins).

Insulin has a trophic effect on bone and at physiological levels increase collagen synthesis (Hosking, 1993). Deficiency, as seen in type I diabetes mellitus, is associated with low bone mass. Insulin-like growth factors, IGF-1 and IGF-2, stimulate collagen, non-collagen proteins and deoxyribonucleic acid (DNA) synthesis as well as replication of bone cells, chondrocytes and increase the production of matrix constituents (Lynch et al, 1989). Glucocorticosteroids, at physiological concentrations, potentiate the IGF-1 effects on collagen

synthesis with an increase in the number of somatomedin receptors in bone cells (Lukert & Raisz, 1990).

1.1.8(i) Growth hormone and Growth factors.

Growth hormone stimulates growth of the skeleton by increasing synthesis and secretion of somatomedins (Kotake et al, 1996). Growth hormone is most effective on bone growth during infancy and childhood with reduced influence during adulthood and in the elderly. Other growth factors such as epidermal (EGF), fibroblasts (FGF) and platelet-derived (PDGF) also increase bone growth by stimulating the proliferation of bone cells and increasing protein synthesis. However, they also stimulate bone resorption mediated through prostaglandins although EGF can also independently stimulate resorption (Konttinen et al, 1997).

1.1.8(j) Thyroid hormone.

Thyroid hormone stimulates cartilage growth directly and synergistically with somatomedins. In hyperthyroidism, there is an increase in bone turnover with hypercalcaemia and a decrease in PTH, 1,25-dihydroxy-vitamin D and intestinal calcium absorption which, if prolonged, may lead to osteoporotic bone changes (Barran, 1991). Thyroid hormone deficiency decreases the rate of bone turnover and delays growth generally.

1.1.8(k) Prostaglandins (PGs).

These are synthesised and secreted by many cells including bone and macrophages. There are several known PGs; the most common PG influencing bone is PGE_2.. Prostaglandins have biphasic effects on bone since PTH stimulates production of PGE_2 whilst 17β oestradiol reduces PTH secretion and inhibit PGs production (Yoshikuwa, 1998). Akatsu et al, (1991) reported that prostaglandin E_2 directly stimulates bone resorption and is associated with

the osteolysis and hypercalcaemia in neoplasms but more recently Harrison et al, (1994) noted that PGs do not act directly on osteoclast but as local intermediary regulators to influence the coupling between osteoblasts and osteoclasts. However, indomethacin, a prostaglandin synthetase inhibitor, reverses the increased bone resorption seen with prolonged immobilisation (Thompson & Rodan, 1986). Interleukin IL-1 prevents synthesis of collagen partly mediated by prostaglandins (Harrison et al, 1994). Prostaglandins also stimulate bone growth in the presence of cortisol, by increasing replication and differentiation of osteoblast precursors, mediated by cyclic AMP and insulin-like growth factors (Gronowicz et al, 1994). Endogenous PGs promote healing of bone fractures and contribute to bone mass through impact loading which itself increases local production of growth factors (Yang et al, 1998).

1.1.8(l) Cytokines.

Cytokines are cell mediators involved in intercellular communications. Several cytokines and their roles in bone turnover have been identified (Konttinen et al, 1997; **appendix 4).** Interleukin-1 (IL-1) stimulates the synthesis and release of enzymes such as collagenase, stromelysin and plasminogen activator involved in degradation of bone collagen matrix. It also increases the level of prostaglandins (Kirkham, 1991). The action of tumour necrosis factor (TNFα) is mediated by a transcription nuclear factor-kappa B (NF-kappa B) to increase production of cytokines and cell adhesion molecules to promote bone resorption and inflammation (Kurokouchi et al, 1998).

1.1.9. Environmental factors, diet and life style.

1.1.9(a) Dietary calcium.

Low dietary intake of calcium reduces the formation of hydroxyapatites and increases the risk of fractures (Petridou & Karpathios, 1997). Bunker, (1994) proposed that high intake of milk and other dairy products containing calcium during childhood and early adulthood are vital for healthy bone. Johnson et al, (1992) estimated that calcium demands for skeletal growth during pre-pubertal stage is greater than that at any other time when as much as 1600 mg / day of calcium may be necessary to meet the adolescent's calcium requirement to achieve optimum peak bone mass (Kanders & Demster, 1988). Low calcium intake by pre-pubertal females will seriously compromise their accrual of bone mass (Chan, 1991). Adequate dietary calcium intake, therefore, appears crucial for achieving maximum peak bone mass and minimising age-related bone mass loss (Cumming, 1990). Average calcium intakes for many teenagers, particularly females, is lower than the recommended daily requirement -1500 mg / day (Porthmouth et al, 1994).

Calcium supplements, in women up to 10 years after menopause, appear to slow down the rate of bone loss and can give maximum protection particularly if taken with hormone replacement therapy (Schneider et al, 1997). There is an increased loss of calcium in post- menopausal compared to pre-menopausal women (Elders et al, 1991). Bone mass losses in men, due to dietary calcium depletion, is not conclusive. Calcitonin and vitamin D increase calcaemia whilst PTH is inversely related to serum calcium level (Rizoli & Bonjour, 1998). Kelly et al, (1990) proposed that calcium intake should be seriouly considered as an independent predictor of bone mineral density.

Calcium balance, generally, reflects the degree to which bone formation is coupled with resorption; with a negative balance, bone resorption exceeds formation and vice-versa (Cumming, 1990). Calcium homeostasis depends upon

several factors such as the amount of calcium in the diet, absorption rate from the gastro-intestinal tract and excretion via urine and faeces.

1.1.9(b) Dietary magnesium.

The adult human body contains about 25g of magnesium of which up to 50% may be in bone (Porth, 1994). Magnesium is required for the synthesis of 1,25 dihydroxyvitamin D, the release of PTH, and it is a co-factor for alkaline phosphatase production by osteoblast (Leone & Rezende, 1998). Magnesium deficiency can result from poor dietary intakes, as in chronic alcoholism or in teenagers on voluntary dietary restrictions. Magnesium maintains a low level of intracellular calcium ion by competing for membrane binding sites with calcium (Ryan, 1991). Elevated magnesium may also promote calciuria by suppressing PTH production (Durlach & Collery, 1984). Deficiency of magnesium may lead to reduced bone magnesium and abnormal crystal formation and possibly even osteoporosis if not corrected in time (Cohen & Kitzes, 1981). However, boron promotes bone formation at lower level of magnesium (Neilsen, 1992)

1.1.9(c) Dietary phosphates.

Phosphate, a major element in hydroxyapatite, is a key inorganic constituent of bone (Marieb, 1997). Serum level of phosphate acts as one of the regulators for the rate of renal production of $1,25(OH)_2D_3$. During chronic hypophosphataemia, defective bone mineralisation occurs (Cooper et al, 1996). Low dietary phosphate intake reduces intestinal absorption of calcium due to reduction in 1,25 (OH_2) D_3 production (Portale et al, 1986). Elderly eskimos, with a habitually high intake of proteins and phosphorus and low calcium intakes, have been associated with an increased loss of cortical bone (Mazess & Mather, 1974). However, pre-and post-menopausal lacto-ovo-vegetarians lose less cortical bone than age-matched omnivorous as they have a higher dietary intake of proteins and phosphorus (Reed et al, 1994).

1.1.9(d) Dietary sodium and proteins.

The effects of dietary sodium and protein on bone are unclear. Need & Morris, (1991) proposed that excess intakes of sodium may contribute to low bone mass through obligate urinary calcium loss. Orwell, (1992) and Cooper et al, (1996) found a positive association between proteins intakes and bone density in pre-menopausal but not in post-menopausal women.

1.1.9(e) Vegetarians / non-vegetarians.

Male and female vegetarians lose less bone minerals than their omnivorous counterparts as a dietary cocktail of animals proteins and fibres of omnivores decreases the absorption of calcium and causes hypocalcaemia due to chelation of calcium and calcium - phosphate imbalances (Spencer et al, 1983). Increased intake of purified proteins dramatically increases urinary excretion of calcium as a result of a raised glomerular filtration rate and decreases fractional renal tubular resorption of calcium. In comparison, normal diet containing a balanced phosphate intake may actually lower the rate of calcium excretion (Barzel & Massey, 1998). There is also increased bone loss in omnivorous females over the age of 50 years, compared to lacto-ovo-vegetarians of same age but no significant differences in 20, 30 or 40 year olds (Marsh et al, 1980). In contrast, reduction in dietary protein causes progressive bone growth retardation due to decreased plasma concentration of IGF-1 (Leili & Scanes, 1998).

1.1.9(f) Vitamin supplements.

Vitamin A, a fat soluble vitamin, is formed from provitamin carotene in the guts and is abundant in fish, liver and fortified foods. It facilitates synthesis of chondroitin sulphate, a substance that intermingles with collagen fibres giving plasticity to the organic matrix (Marieb, 1997). Vitamin C, a water soluble vitamin found in citrous fruits and green vegetables, promotes the

addition of hydroxyl groups to proline and the formation of cross-linkages between collagen molecules to add tensile strength to the bone matrix (Devlin, 1997). It is also an anti-oxidant and therefore bonds free radicals which otherwise can damage plasma membrane of various cells. Vitamins B_6, found in meat, fish and vegetables, is important for glycogenesis and is involved in several amino acid metabolism and conversion of tryptophan to niacin which is known to inhibit cholesterol synthesis. Depletion may result in reduced IL-2 levels which may impair the immune system as T-helper cells production is already reduced in the elderly (Miller & Stutman, 1981). Vitamins B_6 and B_{12}, found in meat and fish and some dairy products, are involved in metabolising methionine to homocysteine which, in turn, is converted to cystathione. Hyperhomocysteinaemia is elevated in B_{12} and folate deficiency and is associated with osteoporosis (Mc Cully, 1993). Healthy elderly people have higher plasma level of homocysteine than younger people probably related to age-associated impairment of their diet or reduction in absorption of these vitamins from the gastrointestinal tract (Joosten et al, 1993).

Vitamin D, a fat soluble vitamin produced by the action of ultra-violet light on 7-dehydrocholecalciferol, is converted in the kidneys to 1,25 hydroxy-vitamin D which facilitates absorption of calcium from the guts by inducing local synthesis of calcium-carrier proteins (Zerwekh & Reed, 1998). However, an increase in vitamin D causes excess bone destruction through activation of osteoclast (Bataille & Fardelline, 1998). Dietary supplements of calcium and vitamin D moderately reduce bone loss in the femur, neck bones, spine as well as reduction in the overall incidence of non-vertebral fractures in men and women over 65 years old (Dawson-Hughes et al, 1995).

1.1.9(g) Alcohol consumptions.

It is not clear whether 'social drinking'(20-30g alcohol / week) increases bone turnover, although Sampson, (1998) reported a negative linear

relationship between alcohol intake and bone density. However, alcohol intake of 70-140 g / week decreases bone mass in pre-menopausal (Albers, 1990) and post-menopausal women (Hernandez-Avilla et al, 1991).

There is a decrease in bone mass in heavy drinkers compared to non-drinkers and moderate drinkers (Hansen et al, 1991). In many chronic alcoholics, testosterone production is reduced (Slemenda et al, 1992) although other work indicate no reduction in serum testosterone or cortisol (Peris et al, 1992). Chronic alcoholism frequently leads to bone loss in the spine and femoral neck and the amount of bone lost is proportional to the duration of alcohol intake (Birdwood, 1996). Alcohol depresses bone formation by reducing Gla-protein level, an index for bone formation as it is produced by osteoblasts (Peris et al, 1992) while Feitelberg et al, (1987) associated these deleterious effects of high alcohol consumptions on bone to (a) a direct effect on osteoblast, (b) an indirect effect on osteoblast by inducing hypogonadism particularly in men, (c) reduced mobility and (d) low protein and calcium dietary intakes as well as reduced absorption from the gastrointestinal tract.

1.1.9(h) Caffeine consumptions.

Moderate caffeine intake reduces bone density and increases susceptibility to fractures (Keil et al, 1990) and excessive caffeine accelerates the development of osteoporosis in both sexes (Cooper et al, 1992). Caffeine intake may also offset the beneficial effects of oestrogen on calcium metabolism (Holligberg, 1985) and increase urinary calcium excretion in women taking oestrogen supplements (Massey & Wise, 1984). In addition, excessive caffeine ingestion may increase excretion of magnesium, sodium and chloride. Daily intake of 2 cups of caffeine may result in as much as 22 mg / day net loss of calcium in menstruating 35-45 year old females. If these subjects carry on drinking coffee at this rate, it may cause a negative calcium balance of up to 40 mg / day by the post-menopausal period which may account

for as much as 1-1.5 % loss of bone mass annually (Heaney & Recker, 1986).

1.1.9(i) Smoking.

Smoking is associated with reduced bone mass as well as an increase risk of related fractures in both sexes (Valimaki et al, 1994). Smoking reduces circulating oestrogen level by converting it into 2-methoxyestrone which not only lacks oestrogenic properties but also blocks oestradiol receptors (Birdwood, 1996). Hansen et al, (1991) proposed that bone loss through smoking is not due to the cigarette smoke itself but mediated through premature menopause, which reduces body weight and enhances metabolic breakdown of oestrogen. Reid, (1997) found a good correlation of low body weight to loss of bone mass in females.

Teenage smoking is increasing particularly among young female college students, but very little research is available on whether there is any direct link with delayed bone growth (Mazess & Burden, 1991). However, smoking in older subjects in both sexes, decreases bone mineral density (Hollenbach et al, 1993). Oestradiol secretion ceases prematurely in heavy longstanding smokers and results in an early onset of post-menopausal bone loss (Pocock et al, 1989).

1.1.9(j) Physical activities.

The effects of exercise on bone is related to sites, age at which exercises started, hormonal status and amount and types of exercise (Smith & Gillian, 1996). No exercise results in bone loss through immobility (Krolner & Toft, 1983). Moderate to highly physically active individuals have a larger bone mass than sedentary persons (Brahm et al, 1998). Physical activities such as cycling, walking the dog and swimming are unlikely to increase bone mass significantly as they are not weight-bearing exercises (Basssey, 1995). Subjects who engaged in brisk walking, jogging for over 30 minutes, played football, rugby

and performed gymnastics at least once a week, show an increase in bone mass (Allen, 1994).

High intensity physical activities in children and in young adults correlate with significant bone mass increases and may be a determinant of peak bone mass (Valimaki et al, 1994). Postmenopausal women show higher bone mass when subjected to high load and low repetition strength training regimes compared to low load and high repetitions group (Kerr et al, 1996). On the other hand, excessive strenuous physical exercise can reduce bone mass through irregularities in menstrual cycle with a reduction in oestrogen and progestrone levels in females and testosterone levels in men (Alfredson et al, 1997). However, varied and high impact exercise have only a modest beneficial effect on bone loss (Gutin & Kasper, 1992), while walking does not increase bone mass (Cavanagh & Cann, 1988). On the other hand, Alekel et al, (1995) concluded that physical activities of daily living, such as walking, may have a positive impact on bone mass regardless of menstrual status. However, when the exercise programme is stopped, the bone mass gained, can be loss indicating a reversible process (Forwood & Burr, 1993).

The mechanism by which mechanical strain exerts its physiological effect on bone remains unclear but Allen, (1994) suggested that the strain causes changes in ionic, for example calcium, fluxes in osteoblasts which control local regulation of cytokines, growth factors such as IL-2 and 6 , insulin-like growth factors and prostaglandin E_2 . Mechanical stress also leads to the attraction of positive ions including H^+ in extracellular fluid (ECF) onto the crytalline hydroxyapatites making the ECF more alkaline (Snow-Harter et al, 1992). This increased alkalinity enhances the production of alkaline phosphate which feeds the osteoblast with substances for bone formation. On the other hand, reduced exercise, may decrease the attraction of H^+ in the ECF with a subsequent increase the acid medium which increases osteoclastic activities (Nielsen et al, 1994).

1.1.9(k) Reproductive history.

During pregnancy, oestrogen level increases and therefore may enhance bone mass but the evidence appears inconclusive (Nguyen et al, 1995. High parity and lactation periods have been associated with an increase in bone mass with a subsequent decrease in the risk of fractures (Alderman et al, 1986) either due to the increased weight gain of pregnancy or through increased oestrogen level (Nguyen et al, 1995). Ortega et al, (1998) reported that although the dietary calcium intake of about 75% of pregnant women fails to meet the national recommended requirement, their serum calcium levels remain within the normal ranges and may indicate a depletion in calcium stores. Pregnant women with low calcium, have reduced calcium levels in their breast milk. However, breast feeding provides a transient and reversible decrease in bone mineral density during nursing, but normally there is no lasting detrimental effect on bone mass of the mother as long as the daily dietary calcium intake does not fall below 1200mg / day (Lopez et al, 1996).

1.1.9(l) Diseases.

Several diseases can influence bone structure and functions which can lead to weakness and increased risk of fractures.

1.1.9(l) i Cushing's Syndrome.

Patients with Cushing's syndrome have excess glucocorticoids which can contribute up to 50% to the incidence of osteoporosis (Ross & Linch, 1982). This is compounded by a decrease in sex hormones as a direct result of elevated glucocorticoids through decreased influence of gonadotropin-releasing hormones on the pituitary gland which inhibit oestradiol and testosterone secretion by the ovaries and testes respectively (Yeap & Hosking, 1995).

1.1.9(l) ii Thyrotoxicosis.

Thyrotoxicosis, a disease in which the level of thyroxine hormone is increased, is associated with bone resorption and osteoporosis. Patients suffer from hypercalciuria as PTH, 1,25 dihydroxy vitamin D and calcium absorption rates are decreased (Barran, 1991). Serum type 1 collagen, released from collagen breakdown, is increased in all ages in which thyroxine level is elevated (Harvey et al, 1991).

1.1.9(l) iii Rheumatoid Arthritis.

In up to 80% of cases of rheumatoid arthritis, an autoimmune disorder, there is an increased bone resorption with reduced bone density and weakening of bone as a result of mature collagen breakdown and release of pyridinium cross-links (Siobel et al, 1989). Bone damage can be compounded further if prescription of glucocorticoids as anti-rheumatoid agent is not monitored (Porth, 1994).

1.1.9(l) iv Inflammatory bowels diseases.

Extensive inflammatory bowels diseases such as Crohn's disease and ulcerative colitis, can lead to malabsorption of calcium, vitamin D and other vital nutrients and minerals necessary for the maintenance of existing bone as well as production of new bone (Comspton et al, 1987). If untreated, 3% to 5% of sufferers eventually develop osteoporosis through an increase in the levels of calcitonin, PTH and IL-6 (Konttinen et al, 1997). Again, many patients are prescribed glucocorticoids to control the progress of the disease, an increased serum cortisol may lead to increased bone damage.

1.1.9(l) v Hepatic disorders.

Hepatic conditions, such as chronic alcohol abuse, are associated with decreased bone formation due to secondary malnutrution, hypogonadism,

reduced vitamin D and calcium intake (Lalor et al, 1986) These diseases may increase bone breakdown due to raised acid phosphatase level as well as increase collagen release of hydroxyproline (Reddy & Enwemeka, 1996) and decrease in mineralisation and bone mass (Reid, 1997). Ethanol is also toxic to osteoblasts (Guanabens et al, 1990).

1.1.9(l) vi Diabetes Mellitus.

Insulin is necessary to ensure glucose is available within most cells for the production of adenosine triphosphates (ATP). It increases intracellular amino acid accumulation (Yeap & Hosking, 1995) and bone collagen synthesis through stimulation of insulin receptors in osteoblasts (Levy et al, 1986). Long standing, poorly controlled diabetes with renal complications, may increase the risk of developing osteoporosis by suppressing osteoblasts proliferation and its response to PTH and vitamin D (Kumeda et al, 1998).

1.1.9(m) Drugs and hormone therapy.

Long term use of corticosteroids and thyroxine is associated with bone loss (Saito et al, 1997). Manogalas & Jilka, (1995) reported that thyroxine, some anti-convulsants and antacids which contain aluminium, may reduce bone mass in women who are on a low calcium diet. Some diuretics, such as the thiazide group, have been associated with a lower rate of bone loss (Cualey & Cummings, 1993) and higher bone density (Wasnich & Davis, 1980).

Hormone replacement therapy (HRT) aimed at replacing the loss of sex hormones following menopause, is effective in reducing loss of bone mass. Although HRT has been in practice for many years, the mechanism whereby bone loss is reduced remains unclear. However, oestrogen within the HRT, reduces the production of parathyroid hormone and increases the synthesis of 1,25 dihydroxyvitamin D resulting in increased absorption of calcium from the small intestines (Rozenberg et al, 1995). It acts on osteoblast to increase bone

cells proliferation and collagen synthesis (Horowitz, 1993) as well as increasing apoptosis of osteoclast and therefore increases osteoblast activity (Perkins et al, 1994). HRT suppresses the release of IL-1 and tumour necrosis factor from leucocytes suggesting a possible link between cytokine production and sex hormone deficiencies (Cutolo, 1997).

1.1.10. BORON AND BONE TURNOVER.

1.1.10(a) Boron.

Boron is an ubiquitous element in volcanic rocks, water, air and the earth's crust. Soil contains boron ranging from 2-100 μg / g, rock 5-100 μg / g, seawater 0.5-9.6 μg / ml and fresh water 0.01-1.5 μg / ml (Wood, 1994). Boron enters the human food chain via water, fruits, green vegetables and meats. Associated with pectin, it is essential for vascular plants as a component of cell walls as it stabilises the cell membrane. It is required for the efficient growth of pollen tubes and is involved in membrane transport, stimulation of H^+-pumping ATPase activity and K^+ uptake. Boron may be involved in cerebral function via its effects on the transport across membranes and therefore may influence behaviour (Naghii & Samman, 1997). It affects the synthesis of the extracellular matrix and is beneficial in wound healing and some boronated compounds have been shown to have potent anti-osteoporotic activities in animals (Hunt, 1994). Boron concentration greater than 9000 μg/g in the soil, is toxic to plants. Boronated derivatives are also used as herbicides.

A physiological level of boron reduces urinary excretion of calcium and magnesium. Boron increases the influence of 17 β-estradiol on calcium utilisation during bone mineralisation (Neilson, 1992). Dietary supplement of boron increases serum concentration of testosterone and reduces the risks of bone defects but supplement of 9000 μg/g has toxic effects on the reproductive functions and can lead to atrophy of the gonads and infertility (Chapin et al, 1994). Deficiency of boron has been linked to increased loss of bone minerals and reduced bone growth (Loomis & Durst, 1992).

1.1.10(b) Chemistry of boron (B).

Boron is the 5th element in the periodical table and the only non-metal in Group III and therefore it has many similarities to its neighbours, carbon (C) and Silicon (Si). However, it differs from C and Si because it has one less (i.e.3) valence electron. Its ground-state electronic configuration is [He] $2s^2 2p^1$ with the first 3 ionisation energies of 800.5, 2426.5 and 3858.7 kJ mol^{-1}. There are two stable naturally occurring boron isotopes, ^{10}B and ^{11}B, which have a relative mass of 10.013 and 11.02 respectively (Abou-shakra et al, 1989). The name boron reflects its source (Bor-ax) and its similarity to (Carb-on) i.e. bor-on = boron.

Boric acid is a very weak acid with a published pKa_1 of 9.24 and pKa_2 of 12.74 (Dean, 1979). The electronic configuration, $2s^2\ 2p^1$ reflected in a predominantly tervalence and bond energies, is such that there is no tendency to form univalent compounds. Boron will react with fluoride at normal temperature and is superficially attracted by oxygen but is otherwise inert.

Boron was first isolated from borax and has since been converted into boric anhydride, borax anhydrous, boric acid, ulexite, colmanite, borax (Tincal), borax pentahydrate, boron oxides, borates as well as esters of boric acid and compounds such as borides, boranes and carboranes (Sprague, 1992).

Boron has evolved from its initial use in the gold, glass and food industries at the beginning of this century, to become an important element for normal growth and development. Its commercial values have been exploited to produce toughened glass, ceramics and bleaches for household consumption (Smith, 1986). During the 1980s, boron was identified as an important element in animal and human diet with several possible physiological functions including influencing the properties of cell membrane and maximising the functions of various hormones involved in calcium homeostasis (Blevins & Lukaszewski, 1994).

1.1.10(c) Physiological roles of boron.

While the U.S. diet is reported to contain 1 mg of boron per day (Neilsen, 1992) and Chilean diet about 20 mg (Barr et al, 1993), the average dietary level is between 2.4-4 μg / per day (Iyenger et al, 1988). Mixed diets provide about 1.5-10 mg of boron per day but with diets rich in vegetables and fruits, a higher intake can be achieved (Naghii & Samman, 1993). Boron accumulates in several tissues including bone. 45-75 years old human bones contain between 16-138 μg / g (Alexander et al, 1951), blood 0.04 - 0.36 μg / ml and urine 0.040 - 6.6 μg / ml (Imbus et al, 1963). A more recent work suggests blood level averages 0.097 μg / ml (Clarke & Webber, 1987) and urine 4.3 μg / ml (Abou-Shakra et al, 1989). Liver and brain tissues have 3.5 and 4.3 fold increases compared to fatty tissues (Moseman, 1994). **Appendix 2** list the boron levels in various human tissues and body fluids.

Boronic acids, derivatives of boron, are highly effective in binding serine proteases such as Hageman factor and thrombin to reduce their activities and bind with coagulative factors such Ixa, Xa, XIa, XIIa, to increase blood clotting time (Kettner et al, 1990). Mean cell haemoglobin can be low with an elevated red blood cell count when dietary boron intake is low but when the diet is supplemented with boron, the binding of transferrin to high affinity surface receptors of the plasma membrane of the red blood cell is increased (Nielsen & Mullen, 1991). This is the first step in the red blood cell accumulation of iron during haem synthesis within bone marrow which is vital for the production of healthy haemoglobin (Kaplan, 1983).

Boron influences electrical activities in the brain and a low intake in mature rats increases the amplitude of brain waves at high frequency and increases the proportions of total amplitude at low frequency (Penland & Eberhardt, 1993). Similar results were obtained in healthy elderly humans where low boron intake was significantly associated with a reduction in

performance of various cognitive and psychomotor tasks (Penland, 1994).

Animal extracellular matrix and plant cell wall have several similar properties. Fibrillar framework of cellulose (plant) or collagen (animal) is maintained in a gel of polygalacturinic acid and rhamnogalacturonate (plants) or proteoglycans (animal), and both are stabilised by structural proteins (Hirsinger & Jamet, 1997). The addition of boron to culture medium increases the production of proteoglycan, collagen and total proteins (Benderdour et al, 1997).

Supplementing diet with boron increases serum 17β oestradiol during oestrogen therapy. However, it is not clear whether boron increases oestrogen absorption from the gastro-intestinal tract, reduces urinary excretion or delays breakdown (Nielsen, 1992). The increased blood level of boron is synonymous with an increase in copper and ceruminoplasmin levels during oestrogen therapy indicating perhaps that boron may be mimicking oestrogen (Davis & Mertz, 1987).

1.1.10(d) Boron and ageing.

Work on Drosophila, shows that very low as well as very high boron diets accelerated the ageing process. Boron levels are raised during the developmental stage, in apple peel and in egg shell and may possibly be related to some protective function during growth and development stages and may increase resistance during the ageing process (Massie, 1994). A very high boron level is toxic to the gonads and may reduce bone mass through reduced sex hormones.

A physiological amount of dietary boron modulates energy substrate utilisation and influences the roles of vitamin D_3 in cartilage mineralisation for which large energy provision is required. 17β oestradiol and boron actions are interrelated either by increasing each other's potentials or by mimicking each other's functions particularly those that alter plasma membrane function in the

production of red blood cells and bone cells (Benderdour et al, 1997). Dietary intake of 0.5 mg / day of boron provides optimum maintenance of cellular integrity, various enzymes, minerals and hormone activities for health (Nielsen et al, 1987). In addition, this amount which can be easily obtained from diets rich in fruits and vegetables, induces changes in postmenopausal women consistent with prevention of calcium loss and bone demineralisation (Volpe et al, 1993). Although boron is easily absorbed from the gastrointestinal tract and excreted in urine, uptake into the kidneys, liver, brain and particularly bones can occur, and in chronic conditions, may have adverse health reactions (Litovitz et al, 1988).

Several studies in animals suggest that boron and their derivatives possess anti-osteoporotic effects but these have not been investigated in humans.

1.1.10(e) Boron and bone turnover.

Chicks fed with boron, fluoride and nickel, have showed an increased bone growth (Hunt & Neilsen, 1981). Boron deprivation depresses the growth of chicks when dietary cholecalciferol is deficient due to an interaction between boron and cholecalciferol affecting plasma alkaline phosphatase activity (Hunt & Neilsen, 1981).

Responses to stressors are markedly reduced when the boron level is low (Penland, 1994). During low magnesium, calcium and cholecalciferol levels, effective functioning of the skeleton, brain and kidneys will be reduced if the level of boron is lower than the physiological ranges (Mc Coy et al, 1994). However, boron supplements of 3 mg / day increases the levels of plasma calcium, 1,25 hydroxyvitamin D, β-oestradiol and testosterone in subjects with low boron level (Mastromatteo & Sullivant, 1994) while urinary calcium excretion, serum calcitonin and osteocalcin concentrations will be reduced (Nielsen, 1990). The precise physiological role of boron in bone is not clear but

the fact that boron also decreases the level of calcitonin, a hormone generally considered to prevent bone loss, is contrary to expectations (Nielsen, 1990).

Increasing dietary boron decreases elevated plasma glucose concentration by 29 % compared to only 6 % in a vitamin D_3 control group (Muessig & Hunt, 1991). When chicks' diet, deficient in vitamin D_3 and magnesium, is supplemented with boron, the process of cartilage calcification increases due to increases in plasma calcium and magnesium levels (Hunt, 1994).

In women between 48 - 82 years old, boron supplements in their diet reduce urinary excretion of calcium and magnesium as well as elevating blood 17β oestradiol and testosterone levels. However, rats given increased boron (300 mg / L) in drinking water show an 30% reduction in plasma alkaline phosphatase activity and reduced volume of spongy and osteoid bone tissues suggesting an underfunctioning of osteoblast (Seal & Weeth, 1980).

1.1.11. Cortisol and bone.

1.1.11(a) Biosynthesis, metabolism and excretion of cortisol.

During stress, a large amount of cortisol is produced and released by the adrenal cortex. Stress increases adrenocorticotropin hormone from the pituitary gland which stimulates the synthesis and release of cortisol from the fasciculata cells of the adrenal cortex. Cortisol secretion can also be stimulated by activation of the hypothalamus-pituitary-adrenal axis. About 80 % of cortisol circulates in plasma as 17-hydroxycorticoids and about 20 % as cortisone and 11-deoxycortisol. The liver concentrates cortisol into a water soluble form, about 20% of this is excreted in faeces and the rest is filtered by the kidneys of which 90% is reabsorbed and the rest excreted in urine.

Blood cortisol can be free or bound to globulin and albumin. Under 1 % of total blood cortisol is free and available for renal filtration. During increased stimulating of the adrenal cortex, the cortisol level increases, and once the maximum binding capacity of these elements has been reached, the free cortisol level will increase and therefore urinary excretion will also increase.

1.1.11(b). Cortisol and bone turnover.

During stress, a large amount of cortisol is produced very quickly to help the body cope with and adapt to the damaging effects of the stressor **(fig.1.8).** An individual who is stressed either displays an active or passive behavioural response; the active response is associated with high neurosympathetic activities in which large amounts of catecholamines are produced to mediate coping strategies while the passive response produces large amounts of cortisol to sustain these over a longer period (Ohl & Fuchs, 1999).

Cortisol has a biphasic effect on bone. Physiological concentration maintain osteoblast functions and increases production of collagen by raising the number of somatomedin receptors to promote somotomedin-induced

collagen formation. In contrast, it can also inhibit replications of cells which synthesise type-1 collagen and down regulate collagen gene expression in osteoblast (Canalis, 1983) as well as decreasing bone mass (Hosking, 1993). Bone mass is also lost with continued administration of the synthetic prednisolone possibly mediated by an increase in PTH in response to impairment of intestinal calcium absorption (Lukert & Raisz, 1990).

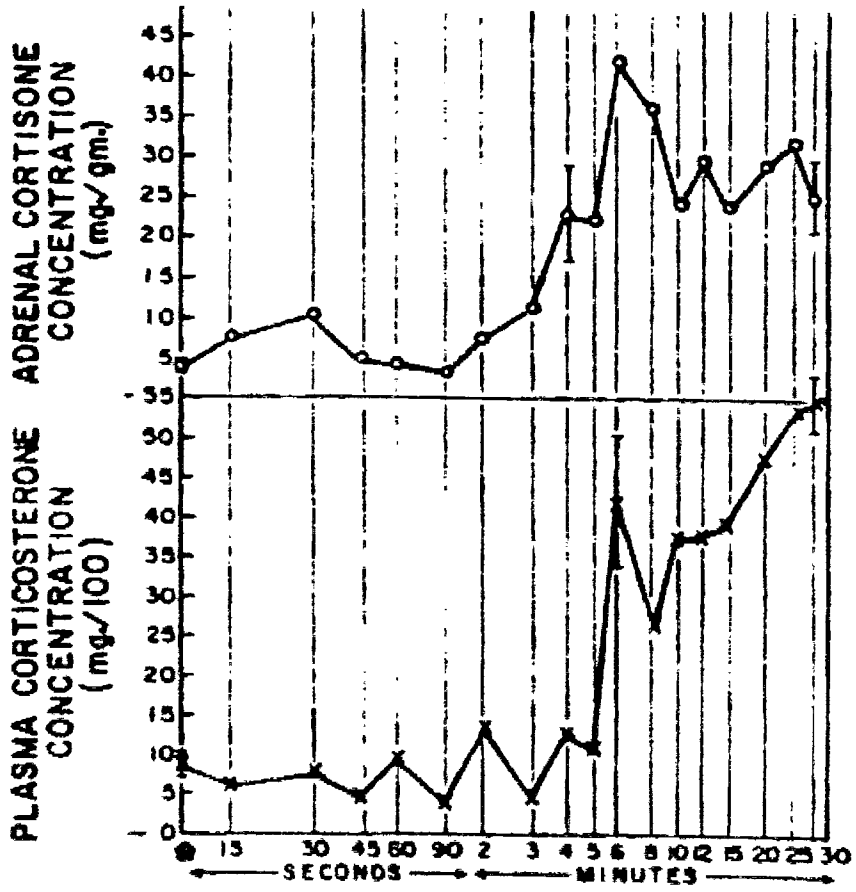


Fig. 1.8. Cortisol production during fracture of the tibia and fibula (stressor) (Guyton A. 1997).

Raised glucocorticoids slow down maturation of osteoblasts, depress calcium and phosphate absorption from the alimentary tracts and reduce calcium and phosphate reabsorption from the glomerular filtrate. This negative calcium and phosphate balance is followed by secondary hyperparathyroidism (Libanati & Baylink, 1992). Cortisol magnifies the effects of catecholamines in inducing vasoconstriction in extremely stressful situations (Ohl & Fuchs, 1997).

When synthetic cortisol compounds are administered to patients, other physiological responses such as anti-inflammatory and immunosuppression have been reported (Bray et al, 1994). Glucocorticoid decreases insulin-like growth factor 1 (IGF-1) and messenger ribonucleic acid (mRNA) reducing collagen synthesis and bone formation (Delany et al, 1995). On the other hand, Kream et al, (1997) and Pandipati et al, (1988) suggested that glucocorticoids stimulate bone formation by inducing differentiation of a preosteoblast progenitor into osteoblast and by increasing transportation of sodium-dependent ascorbic acid into osteoblasts, an essential step in osteoblast differentiation.

1.1.12. Catecholamines and bone.

1.1.12(a) Biosynthesis, metabolism and excretion of catecholamines.

Noradrenaline, adrenaline, dopamine and serotonin are released during stress (Clancy & McVicar, 1995; **appendix 3).** Tyrosine is the major precursor of noradrenaline, adrenaline and dopamine **(fig.1.9),** while serotonin is produced from tryptophan.

Noradrenaline and adrenaline are synthesised from tyrosine in adrenalinergic nerve terminals and in the adrenal medulla by tyrosine hydroxylase using molecular oxygen, reduced pteridine cofactor and ferrous ion. They are stored in local vesicles and if not taken up, some will spill over into blood, while the rest will be metabolised by monoamine oxidase (MAO) and catechol-O-methytransferase (COMT) into the biologically inactive compound 3 methoxy-4-hydroxymandellic acid. This element represents 95% of catecholamines in urine and about 5% is free catecholamines (St. John-Dixon & Woolf, 1988). The levels of these hormones in blood are largely influenced by their production from the adrenal medulla and spill over from adrenergic nerve endings.

Dopamine is made from tyrosine in dopaminergic neurones by tyrosine

hydroxylase using reduced pteridine cofactor, molecular oxygen, ferrous ion and L-dopa decarboxylase. Significant amount will reach the blood but most of it is stored in locally vesicles and released into blood during stress. It is metabolised by MAO and COMT into 3-methoxy-4-hydroxyphenylacetic acid and homovanillic acid and less than 5% excreted in urine as free dopamine.

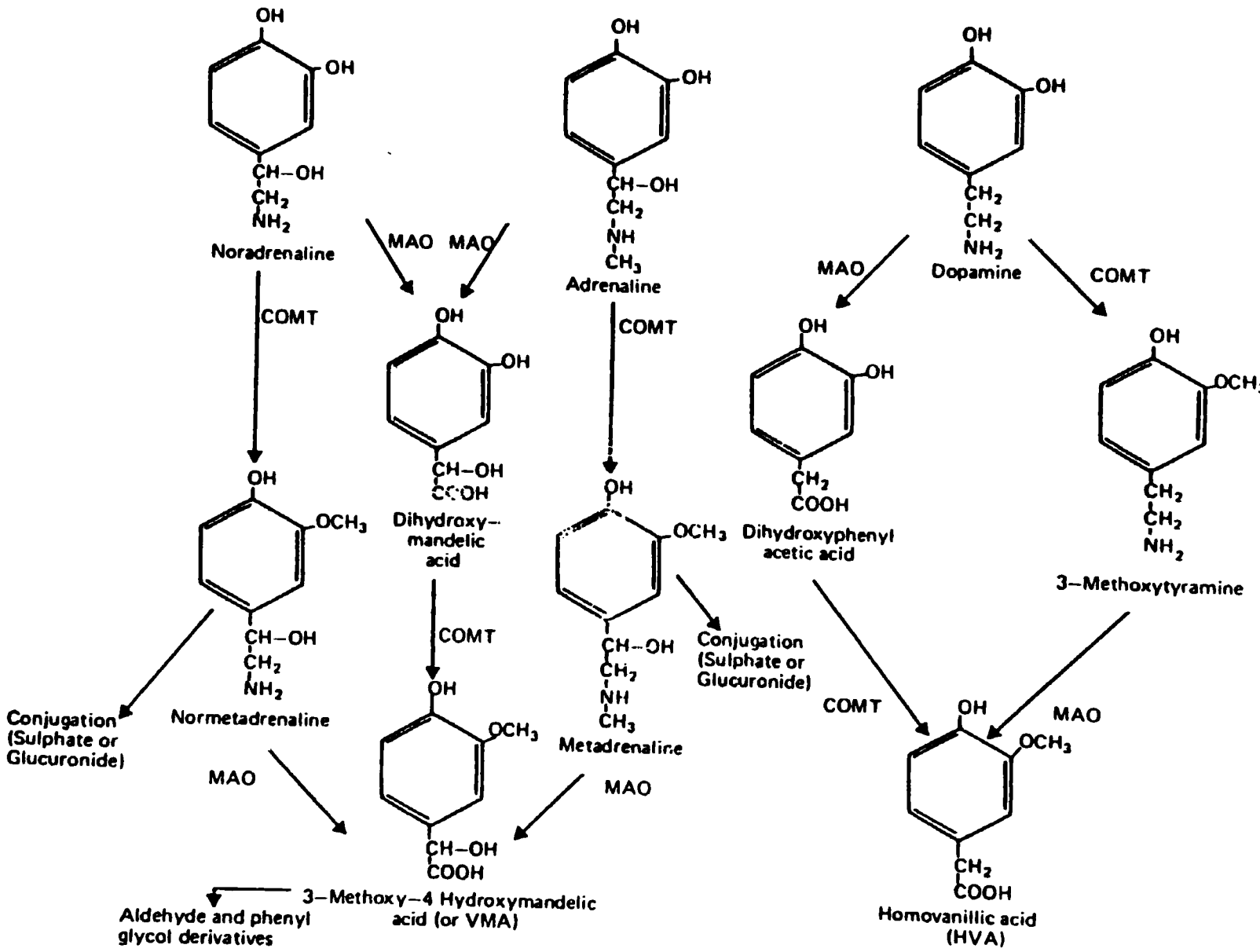


Fig. 1.9. The metabolism of catecholamines. (Hall R. & Besser M. 1989).

Serotonin is biosynthesised from tryptophan by tryptophan hydroxylase in cytoplasm of serotonin neurones and in chromaffin cells in the presence of

molecular oxygen and reduced pteridine cofactor and stored locally (Devlin, 1997; **fig.1.10).**

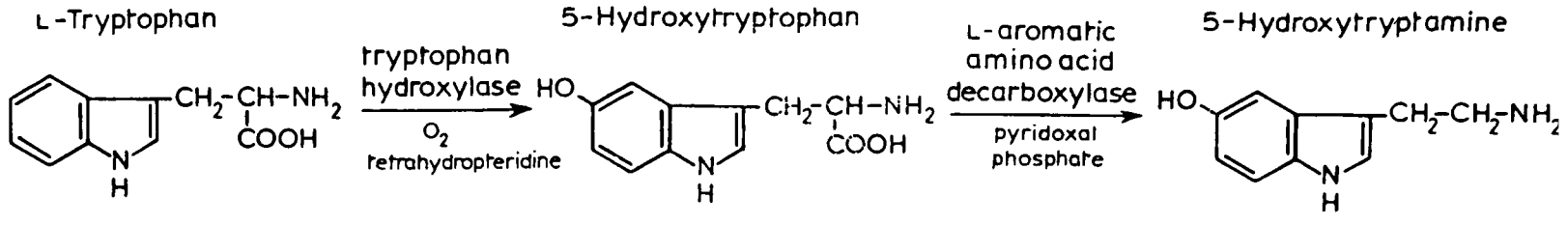


Fig.1.10. The biosynthesis of serotonin (Hall R & Besser M. 1989.

Some of this will be stored locally in neurons but almost all circulating serotonin concentration is contained, in special granules, within platelets. During platelets aggregation, serotonin is released which causes local vasoconstriction (Riddle et al, 1981). Serotonin may also enter blood as a spill over from neurones secreting serotonin as the neurotransmitter. Excess is inactivated by MAO and excreted as urinary 5-hydroxyindolacetic acid (5-HIAA) and represents the bulk of plasma serotonin as a very small amount appears as free serotonin (Greenspan 1991)

1.1.12 b. Catecholamines and bone turnover.

Prolonged stress increases the level of these neurotransmitters and hormones (Clancy & Mc Vicar, 1995). High levels of these chemicals, have been linked to certain diseases such as stomach ulcers. There is very little information available on the direct or indirect effects of these hormones on the bone. Much of what is available refers to bone changes mediated through cytokines which can be influenced by these chemicals. Rheumatoid arthritis may also result from increased productions of several lymphokines in direct response to stress hormones (Kirkham, 1991). IL-1 increases production of adrenocorticotrophin (ACTH) (Porth, 1994), IL-6 (Jirik et al, 1989),

prostaglandins-E_2 and -I_2 (Rossi et al, 1985) and stimulation of chondrocyte stromelysin which, collectively increase bone resorption and reduce collagen synthesis (Harris Jr., 1990). Noradrenaline, adrenaline and cortisol are associated with inhibition of fibroblast proliferation in human subjects in surgical stress (Saito et al, 1997).

Recent work by Cologer- clifford et al, (1999) showed that inter-male offensive and aggressive behaviour is facilitated by gonadal steroids but inhibited by serotonin. Administration of serotonin precursor L-5-hydroytryptophan elevates cortisol production (Hudgel & Gordon, 1997). High level of serotonin cause generalised deposition of calcium in the kidneys and cutaneous tissues (Klimiuk et al, 1989). The dopamine metabolite, homovannilic acid, is elevated in alcoholics who relapsed indicating that increased dopamine may be associated with an increased alcohol level.

Should catecholamine levels become higher, for example during stress, a corresponding increase will be seen in urine. Similarly, as the free blood level of serotonin is increased so would the urine level. In addition, free and conjugated catecholamines are passed in urine which reflect plasma levels.

1.1.13. OSTEOPOROSIS.

Osteoporosis is a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissues leading to enhanced bone fragility and a consequent increase in risk of fractures (Consensus Development Conference, 1993). There are parallel losses of both organic matrix and mineral contents. The composition of osteoporotic bone remains the same but its mass is reduced. These diseases can be classified as primary or secondary based upon associated pathologies. The rate of osteoporotic fractures is generally increasing, particular fractures of the hips which is associated with the highest mortality and morbidity. About 25% of those who have hip fractures die within 6 months while about 50% are incapacitated **(Fig. 1.11).**

The National Osteoporotic Society, in its annual report of 1998/1999, reported over 3 millions sufferers in the United Kingdom with 1 in 3/4 women and 1 in 12 men falling victim to this progressive bone disorder resulting in 60,000 hip fractures, 50,000 wrist fractures, 40,000 spinal fractures and over 50,000 other fractures annually. This report suggests that the incidence of osteoporosis which is higher in post-menopausal white women and in the elderly of both sexes than black men and women, is increasing every year. The incidence of osteoporosis in children is non-existent. The National Health Service (NHS) spends over £980 millions every year just coping with the basic consequences of these fractures.

1.1.13(a) Primary Osteoporosis.

Primary osteoporosis is subdivided into juvenile, pre-menopausal, post-menopausal and senile osteoporosis.

Juvenile Osteoporosis.

The cause of juvenile osteoporosis is unknown. Experimental trials with

restricted phosphorus intake in the diet or administration of small doses of phosphorus-binding agents gives partial relief in some cases (Jowsey & Johnson, 1972).

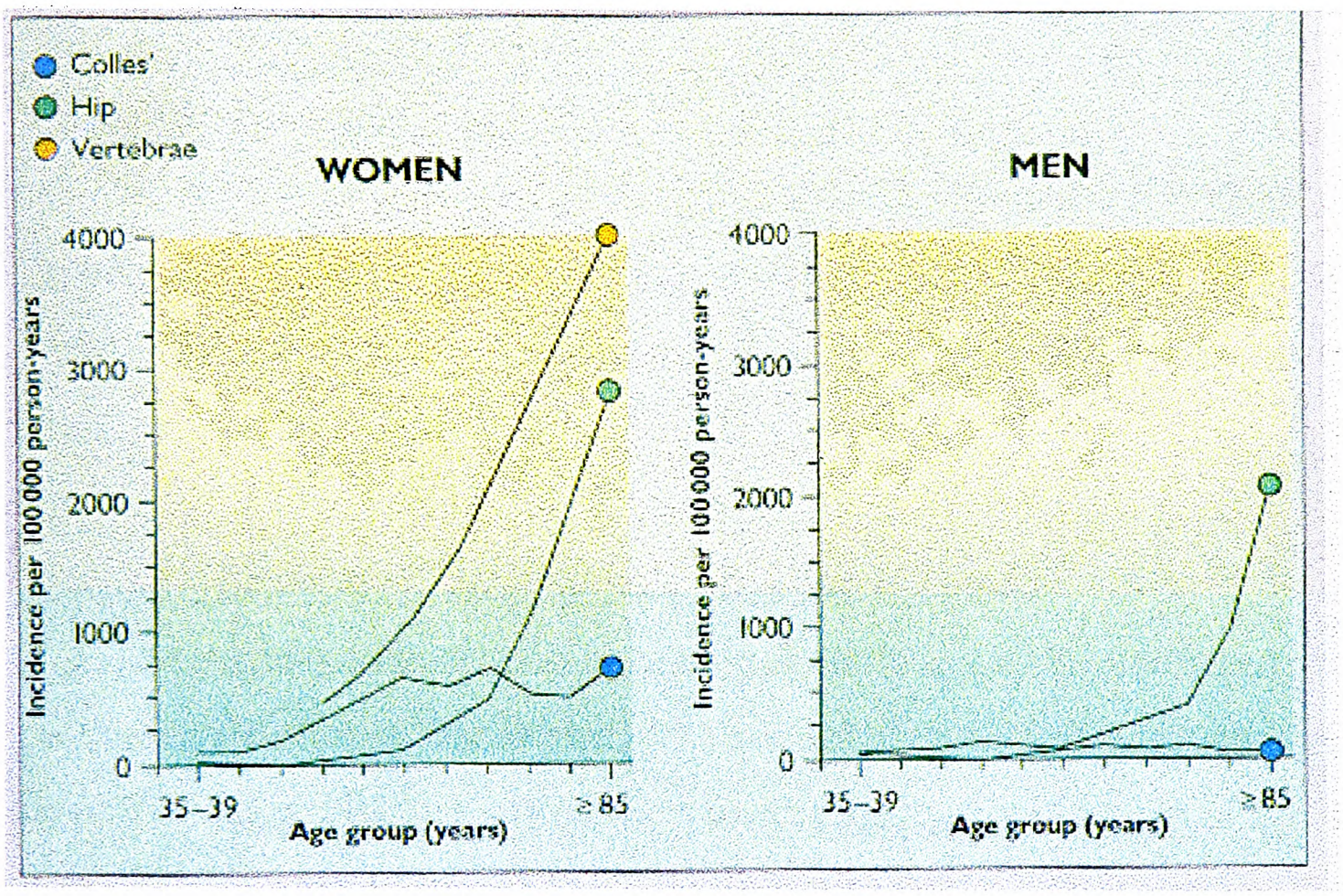


Fig. 1.11. shows the increased rate of three common fractures in men and women plotted against age.

(Source: National Osteoporotic Society, 1994/1995).

Young adults and pre-menopausal Osteoporosis.

Osteoporosis also affects young adults with sex hormones deficiencies or who have had surgical removal of the ovaries or testes in which the levels of these hormones are severely curtailed.

Post-menopausal Osteoporosis (Type 1).

Type I osteoporosis occurs predominantly in menopausal and oophorectomised women. There is an accelerated and disproportionate loss of

trabecular bone coupled with oestradiol deficiency. Spinal osteoporosis and Dowager's hump are common due to weakening of the vertebrae. Osteoporosis also affect males who lack testosterone. The incidence in males is 5% compared to 100% of post-menopausal women.

Senile Osteoporosis (Type 2).

Type 2 osteoporosis causes a proportionate loss of both cortical as well as trabecular bone tissues in people over the age of 75 years (Riggs & Melton, 1986). Elderly women are, again, at greater risks from related fractures than are men.

1.1.13(b) Secondary Osteoporosis (Disease-induced).

Secondary osteoporosis occurs when there is a well established disease-related risk factor for fracture or low bone mass. Several diseases and drugs, used in medical practice, are strongly implicated in the aetiology of secondary osteoporosis. These are listed below **(table 1.1).**

1.1.13(c) Osteoporosis and bone changes.

Osteoporotic bones remain, characteristically, the same in their outward appearances as those of healthy bones but their strength is reduced. There are minor adaptable characteristics in these bones although the trabecular and cortical bones retain their internal structures. While bone tissues are lost from cortical as well as trabecular tissues reducing bone mass overall, the remaining bone tissues become thicker to compensate for the weakness **(Fig. 1.12a & b).**

Table 1.1. Aetiology of osteoporosis.

Genetic

1. Female gender
2. Advancing age
3. Race
4. Petite and lean body mass
5. Positive family history
6. Early menopause

Lifestyles

1. Inactivity (bed rest, weightlessness)
2. Excessive exercise (secondary amenorrhoea)
3. Smoking
4. Alcoholism

Dietary disorders

1. Milk intolerance
2. Vit. D deficiency (ricket)
3. High protein diet
4. Lifelong low dietary calcium
5. Vit.C deficiency (scurvy)
6. High protein diet

Sex hormone deficiency

1. Premature or surgical menopause
2. Testosterone deficiency
3. Amenorrhoea / polycystic ovaries

Endocrine disorders

1. Cushing's diseases
2. Thyrotoxicosis
3. Addison's disease
4. Hypopituitarism
5. Diabetes Mellitus
6. Acromegaly

Bone and bone marrow diseases

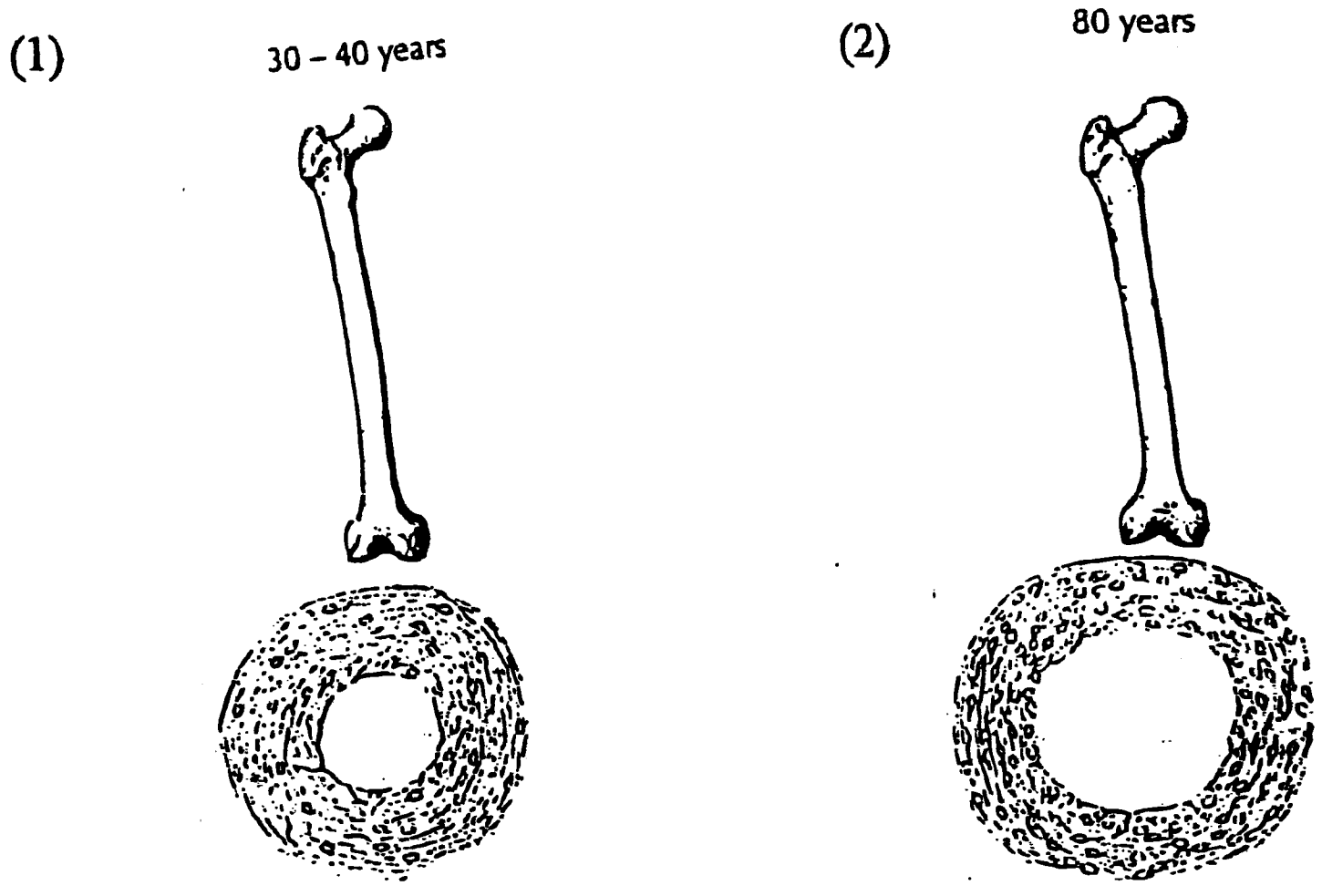
1. Metastatic carcinoma
2. Myeloma
3. Haemophilia
4. Leukaemia
5. Lymphoma
6. Thalasaemia

Kidney or liver diseases

1. Renal failure / dialysis
2. Hepatic / cirrhosis
3. Aluminium overload

Drugs

1. Glucocorticoids
2. Heparin
3. Immunosuppressants (methotrexate)
4. Anti-convulsants
5. Antacids (aluminium-based)



Figs. 1.12(a) Cortical bone: bone tissues lost from inside of bone and small amount of bone tissue added externally to adjust in old age (Birdwood G, 1996).

(1. tissue loss from inside bone 2. tissue added on external surface).

The collagen matrix is normal in appearance and fully mineralised but matrix volume is depleted with slower mineralisation. Bone remodelling continues to be controlled and influenced by the same factors involved in normal bone formation, but, osteoclastic bone resorption is slightly raised and progressively increases, a common feature of age-related osteoporosis. These cells continue tc remodel osteoporotic bone and strengthen existing bone **(Figs. 1.13(a&b) & 1.14(a&b)**. Trabecular bone mass may fall by between 6-8% per decade startin from about the fourth decade of life in both sexes but is more steep in postmenopausal females. Cortical bone mass is reduced by 3% by the age of 45 in both sexes to 9% by mid-seventies.

(1) (2)

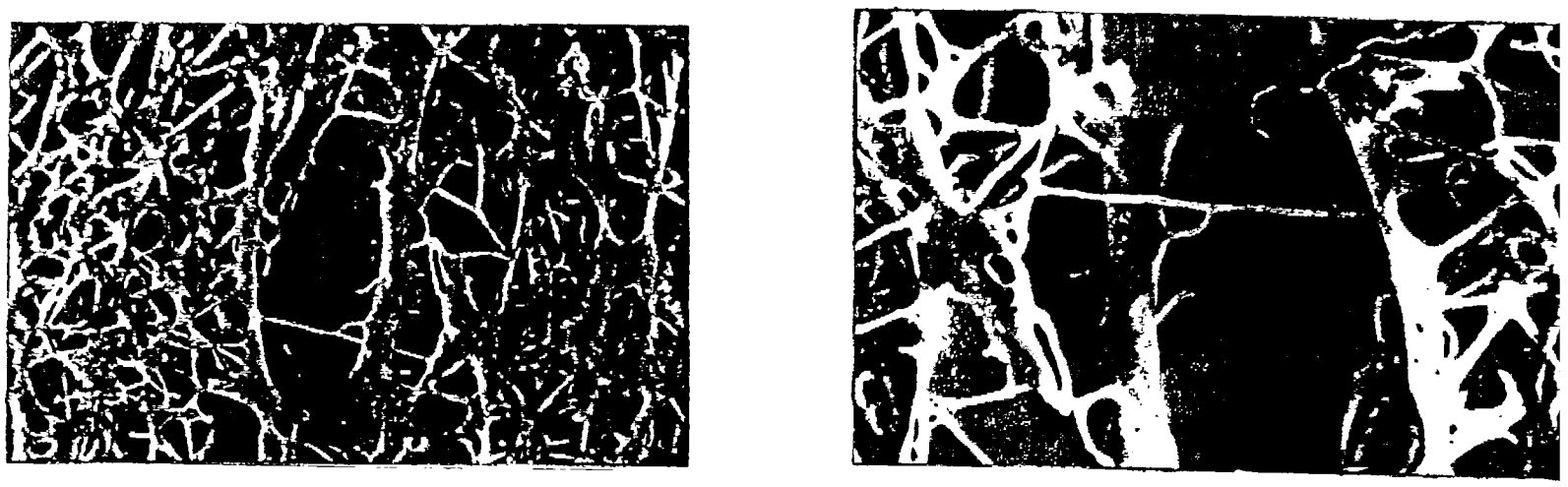


Fig. 1.12(b) Trabecular bone: bone tissues lost and become thinner; thickening in old bones to compensate for lost tissues. (1. as the tranverse trabeculae become progressively thin and disrupted, 2. thickening of some vertical trabeculae compensate for the loss).

There is also a continuous fall in total body calcium following menopause when sex hormone production is simultaneously reduced. In osteoporotic bone, the tiny but rigid plates forming the honeycomb matrixes gradually become thinner and rod like, and the spaces between them become larger. The bone thus becomes more porous, less dense, weaker and fractures easily from minor traumas and stresses that would ordinarily have no ill effects.

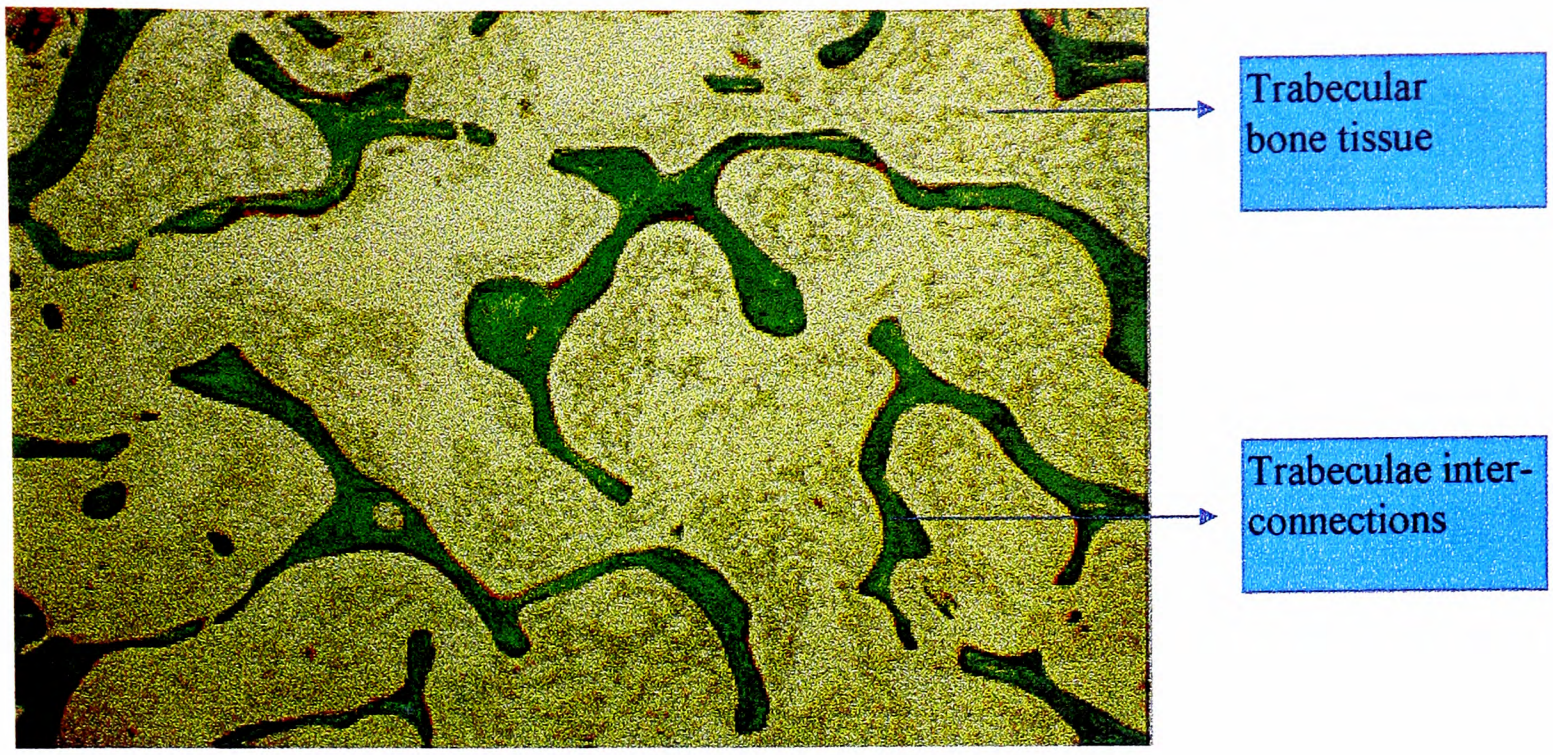


Fig. 1.13(a) Normal trabecular bone elements.

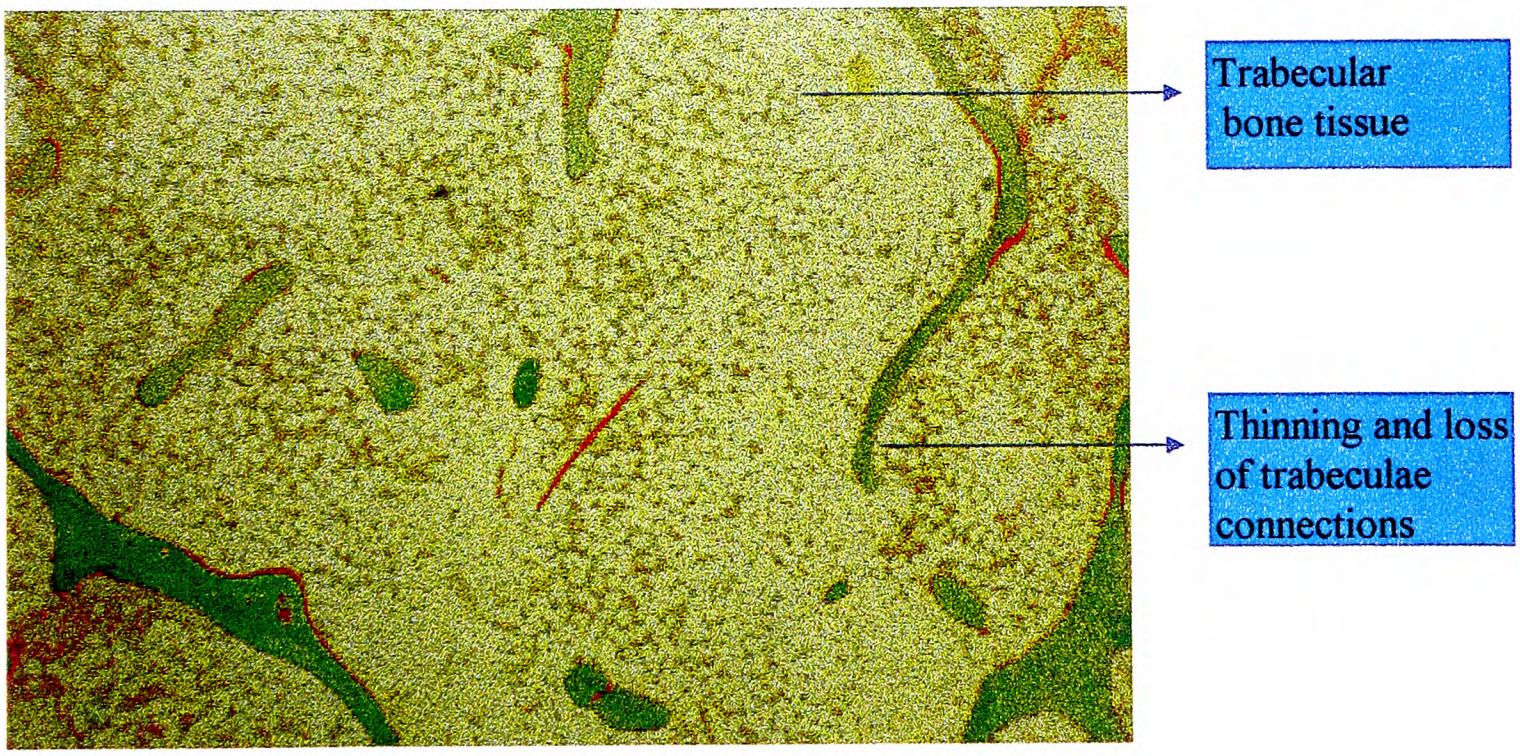


Fig. 1.13(b). Osteoporotic trabecular bone tissues showing loss of trabecular elements.

(Source: 1.13(a&b):An atlas of osteoporosis by J.C. Stevenson & M.S. Marsh, 1992).

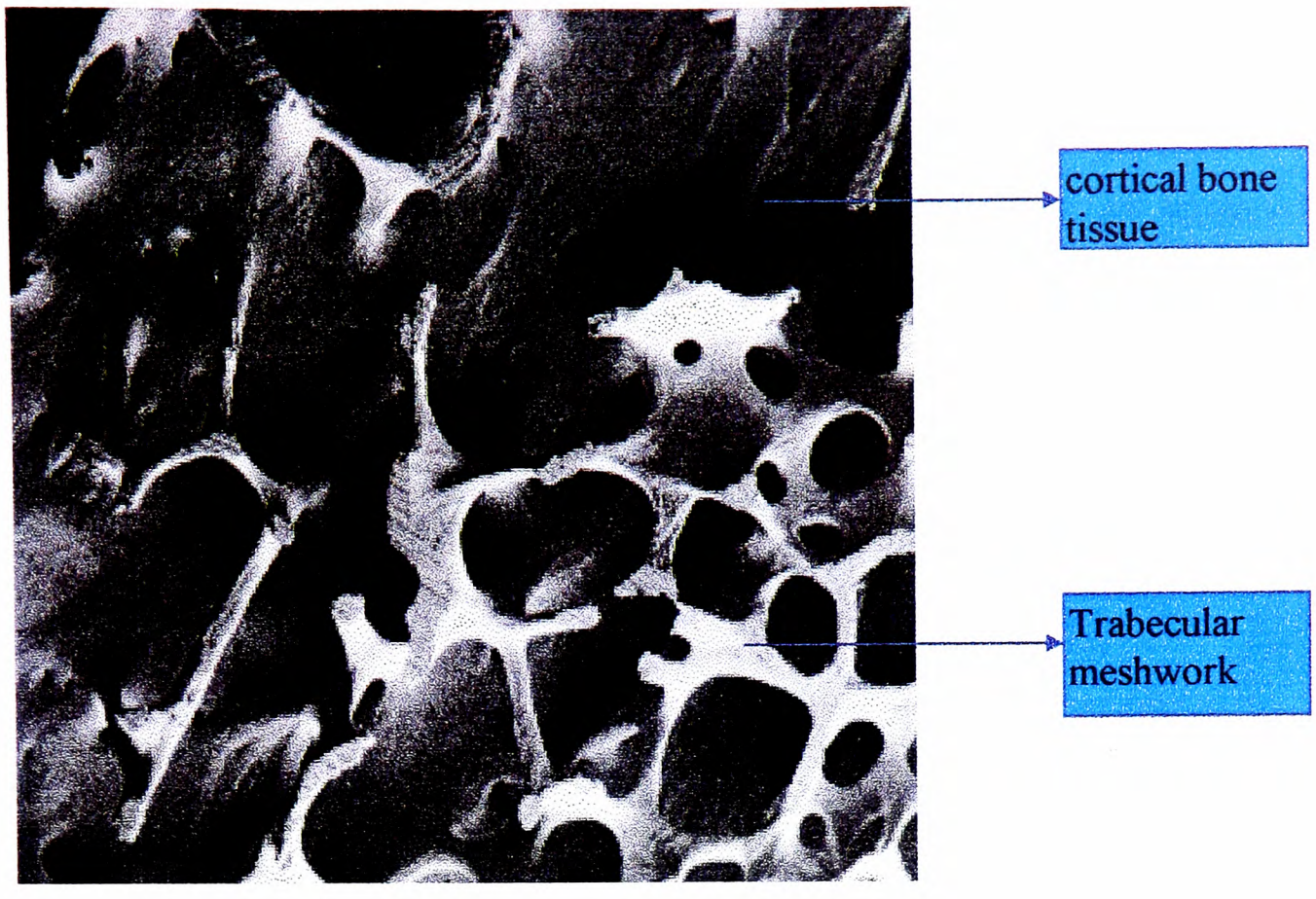


Fig. 1.14(a). Normal cortical bone elements.

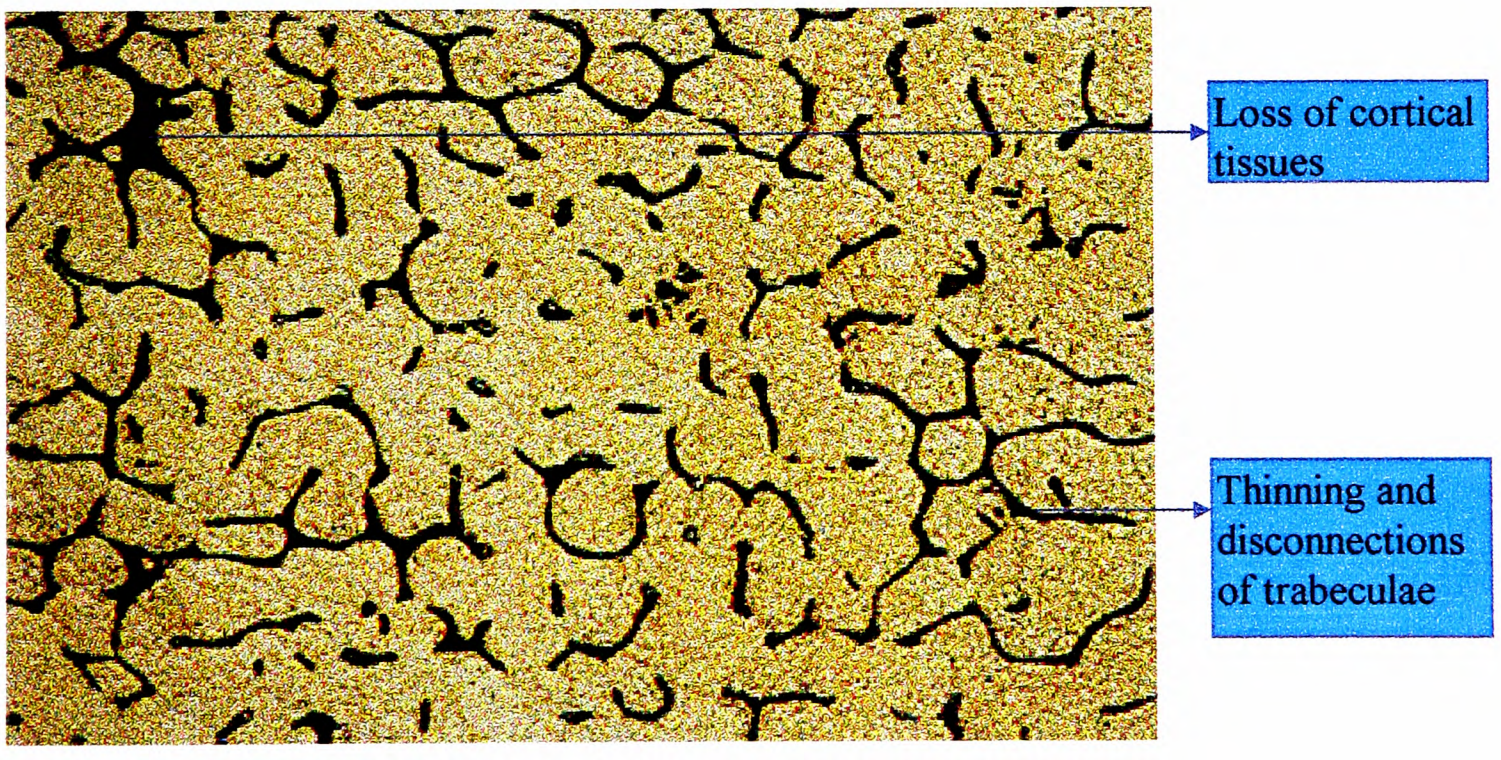


Fig. 1.14(b). Osteoporotic cortical bone tissues.

(Source: 1.14 a&b, Parsons V, 1980).

Over time, minute crushed fractures occur in the vertebrae of the spine and curve the spine resulting in the humped back and a bent posture which can be observed in many elderly people afflicted with osteoporosis (Guyton, 1997). The bones of the hip and forearm are also especially vulnerable to fractures. The other symptoms of osteoporosis are loss of height and back pain.

1.1.14. Costs of dealing with osteoporosis and related fractures.

Osteoporosis is recognised as a multifactorial and complex disorder characterised by an asymptomatic reduction in the quality of bone mass per unit volume. When bone mass becomes too low, structural integrity and mechanical support are not maintained and fractures occur with minimum trauma. These fractures typically occur at the hip , in the vertebral column and forearms (Compston, 1996). It causes loss of height and chronic pain and may result in severe disability with up to 50% of sufferers who sustain fractures not able to lead an independent life. The fear of fractures, spinal deformities, loss of self-confidence and low self-esteem, social isolation, depression and poor quality of life are in general some of the very common consequences of this chronically debilitating disease.

It is difficult to assess the real costs in dealing with fractures associated with osteoporosis. Undoubtedly, the cost varies from country to country. However, what seems undisputed is that this cost is significant and is increasing annually (Norris, 1992). About £742 millions was spent dealing with osteoporotic fractures in the United Kingdom in 1994 (Department of Health, 1994) and in 1998/1999, the cost increased to £980 millions (National Osteoporotic Society Annual Report, 1998/1999). In America , the costs of treating fractures in women 45 years old alone increased from 7.2 billions dollars in 1993 to 10 billion dollars a year later. About 50% of this cost was incurred through hospitalisation and 40% for nursing home care but the bulk was for fractured hips (Randell et al, 1995).

Cost is driving the choice of prescription of medical care as the NHS moves towards a market oriented status. With the emergence of purchaser - provider practice, many purchasers of health care are selecting

better cost effective and non-invasive medical care. Osteoporosis results from multiple factors and remains very difficult to treat and as such, prevention of fractures should remain the focus for providing a better quality of life for sufferers. Exercise, modification of the diet and lifestyle may provide a better and more appropriate prevention regime.

1.1.15. Aims of Project.

The causes of osteoporosis can be attributed to many factors including lack of physical activities, hormonal changes and dietary deficiencies such as calcium, magnesium, trace elements and vitamins. In addition, stress and diseases may cause transient reduction in bone mass which may contribute to the causes of osteoporosis. Bone mass increases rapidly at puberty, under the influence of sex hormones and environmental factors until the third decade of life when bone mass reaches its genetically predetermined peak value, thereafter a plateau is maintained before it is reduced, in both genders, with normal ageing. This decline occurs earlier in females due to the reduction in sex hormones at menopause. During this period, bone mass decreases rapidly and bones become weaker as more of the bone elements are lost in urine. During the post-menopausal period, there is a high incidence of fractures, particularly hip in the elderly resulting in a high morbidity and mortality. Preventative measures to promote a greater bone mass or / and slow down the rate of loss can, therefore, reduce the number of fractures and cost to the national health service.

The aims of this investigation was to determine the role of boron and stress hormones in bone resorption in men and women of different age groups, nutritional status, life style and health status using a non-invasive method.

The objectives were:

1) to establish possible relationships between boron and bone markers (calcium , phosphate , magnesium and hydroxyproline) in males and females of 11-20, 20 - 40, over 40 years old and during pregnancy.

2) to evaluate the relationships between boron, nutritions, life style, health status and these bone markers.

3) to analyse the relationships between stress hormones (cortisol, noradrenaline, adrenaline, dopamine), serotonin and selective nutrients.

4) to establish the relationships between boron, stress hormones and the bone markers.

5) to propose preventative strategies, based on nutritional and life-styles changes in order to improve bone mass especially in nurses since they were one group of the population under consideration.

CHAPTER 2.

MATERIALS AND METHODS.

2.0.0. Introduction.

One hundred and seventy two subjects were placed into 4 groups of males and females, between the ages of 11-20, 21-40, over 40 years and a group of women (20-40 years), in their 3rd trimester of pregnancy or up to 5 days post-partum. The subjects were randomly selected from secondary schools, student nurses at the School of Health, University of Greenwich and patients at a local hospital. A validated questionnaire and a non-invasive approach were used to investigate the relationships between stress hormones, boron and bone markers, in human subjects of different age, life style and health status. An early morning urine sample was collected and cortisol, noradrenaline, adrenaline, dopamine, serotonin, boron, hydroxyproline, calcium, phosphate, magnesium and creatinine were determined and correlation established between these analytes.

2.1.0. Rationale for selecting this subject.

Osteoporosis is characterised by severe structural changes to ageing bone resulting in severe pain, restricted movements, fractures and causes enormous sufferings in elderly patients particularly females. There are a number of causes for these diseases and as certain dietary factors and life style predispose to these defects, an alternative approach, based on preventative measures, may be more effective in reducing the incidence of osteoporosis and therefore, the cost to the National Health Service.

2.2.0. Questionnaire (appendix 1).

Questionnaire is a relatively low cost data collection and processing tool while still maintaining a relatively large sample size. It gave anonymity to respondents as a third party was involved and avoided interviewer bias (Dempsey & Dempsey, 1996). However, the answers were treated cautiously as the respondents were volunteers with no obligation and may give inaccurate or misleading answers. Accordingly a pilot study was conducted on a small sample of subjects on its design, layout and questions to reduce bias, ambiguity, influence and response set. 27 questionnaires were discarded because of incompleteness or inaccuracies and inconsistencies mentioned in the discussion.

The questionnaire had 38 questions on personal details, occupation, health, disease, life style and dietary habits and an additional 12 questions on the menstrual and reproductive history. This information was then used to compare the relationships between the details provided in the questionnaire and the roles of boron and stress hormones in bone turnover. An information sheet and a consent form were included with each questionnaire before distribution to all subjects. At each school, the project was discussed with the head teacher, parents and pupils.

While age (Beck et al, 1993), gender (Finkelstein et al, 1987), cultures (Gilsanz et al, 1991), amount and type of exercise (Kerr et al, 96), alcohol (Peris, 1992) and caffeine (Keil et al 1990) influence bone mass, nutrition provides important nutrients, such as calcium and vitamins, necessary for healthy bone formation. Green vegetables, fruits and dairy products, provide substrates for production of bone proteins and micronutrients necessary for satisfactory mineralisation. Reproductive history, numbers of children borne, menopausal status and HRT also influence bone turnover (Marcus, 1996) whilst, medical anomalies such as diabetes and Cushing's syndrome, can cause bone

damage by interfering with bone remodelling (Birdwood, 1996).

2.3.0. Urine.

Due to ethical limitations, bioclinical assay on urine was selected as the most reliable method to obtain the relevant data. Early morning urine sample was best to determine cortisol level due to diurnal variations in its secretion (Trans et al, 1999). Ideally, 24 hour urine collection is better but early morning spot test urine is the next best option as generally there are no significant difference in urinary analytes between these samples (Wittert & Nordin, 1998 & Stein et al, 1999). Also, 24 hours urine collection is difficult to obtain (Gawenlock, 1988). Boron is not stored in the body and any excess is excreted in urine within 120 hours of ingestion (Jensen et al, 1984). Urinary calcium, magnesium and phosphate are reliable representatives of their serum levels (Welch et al, 1990). Hydroxyproline is a reliable bone resorption marker although it can also represent other collageneous breakdown. Spot urine sample is regularly used to monitor bone breakdown rate in medical practice as it is less expensive to carry out and is a valuable indicator of the effectiveness of medical intervention in myeloma, a bone breakdown disorder (Inoue et al, 1996).

2.4.0. Access and ethical approval.

A proposal for the study was submitted to the Higher Degree Research and the Ethic Committees for approval. Written permission from the Bexley Health Authority Research and Ethics Committee and permission from Queen Mary's Sidcup NHS Trust were secured as patients from this Trust formed the bulk of the over 40 year old subjects. Written consent from each participant was obtained. If the participant was a minor, then parent / guardian and teacher were required to give consent.

2.5.0. Urine analysis.

2.5.0(a) Calcium and Magnesium.

Preparation of reagents and standards.

(a) Trichloroacetic Acid (TCA). A 5% TCA solution was prepared by dissolving 25g of TCA granules (molecular weight, Mwt. 163.39) in 500 ml of double distilled water.

(b) Strontium Chloride. 2.76g of Strontium Chloride (Mwt.266.62) was added to this solution before the final solution was made up to 500 ml.

Serial dilutions of a calcium (0.5, 1, 2, 4 and 5 mg/L) and of magnesium standards (0.1, 0.2, 0.3, 0.4 and 0.5 mg/L) were prepared using a stock solution of 1000 μg/g (1000 mg/L).

Procedure.

The assay was carried out using the SP-9 (Phillips) Atomic Absorption Spectrophotometer (AAS). Before the analysis, the instrument was calibrated and a blank, double distilled water, was used to zero the spectrophotometer.

Calcium: 0.1 ml of urine was diluted with 5 ml of TCA to obtain dilution of 51 times. The calcium lamp of the AAS was set at a current of 7 units, with a band width of 1 and wavelength 422.7 nm. A typical calibration graph is shown in **figure 2.1.**

Magnesium: All urine samples were diluted 300 times using the TCA solution. The magnesium lamp current was set at 3 units, wavelength 285.2 nm. and band width at 7.

Fig.2.1. shows a typical calibration response for calcium.

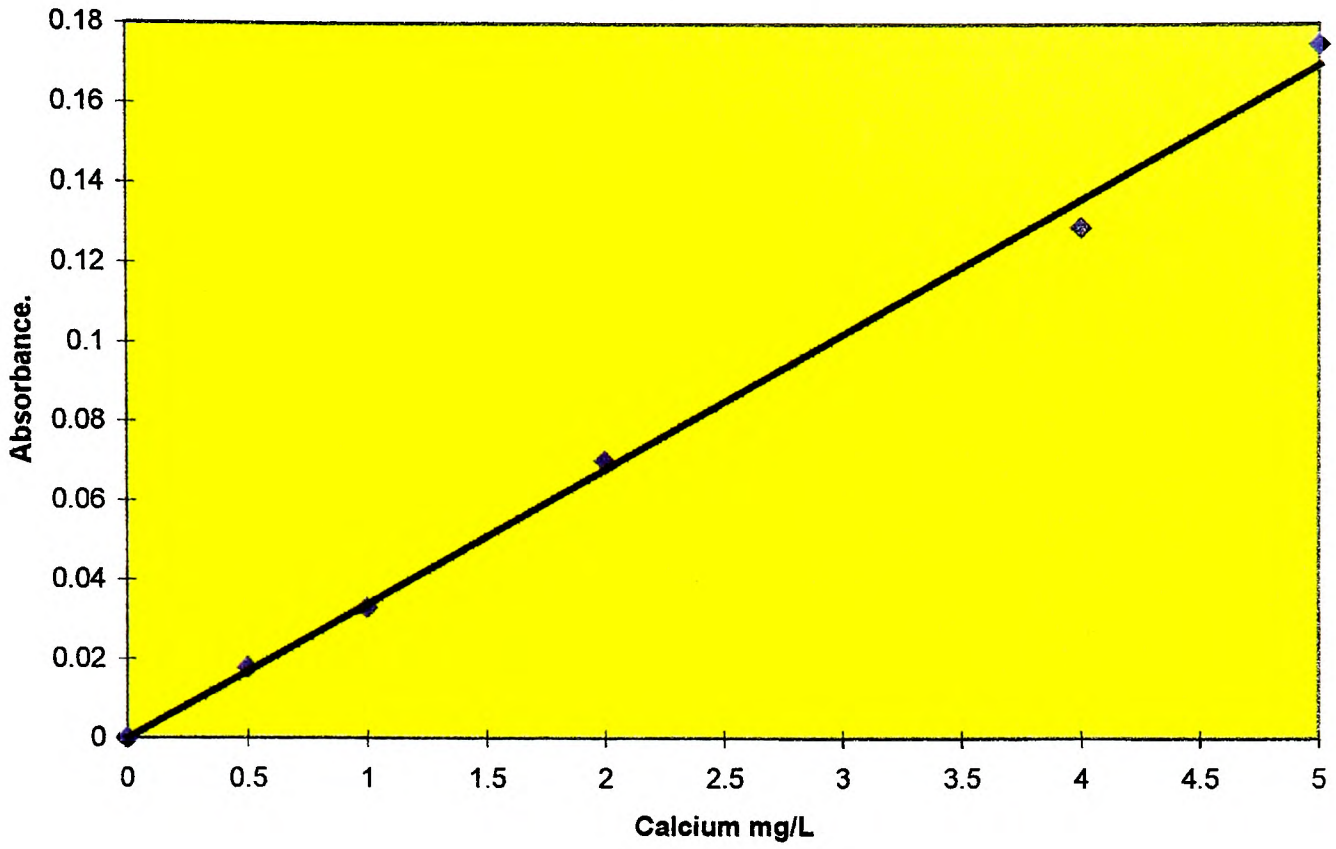
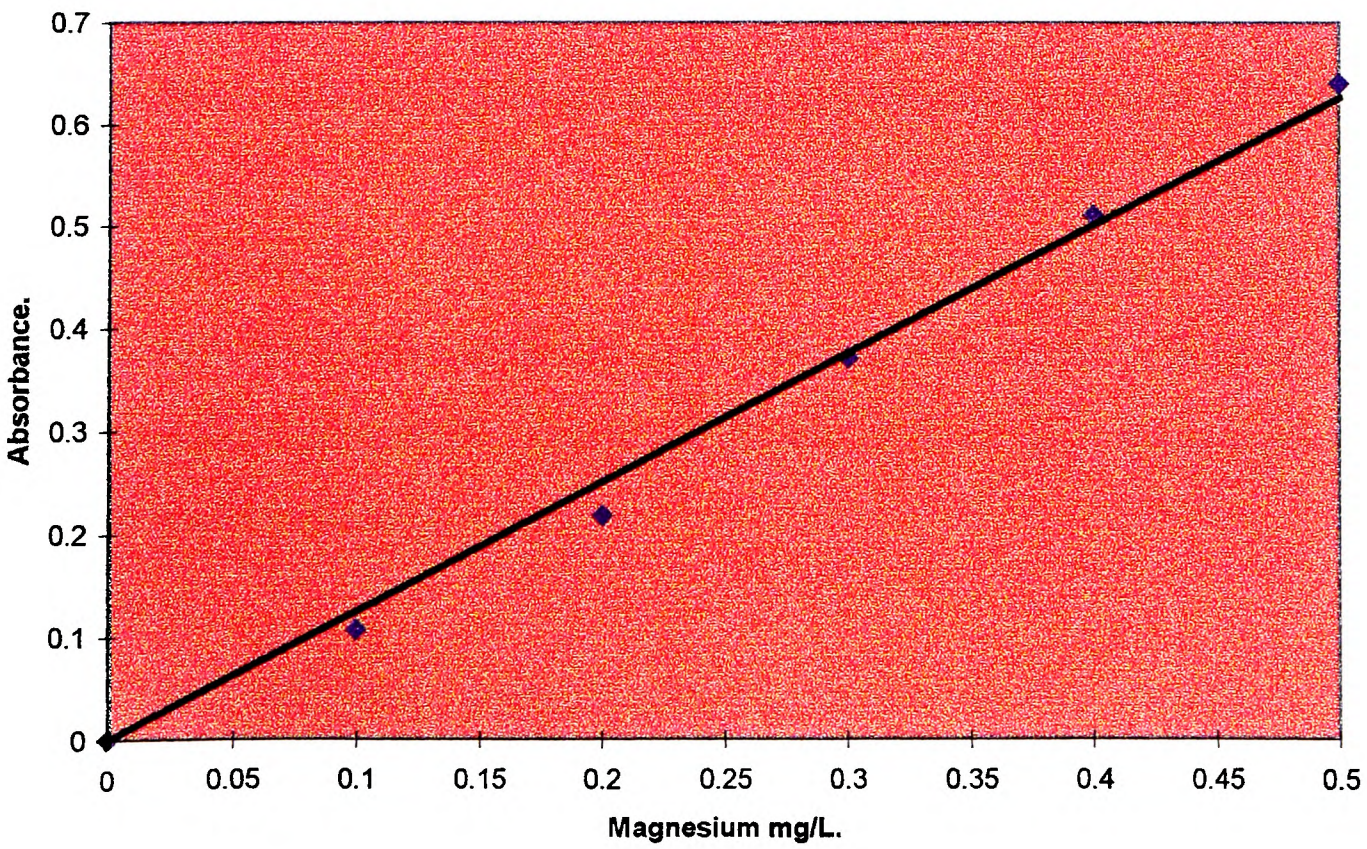


Figure 2.2. shows a typical calibration graph. Each urine sample was aspirated into the AAS and the concentration of urinary calcium and magnesium deduced from the linear range of the calibration curve.

Fig. 2.2. shows a typical calibration curve for magnesium.



A standard was introduced, after every 10 urine samples to ensure reliability of the procedure. The percentage coefficient of variation (%CV) of calcium and magnesium were estimated from 10 analyses of each analytes. At 2.0 mg/L this was 1.2 for calcium and at 0.2 mg/L, it was 2.8 for magnesium.

2.5.0(b) Phosphate.

Preparation of reagents and standards.

(a) Copper acetate buffer (pH 4). 1.25g copper sulphate crystals (Mwt. 159.60) and 23g of sodium acetate (Mwt. 82.03) were dissolved in a final volume of 500 ml acetic acid in the fume cupboard due to the potential risks of toxic fume inhalation.

(b) Ammonium molybdate. 12.5g of ammonium molybdate (Mwt. 1235.9) was dissolved in 250 ml of double distilled water and stored at 4°C until required.

(c) Rhodol solution. 2g of paramethyl-amino-phenol sulphate (Mwt. 334.39) was dissolved in 80 ml of double distilled water. 10 ml of hydrated sulphite with 7 water (Mwt. of solid, 252.19) was added and the solution was made up to 100 ml and stored in a dark bottle at 4°C until required.

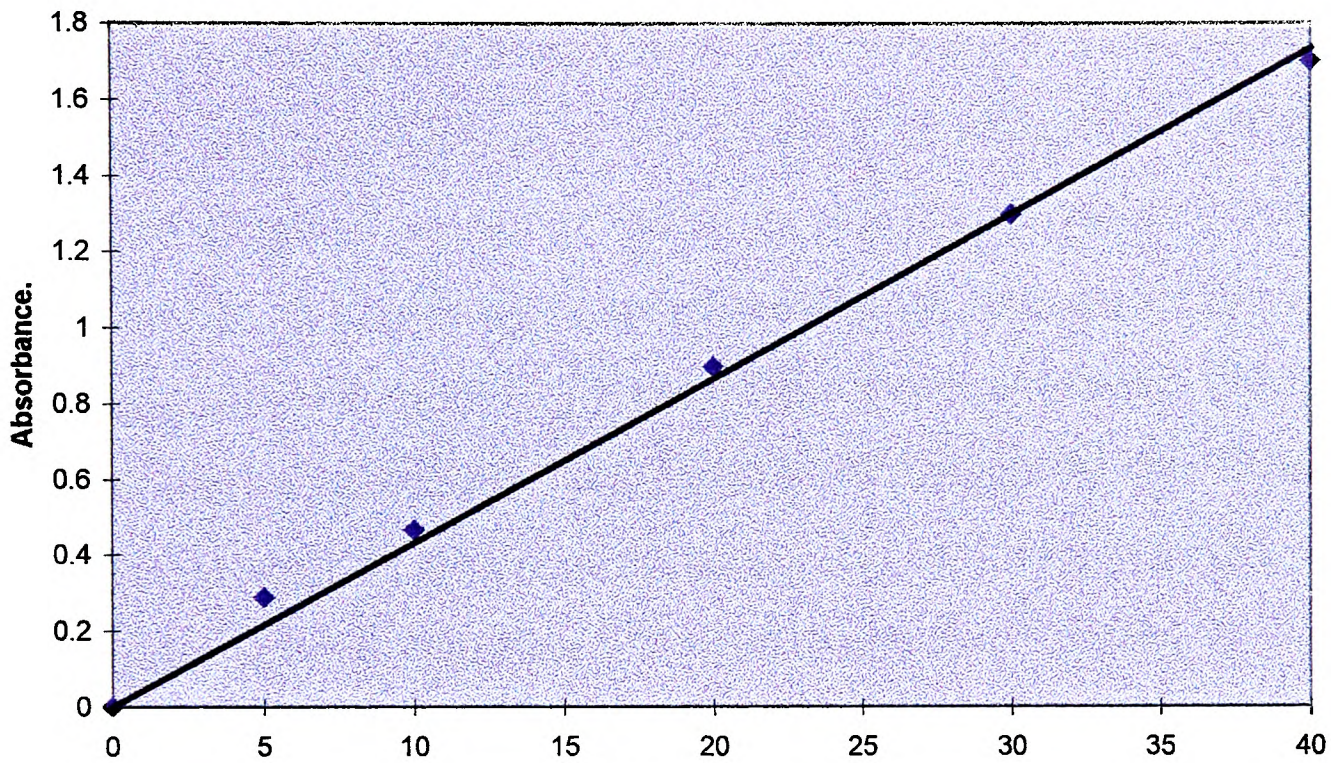
(d) Standards. 2.1935g of potassium dihydrophosphate (Mwt. 136.09) was dissolved and made up to 500 ml with double distilled water to give 1g/L of phosphate. Serial dilutions of 5, 10, 20, 30, and 40 mg /L were made from this stock.

Procedure.

1 ml of the blank solution (double distilled water), standard solutions or urine samples, which were diluted 51 times with 5% Trichloroacetic acid (TCA) solution, was added to 3 ml of copper acetate

buffer containing 0.5 ml of the ammonium molybdate. 0.5 ml of the Rhodol solution was then added to these tubes, thoroughly mixed and allowed to stand for 5 minutes.

Fig. 2.3. shows a typical response for phosphate standards.



A blue colour change in the samples was noted which indicated the presence of phosphate. The blank was used to zero the Unicam8625 UV/VIS spectrophotometer and the absorbance read at 880 nm. A linear range was established from the standards curve (**fig. 2.3**) and the phosphate level in each sample deduced. At 20 mg/L, the %CV was 4.3; n=8.

2.5.0(c) Boron .

Preparation of standards for boron.

Stock boron (Orthoboric Acid- $H_3 BO_3$ - Mwt. 61.83) of 1000 ppm was used to produce serial dilutions of 0.25. 0.5, 1, 2 and 4 µg / L of

boron.

Procedure.

Urinary boron was determined using Inductive Coupled Plasma Mass Spectrophotometer (ICP-MS). No pre-preparation of the urine samples was necessary before analysis. The lamp was set at 14 mm , just above the plasma level to provide the best detection limit and the method validated. Power rate (Kwt) of between 1 and 5 were tested and 3 Kwt gave the best response. Channel A was selected for ultra violet detection. To check the effects of sodium on the boron absorbance, standard boron solutions of 0.25, 0.5 and 4 μg / L were ran with the same concentration of added sodium (150 mmol / L). 3 recordings were made under these conditions and boron response was between 97.3% and 99.8% . Since, there was no significant effects of the sodium on the boron response, these parameters were, therefore, adopted for routine analysis.
boron. At 1μg/L, the %CV was 2.9; n=10.

Fig. 2.4. shows a typical calibration curve for boron.

A blank of distilled water was used to zero the spectrophotometer and using the serial dilutions above, a linear range was obtained. Fig.2.4 shows a typical standard curve for boron. One of the standard dilutions was run after every 10 urine samples followed by washing of the column with double distilled water to remove residual contaminant.

2.5.0(d) Hydroxyproline.

Preparation of reagents and Standards.

(a) Oxidant solution. 500 ml of a 7% (7 gm/100 ml) aqueous solution of chloramine T (Mwt 227.65) was made up, using 35 gm of chloramine T dissolved in 500 ml. of double distilled water. This was stored in a dark bottle in cold room at 4 ° C until required.

b) An acetate / citrate buffer at pH 6 was made by dissolving 57 gm. of sodium acetate ($3H_20$), (Mwt 82.03), 37.5 gm. of sodium citrate (Mwt 60.05) and 5.5 gm. of citric acid (Mwt 210.14) in 385 ml. of isopropanol which was finally made up to one litre with double distilled water. Just before the start of a series of hydroxyproline determination, the aqueous chloroamine T solution and the acetate/citric buffer were mixed in the proportions of 1: 4 to form the oxidant solution.

(c) Ehrlich's reagent solution. P-dimethylamino-benzaldehyde - methol (Mwt 149.19) was dissolved in 70% perchloric acid (Mwt 100.46) in a proportion of 2 gm. to 3 ml. of acid. This solution was made up fresh before the start of each series of analysis.

(d) Isopropanol.

Before the start of each series of determinations, solutions (c) and (d) were mixed in the proportions of 3 volumes of (c) to 13 volumes of (d) to form the final Ehrlich's solution.

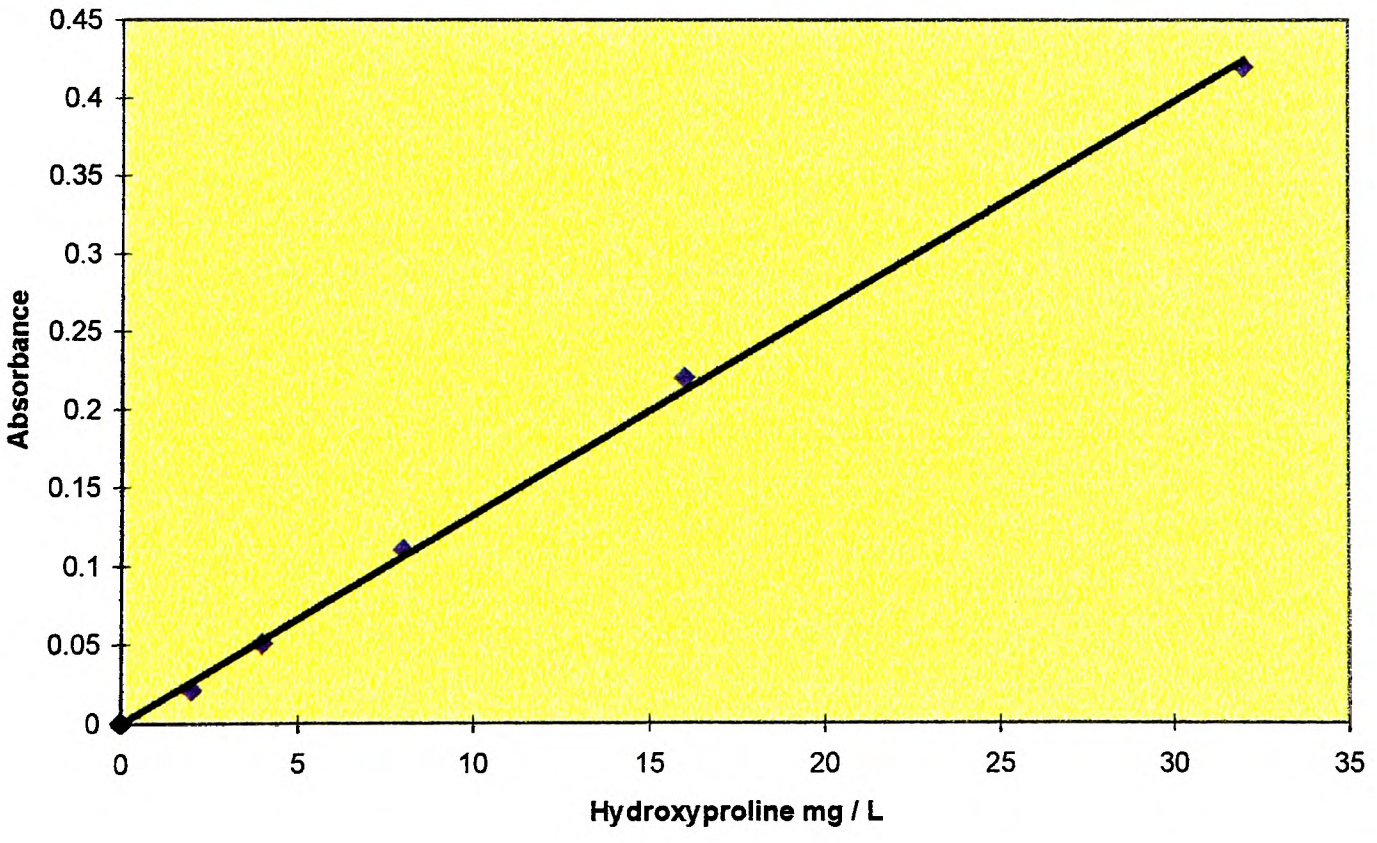
0.04 gm. of hydroxyproline (Mwt 131.11) was made up to 100 ml.

using double distilled water containing 0.001 mol/L HCl as a preservative, and stored at 4° C. Serial dilutions of 2,4,8, 16 and 32 mg / L were made from the standard hydroxyproline solution using double-distilled water.

Procedure

1 ml. of each standard, sample or distilled water (in the case of the reagent blank) was pipetted into clean labelled boiling tubes. To each boiling tube, 2 ml. of isopropanol and 1 ml of oxidant solution were added, mixed thoroughly and left to stand for five minutes. 13 ml of the final Ehrlich solution was then added to each tube, again mixed well before incubating at 60°C in a water bath for 30 minutes. The tubes were then cooled under running tap water for 2-3 minutes and diluted, with isopropanolol, to 25 ml, in standard volumetric flasks.

Fig.2.5. shows a typical standard curve for hydroxyproline.



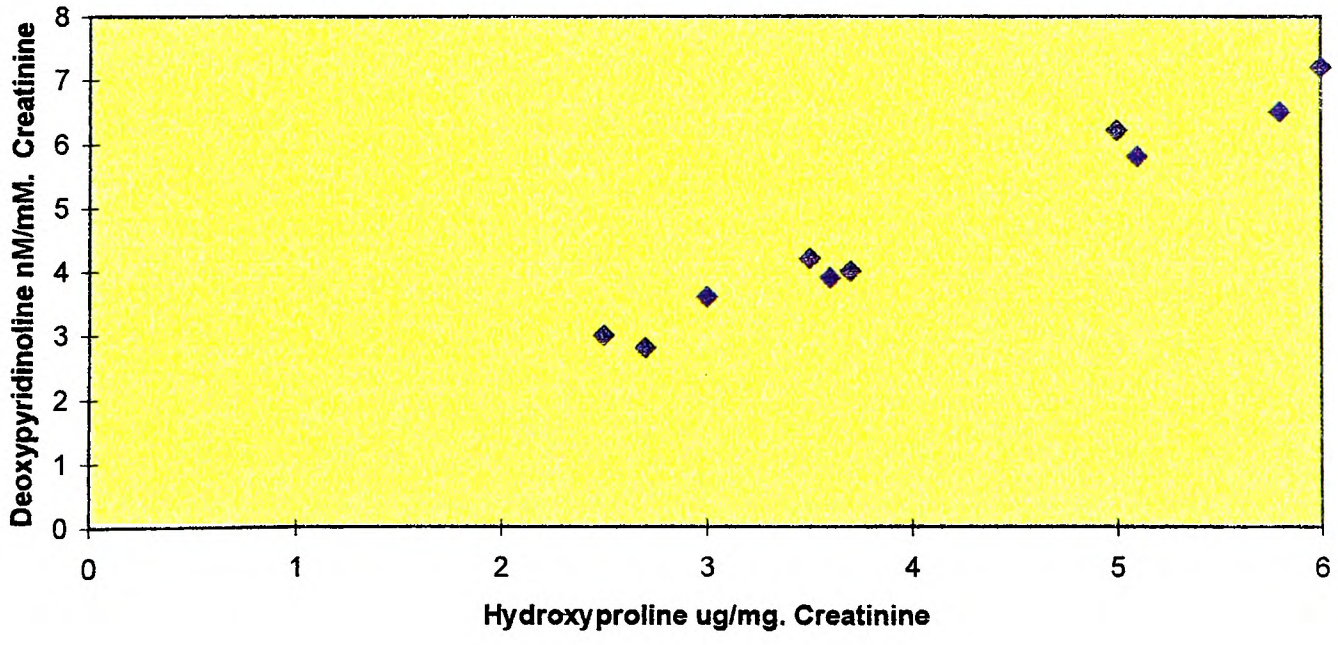
The colour change from yellow to red was observed indicating the presence of hydroxyproline. A Unicam 8625 UV/VIS spectrophotometer

was used to determine the absorbance at 550 nm and a standard calibration curve was obtained from which the concentration of hydroxyproline in each urine sample was determined. **Figure 2.5** shows a typical reference curve for hydroxyproline. At 16.0 mg/L, the %CV was 4.1; n=6.

2.5.0(e) Deoxypyridinoline analysis.

Deoxypyridinoline was determined in selected urine samples using a competitive enzyme immunoassay (Pyrilinks-D, MetroBiosyntems. USA), and compared to hydroxyproline levels. Free deoxypyridinoline, derived mainly from type 1 collagen of bone by the enzymatic action of lysyl oxidase on acid lysine, is released into the circulation before being excreted in urine. **Fig. 2.6.** shows that both, hydroxyproline and deoxypyridinoline are good markers of bone resorption ($r^2 = 0.9792$). Therefore, urinary hydroxyproline was determined as the index of bone resorption in all urinary samples.

Fig. 2.6. shows the relationship between urinary deoxypyridinoline and hydroxyproline.($r^2 = 0.9792$)

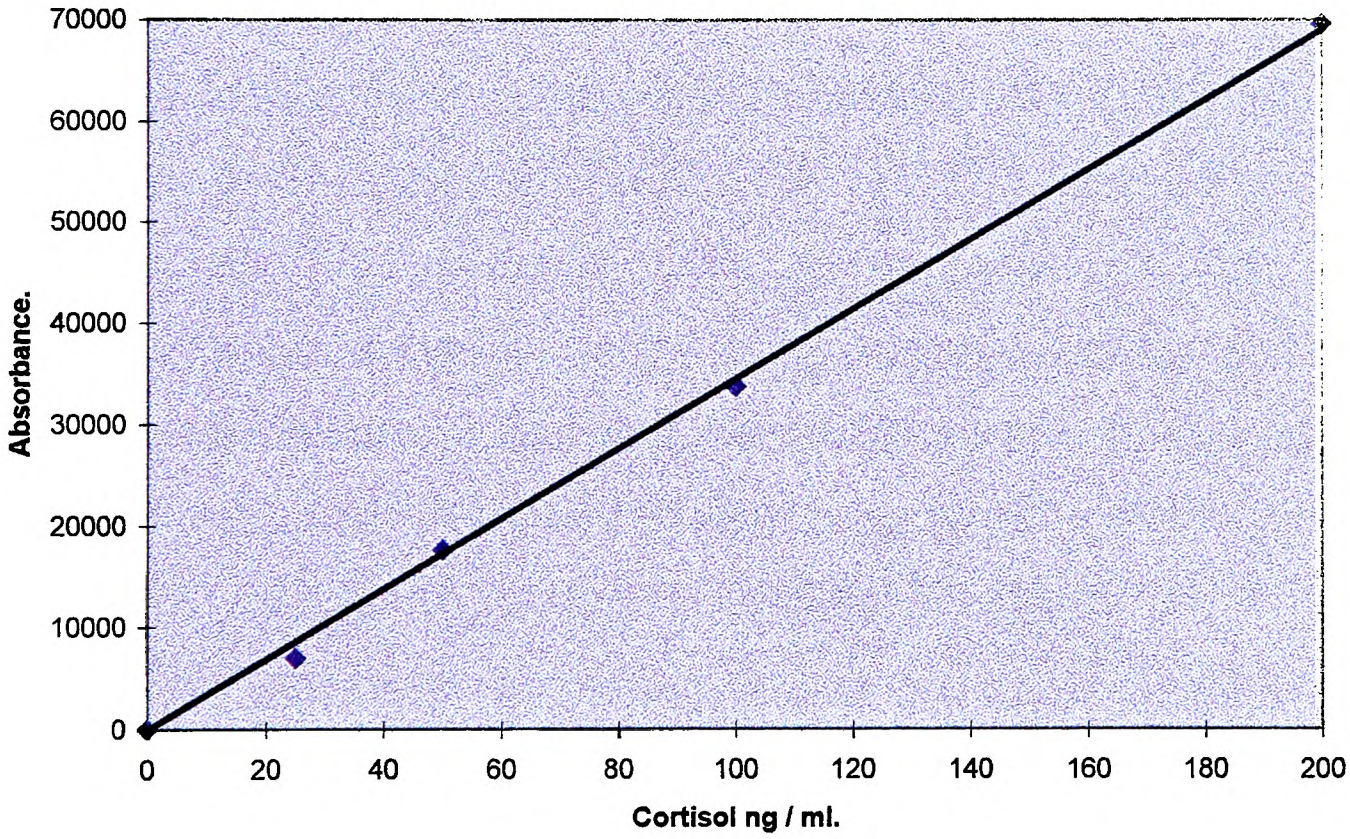


2.5.0(f) Cortisol.

Preparation of standard cortisol.

A stock solution of hydrocortisone was made by dissolving 10 mg of purified hydrocortisone (11 , 17 , and 21 trihydroxyprega- 4ene-3,20 dione, Mwt 362.5) in 10 ml of HPLC grade methanol from which final dilutions of 25, 50, 100 and 200 ng/ml were made. Each dilution was injected into the column and a graph of peak height recorded against hydrocortisone concentration was plotted (**figure 2.7**) from which the urinary cortisol concentration was obtained.

Fig.2.7. shows a typical calibration curve for cortisol.



Preparation of urine samples.

All glass-ware was acid washed and rinsed with HPLC grade reagents.

1) 2 ml of urine was mixed with 5 ml of HPLC grade dichloromethane in a glass centrifuge tube. The mixture was vortexed and then centrifuged

for 10 minutes at 4000 r.p.m.

2) The top aqueous layer was removed using a glass pasteur-pipette and discarded.

3) The remaining lipid layer was washed three times using doubled distilled water :-

a) 1 ml of the deionised water was added to the lipid layer, vortexed and centrifugation at 4000 r.p.m. for another 10 minutes. The top layer was removed and discarded after the each wash.

4) After the final wash, the bottom layer was then reduced to dryness using nitrogen and stored at -20^0 C until required for analysis, within 2 weeks. Due to the dangers of dichloromethane fumes , the entire procedure was carried out in the fume cupboard.

Preparation of the mobile phase.

The mobile phase was prepared by mixing HPLC grade methanol and double distilled water in a ratio of 6:4. The HPLC system consisted of a spherisorb 50DS column of 25 cm x 4.6 mm, a LC-RA liquid chromopac pump, a Pye Unicam PU 4025 ultraviolet detector at 254 nm with an output filter at 0.5 second, range of 0.01 and back off course setting at 5 and a 33924 Hewlett Packard integrater. The mobile phase was gassed with helium at a fast flow rate for 20 minutes then continuously at a lower rate. A blank sample of pure HPLC grade methanol, a sample of mobile phase and serial dilutions of standards were ran before the samples were analysed. One of the standard solutions was placed , randomly, among the urine samples to ensure the set parameters were not altered during the run.

Procedure.

The dried urine extract was resuspended in 75µl of the mobile phase solution and 20µl of the sample used for the analysis. The mobile phase was pumped through the HPLC system (**fig 2.7a**) at a flow rate of

FLOW DIAGRAM OF THE HPLC SYSTEM .

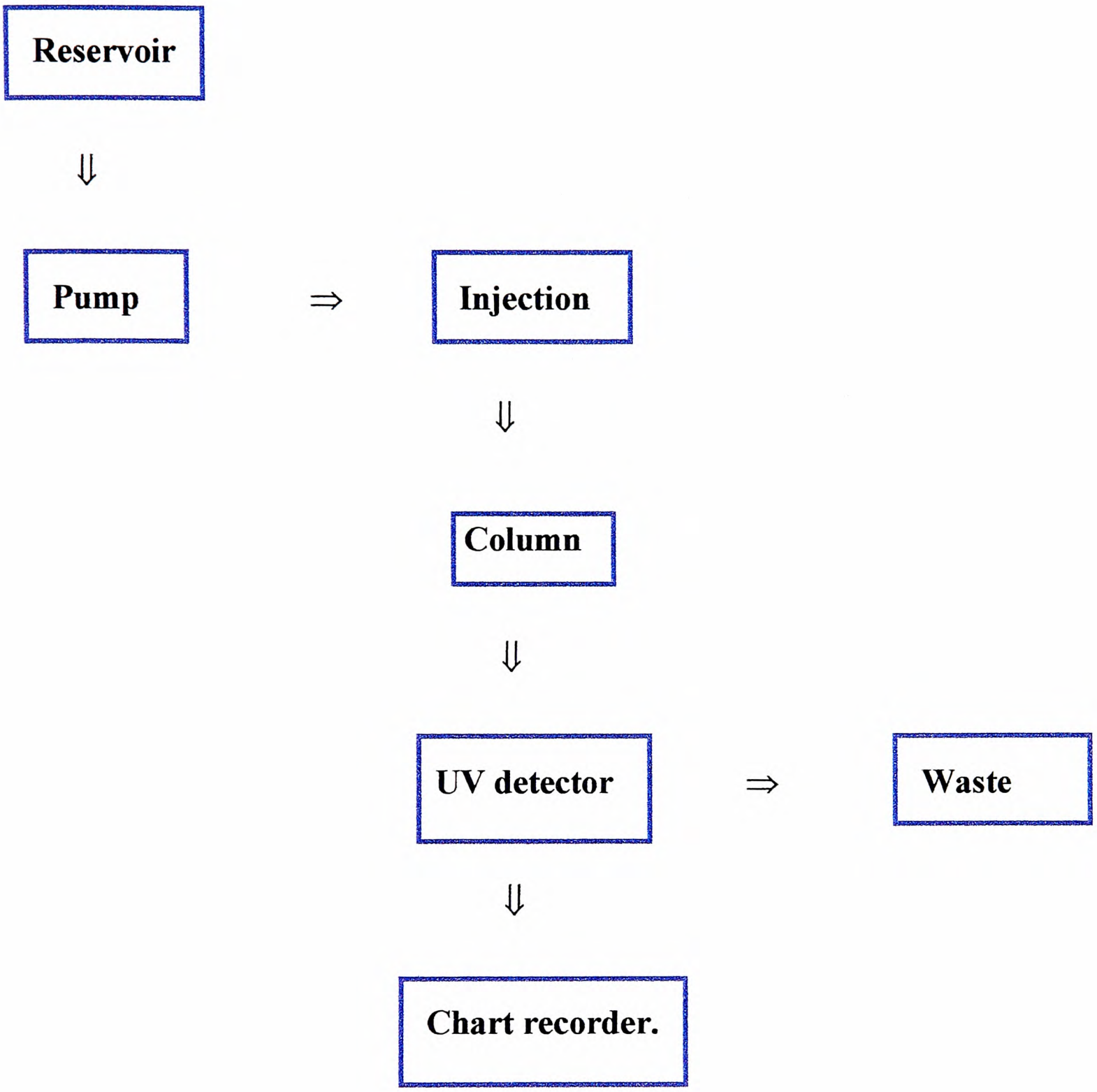


Fig.2.7a shows a diagrammatic flow chart of the HPLC system analysis.

1.5 ml per minute. The samples were injected using a Hamilton microlitre syringe through a rhoedyne 7125 valve, fitted with a 20 µl loop. The absorption was recorded on a Hewlett Packard 33924

integrator at a chart speed of 5 mm per minute.

2.5.0(g) Catecholamines.

Urinary catecholamines were determined by also using the high performance liquid chromatography (HPLC). Catecholamines were extracted from urine using activated alumina and separated by reverse phase ion-pair chromatography with an electrochemical detector.

Equipments.

Waters Intelligent Sample Processor (WISP^{TM}) 710B was used with an automatic sample injection module incorporating a WISP cooling module, a Hichrom 5μm analytical column 250x4.6 mm (serial number S50DSZ-11873), Water's (model 590) water pump with a programmable solvent delivering system, a Hewlett Packard integrater (model 3392A) and an electrochemical detector, (L-ECD-6A) set at STD, range of 32 and potential of +0.76 mV.

Collection of urine samples for catecholamine analysis

7.5 ml of the early morning urine samples collected from all subjects was transferred to clean labelled plastic tube containing 0.5 ml of concentrated hydrochloric acid (11 mol/L) to prevent bacterial growth and stored in a freezer at -20°C. All the reagents and chemicals used were HPLC-grade and obtained from Sigma.

Preparation of the urine sample.

1) The acidified urine samples were thawed at room temperature.

2) 5 ml of the thawed urine was transferred into a clean 50 ml beaker.

3) 15 ml of di-Sodium EDTA (1g/L) was added to the urine and filtered

4) The pH of the mixture was adjusted to 6.5 using NaOH.

5) 100μl of the internal standard DHBA (5 mg/L) was then added

to the mixture.

6) The mixture of urine, EDTA and DHBA was poured over a column of ion- exchange resin (Bio-Rex 70).

7) The column was then washed twice with deionised water and the eluate discarded.

8) The catecholamines and 5 HT attached to the ion-exchange resin were then eluted with 5 ml of boric acid (40g/L), followed by 5 ml of perchloric acid (0.5 mol/L). The final eluates were collected and stored at -20^0 C and used within 2 weeks.

Bio-Rex 70 Cation-Exchange Resin.

Bio-Rex is a weak cation-exchanger with a carboxylic acid functional group which binds positive charged catecholamines and 5 HT in urine . In order to purify the urine samples "clean resin" was used. The flask containing the "clean resin" was shaken to ensure thorough mixing . 5 ml of this resin was then added to the column and allowed to settle to the 3 ml mark on the 5 ml Econo-column (**fig. 2.8**). The used resin was recycled by washing it out of the column with water into a container and treated as follows:

1) After it was settled, the top layer of water was discarded.

2) Hydrochloric acid (3 mol/L) was added to the resin in ratio of 2:1 and thoroughly mixed for 10 minutes and allowed to settle . The top layer of hydrochloric acid was removed and discarded .

3) Step 2 was repeated using sodium hydroxide (3 mol/L) , then with acetic acid (3 mol/L) and finally using phosphate buffer (0.1 mol/L)

4) The pH of the "cleaned resin" was adjusted to 6.5 . and stored until ready for use .

Preparation of the mobile phase buffer.

This buffer contained the followings:

1) 15.6g sodium dihydrogen phosphate (0.1 mol/L)

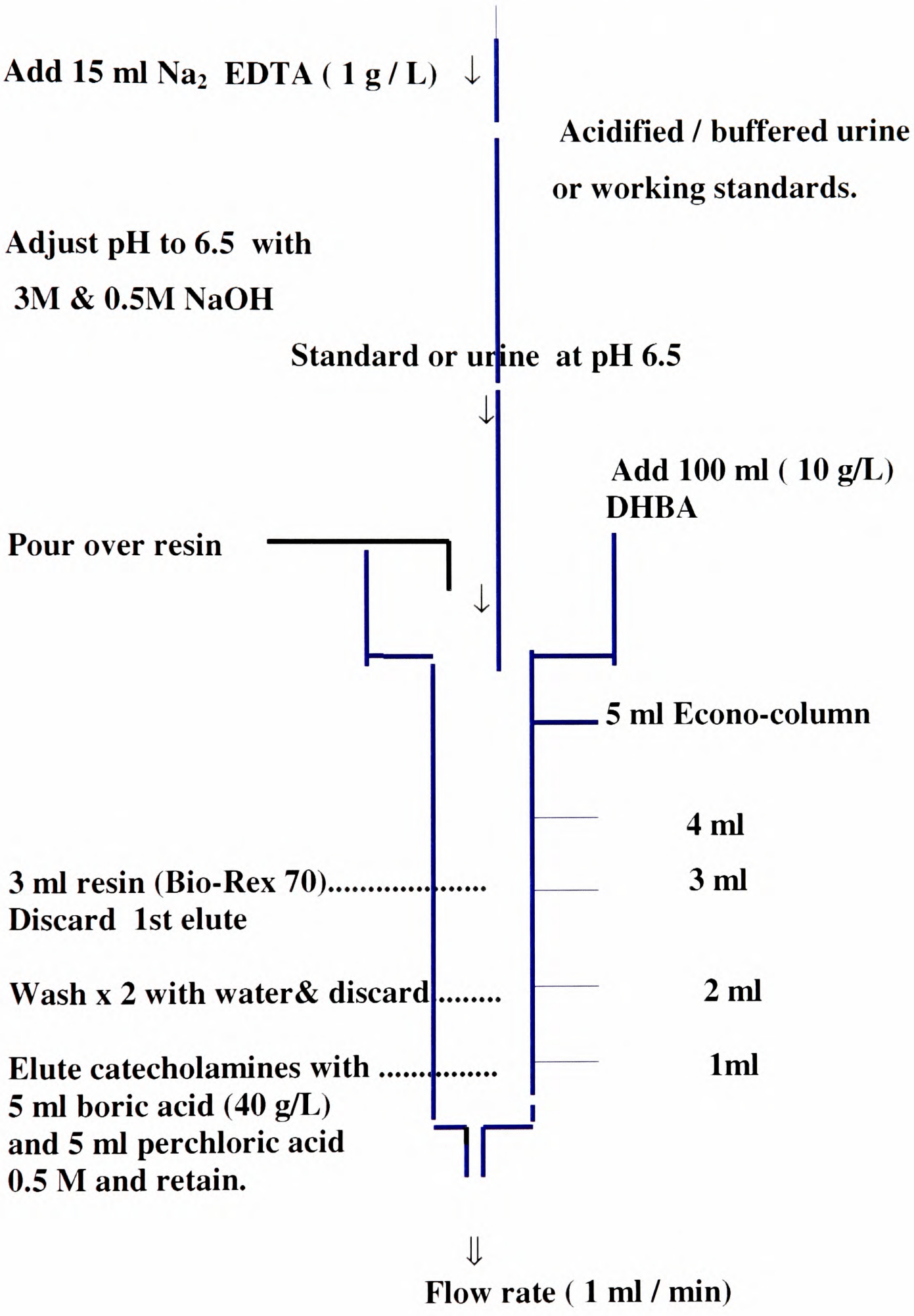


Fig.2.8. Showing the purification steps of urine for HPLC analysis.

(Source: Modified from Makarem 1992).

2) 0.0372g EDTA (0.1 mol/L)

3) 0.065g sodium octanesulphonic acid (0.3 mol/L) and

4) 140 ml HPLC-grade methanol.

5) The pH was adjusted to 3.4 and the solution made up to 1 litre with de-ionised water and pH checked again to ensure it was 3.4.

Preparation of Catecholamines Stock Standards.

A fresh stock solution of standard catecholamines was prepared by dissolving 0.015g of noradrenaline (Mwt. 319.3), 0.015g of adrenaline (Mwt. 333.3) and 0.0043g of dopamine (Mwt. 189.6) in 500 ml of perchloric acid (0.1 mol/L). A separate standard for serotonin (Mwt 212.7) was made by dissolving 0.0043g of 5 HT in 10 ml and diluted 500 times in HPLC-grade perchloric acid (0.1 mol/L) containing the anti-oxidant, sodium sulphite (0.4 mmol/L). From these standards, five serial dilutions were prepared using 100 , 200 , 400, 800 and 1000 µl for NA and AD, five dilutions of 500, 1000, 1500, 2500 and 3000 µl for DA and 100, 150, 200, 250 and 300 µl for serotonin (5HT). 100 µl of dihydrobenzylamine (DHBA) was added to each standard as an internal standard and made up to 10 ml (**Table 2.1**). These final working standards were injected prior to and at intervals during the analysis of urine samples to monitor reproducibility of the individual catecholamine response. **Figures 2.9, 2.10, 2.11** and **2.12** show a typical standard graph for these catecholamines and serotonin respectively. At 200 ng/L, %CV was 4.1 and 3.8 for NA and AD respectively; n=5.

Fig.2.9. shows a typical standard graph for noradrenaline

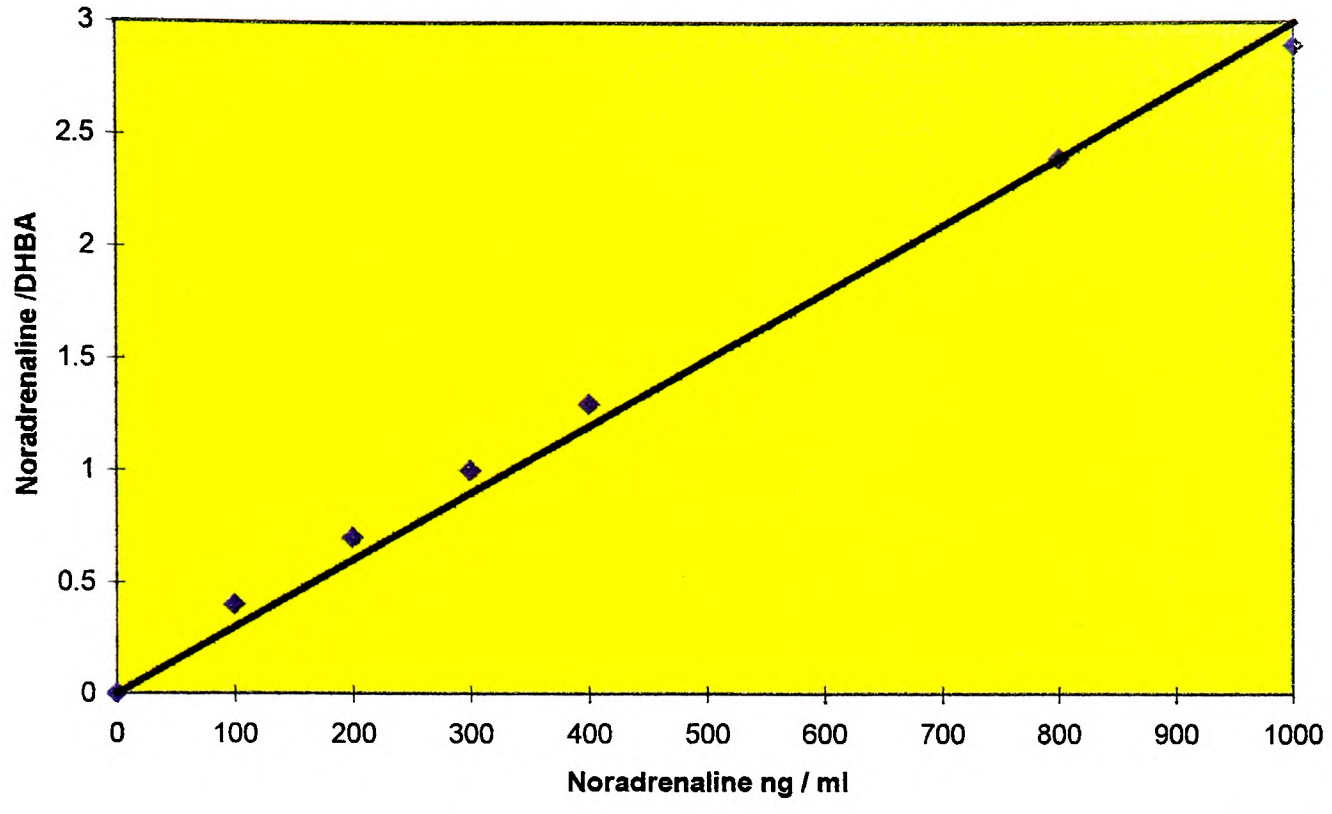


Fig.2.10. shows a typical standard curve for adrenaline.

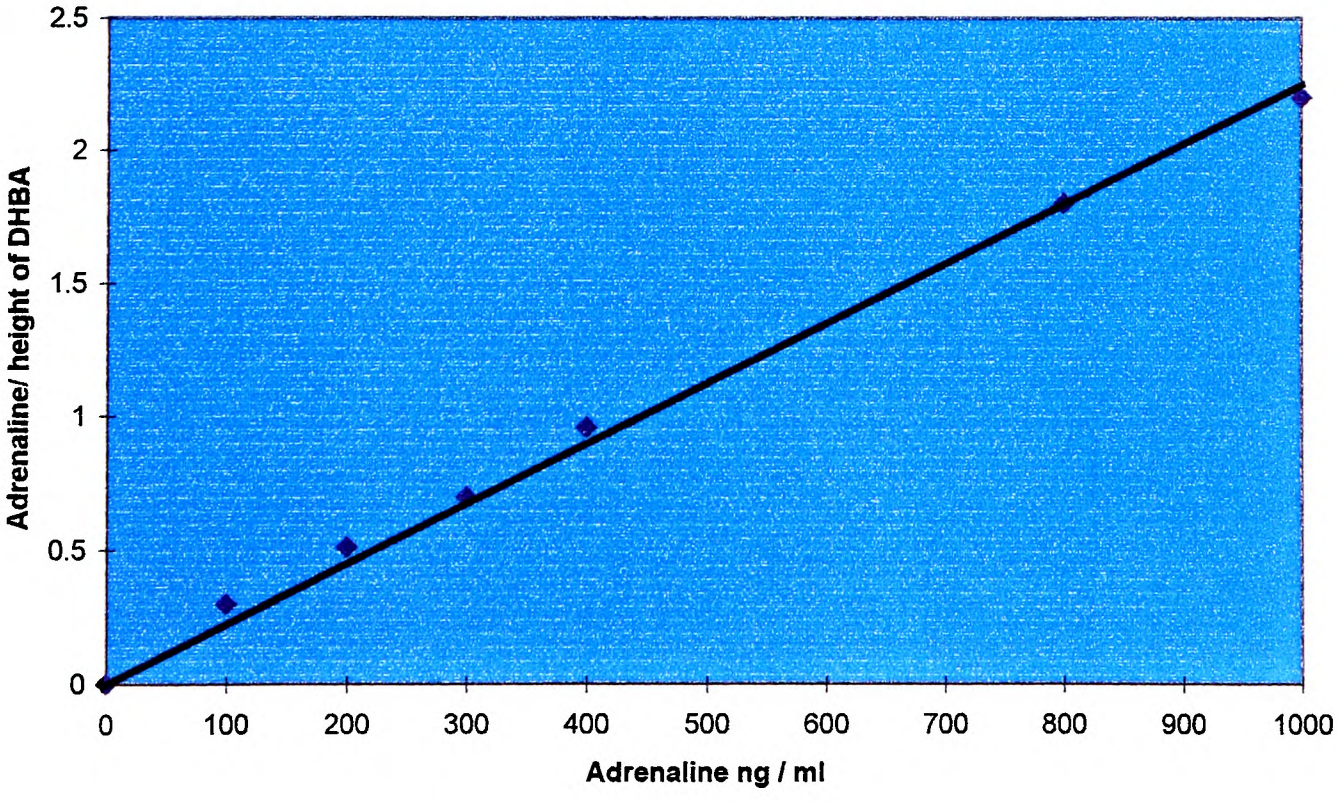


Fig. 2.11. shows a typical standard graph for dopamine.

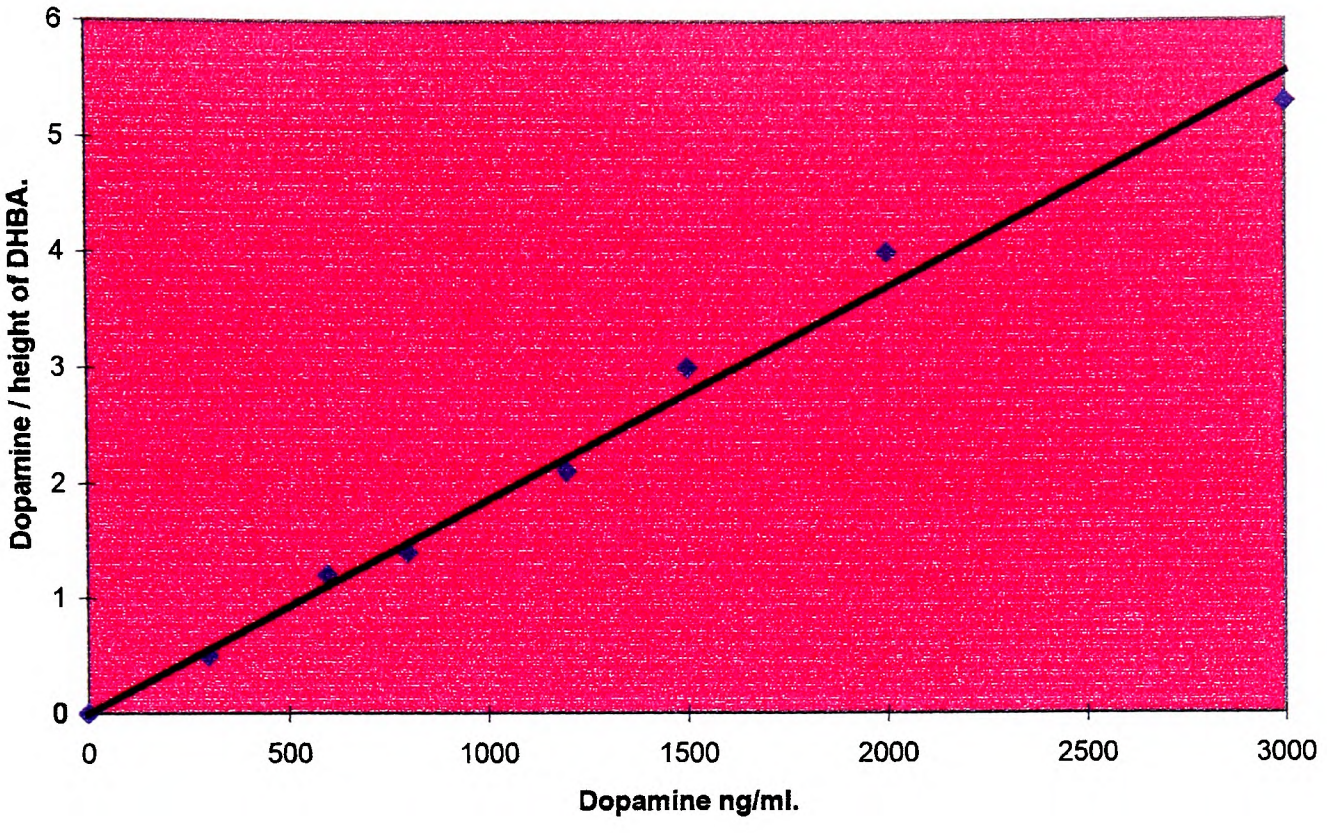


Fig.2.12. shows a typical reference curve for serotonin.

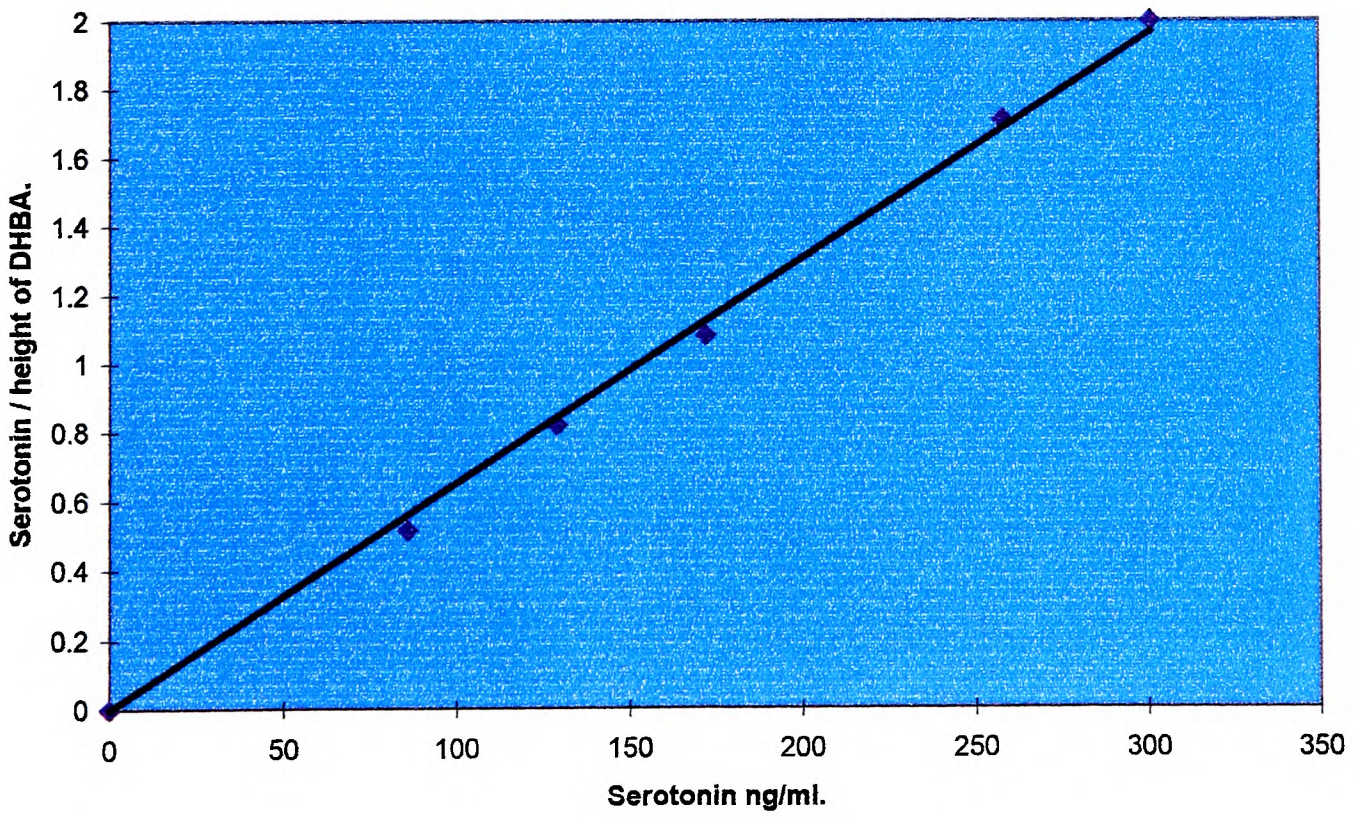


Table 2.1. The volumes of working standards of catecholamines and serotonin used.

No.	DHBA volume µl	NA & AD stock µl	DA stock µl	5 HT stock µl
1	100	100	500	100
2	100	200	1000	150
3	100	400	1500	200
4	100	800	2500	250
5	100	1000	3000	300

(All made up to a final volume of 10 ml).

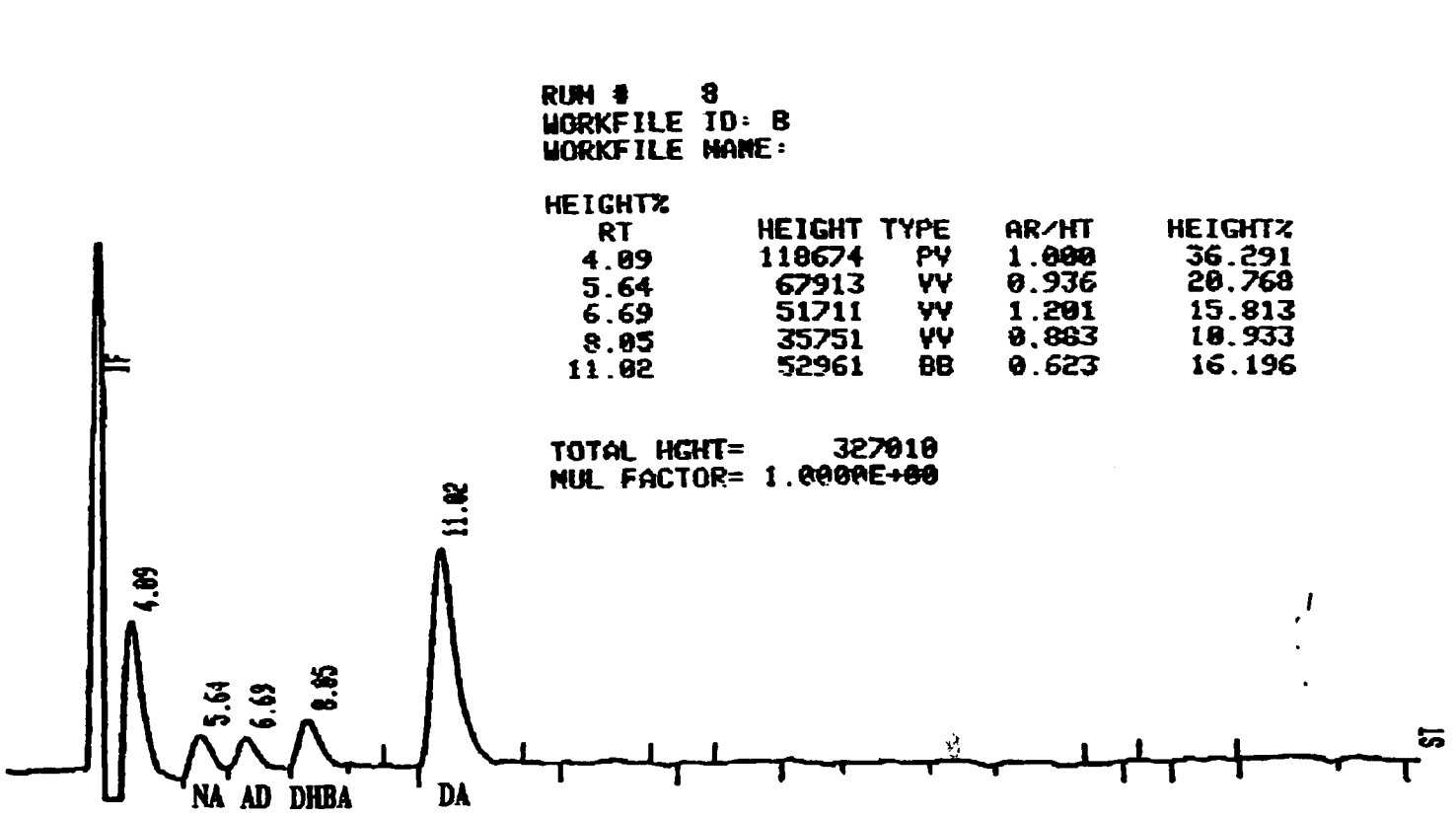


Fig. 2.13. A typical chromatographic recording of urinary NA, AD and DA.

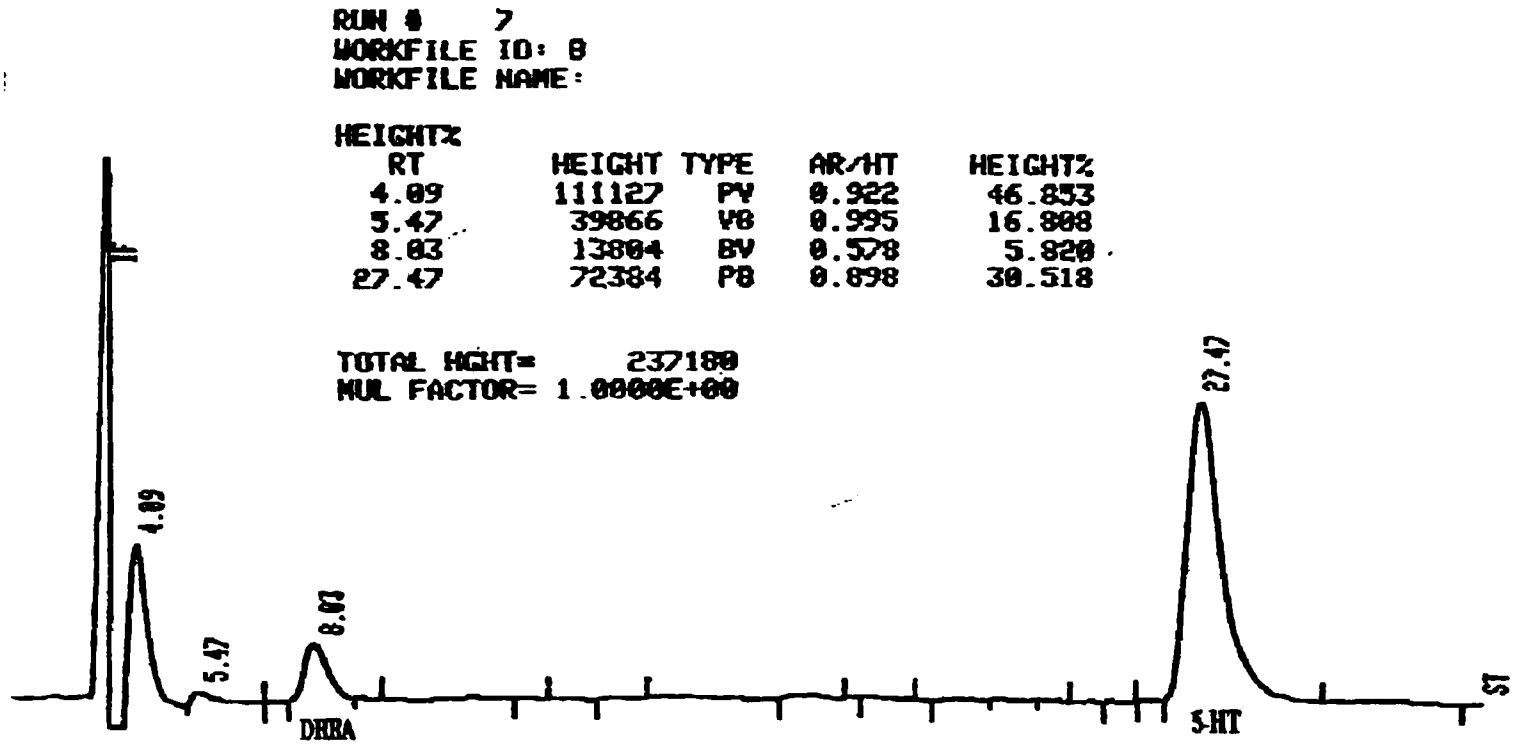


Fig.2.14. shows a typical chromatographic recording of urinary serotonin (5 HT).

2.5.0(h) Relationship between plasma and urinary catecholamines.

Previously, it was shown that urinary catecholamines (NA, AD and DA), increased as plasma levels were raised after exercise (30 minutes at moderate intensity). **Table 2.2.**, clearly, indicates that urinary catecholamines may reflect plasma levels. These bioamines are released into blood from the adrenal medulla and as spillover from the sympathetic nerve endings and cleared by the kidneys. As a result, urinary catecholamines were used as a good biochemical index of stress in all subjects. This also reduces the effects of stress from taking blood samples.

Table 2.2. shows catecholamines level in plasma and urine before (1) and after (2) moderate exercise.

Catecholamines		Plasma (ng/ml)	Urine (ug.g creatinine)
NA	**(1)**	0.35	38
	(2)	0.62	64
AD	**(1)**	0.04	2.3
	(2)	0.10	5.2
DA	**(1)**	13.7	140
	(2)	20.7	200

2.5.0(i) Creatinine.

Preparation of reagents and standards.

(a) Picric acid (0.04 mol/L) . Liquid form of picric acid (Mwt. 229.11) was used instead of the volatile powered form.

(b) Sodium hydroxide (0.75 mol/L). 3 g of sodium hydroxide (Mwt. 40) was dissolved in 100 ml of double distilled water.

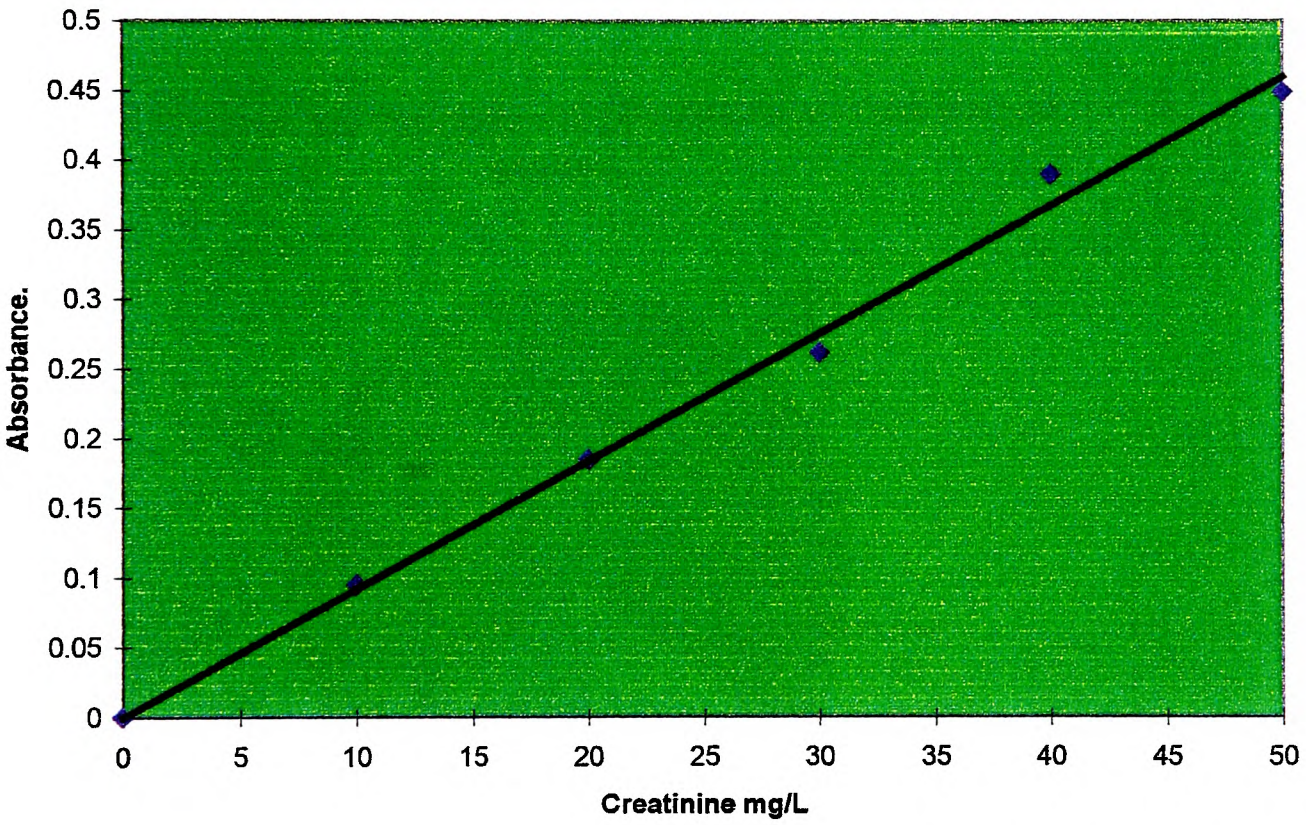
(c) Creatinine standard solution. 0.1 g of creatinine (Mwt. 113.12) was dissolved in 50 ml of double distilled water. Serial dilutions of 10, 20, 30, 40 and 50 μg/ml were made.

Procedure.

All reagents and urine samples were prepared in the fume

cupboard. Double distilled water was used to dilute the urine samples 50 times and also to prepare a blank sample to zero the Unicam 8625 UV/VIS spectrophotometer. 1 ml of picric acid was added to 3 ml of blank, standards or urine samples followed by 1 ml of sodium hydroxide and shaken vigorously. After 15 minutes, the absorption of the blank, standards and urine samples were recorded from the spectrophotometer at 500 nm. **Figure 2.14** shows a typical calibration curve for creatinine. At 20 mg/L, the %CV was 3.95; n=10.

Fig. 2.15. shows a calibration graph for creatinine



2.6.0. Statistical analysis.

Excel and Mintab were used for the analysis of data. Analysis of variance (ANOVA), t-test and Pearson's correlation coefficient were used to establish significance at 5 % level. All values were standardised as a ratio to creatinine and expressed as mean ± standard error of mean.

CHAPTER 3

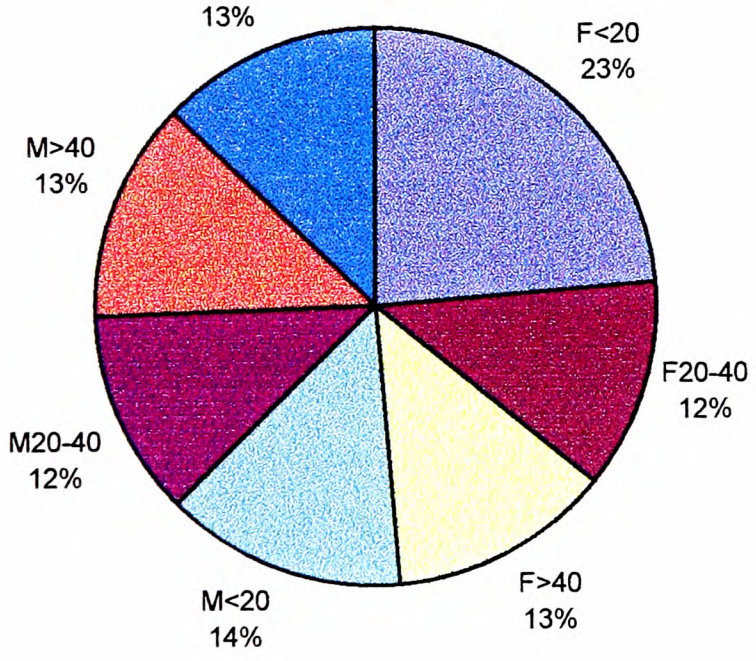
RESULTS

3.0.0. Introduction.

The total number of subjects (n= 172, **Fig. 3.1**) were placed into 3 main groups; women (F, n=84), pregnant women (P, n=21) and men (M, n= 67). These groups were further divided into females 11-20 years old (F<20; n=41), 20-40 years old (F20-40; n=21), and 40 years old and above (F>40; n=22). Similarly, the male groups were 11-20 years old (M<20; n=24), 20-40 years old (M20-40; n=21) and 40 years old and above (M>40; n=22).

Fig.3.1. shows the distributions of subjects into the various groups.

p



The subjects in F<20 and M<20 were students from local secondary schools, F20-40 and M 20-40 were students nurses from the School of Health, University of Greenwich. The pregnant women and 50 % of F>40 and M>40 were patients from the local hospital and the other 50 % were nurses from the University.

Urinary levels of calcium, magnesium, phosphate, boron, hydroxyproline, cortisol, noradrenaline, adrenaline, dopamine and serotonin were analysed and related to age, gender and pregnancy. Bone markers (calcium, magnesium, phosphate and hydroxyproline) were then correlated with stress hormones (cortisol, noradrenaline, adrenaline and dopamine), serotonin and boron. All analytes were compared between alcohol / low-alcohol drinkers, stressed / non-stressed, those performing more / less than 3 sessions of weight-bearing exercise per week, vegetarians / non-vegetarians, vitamins supplements (multivitamins and vitamins C) takers / non-takers, those suffering / not suffering from the bone diseases (osteoarthritis -O/A and osteoporosis-O/P), those with / without a family history of these bone diseases, smokers / non-smokers, those on / not on contraceptive pill, women with / without previous pregnancy, those with / without hysterectomy, pre-menopausal / post-menopausal women, those on / not on HRT and subjects taking / not taking anti-inflammatory drugs (aspirin and voltarel) within the same group, between genders of the same age and between pregnant and non-pregnant women of 20-40 years old group. All urinary analytes were expressed as ratio to creatinine.

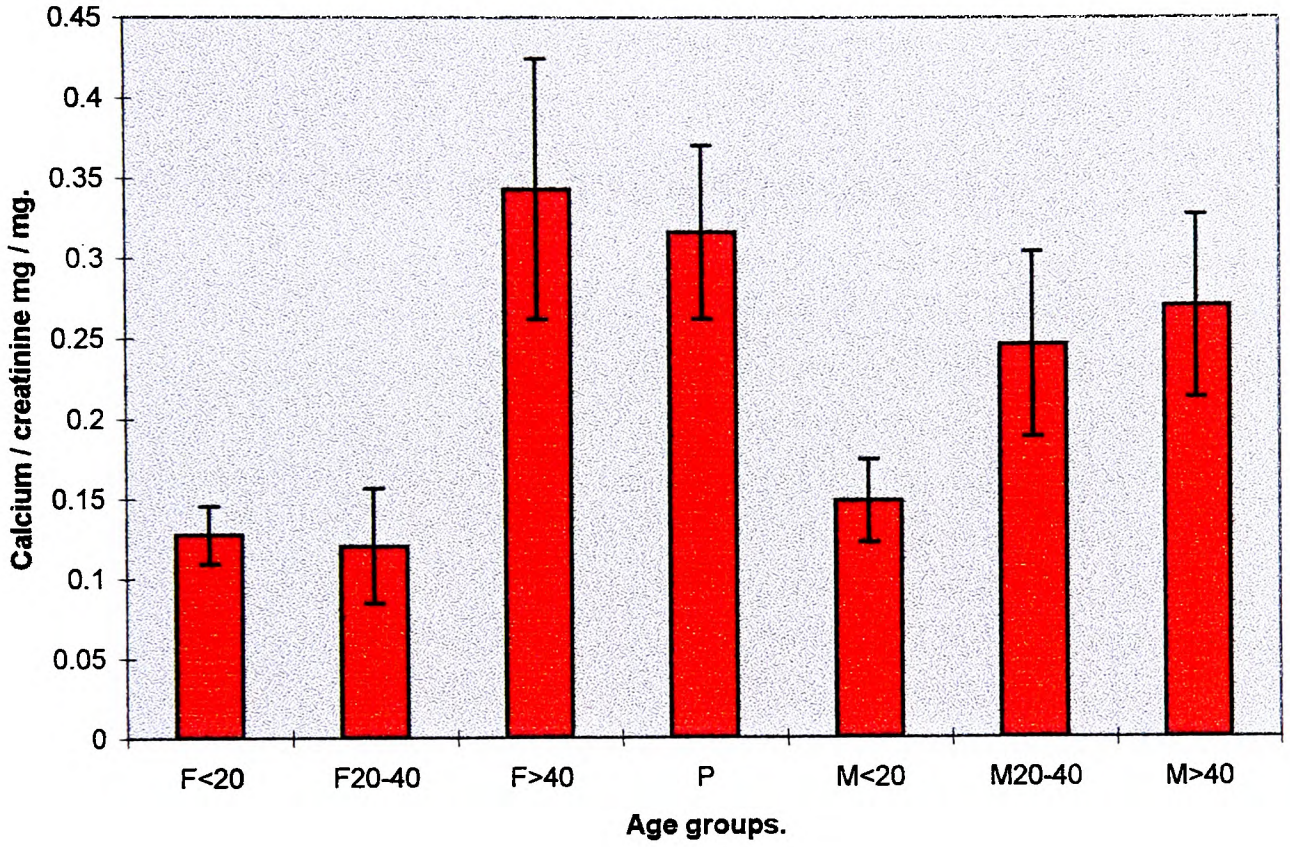
3.1.0. The effects of age, gender and pregnancy on urinary analytes.

3.1.0(a) Urinary calcium.

In general, calcium levels increased with age in both genders, but significantly greater in the pregnant women compared to F <20, M <20 and F20-40 ($P<0.05$). It was highest in F>40 followed by pregnancy (**fig. 3.2**). In F>40, it increased significantly by 62% and 65% ($P<0.05$) when compared with F<20 and F20-40 respectively. In M>40, it increased by 45% when compared with M<20 ($P<0.05$) and by 10% compared with M20-40. The levels in M<20 were 14% higher than in F<20; in M20-40 it was 51% higher than in F20-40 and in M>40 it was 17% lower than in F>40. In pregnant women it was

significantly higher (P<0.05) than in F<20, F20-40 and M<20 and 31% and 43% (P<0.05) higher than in men and non-pregnant women of similar age respectively.

Fig.3.2. showing urinary calcium in the different age groups.



3.1.0(b). Urinary magnesiun.

While urinary magnesium levels increased in women and decreased in men with age, this was not significant. Magnesium levels in F<20 were 0.137 ± 0.016, F20-40 0.124 ± 0.041, F>40 0.183 ± 0.041, P 0.125 ± 0.019, M<20 0.148 ± 0.021, M20-40 0.0140 ± 0.033 and M>40 0.111± 0.014 mg / mg creatinine. Urinary magnesium levels decreased with age, in male but increased in female subjects.

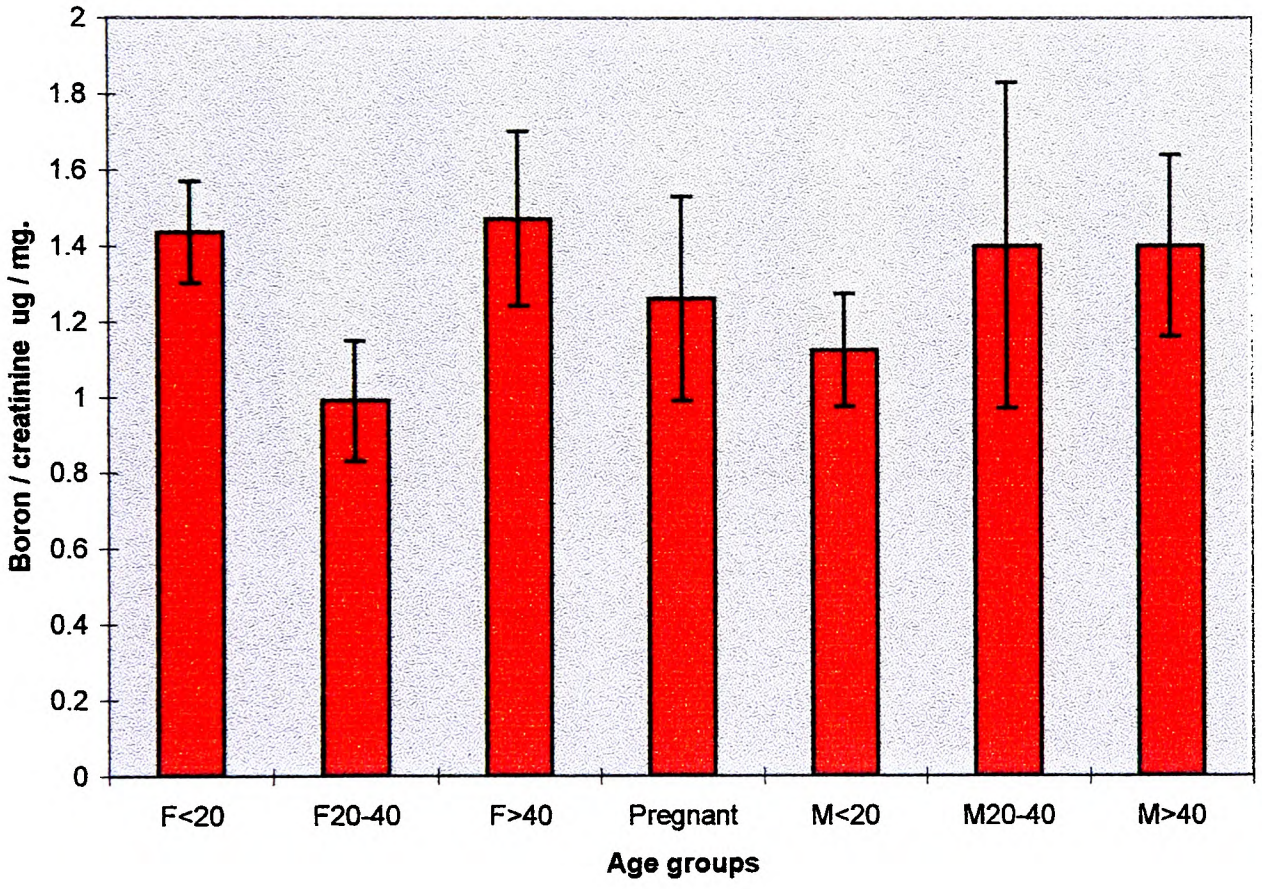
3.1.0(c). Urinary phosphate.

Urinary phosphate increased with age in both sexes and was highest during pregnancy. The levels in F<20 were 1.071 ± 0.083, F20-40 1.197 ±

0.150, F>40 1.560 ± 0.240, P 1.800 ± 0.410, M<20 1.395 ± 0.170, M20-40 1.318 ± 0.210 and M>40 1.760 ± 0.290 mg / mg creatinine. These were highest in pregnant and lowest in non-pregnant women but none of these was statistically significant.

3.1.0(d). Urinary boron.

Fig.3.3. shows urinary boron in the different age groups.

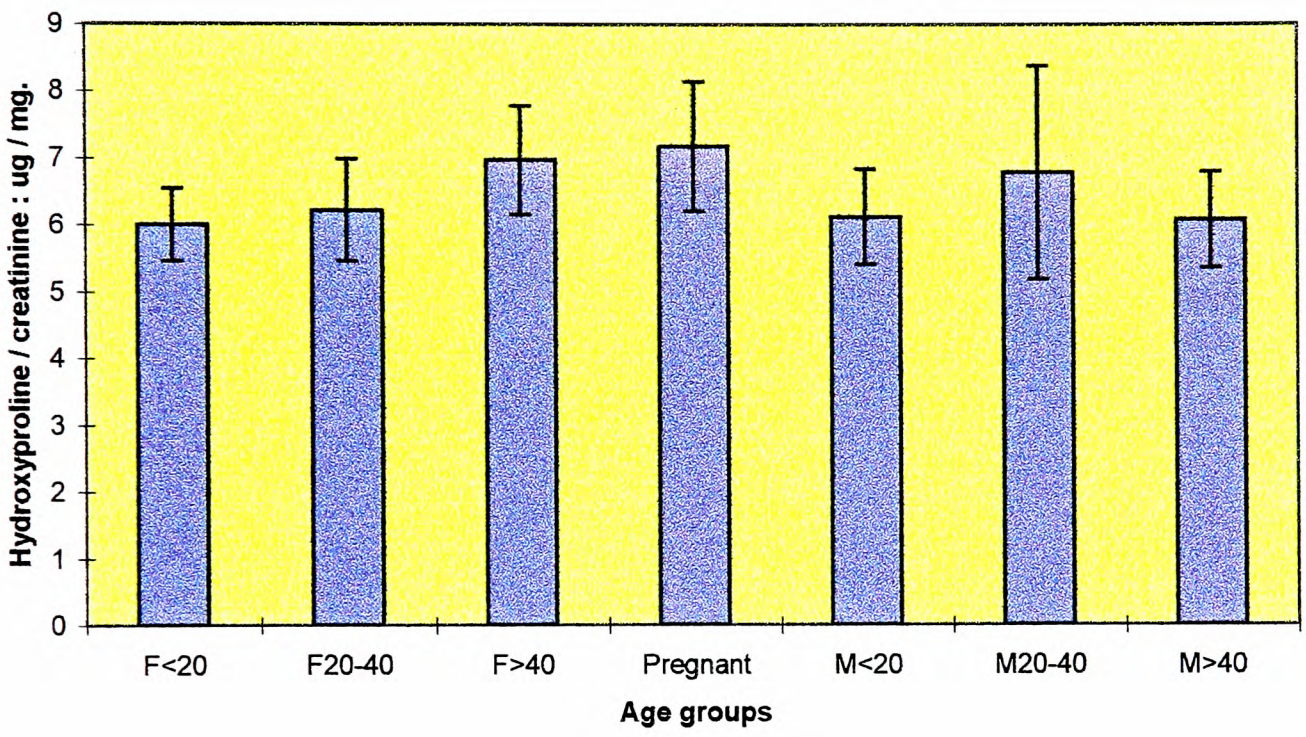


In general, there was no significant change in urinary boron with age in both genders. The levels initially decreased in F20-40 but increased in F>40 compared to F<20 and increased in male subjects with age from M<20 to M20-40. Boron level during pregnancy was not significantly different to those in F20-40 and M20-40 (**fig.3.3**). The differences in boron levels in females, males and pregnant women were not significant (P>0.05).

3.1.0(e). Urinary hydroxyproline.

While urinary hydroxyproline levels increased in women with age, they decreased in men and were highest during pregnancy but none of these were statistically significant (**fig.3.4**).

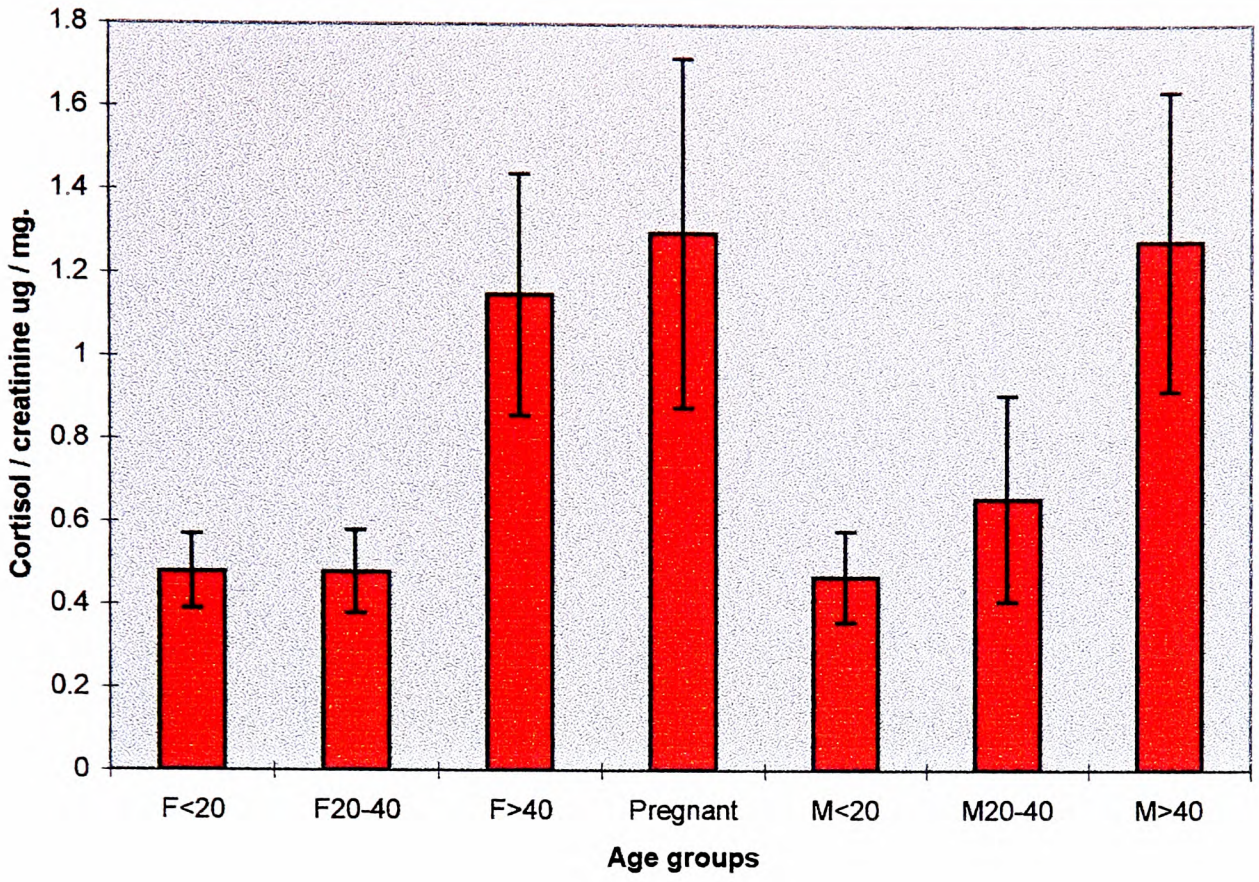
Fig. 3.4. shows urinary hydroxyproline levels in the different age groups.



3.1.0(f). Urinary cortisol.

Urinary cortisol levels increased with age in both genders. These levels were highest in pregnant women compared to the non-pregnant females (F20-40) and men of the same age groups **(fig 3.5).** In F>40, they were significantly higher ($P<0.05$) than in F<20 and F20-40. There was a 64% increase ($P<0.05$) in M>40 compared to M<20, 49% compared to M20-40 and 30% in M20-40 compared to M<20. Cortisol level was 63% higher in pregnant women compared to non-pregnant women (F20-40, $P<0.05$) and 49% compared to men of similar age group ($P<0.05$). There was no gender differences between F<20 and M<20 and F>40 and M>40.

Fig.3.5. shows urinary cortisol in the different age groups.



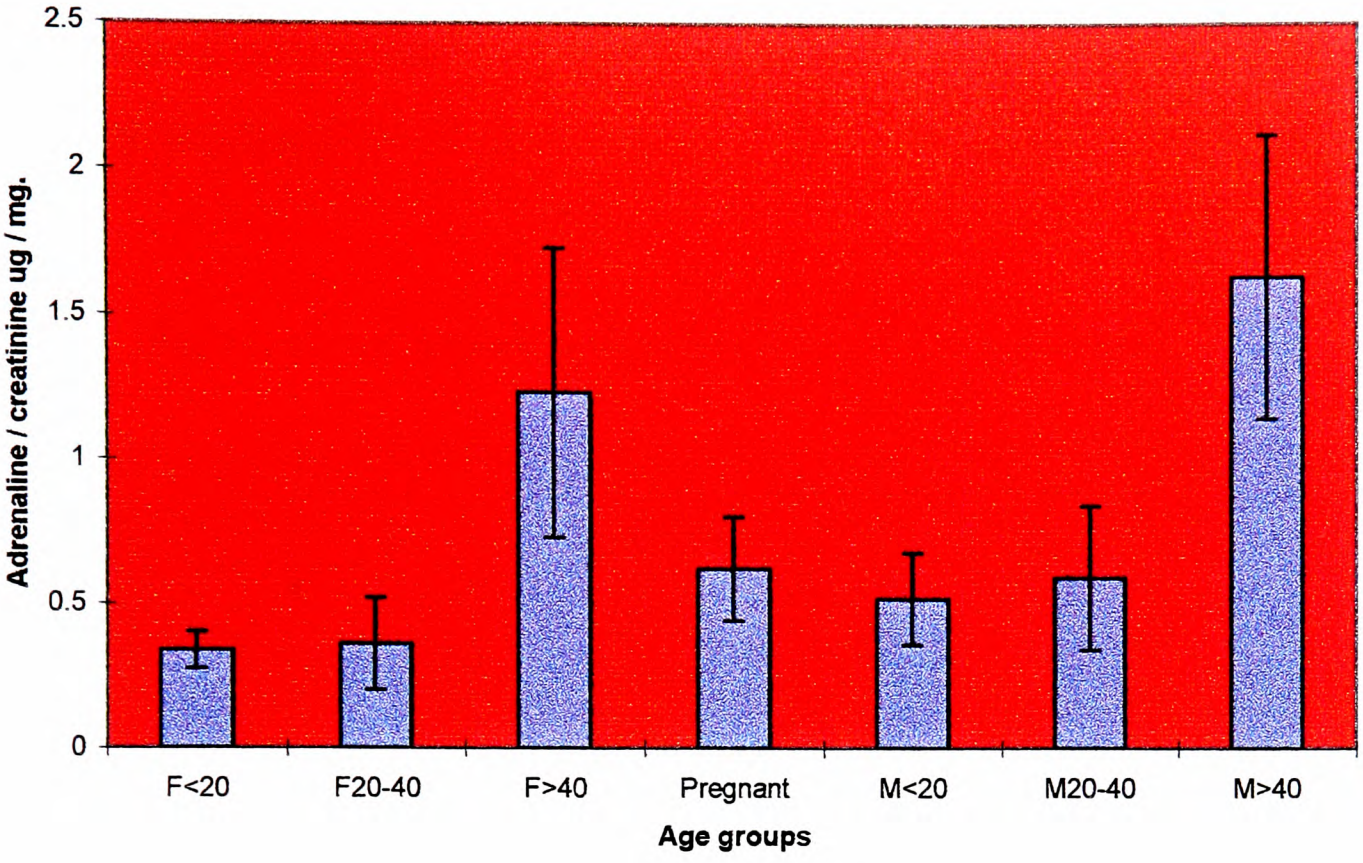
3.1.0(g). Urinary noradrenaline.

While these levels in F<20 and F>40 were very similar, they were lower in F20-40 and, in men, decreased with age. These levels were slightly higher during pregnancy compared to F20-40. In F<20 they were 0.343 ± 0.088, F20-40 0.210 ± 0.042, F>40 0.364 ± 0.140, P 0.281 ± 0.085, M<20 0.270 ± 0.047, M20-40 0.349 ± 0.130 and M>40 0.313 ± 0.064 μg / mg creatinine. These levels decreased by 32% (P=0.058) in M20-40 compared to M<20 but none of these differences were statistically significant.

3.1.0(h). Urinary adrenaline.

As with urinary cortisol levels, adrenaline increased with age in both genders. The levels in pregnancy were not significantly different from those of F20-40 and M20-40 **(Fig. 3.6).**

Fig.3.6. shows urinary adrenaline in the different age groups



These levels were 68% ($P<0.05$) higher in M>40 than in M<20, 64% higher than in M20-40 ($P=0.07$) and 12% in M20-40 compared to M<20. In pregnant women they increased by 46% compared to F<20, 42% compared to F20-40, 17% compared to M<20 and 5% compared to M20-40 but decreased by 49% ($P=0.27$) and 61% ($P=0.61$) in F>40 and M>40 respectively. In males, these levels were significantly higher than in females ($P<0.05$) but not in pregnant women.

3.1.0(i). Urinary dopamine.

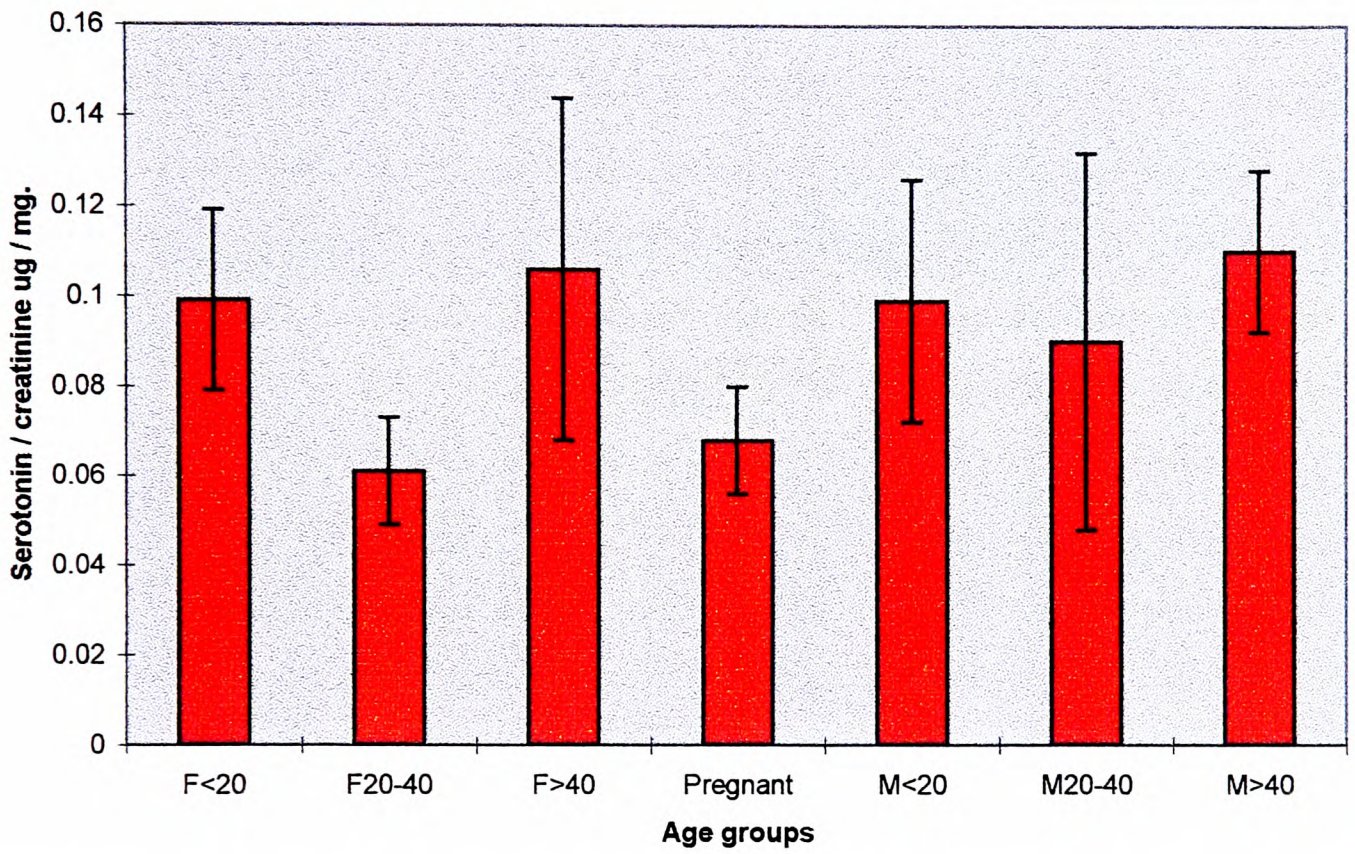
Urinary dopamine in F<20 were 2.760 ± 0.380, F20-40 1.320 ± 0.160, F>40 2.690 ± 0.700, P 1.661 ± 0.248, M<20 2.840 ± 0.600, M20-40 1.526 ± 0.200 and M>40 1.621 ± 0.210 μg / mg creatinine. Urinary dopamine levels did not change with age in female but decreased in male subjects. In F20-40, these were 51% lower ($P = 0.06$) than in F>40 and 52% lower ($P<0.05$) than in F<20 and in M20-40 they decreased by 43% ($P<0.05$) and 6 % compared to

M<20 and M>40 respectively.

3.1.0(j). Urinary serotonin.

Urinary serotonin levels decreased in F20-40 compared to F<20 but

Fig.3.7. shows urinary serotonin in the different age groups

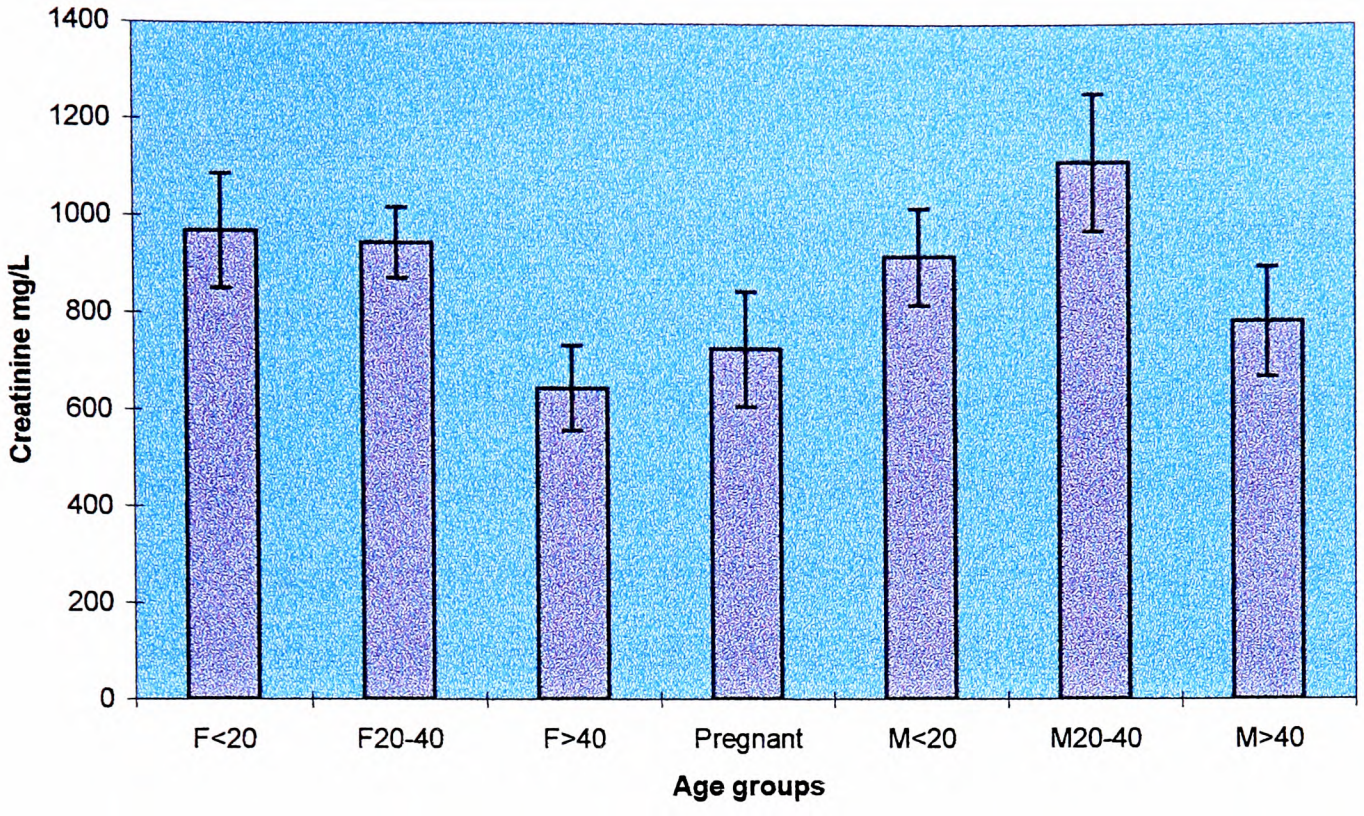


increased slightly above those of F20-40 in F>40. Similar patterns of serotonin excretion were observed in the male subjects. The levels during pregnancy were slightly higher than those in F20-40 (**fig.3.7**). These were not significant (P>0.05).

3.1.0.(k) Urinary creatinine.

In women, excretion of creatinine decreased with age, but increased in M20-40 but decreased in M>40 (**fig. 3.8**). The decreases in F>40 compared to F<20 and F20-40 were significant (P<0.05).

Fig. 3.8 shows urinary creatinine in the different age groups



3.2.0. Correlation between urinary boron, stress hormones, serotonin and bone markers.

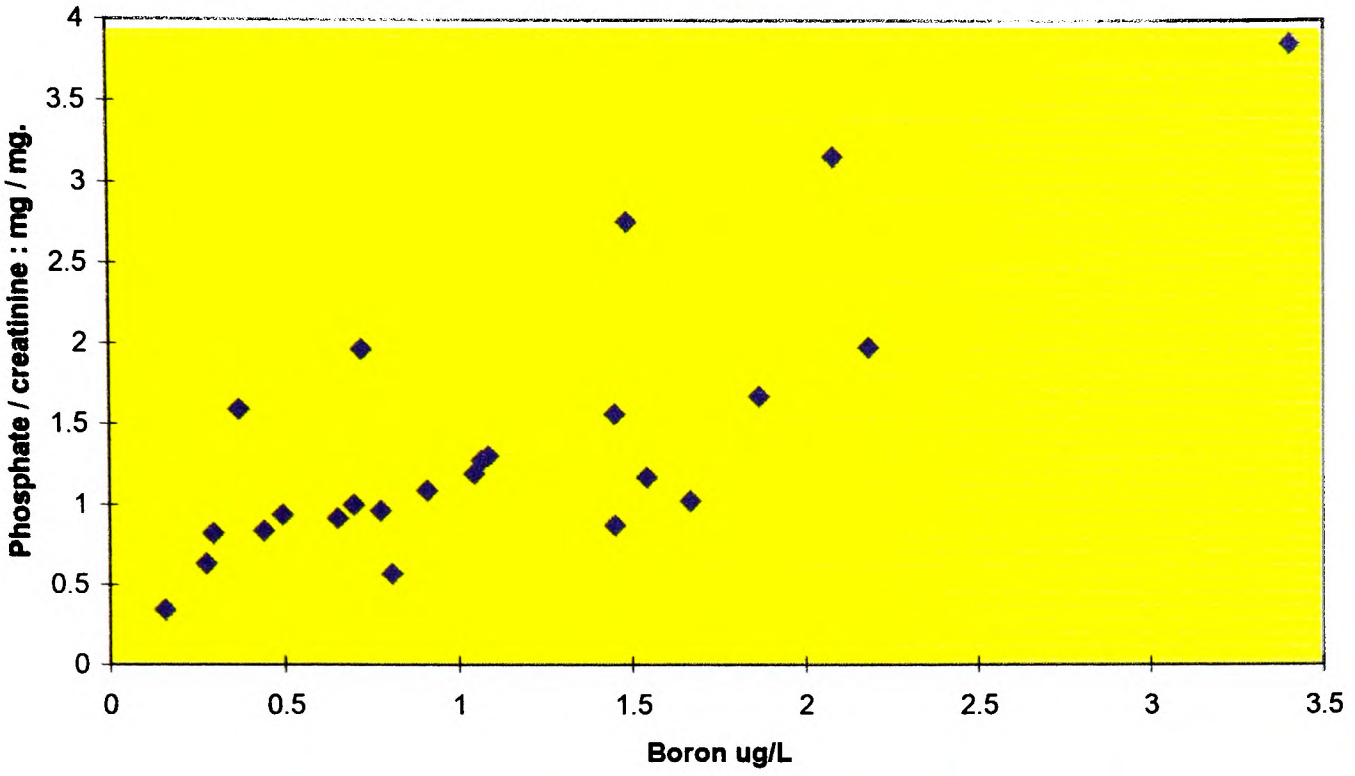
3.2.0(a). Introduction.

In this section, urinary levels of boron and stress hormones (cortisol, noradrenaline, adrenaline, dopamine) and serotonin were related to the urinary levels of well-known bone markers (calcium, magnesium, phosphate and hydroxyproline). The levels of boron were also correlated with those of the stress hormones and serotonin of the main groups of males, females and pregnant women and of the sub-groups (F<20, F20-40, F>40, M<20, M20-40 and M>40).

3.2.0(b). Urinary boron and bone markers.

There was no significant correlation between urinary boron and calcium in any of the main groups, however, there was a positive trend in the pregnant group ($r^2 = 0.105$), the main male group ($r^2 = 0.143$), M20-40 ($r^2 = 0.199$), M>40 ($r^2 = 0.240$) and F<20 ($r^2 = 0.159$). There were positive correlations between urinary boron and magnesium in F20-40 ($r^2 = 0.468$, P<0.025), M20-40 ($r^2 = 0.510$, P<0.01), M<20 ($r^2 = 0.6991$, P<0.0005,), positive trend in F>40 ($r^2 = 0.183$) and between boron and phosphate in the main male group ($r^2 = 0.334$, P<0.005), M<20 ($r^2 = 0.6208$, P<0.005, **fig. 3.9),** M20-40 ($r^2 = 0.580$, P<0.005) and M>40 ($r^2 = 0.365$, P<0.05). There was no significant correlation between boron and hydroxyproline in any of the groups.

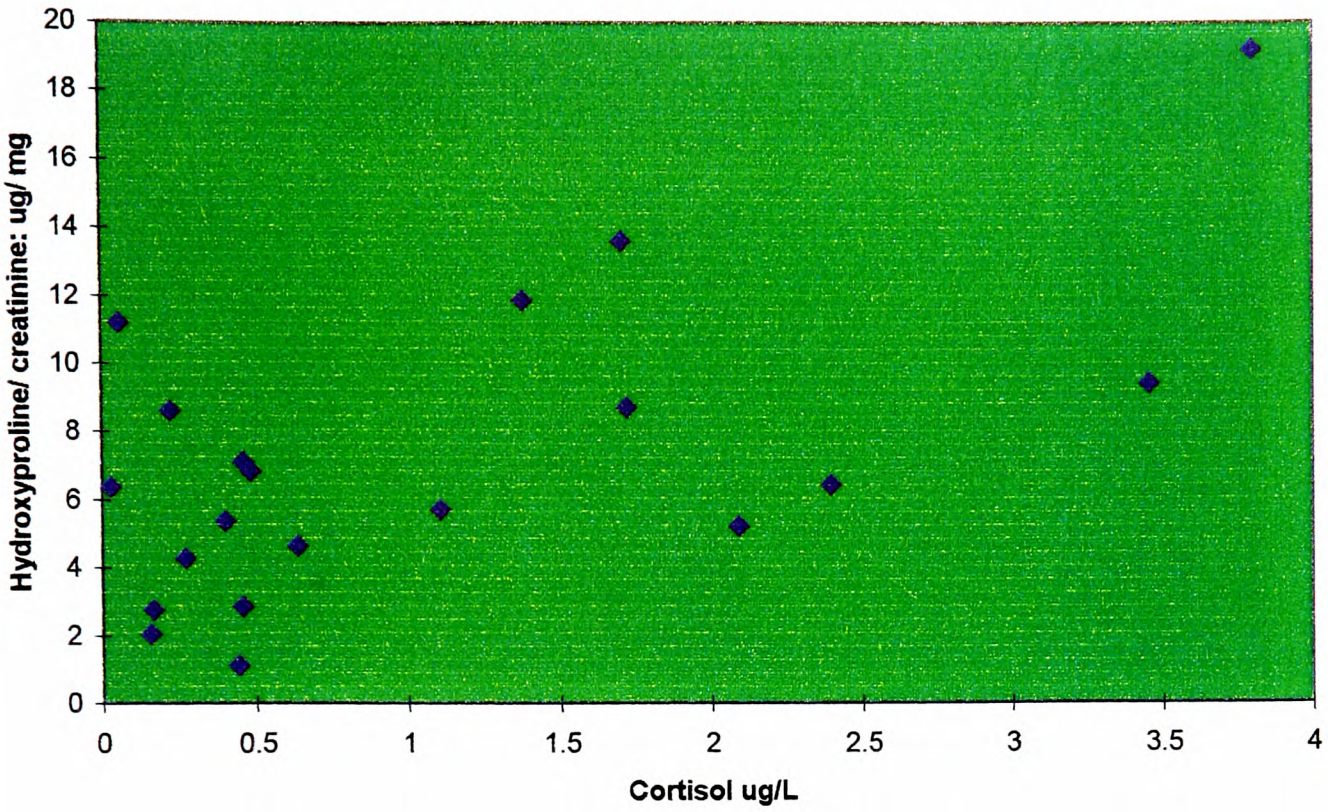
Fig.3.9 shows the relationship between urinary boron and phosphate in M<20 (n = 24, r^2= 0.6208).



3.2.0(c). Urinary cortisol and bone markers.

There was positive correlation between urinary cortisol and calcium in the main male group (r^2 = 0.6136, P<0.0005) particularly in M>40 (r^2 = 0.583, P<0.005), M<20 (r^2 = 0.4110, P<0.05), and positive trends in M20-40 (r^2 = 0.157), F20-40 (r^2 = 0.159) and P (r^2 = 0.138). With magnesium, the correlation in the main male groups were significant, (r^2 = 0.325, P<0.005), M20-40 (r^2 = 0.469, P<0.025) M>40 (r^2 = 0.410, P<0.05) and positive trends in F<20 (r^2 = 0.248), while with phosphate also in the main male groups (r^2 = 0.4732, P<0.0005) M>40 (r^2 = 0.586, P<0.005) with positive trends in P (r^2 = 0.296). The correlation between hydroxyproline and cortisol was also significant in P (r^2 =0.3788, P<0.05, **fig.3.10**) and in M20-40 (r^2 = 0.562, P<0.005).

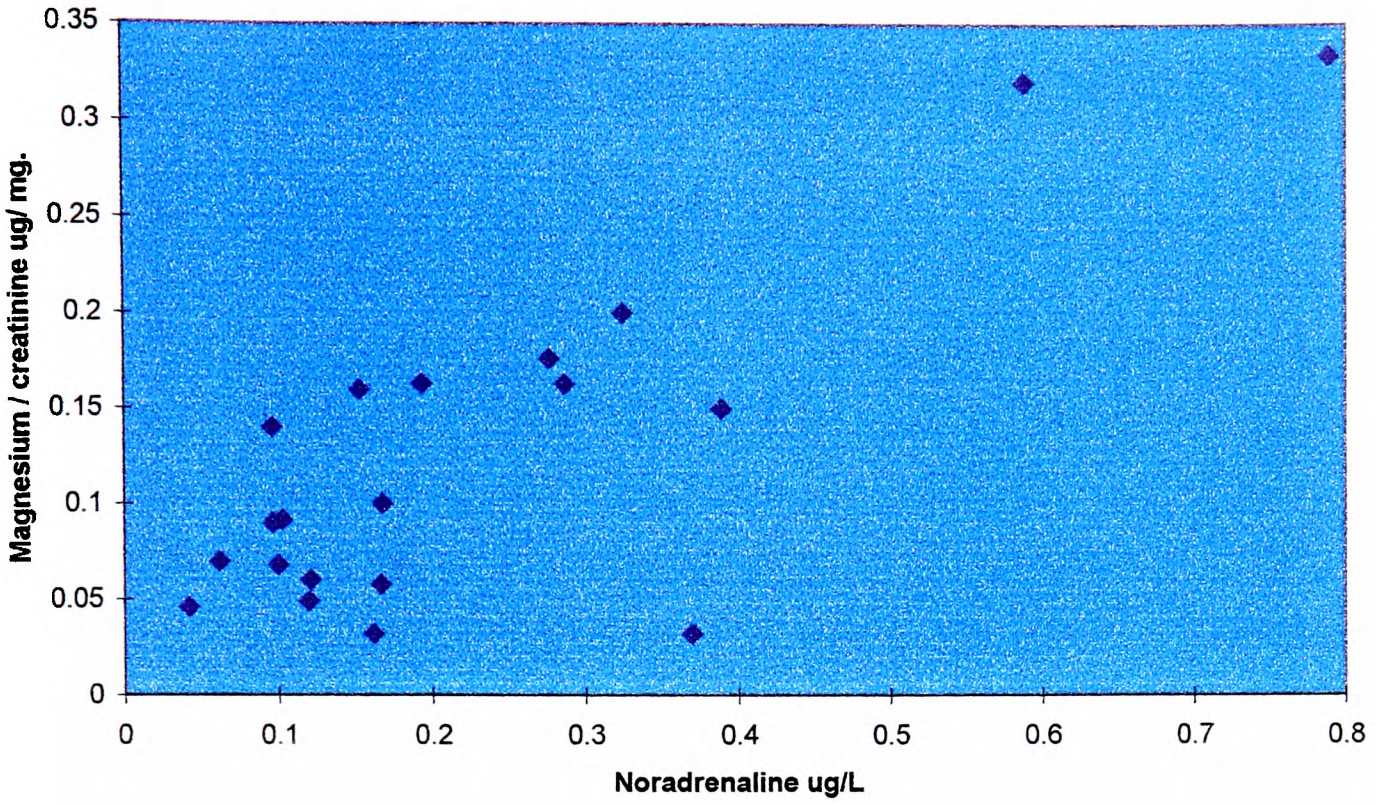
Fig.3.10. shows the relationship between urinary cortisol and hydroxyproline in pregnancy (n = 21, r^2 = 0.3788).



3.2.0(d). Urinary noradrenaline and bone markers .

There was significant correlation between noradrenaline and calcium in the main male group (r^2 = 0.264, P<0.025) but not in the female or in the pregnant groups. In M>40, it was (r^2 = 0.372, P<0.05), F>40 (r^2 = 0.606, P<0.005), P (r^2 = 0.580, P<0.005) with positve trends in the M20-40 (r^2 = 0.328), M<20 (r^2 = 0.331) and F20-40 (r^2 = 0.338). There was also significant correlations between noradrenaline and magnesium in P (r^2 = 0.656, P<0.0005, **fig.3.11**) and M20-40 (r^2 = 0.365, P<0.05) but not in the other groups although positive trends were obtained in M>40 (r^2 = 0.265) and in F20-40 (r^2 = 0.174). In addition, significant correlations were obtained between urinary noradrenaline and phosphate in F20-40 (r^2 = 0.519, P<0.01) and P (r^2 = 0.506, P<0.01) with trends in the main male and M20-40 (r^2 = 0.245). No significant correlation was obtained for noradrenaline and hydroxyproline in any the groups.

Fig.3.11. shows the relationship between urinary noradrnaline and magnesium in pregnant women (n = 21, r^2 = 0.6563)

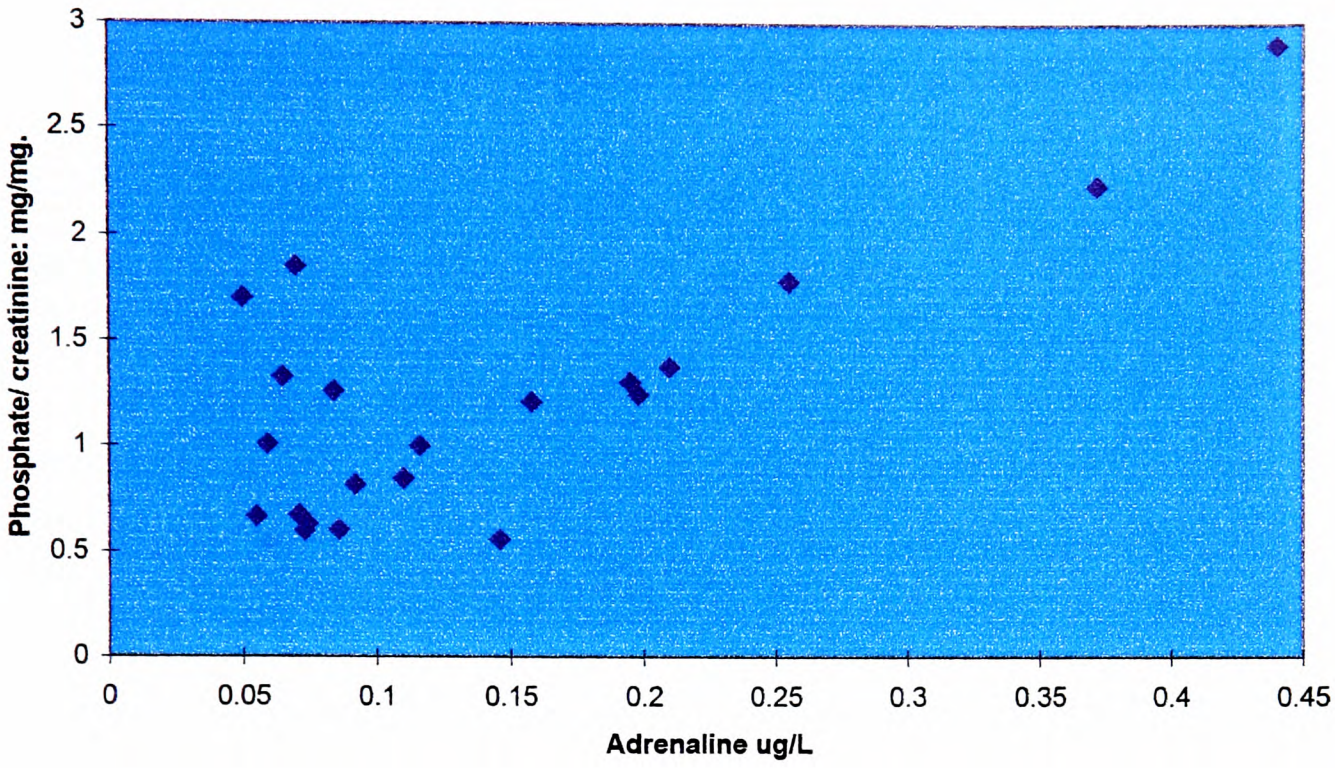


3.2.0(e). Urinary adrenaline and bone markers .

There was a significant correlation between urinary adrenaline and calcium in M>40 (r^2 = 0.563, P<0.005) and trends in F>40 (r^2 = 0.279) and in P (r^2 = 0.238) but not in the other groups. Correlation between adrenaline and magnesium in P was (r^2 = 0.392, P<0.05) and between adrenaline and phosphate in F>40 (r^2 = 0.530, P<0.01), M20-40 (r^2 = 0.568, P<0.005, **fig.3.12**), M>40 (r^2 = 0.468, P<0.025) and in the main female group (r^2 = 0.240, P<0.025). with trends in F20-40 (r^2 = 0.290).

In addition, significant correlation between urinary adrenaline and hydroxyproline in M20-40 (r^2 = 0.380, P<0.05) was obtained. The other results were not statistically significant.

Fig.3.12. shows the relationship between urinary adrenaline and phosphate in M20-40 (n = 21, r^2 = 0.568).



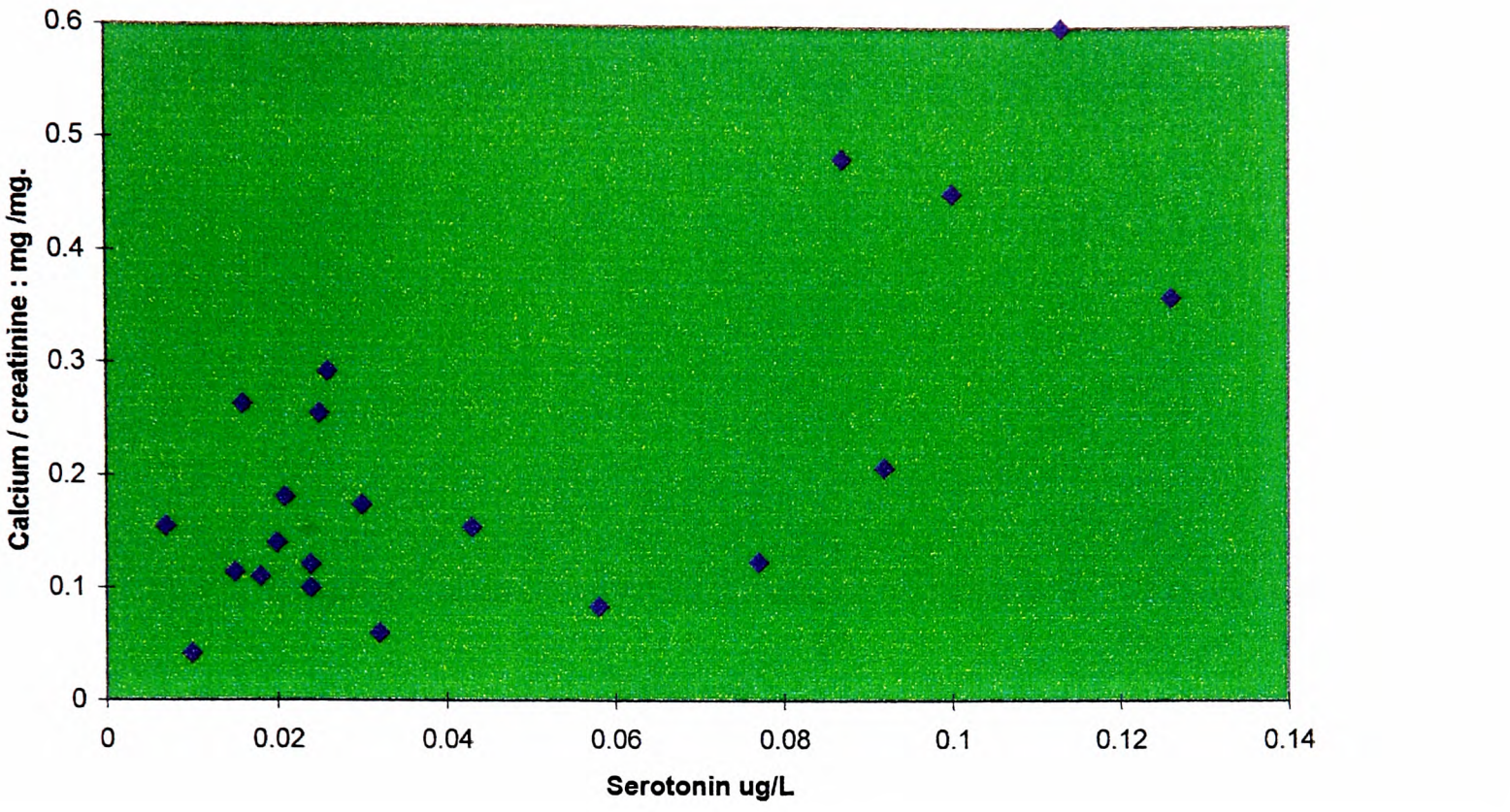
3.2.0(f). Urinary dopamine and bone markers.

No significant correlation was obtained for dopamine and calcium in any group, but positive trends were obtained in M>40 (r^2 = 0.328), F<20(r^2 = 0.218), M<20 (r^2 = 0.25), M20-40 (r^2 = 0.2) and P (r^2 = 0.294). Similarly, there was no significant correlation between dopamine and magnesium or hydroxyproline but there was significant correlation between urinary dopamine and phosphate in M>40 (r^2 = 0.405, P<0.05) with trends in M<20 (r^2 = 0.345) and during pregnancy (r^2 = 0.267).

3.2.0(g). Urinary serotonin and bone markers.

Urinary serotonin was positively correlated with calcium in M20-40 (r^2 = 0.4878, P<0.025, **fig. 3.13**) with a positive trends in F>40 (r^2 = 0.300) and F20-40 (r^2 = 0.336) and between serotonin and magnesium in M20-40 (r^2 = 0.232) and P (r^2 = 0.205).

Fig.3.13. shows the relationship between urinary serotonin and calcium in M20-40 (n= 21, r^2= 0.4878).

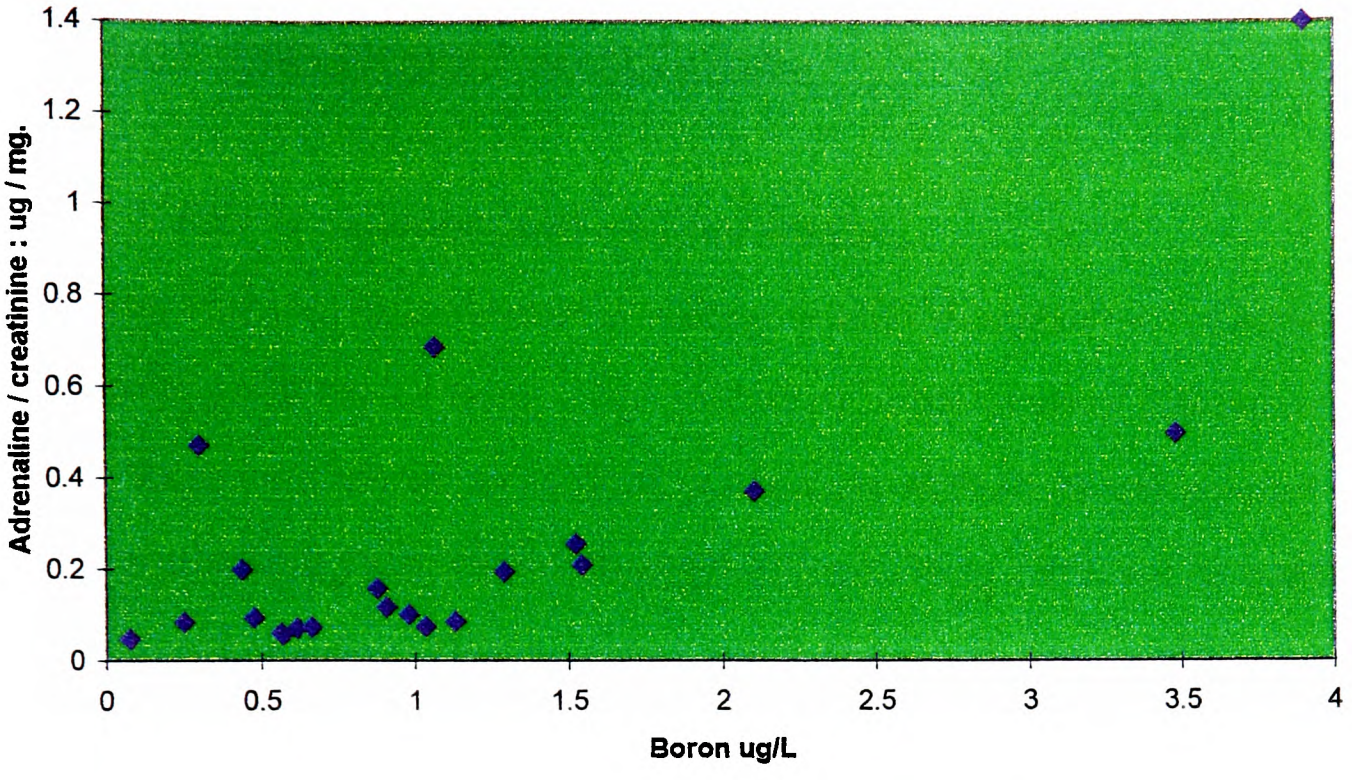


There was, also, significant correlation between serotonin and phosphate in M20-40 (r^2 = 0.419, P<0.05) with trends in M<20 (r^2 = 0.253) only and no significant correlation between serotonin and hydroxyproline in any of the group.

3.2.0(h). Urinary boron and stress hormones.

There were significant correlations between urinary boron and cortisol in M20-40 (r^2 = 0.5458, P<0.005) and the main male group (r^2 = 0.344, P<0.005). However, no significant correlation between boron and noradrenaline was obtained other than positive trends (r^2 = 0.276) and (r^2 = 0.274) in M20-40 and M>40 respectively. But correlation with adrenaline, in M20-40 was significant (r^2 = 0.565, P<0.005, **fig.3.14**) and with dopamine in F>40 (r^2 = 0.405, P<0.05), serotonin in P and M20-40 with r^2 = 0.375, P<0.05 and r^2 = 0.574, P<0.005 respectively. The other results were not statistically significant.

Fig.3.14. shows the relationship between urinary boron and adrenaline in M20-40 (n = 21, r^2 = 0.565).



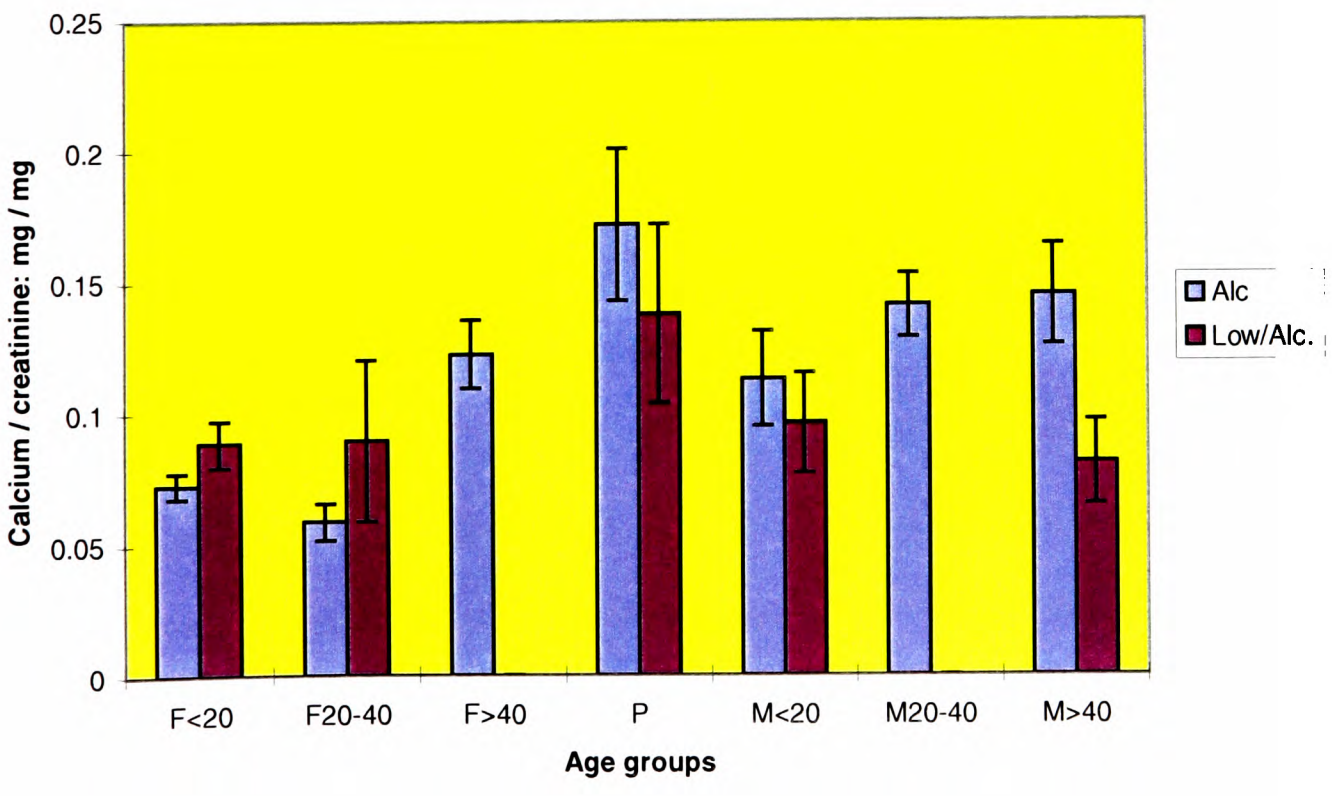
3.3.0. Relationship between urinary bone markers, boron and stress hormones to specific life-styles, nutrients, reproductive factors and bone diseases (osteoporosis and osteoarthritis).

3.3.0.(a). Urinary calcium and alcohol intake .

There were no non-alcohol drinkers in M20-40 and F>40. Subjects drinking over 1500 ml of beer, over 750 ml of wine or over 150 ml of spirits per week were considered as alcohol consumers as their blood alcohol level would be equivalent to or above 80 mg / 100 ml, 35µg / 100 ml of breath or 107µg / 100 ml of urine. This was the level adopted in this study as it was also the level accepted by the police authorities as the legal limit for safe driving (English & Card 1994). Those drinking less alcohol, (levels below 80 mg / 100 ml of blood), was considered as low alcohol / occasional drinkers.

In general, urinary calcium levels increased with age in men and women

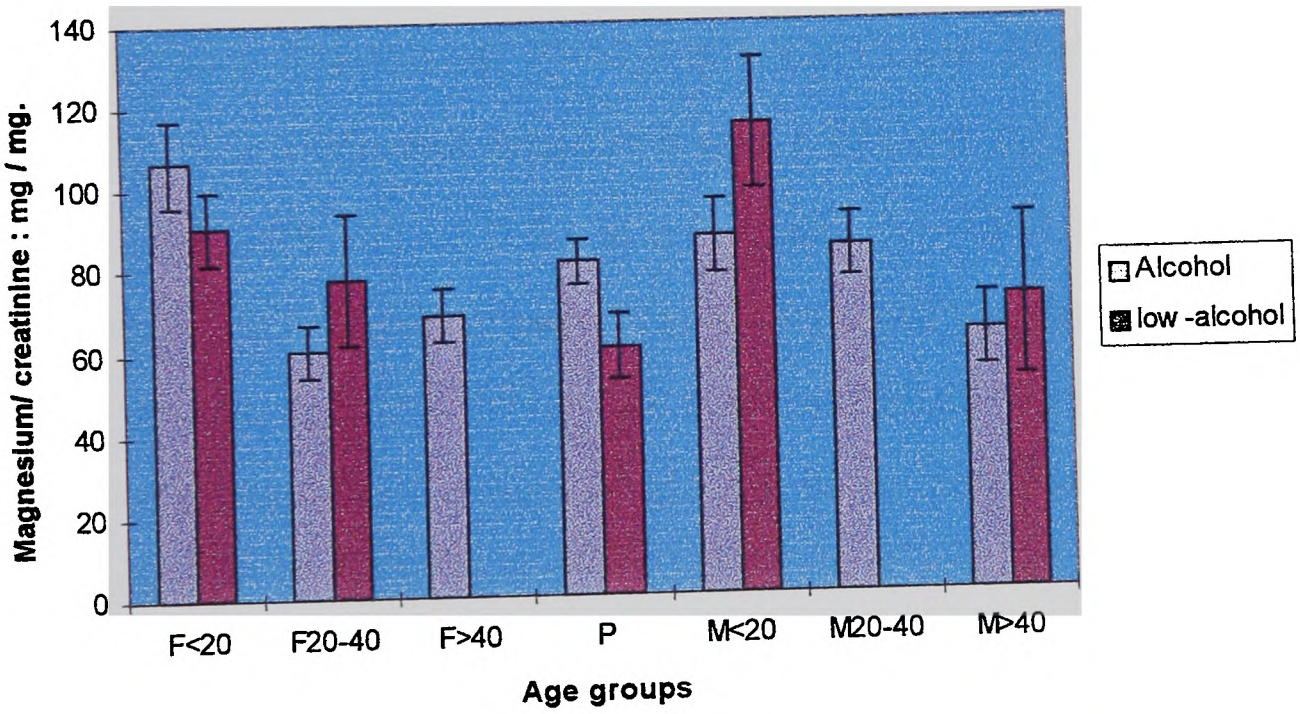
Fig.3.15. Shows urinary calcium levels in alcohol drinkers and low-drinkers.



including during pregnancy with alcohol intake but not in the low-alcohol drinkers (**fig. 3.15**). Male subjects who consumed alcohol excreted more calcium than their females counterparts, however, the levels were highest during pregnancy. Calcium levels in F20-40 who drank alcohol were significantly lower ($P<0.05$) than those in F20-40 and the pregnant group who drank less. In contrast, the levels of the urinary calcium in M>40 alcohol drinkers were significantly higher ($P<0.05$) than those in the lower intake group.

3.3.0(b). Urinary magnesium and alcohol intake.

Fig.3.16 showing urinary magnesium levels in the alcohol / low-alcohol drinkers.



In F20-40 this was significantly ($P<0.05$) lower than in M20-40 and in pregnant women alcohol drinkers **(Fig. 3.16).** Urinary magnesium levels decreased with age in all alcohol drinkers in both genders but not significantly ($P>0.05$).

3.3.0(c). Urinary phosphate and alcohol intake.

The levels of phospate in F<20 alcohol drinkers were 0.776 ± 0.059; M<20 0.897 ± 0.071; F20-40 0.826 ± 0.088; M20-40 0.927 ± 0.079; P 0.745 ± 0.046; F>40 0.774 ± 0.063 and in M>40 0.870 ± 0.100 mg / mg creatinine and the corresponding levels in low-alcohol drinkers were F<20 0.926 ± 0.083; M<20 1.158 ± 0.089; F20-40 0.925 ± 0.091; P 0.891 ± 0.170; and M>40 1.046 ± 0.059 mg / mg creatinine. These were not significant (P>0.05).

3.3.0(d) Urinary boron and alcohol intake.

The mean level of boron in F<20 alcohol drinkers was 0.933 ± 0.106; M<20 0.702 ± 0.109; F20-40 0.898 ± 0.21; M20-40 0.868 ± 0.12; P 0.731 ± 0.14; F>40 0.881 ± 0.13 and M>40 0.692 ± 0.11 while in F<20 low-alcohol drinkers it was 1.225 ± 0.163; M<20 1.0074 ± 0.14; F20-40 0.94 ± 2.0; P 0.654 ± 0.14 and M>40 0.984 ± 0.17 μg / mg creatinine. Overall, urinary boron decreased with age in both genders in all alcohol drinkers. While the male and female low-drinkers excreted more boron than the alcohol groups, these were not significant (P>0.05). However, in F<20 low-alcohol drinkers it was significantly (P<0.05) higher than in the low-alcohol drinking M<20.

3.3.0(e). Urinary hydroxyproline and alcohol intake.

The mean urinary hydroxyproline level in F<20 alcohol drinkers was 4.996 ± 0.589; M<20 4.89 ± 0.99; F20-40 6.9 ± 1.5; M20-40 5.82 ± 1.3; P 4.28 ± 0.65; F>40 4.37 ± 0.52 and M>40 4.27 ± 0.65 μg / mg creatinine while in F<20 low-alcohol drinkers it was 5.41 ± 1.11; M<20 5.45 ± 0.65; F20-40 4.4 ± 0.88; P 5.91 ± 1.3 and M>40 5.32 ± 1.0 μg / mg creatinine. It was increased in F 20-40 and M 20-40

subjects who consumed alcohol compared to the low-drinkers in their respective groups and in alcohol drinkers compared to the low-alcohol drinkers in both genders and in all age groups except in F20-40 but these changes were not significant (P>0.05).

3.3.0(f). Urinary cortisol and alcohol intake.

Urinary cortisol levels in F<20 alcohol drinkers were 0.78 ± 0.13; M<20 0.82 ± 0.17; F20-40 0.99 ± 0.19; M20-40 1.07 ± 0.28; P 1.78 ± 0.76; F>40 1.87 ± 0.49 and M>40 was 1.70 ± 0.43 while in F<20 non-alcohol drinkers, they were 0.93 ± 0.21; M<20 1.08 ± 0.20; F20-40 0.79 ± 0.25; P 1.65 ± 0.49 and M>40 1.21 ± 0.68 μg / mg creatinine. Overall, these were higher in male than in female, but not in pregnant subjects who did not consumed alcohol. However, with age, these levels were significantly increased (P<0.05) in F>40 compared to those in F<20 alcohol drinkers.

3.3.0(g). Urinary noradrenaline and alcohol intake.

These levels in F<20 alcohol drinkers were 1.74 ± 0.51; M<20 1.74 ± 0.26; F20-40 1.14 ± 0.23; M20-40 1.65 ± 0.50; P 1.13 ± 0.07; F>40 1.14 ± 0.23, M>40 1.28 ± 0.22 and in low-alcohol drinkers, these were F<20 1.30 ± 0.27; M<20 1.59 ± 0.34; F20-40 1.52 ± 0.28; P 1.10 ± 0.21 and M>40 5.83 ± 3.00 μg / mg creatinine. They were decreased with age in subjects who consumed alcohol but increased in those with a lower intake. However, these changes were not statistically significant (P>0.05).

3.3.0(h). Urinary adrenaline and alcohol intake.

Urinary adrenaline levels in F<20 alcohol drinkers were 1.12 ±

0.23; M<20 2.86 ± 0.62; F20-40 1.26 ± 0.45; M20-40 1.71 ± 0.033; P 1.14 ± 0.15; F>40 2.60 ± 0.73 and M>40 1.90 ± 0.54 while in F<20 low-drinkers they were 1.80 ± 0.41; M<20 1.76 ± 0.59; F20-40 0.91 ± 0.12; P 2.06 ± 0.59 and M>40 4.98 ± 2.10 μg / mg creatinine. These levels were increased in females but decreased in male alcohol drinkers, In contrast, they were decreased in female and increased in male subjects who consumed less alcohol. There was a clear gender and age difference in adrenaline turnover; M<20 alcohol drinkers had significantly higher (P<0.05) adrenaline level than F<20 but in F>40, it was higher than in M>40 while in the low-alcohol drinkers, it was fairly similar in F<20 and M<20. During pregnancy, alcohol drinkers had a lower adrenaline level compared to the low-alcohol drinkers.

3.3.0(i). Urinary dopamine and alcohol intake.

The mean urinary dopamine levels in F<20 alcohol drinkers were 1.999 ± 0.281; M<20 1.980 ± 0.330; F20-40 1.133 ± 0.190; M20-40 1.232 ± 0.160; P 1.353 ± 0.250; F>40 1.419 ± 0.200 ; M>40 0.980 ± 0.079 and in F<20 low-alcohol drinkers they were 2.259 ± 0.480; M<20 2.380 ± 0.780; F20-40 1.388 ± 0.230; P 1.566 ± 0.300 and M>40 1.210 ± 0.200 μg / mg creatinine. The levels in all alcohol drinkers decreased with age for both genders. In all age groups, these were higher in the low-alcohol drinkers than drinkers but these changes were not significant except between F>40 and M>40 (P <0.05) alcohol drinkers.

3.3.0(j). Urinary serotonin and alcohol intake.

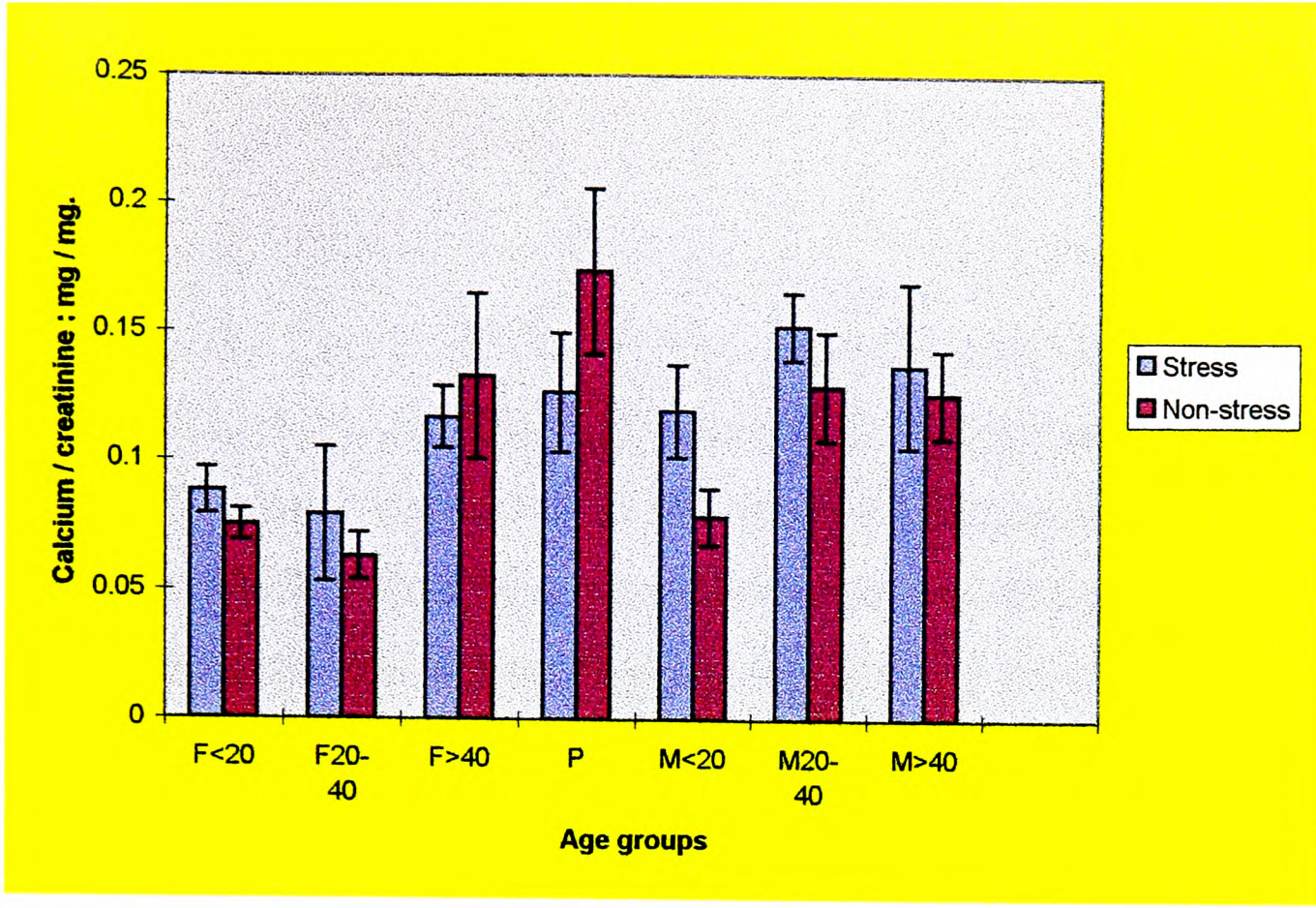
The mean serotonin levels in F<20 alcohol drinkers were 0.080 ± 0.018; M<20 0.088 ± 0.034; F20-40 0.058 ± 0.014; M20-40 0.037 ± 0.006; P 0.045 ± 0.012; F>40 0.037 ± 0.006, M>40 0.055 ± 0.013

ug / mg creatinine while in F<20 low-alcohol drinkers they were 0.058 ± 0.012; M<20 0.112 ± 0.041; F20-40 0.039 ± 0.008; P 0.036 ± 0.005 and M>40 0.080 ± 0.036 ug / mg creatinine. These levels were higher in female than in male alcohol drinkers and decreased in both genders with age. However, these differences were not statistically significant (P>0.05).

3.4.0(a). Urinary calcium and stress levels.

Subjects were asked to assess their level of stress using the questionnaire (appendix 1). In the stressed groups, the levels in F<20 (n=19) were 0.088 ± 0.009; M<20 (n=16) 0.120 ± 0.018; F20-40 (n=10) 0.079 ± 0.026; M20-40 (n=10) 0.153 ± 0.013; P (n=8) 0.127 ± 0.023; F>40 (n=14) 0.117 ± 0.012 and M>40 (n=8) 0.138 ± 0.032 while in the non-stressed F<20 (n=22) they were 0.075 ± 0.006; M<20 (n=8) 0.127 ± 0.017; F20-40 (n=11) 0.063 ± 0.086; M20-40 (n=11) 0.130 ± 0.021; P (n=13) 0.174 ± 0.032; F>40 (n=8) 0.133 ± 0.032 and M>40 (n=14) 0.127 ± 0.017 mg / mg creatinine (**fig.3.17**). In both groups, calcium levels in F20-40 were significantly lower (P<0.05) than in M20-40 and in pregnant women, while in male subjects, these levels were higher in the stress than in the non-stress subjects. However, in the females groups, this was only observed in the F<20 and F20-40.

Fig.3.17. Shows urinary calcium levels in stress/non-stress groups



3.4.0(b). Urinary magnesium and stress levels.

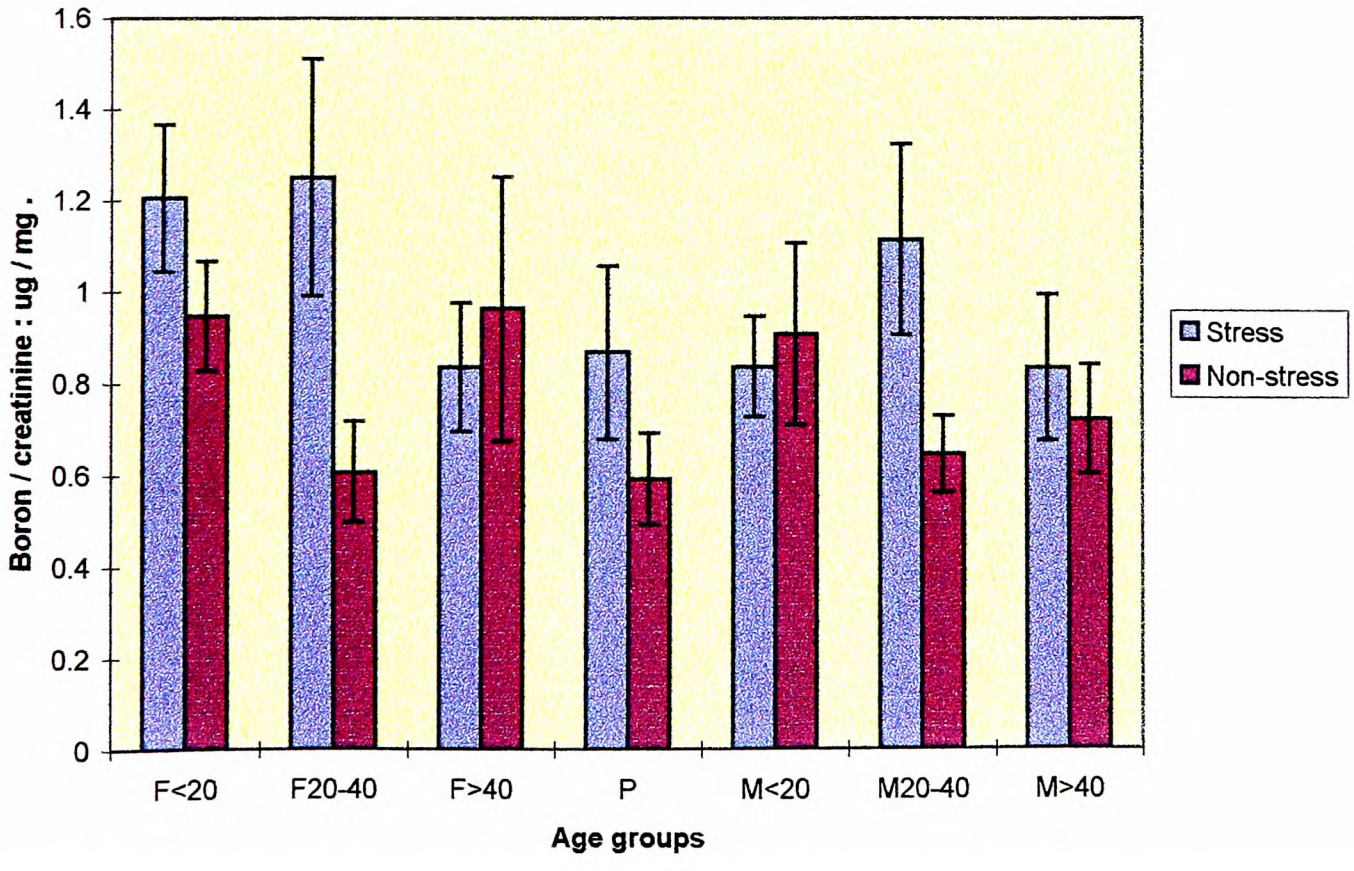
The mean urinary magnesium levels of the stressed F<20 were 0.099 ± 0.011; M<20 0.096 ± 0.011; F20-40 0.073 ± 0.014; M20-40 0.085 ± 0.011; P 0.077 ± 0.009; F>40 0.061 ± 0.007; M>40 0.071 ± 0.017 and in the non-stresed F<20 they were 0.098 ± 0.009; M<20 0.105 ± 0.014; F20-40 0.062 ± 0.007; M20-40 0.084 ± 0.013 ; P 0.068 ± 0.007; F>40 0.083 ± 0.012 and M>40 0.062 ± 0.009 mg / mg creatinine. In general, stressed subjects excreted more magnesium, except in F>40 and pregnant subjects but these changes were not significant (P>0.05).

3.4.0(c). Urinary phosphate and stress levels.

The mean phosphate levels in stressed F<20 were 0.811 ± 0.077; M<20 1.080 ± 0.190; F20-40 0.936 ± 0.094; M20-40 0.965 ± 0.100; P 1.024 ± 0.180 ; F>40 0.787 ± 0.081; M>40 1.080 ± 0.190 mg / mg creatinine and in non-stressed F<20 they were 0.881 ± 0.072 ; M<20 1.098 ± 0.110; F20-40 0.799 ± 0.087 ; M20-40 0.892 ± 0.120 ; P 0.686 ± 0.047; F>40 0.753 ± 0.110 and M>40 0.813 ± 0.061 mg / mg creatinine. The levels decreased in both genders with age in all subjects who were stressed while in non-stressed subjects, they decreased in females but increased in males. Pregnant women who were stressed excreted more phosphate than those not stressed but none of these results were significant (P>0.05).

3.4.0(d). Urinary boron and stress levels.

Fig.3.18. shows urinary boron levels in stress / non-stress groups

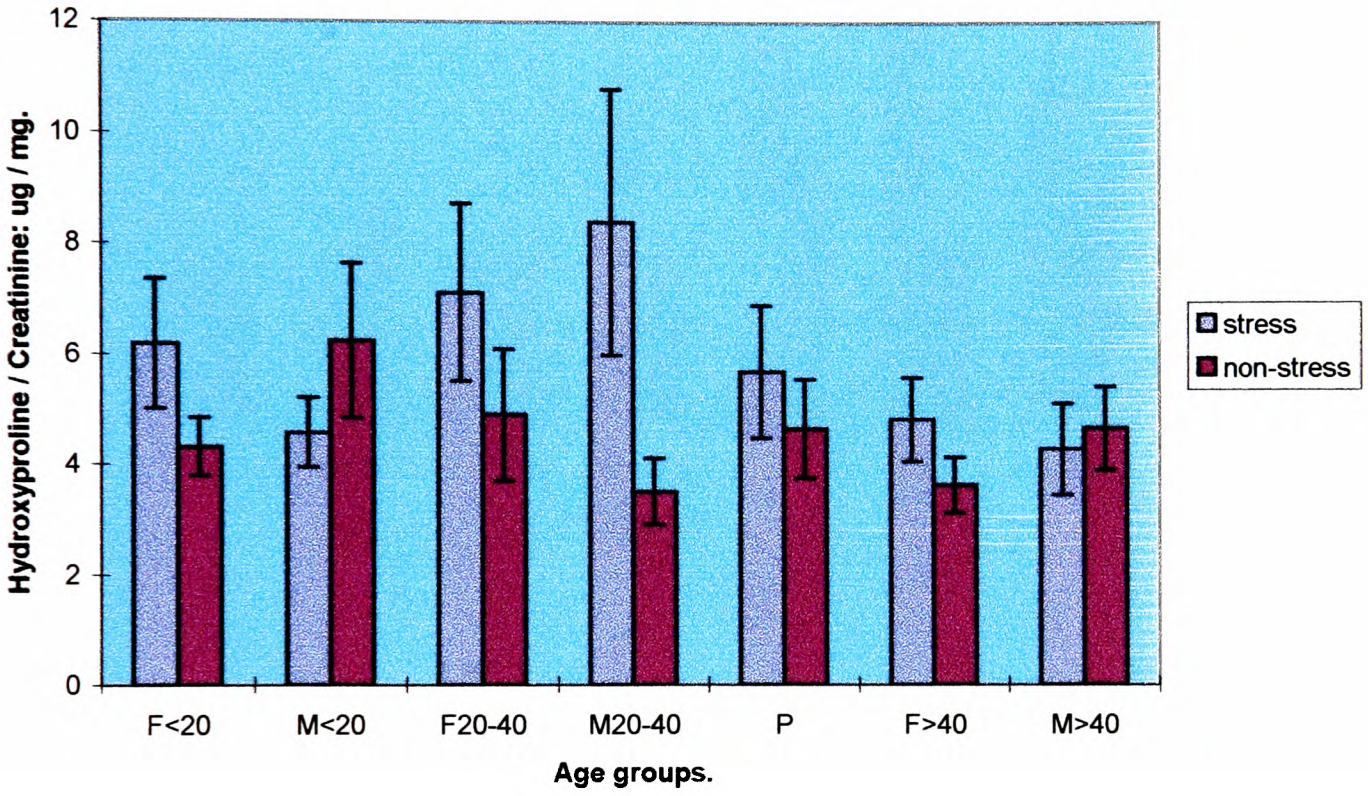


Overall, urinary boron levels were higher in subjects who were stressed compared to those who were not except those in F>40 and

M<20, but, the only changes which were significant (P<0.05) were in stressed F20-40 and M20-40 compared to their non-stressed counterparts in the respective age groups (**fig.3.18**).

3.4.0(e). Urinary hydroxyproline and stress levels.

Fig. 3.19. shows urinary hydroxyproline levels in stress / non-stress groups.



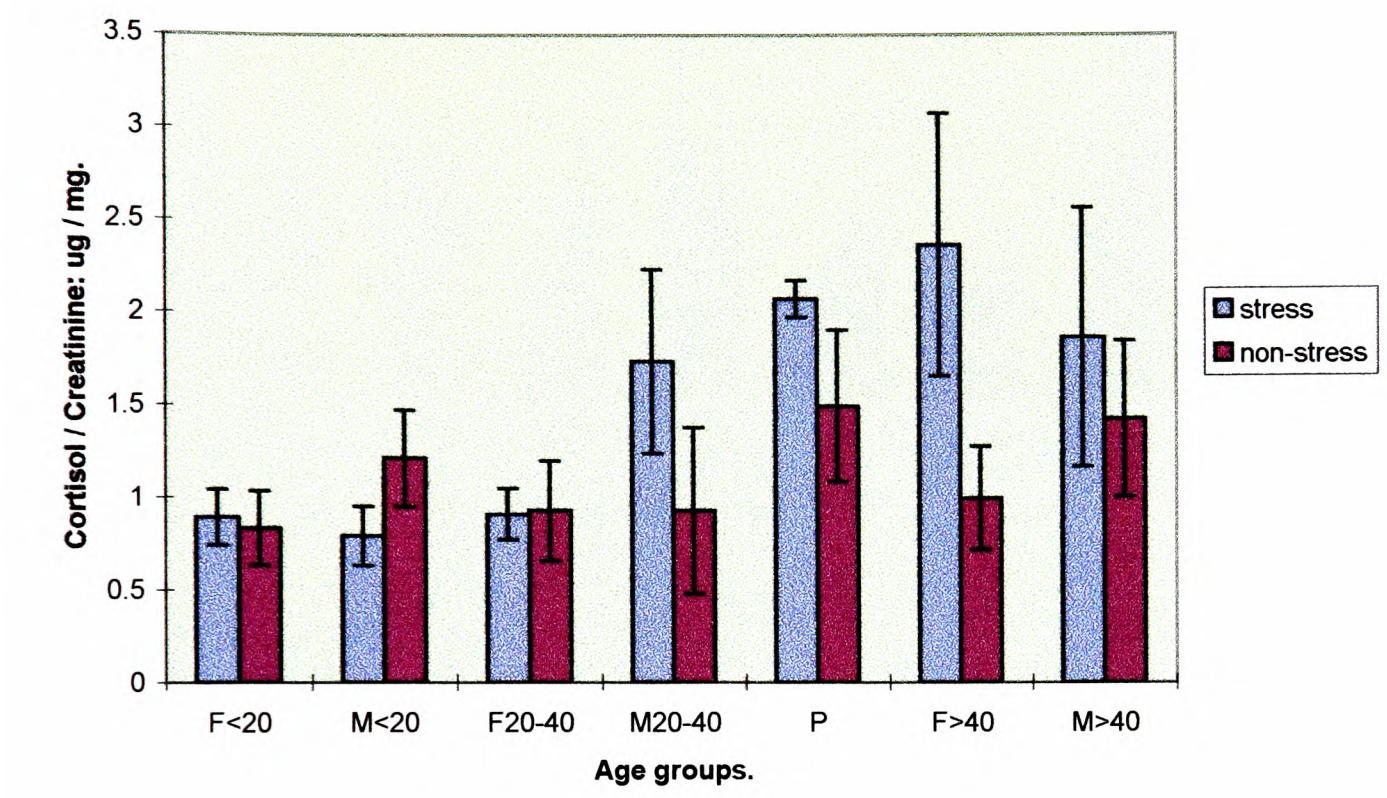
In general, hydroxyproline excretion was higher in the stressed than in the non-stressed subjects except in M<20 and M>40 but these differences were not statistically significant except in M20-40 (P<0.05, **fig. 3.19**).

3.4.0(f). Urinary cortisol and stress levels.

Urinary cortisol levels increased, with age, in male and female subjects who were stressed compared to their respective counterparts but only the levels between the non-stressed pregnant women and the non-

stressed M20-40 and between stressed and non-stressed F>40 were significantly higher (P<0.05, **fig. 3.20**).

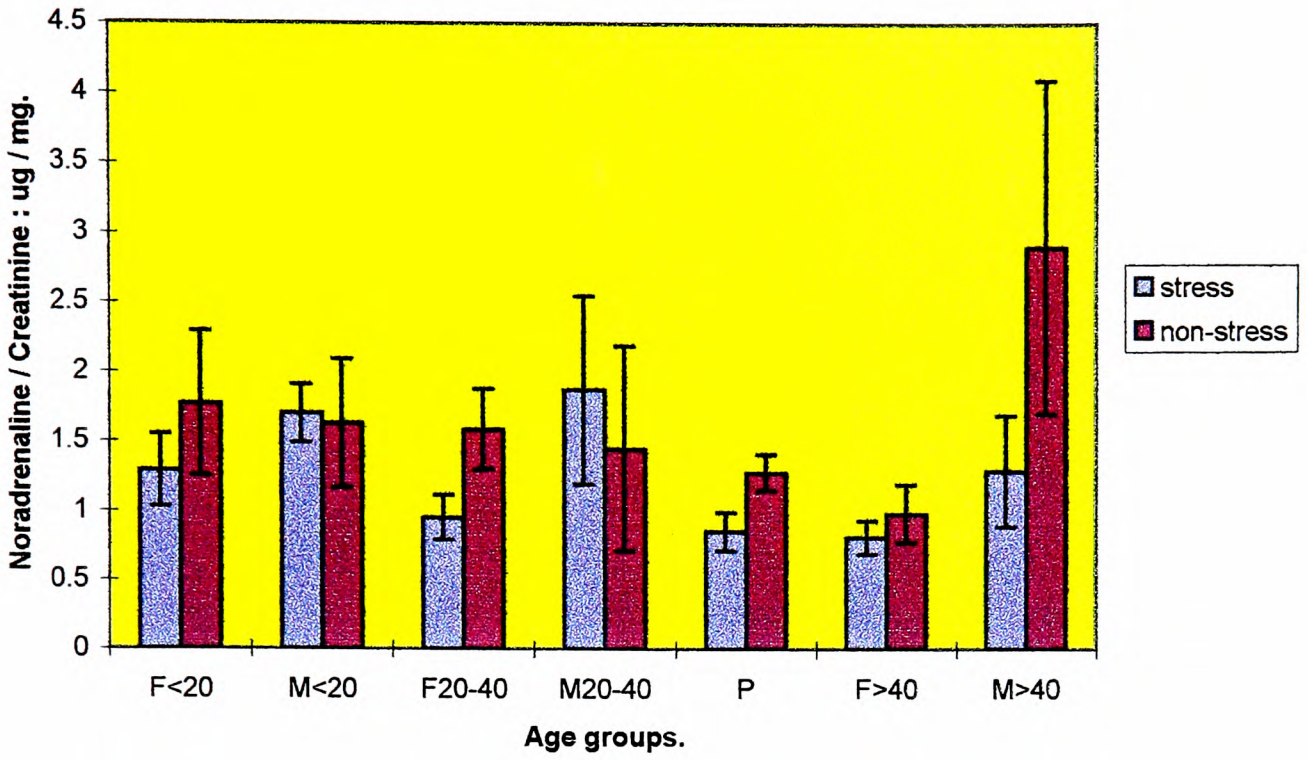
Fig.3.20. shows urinary cortisol levels in stress / non-stress groups.



3.4.0(g). Urinary noradrenaline and stress levels.

These levels increased in non-stressed females in all age groups, M>40 and in the pregnant group compared to their respective stressed counterparts but only the levels between the pregnant women were significantly higher (P<0.05) (**fig.3.21**).

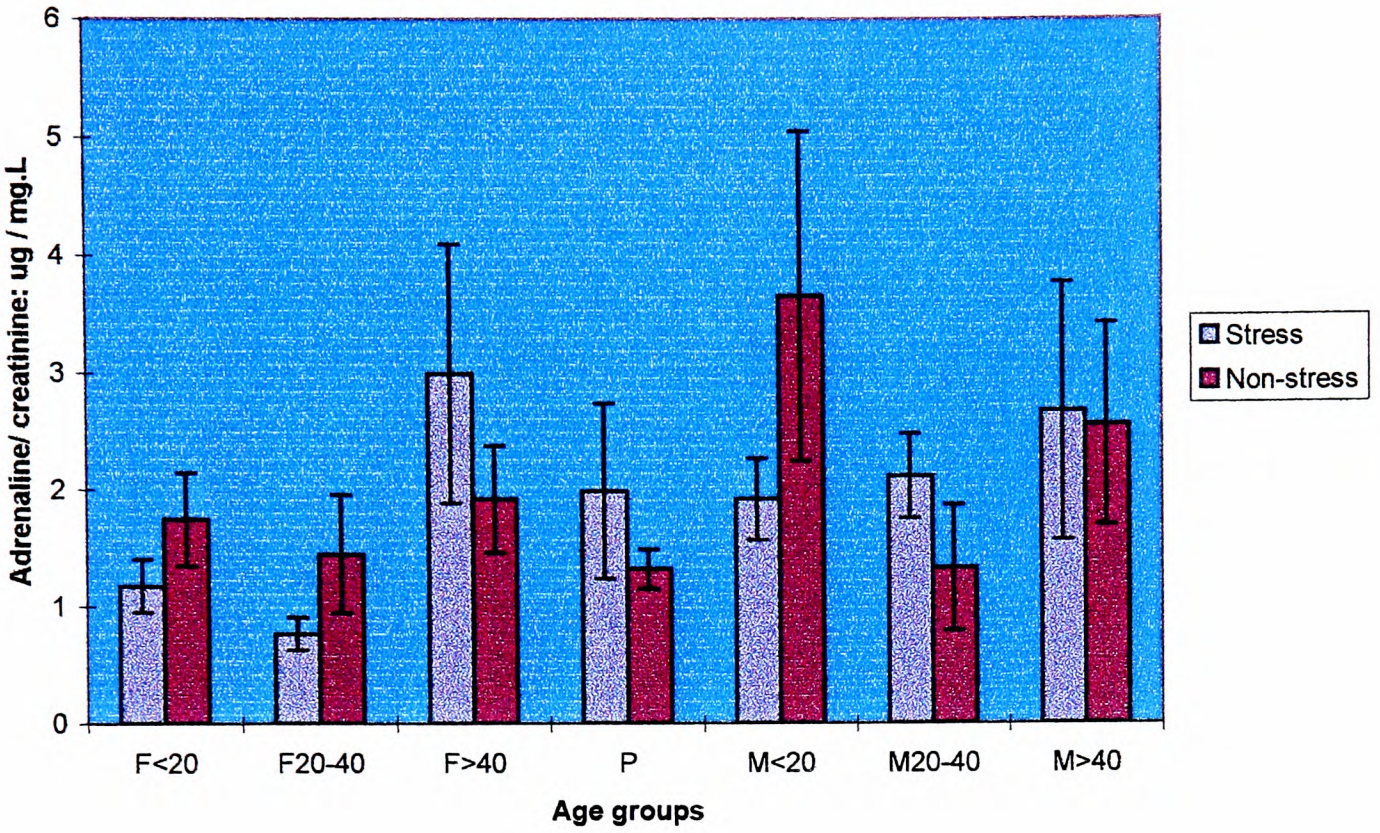
Fig.3.21. shows urinary noradrenaline levels in stress / non-stress groups.



3.4.0(h). Urinary adrenaline and stress levels.

In general urinary adrenaline levels increased with age in the stressed female and male subjects while the levels decreased in male and increased slightly in the female subjects who were not stressed **(fig . 3.22).** In the stressed M20-40, they were significantly higher ($P<0.05$) than those in the F20-40 subjects. Although the levels of adrenaline in the stressed M<20 were higher than those in the female group; these were not statistically significant ($P = 0.083$).

Fig.3.22. shows urinary adrenaline in stress / non-stress groups



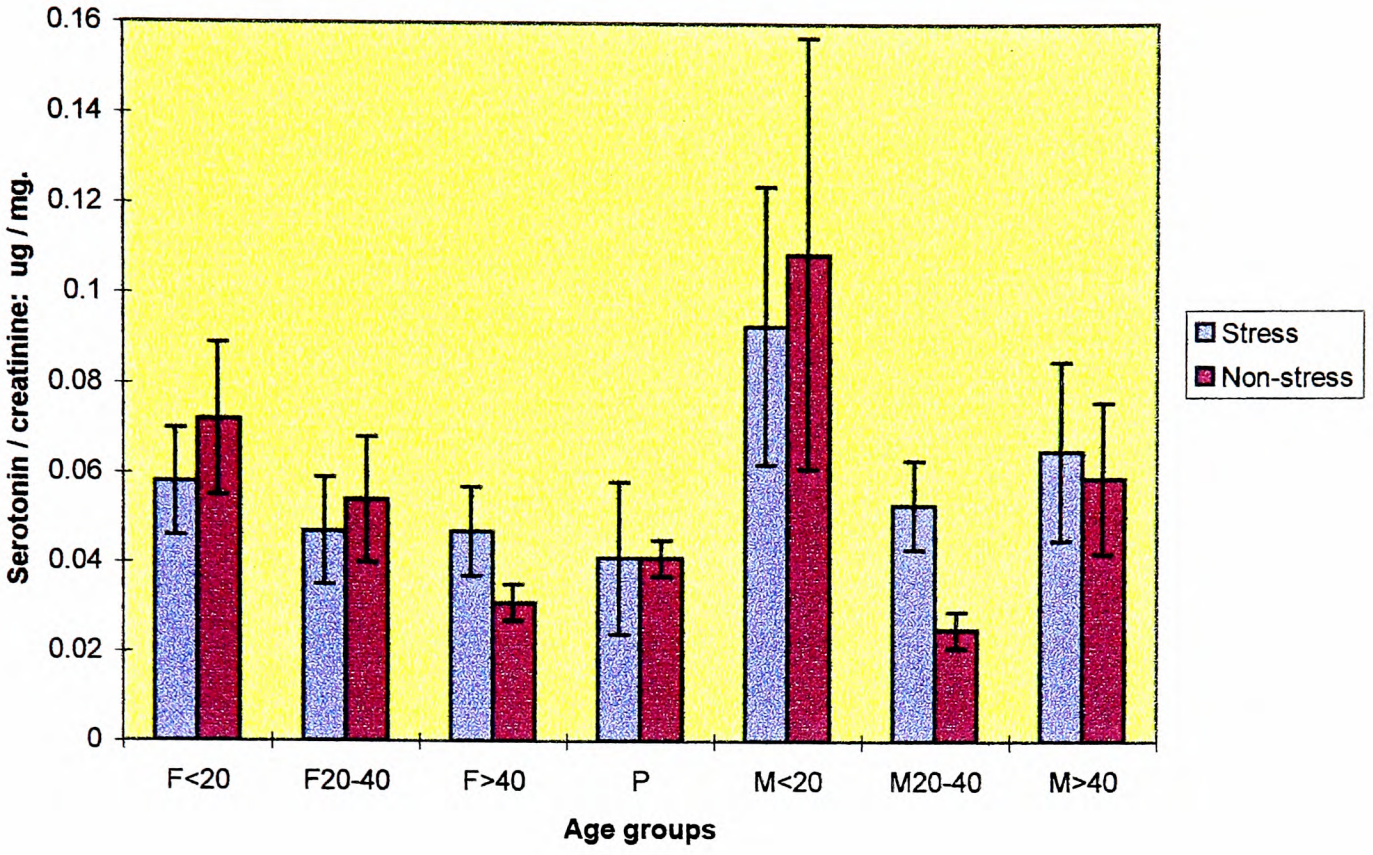
3.4.0(i). Urinary dopamine and stress levels.

The mean levels of dopamine in the stressed F<20 were 2.250 ± 0.390 ; M<20 2.490 ± 0.680 ; F20-40 1.406 ± 0.200 ; M20-40 1.217 ± 0.270 ; P 1.203 ± 0.340 ; F>40 1.397 ± 0.256; M>40 0.894 ± 0.120 and in the non-stressed they were F<20 1.950 ± 0.400 ; M<20 1.680 ± 0.310; F20-40 1.070 ± 0.210; M20-40 1.245 ± 0.210; P 1.610 ± 0.230 ; F>40 1.457 ± 0.350 and M>40 1.113 ± 0.097 µg / mg creatinine. While the levels in all the stressed groups were higher than those in the non-stressed groups, they decreased with age in both genders, but not significantly (P>0.05).

3.4.0(j). Urinary serotonin and stress levels.

Urinary serotonin levels in both groups decreased with age in both genders **(fig.3.23).**

Fig.3.23. shows urinary serotonin levels in stress/non-stress groups



The level in non-stressed M20-40 was significantly ($P<0.05$) lower than that in their stressed counterparts and in the non-stressed pregnant group ($P<0.05$).

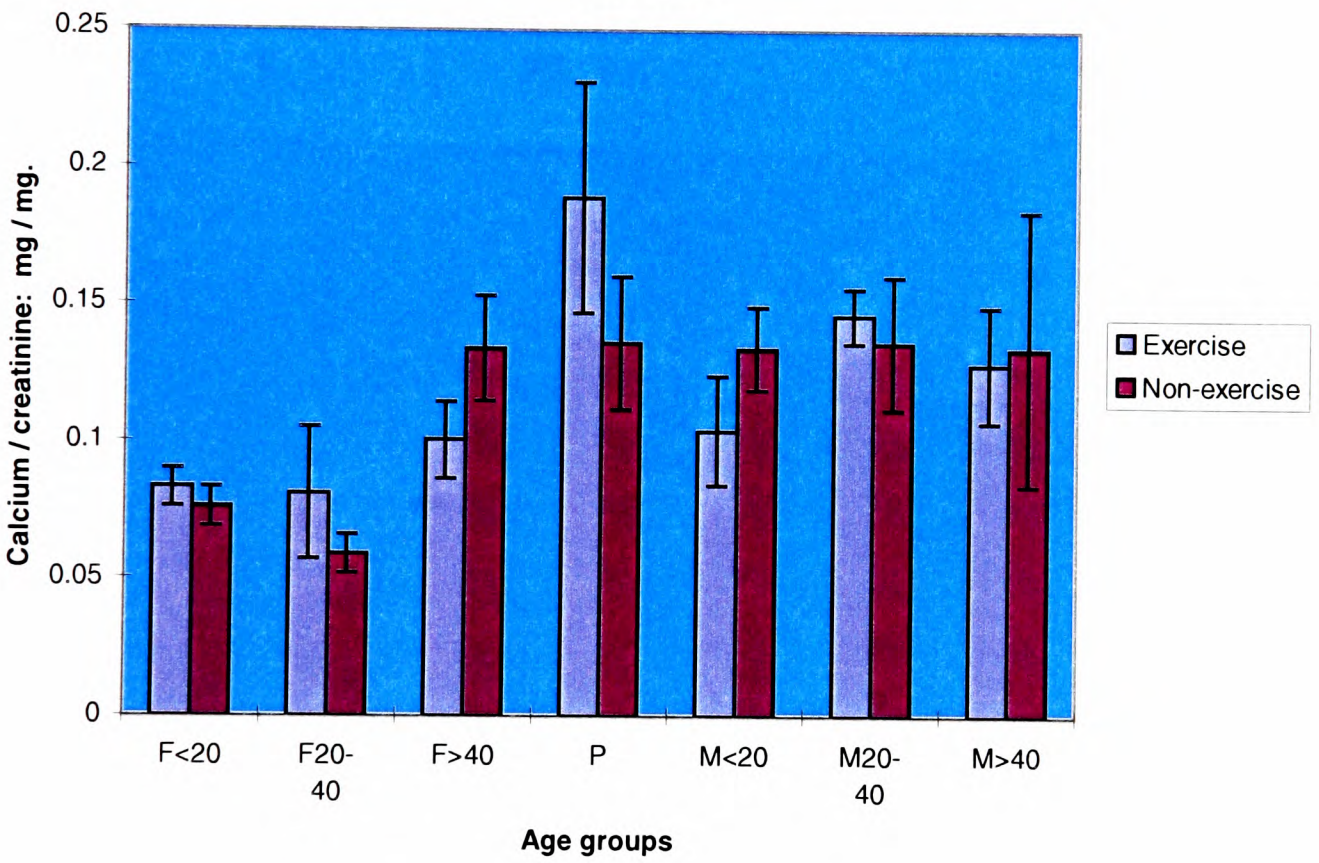
3.5.0(a). Urinary calcium and exercise.

Most of the subjects in this study exercised and therefore two groups were identified. Subjects who took frequent non-weight bearing exercises like swimming, walking the dog, cycling less than 30 minutes and less than 3 times a week were the non-weight bearing exercise group (non-exercise group) while those who played football, rugby, hockey, weight lifting and brisk walking for over 30 minutes, more than 3 times a week for the last 6 months were considered as the weight bearing group (exercise group). Weight bearing exercise were compared against non-weight bearing as these exercises increased bone mass (Allen, 1994).

Mean calcium levels in F<20 who exercised were 0.083 ±

0.007 (n=26); M<20 0.104 ± 0.020 (n=14); F20-40 0.081 ± 0.024 (n=11); M20-40 0.146 ± 0.010 (n=11); P 0.189 ± 0.042 (n=8); F>40 0.103 ± 0.014(n=8); M>40 0.128 ± 0.021 (n=11) and in non-exercising F<20 they were 0.076 ± 0.007 (n=15); M<20 0.134 ± 0.025 (n=10); F20-40 0.059 ± 0.007 (n=10); M20-40 0.136 ± 0.024 (n=10); P 0.136 ± 0.024 (n=13); F>40 0.134 ± 0.014 (n=14) and M>40 0.134 ± 0.025 (n=11) mg / mg creatinine. (**fig.3.24.**).

Fig.3.24. shows urinary levels of calcium in exercise / non-exercise



The levels increased with age in both genders and were higher in male subjects than in non-pregnant women who exercised. In F20-40 they were significantly lower (P<0.05) than those in M20-40 and in the pregnant group. In M<20 it was higher than in F<20 (P= 0.06).

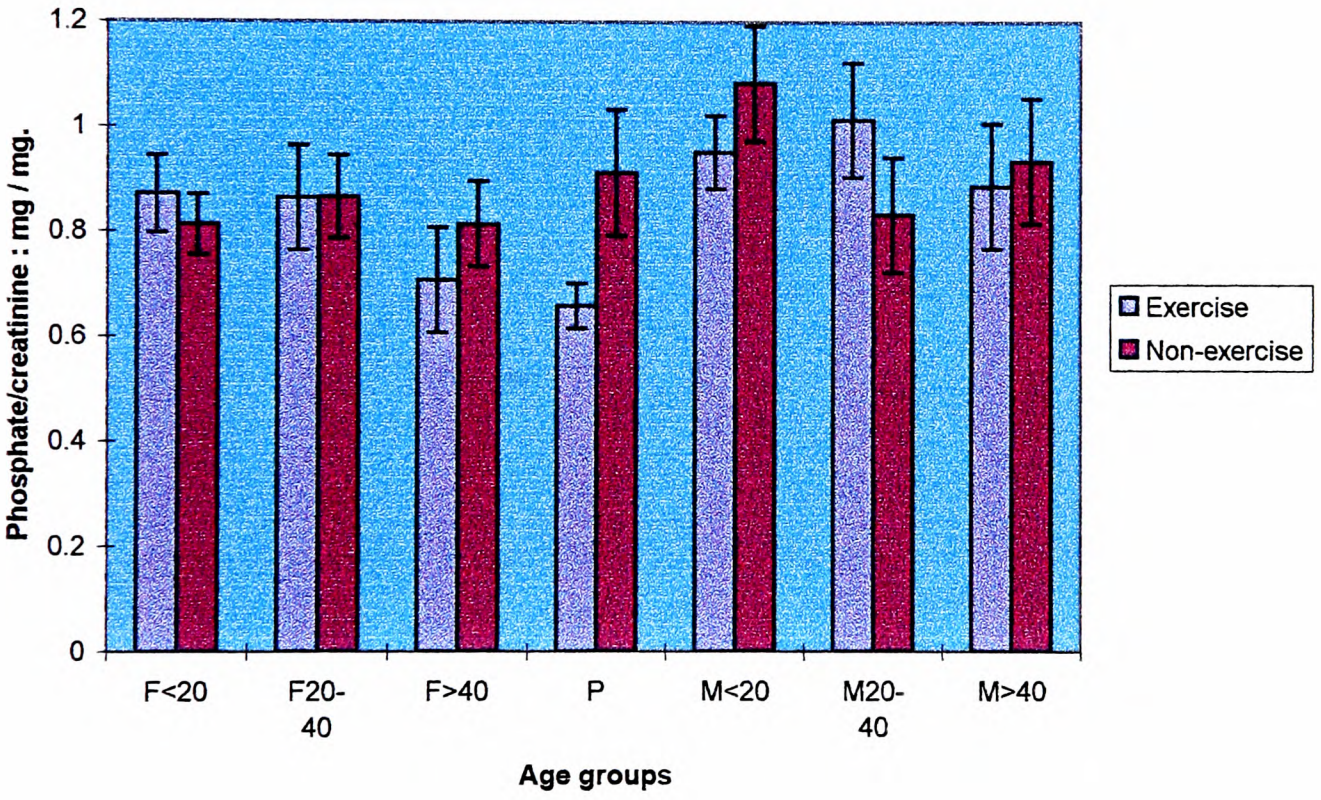
3.5.0(b). Urinary magnesium and exercise.

The mean levels of magnesium in F<20 who exercised were 0.094

± 0.009; M<20 0.092 ± 0.012; F20-40 ± 0.070 ± 0.014; M20-40 0.092 ± 0.012; P 0.075 ± 0.006; F>40 0.078 ± 0.013; M>40 0.068 ± 0.008 and in F<20 who did not exercise they were 0.107 ± 0.011; M<20 0.109 ± 0.013; F20-40 0.064 ± 0.004; M20-40 0.081 ± 0.014; P 0.069 ± 0.008; F>40 0.064 ± 0.007 and M>40 0.063 ± 0.014 mg / mg creatinine. Overall, the levels in both groups decreased with age in both genders, but not significantly (P>0.05).

3.5.0(c). Urinary phosphate and exercise.

Fig.3.25. shows phosphate levels in exercise / non-exercise groups.

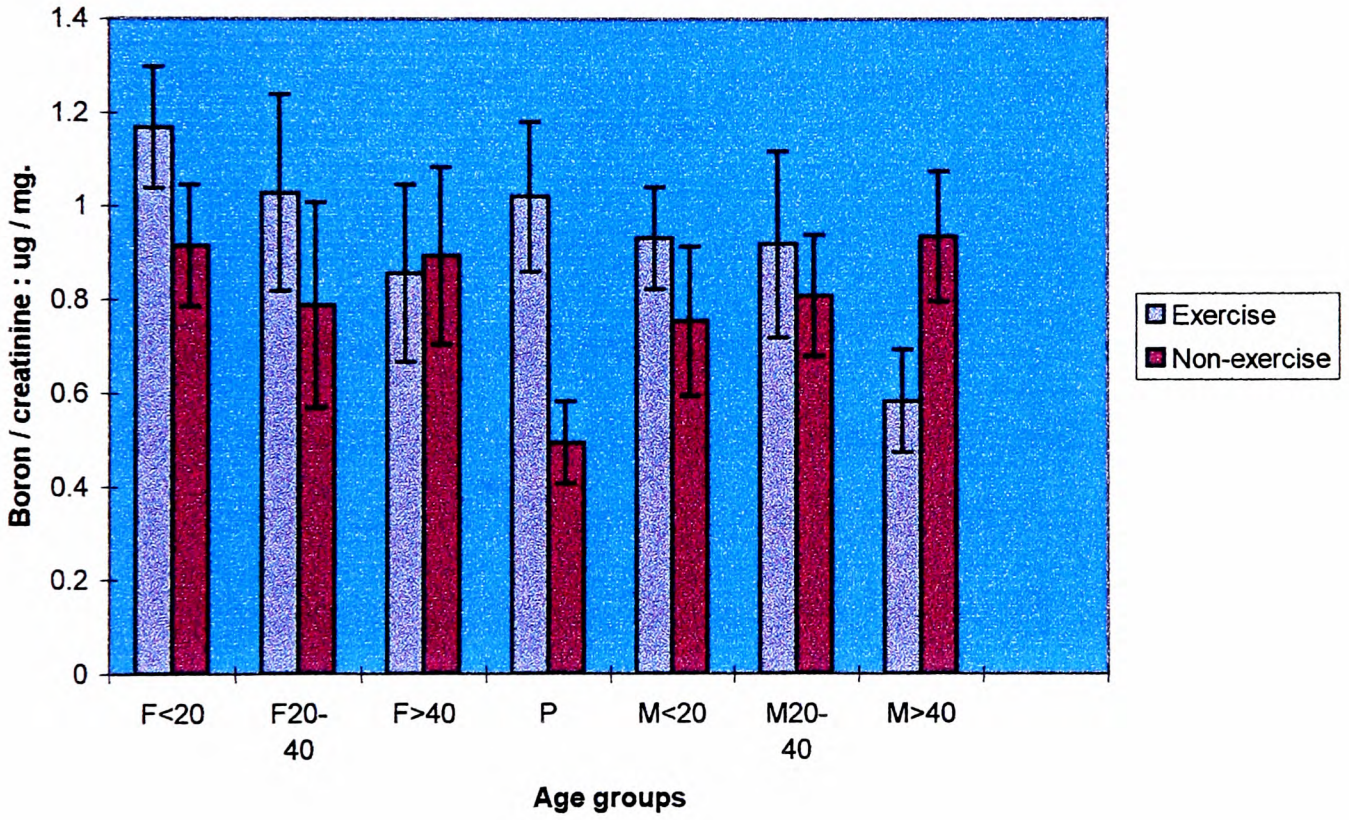


Overall, urinary phosphate levels decreased (P>0.05) with age, in both genders, in the exercising group, while in the non-exercising groups, they decreased in male but not in female subjects. In F20-40 who exercised, it was significantly higher (P<0.05) than that of the pregnant group **(Fig. 3.25).** The level in M20-40 was significantly higher (P<0.05) than that in the pregnant women who exercised

3.5.0(d). Urinary boron and exercise.

Overall, urinary boron levels decreased with age in both genders in subjects who exercised, but increased in male subjects who did not. Pregnant women who did not exercise had significantly lower ($P<0.05$) urinary boron than those who did. M>40 who were not exercising had higher ($P = 0.06$) levels of boron compared to their exercising counterparts (**fig.3.26**).

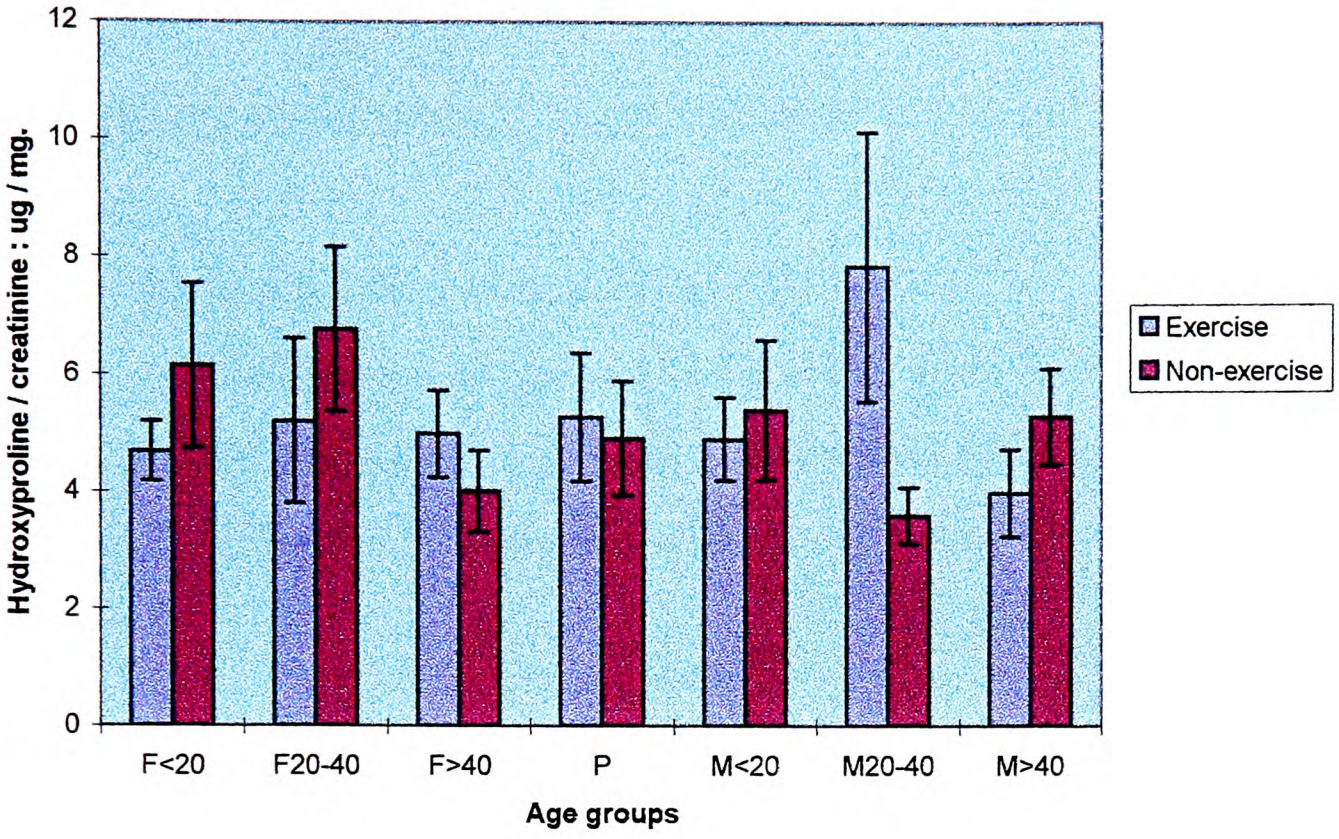
Fig.3.26. shows urinary boron levels in exercise/non-exercise groups.



3.5.0(e). Urinary hydroxyproline and exercise.

M 20-40 subjects who exercised excreted significantly ($P<0.05$) higher levels of hydroxyproline than their counterparts who did not. The other results were not significant **(fig. 3.27).**

Fig.3.27. shows urinary hydroxyproline in exercise/non-exercise groups.



3.5.0(f). Urinary cortisol and exercise.

The mean levels of cortisol in F<20 who exercised were 0.73 ± 0.10; M<20 0.99 ± 0.18; F20-40 1.01 ± 0.24; M20-40 1.16 ± 0.47; P 1.98 ± 0.10; F>40 1.39 ± 0.36; M>40 1.77 ± 0.55 and in those not exercising they were 1.07 ± 0.28; M<20 0.83 ± 0.23; F20-40 0.81 ± 0.18; M20-40 0.96 ± 0.29; P 1.56 ± 0.39; F>40 2.15 ± 0.74 and M>40 1.41 ± 0.50 μg / mg creatinine. In male subjects who exercised, they were higher than their counterparts, while in F<20 and F>40, they were actually lower, but not significantly (P>0.05).

3.5.0(g). Urinary noradrenaline and exercise.

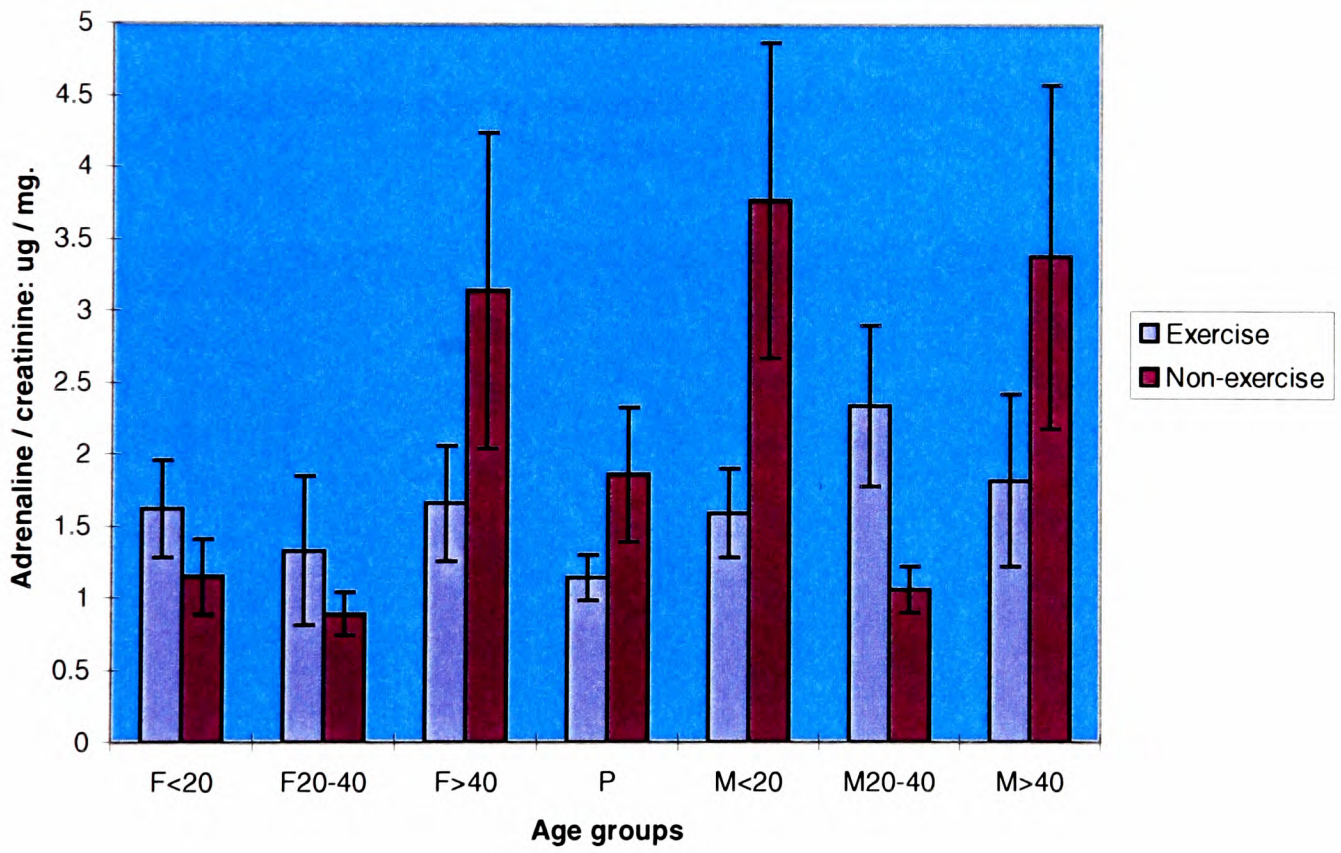
The mean levels of noradrenaline in F<20 who exercised were 1.73 ± 0.42; M<20 1.62 ± 0.23; F20-40 ± 1.36 ± 0.28; M20-40 1.74 ± 0.72; P 1.17 ± 0.14; F>40 0.86 ± 0.16; M>40 2.77 ± 1.40 and in

those not exercising they were 1.17 ± 0.31; M<20 1.77 ± 0.38; F20-40 1.19 ± 0.26; M20-40 1.54 ± 0.71; P 1.08 ± 0.19 ; F>40 0.88 ± 0.14 and M>40 1.86 ± 0.73 µg / mg creatinine. These levels were generally higher in the subjects who exericsed compared to those who did not (P>0.05).

3.5.0(h). Urinary adrenaline and exercise .

All subjects over the age of 40 years, who did not exercise, excreted more adrenaline than their counterparts who did (**fig.3.28**). In M<20 and in pregnant women who did not exercise, they were

Fig.3.28. shows adrenaline levels in exercise / non-exercise groups.



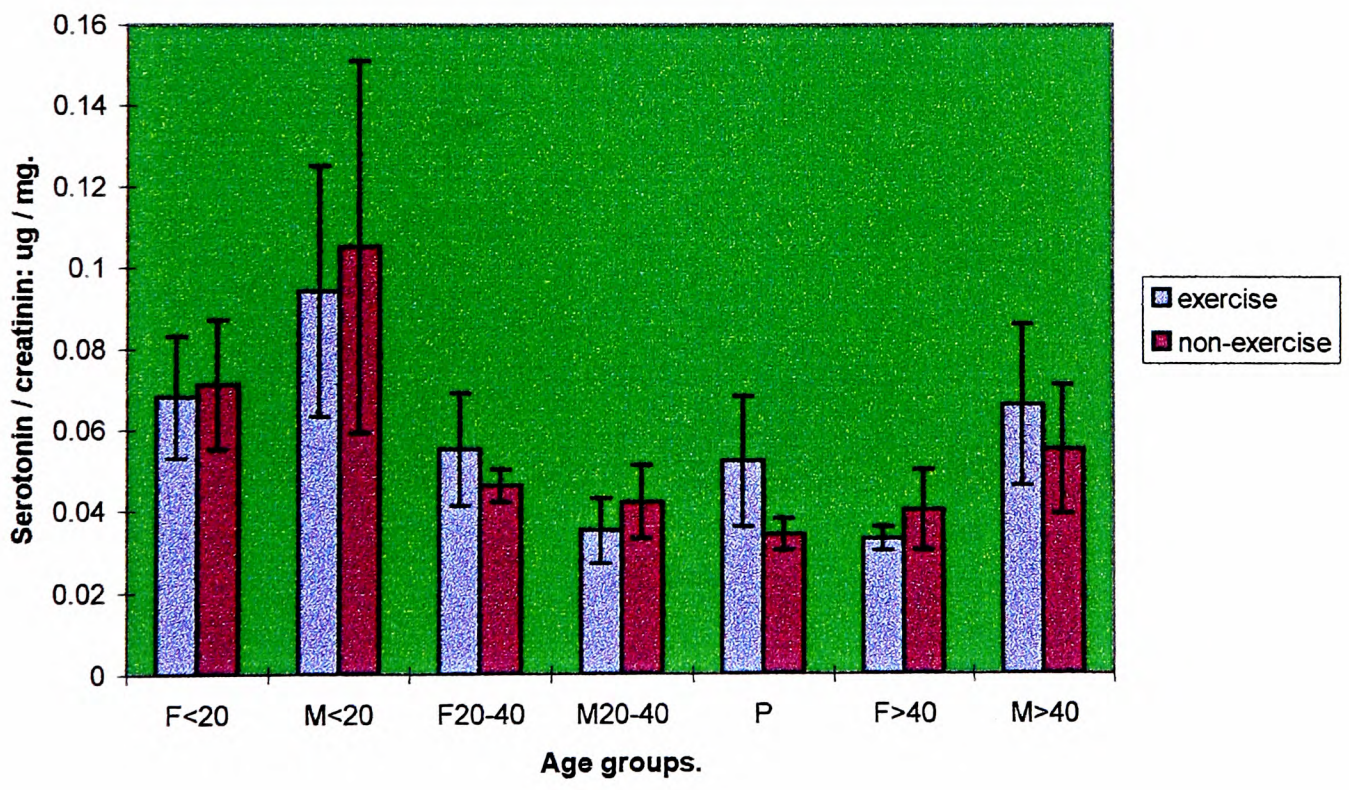
significantly higher (P<0.05) than their respective counterparts. M<20 who did not exercise also had significantly higher adrenaline levels than their counterparts in F<20 while M20-40 subjects who exercised excreted significantly (P<0.05) more adrenaline than those who did not.

3.5.0(i). Urinary dopamine and exercise.

The mean levels of dopamine in F<20 who exercised were 2.100 ± 0.330 ; M<20 2.300 ± 0.750 ; F20-40 1.164 ± 0.140; M20-40 1.072 ± 0.180; P 1.640 ± 0.380; F>40 1.206 ± 0.220; M>40 1.060 ± 0.120 while in those not exercising they were F<20 2.170 ± 0.490; M<20 2.100 ± 0.450; F20-40 1.303 ± 0.280; M20-40 1.407 ± 0.280; P 1.340 ± 0.210; F>40 1.540 ± 0.290 and M>40 1.070 ± 0.095 µg / mg creatinine. The levels were higher in adolescent and middle aged males who exercised compared to those who did not, but not significantly (P>0.05).

3.5.0(j). Urinary serotonin and exercise.

Fig.3.29. shows urinary serotonin levels in exercise and non-exercise groups.



While, urinary serotonin levels decreased in female with age, they increased in male subjects in both groups but not significantly **(Fig. 3.29).**

3.6.0.(a). Urinary analyte levels of vegetarian / non-vegetarian subjects.

F<20 and F20-40 were the only groups with a small number of vegetarian subjects. The results of vegetarians F<20 (n=6) and F20-40 (n=4) and non-vegetarians F<20 (n=35) and F20-40 (n=17) are presented in **table 3.1.** In F<20, noradrenaline, adrenaline and serotonin **(fig. 3.30)** were significantly higher (P<0.05) in non-vegetarians compared to vegetarians. There were no significant changes in the other groups (P>0.05).

Fig.3.30. shows the levels of serotonin in vegetarian and non-vegetarian

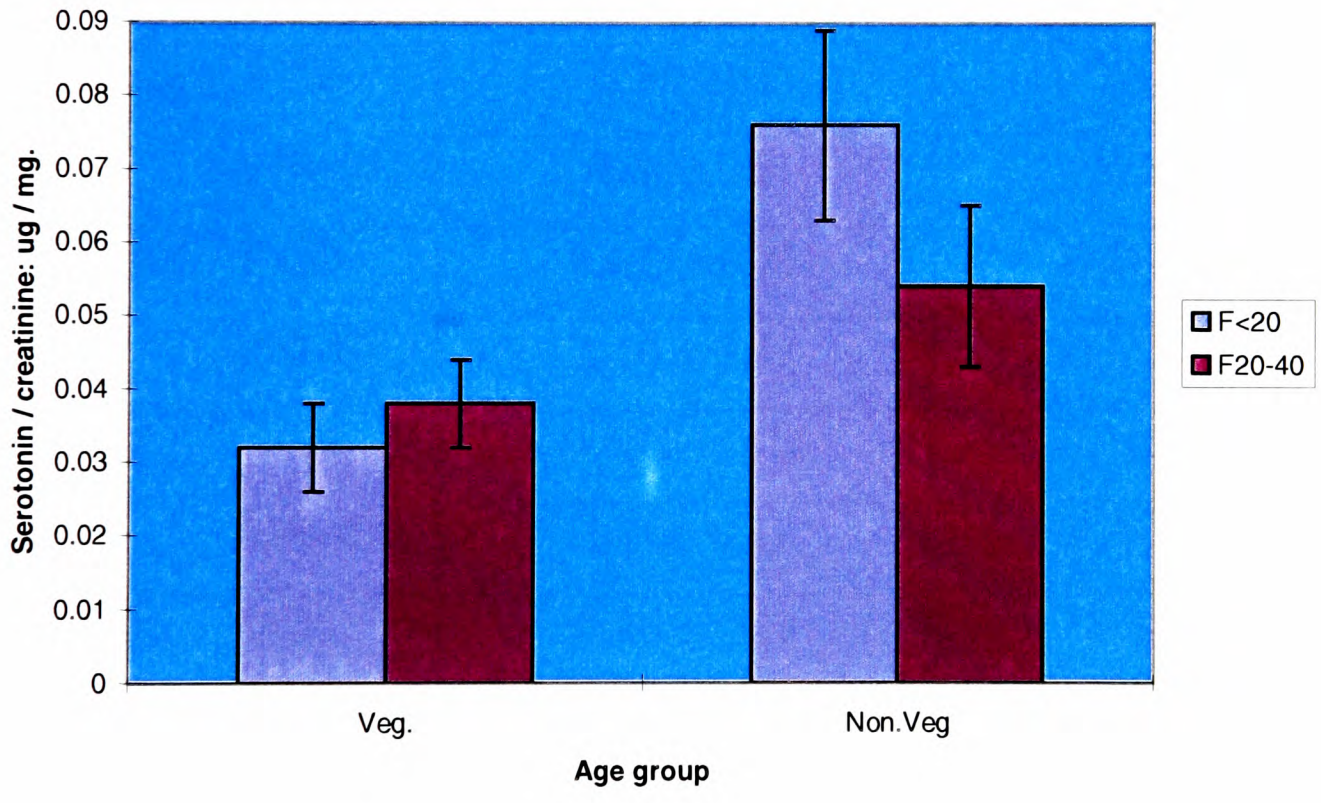


Table 3.1. shows the levels of urinary analytes in vegetarians / non-vegetarians.

Analyte		vegetarians	non-vegetarians
calcium	F<20	0.076 ± 0.010	0.081 ± 0.006
	F20-40	0.123± 0.062	0.059 ± 0.006
Magnesium	F<20	0.123 ± 0.023	0.095 ± 0.007
	F20-40	0.086 ± 0.034	0.063 ± 0.005
Phosphate	F<20	0.761 ± 0.180	0.864 ± 0.053
	F20-40	1.021± 0.420	0.827± 0.065
Boron	F<20	1.433± 0.2	1.014 ±0.11
	F20-40	1.43± 0.42	0.792± 0.15
Hydroxyp	F<20	6.3± 0.81	5.01± 0.7
	F20-40	6.88± 2.3	5.73 ±1.1
Cortisol	F<20	0.679 ±0.21	0.882± 0.14
	F20-40	1.10 ± 0.42	0.871 ±0.16
NA	F<20	0.695 ± 0.14	1.76± 0.34
	F20-40	1.20± 0.41	1.30 ± 0.21
AD	F<20	0.90 ± 0.14	1.54± 0.27
	F20-40	0.689 ± 0.17	1.23 ± 0.34
DA	F<20	1.388 ± 0.300	2.250 ± 0.310
	F20-40	2.556 ± 0.110	1.153 ± 0.180
5 HT	F<20	0.032 ± 0.006	0.076 ± 0.013
	F20-40	0.038 ± 0.006	0.054 ±0.011

(All values μg / mg creatinine except calcium, magnesium, phosphate mg / mg creatinine).

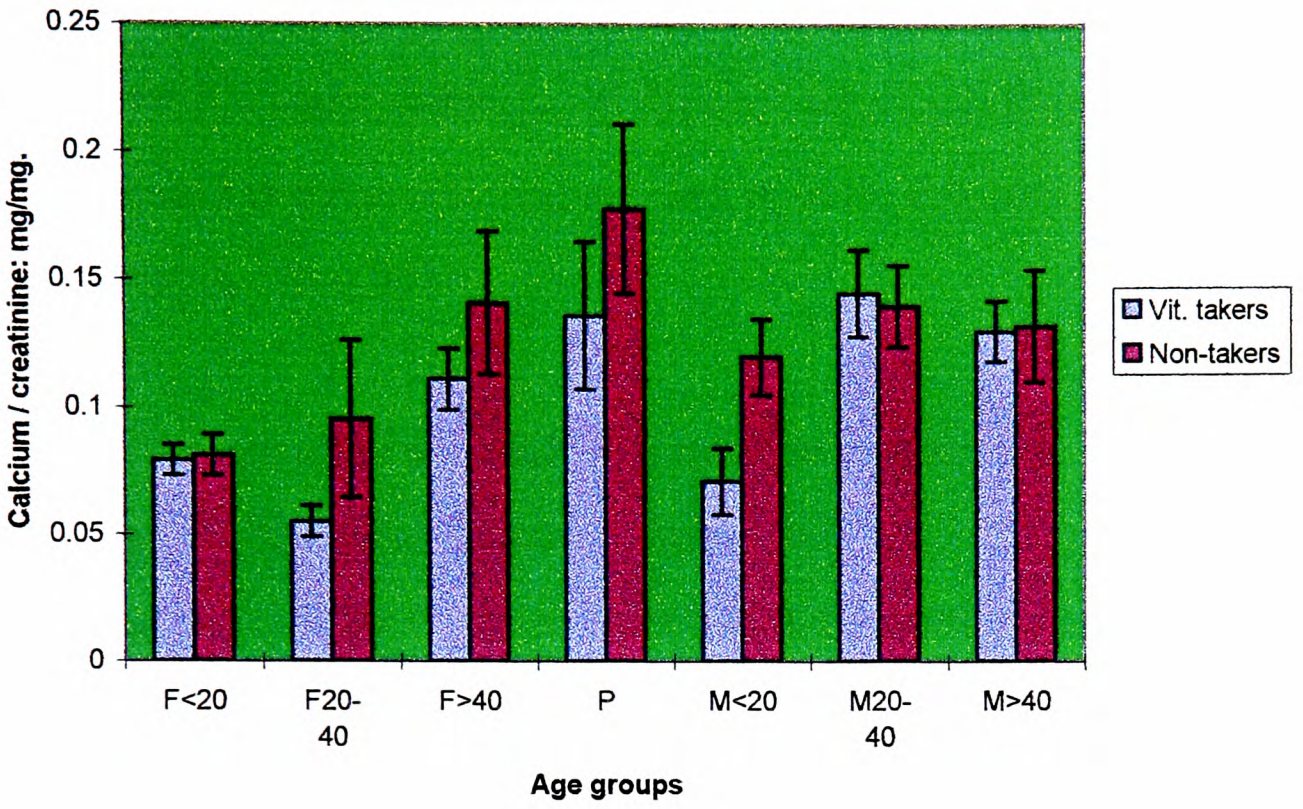
3.7.0(a). Urinary calcium and vitamins supplements.

Subjects taking one or more capsule(s) of multivitamins and vitamin C supplements daily were placed in one group and compared with the group who did not take them. Each multivitamin capsule contained the following: cod liver oil 500 mg, omega-3- nutrients (EPA 42.1 & DHA 37.4 mg), vitamins A 800 μg, D 5μg, E 10 mg, C 60 mg, thiamin 1.4 g, riboflavin 1.6 μg, B_6 2 mg, B_{12} 1μg, niacin 18 mg, folic acid 200 μg and biotin 00.1 mg.

The mean levels of urinary calcium in F<20 (n=17) vitamin takers were 0.079 ± 0.006; M<20 (n=5) 0.071 ± 0.013; F20-40 (n=13) 0.056 ± 0.006; M20-40 (n=6) 0.145 ± 0.017; P (n=10) 0.136 ± 0.029; F>40 0.056 ± 0.006 (n=13) and M>40 (n=14) 0.130 ± 0.012 and in the non-vitamins takers they were F<20 (n=24) 0.081 ± 0.008; M<20 (n=19) 0.120 ± 0.015; F20-40 (n=8) 0.095 ± 0.031 ; M20-40 (n=15) 0.140 ± 0.015; P (n=11) 0.178 ± 0.033; F>40 (n=9) 0.141 ± 0.028 ; and M>40 (n=8) 0.132 ± 0.022 mg / mg creatinine (**fig.3.31**).

In general, calcium excretion was greater in non-vitamin than vitamin supplement takers except in M20-40. In M<20 and in F20-40 non-vitamin takers, it was significantly higher ($P<0.05$) than their respective counterparts. M<20 non-vitamin takers also had significantly ($P<0.05$) higher calcium levels than those in F<20. The levels in F20-40 vitamin takers were significantly lower than those in M20-40 ($P<0.05$) and pregnant women.

Fig.3.31. shows the levels of calcium in vitamin / non-vitamin supplements takers



3.7.0(b). Urinary magnesium and vitamins supplements.

The mean levels of magnesium in F<20 vitamins takers were 0.102 ± 0.013; M<20 0.083 ± 0.022; F20-40 0.062 ± 0.006; M20-40 0.089 ± 0.009; P 0.076 ± 0.007; F>40 0.064 ± 0.009; M>40 0.064 ± 0.010 and in non-vitamins takers they were F<20 0.097 ± 0.008; M<20 0.106 ± 0.009; F20-40 0.075 ± 0.017; M20-40 0.083 ± 0.010; P 0.066 ± 0.007; F>40 0.076 ± 0.010; and M>40 0.066 ± 0.010 mg / mg creatinine. These levels decreased with age in male and female subjects of both groups but were higher (P>0.05) in F20-40 and F>40 who did not take the vitamin supplements compared to their counterparts.

3.7.0(c). Urinary phosphate and vitamins supplements.

Urinary levels of phosphate in F<20 vitamins takers were 0.827 ± 0.085; M<20 1.067 ± 0.170; F20-40 0.850 ± 0.091; M20-40 0.887 ± 0.110; P 0.912 ± 0.150; F>40 0.745 ± 0.091; M>40 0.870 ± 0.065

and in non-vitamins takers they were in F<20 0.865 ± 0.065; M<20 0.989 ± 0.068; F20-40 0.887 ± 0.088; M20-40 0.943 ± 0.100; P 0.707 ± 0.047; F>40 0.816 ± 0.085 and M>40 0.929 ± 0.100 mg/mg creatinine. The levels decreased, with age, in females who took these supplements and increased in those who did not, but in male subjects, these trends were reversed (P>0.05).

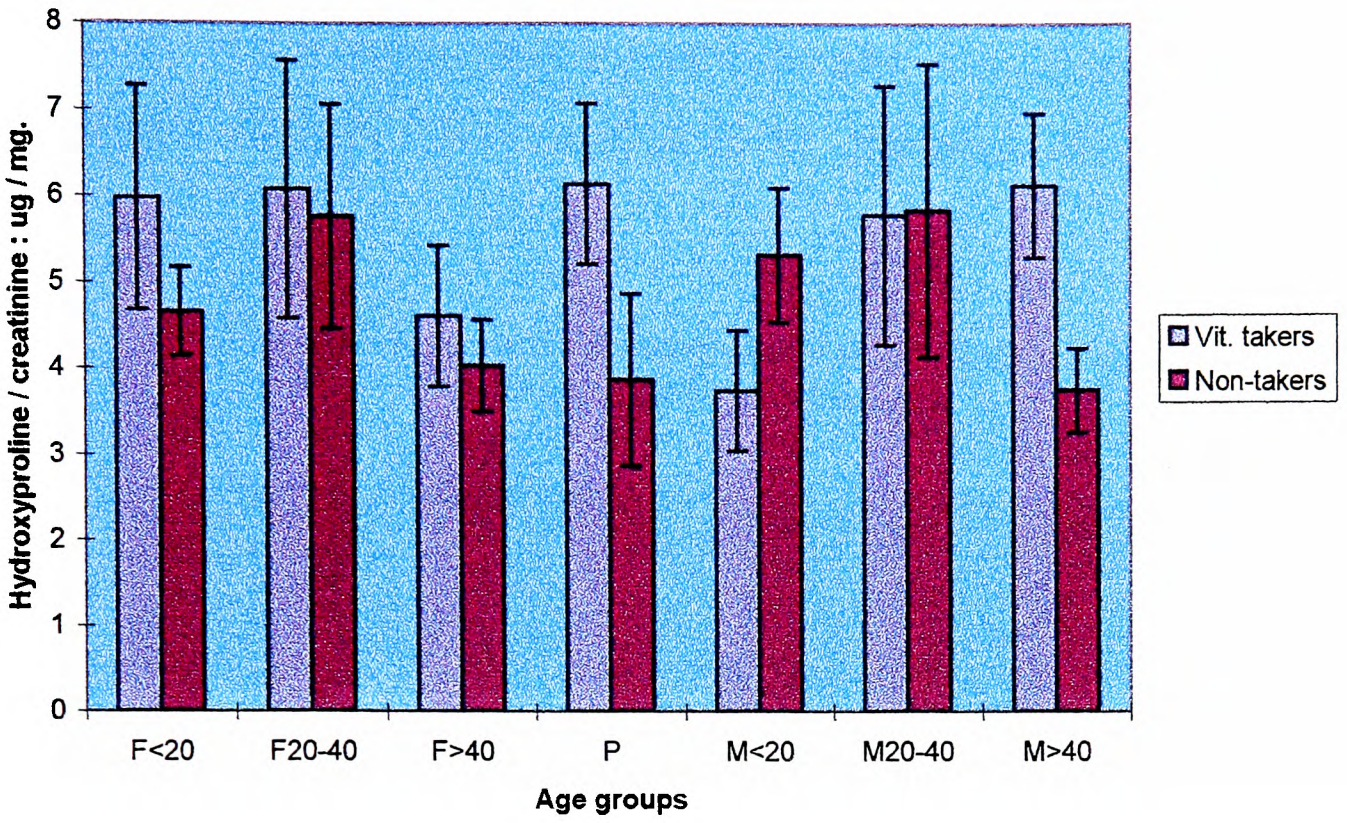
3.7.0(d). Urinary boron and vitamins supplements.

The mean levels of urinary boron in F<20 vitamin takers were 1.276 ± 0.21; M<20 0.795 ± 0.19; F20-40 0.74 ± 0.18; M20-40 0.875 ± 0.13; P 0.688 ± 0.12; F>40 0.766 ± 0.14 and in M>40 0.921 ± 0.14 and in F<20 non-vitamins takers were 0.934 ± 0.25; M<20 0.829 ± 0.11; F20-40 1.19 ± 6 0.25; M20-40 0.862 ± 0.16; P 0.710 ± 0.16; F>40 1.047 ± 0.26 and in M>40 0.683 ± 0.1 g/mg creatinine. The levels decreased, with age, in females who took these supplements and increased in those who did not, but in male subjects, these trends were reversed (P>0.05).

3.7.0(e). Urinary hydroxyproline and vitamins supplements.

Overall, urinary hydroxyproline levels were higher in females of all ages who took the supplements compared to those who did not but, were lower in M<20 who took the supplements while in M>40, they were higher when compared to their respective counterparts (**fig. 3.32**).

Fig.3.32 shows urinary hydroxyproline levels in subjects taking / not taking the vitamin supplements



In M20-40, the two levels were fairly similar, while in M>40 vitamin takers, they were significantly higher ($P<0.05$) than those who did not. The other changes were not significant ($P>0.05$).

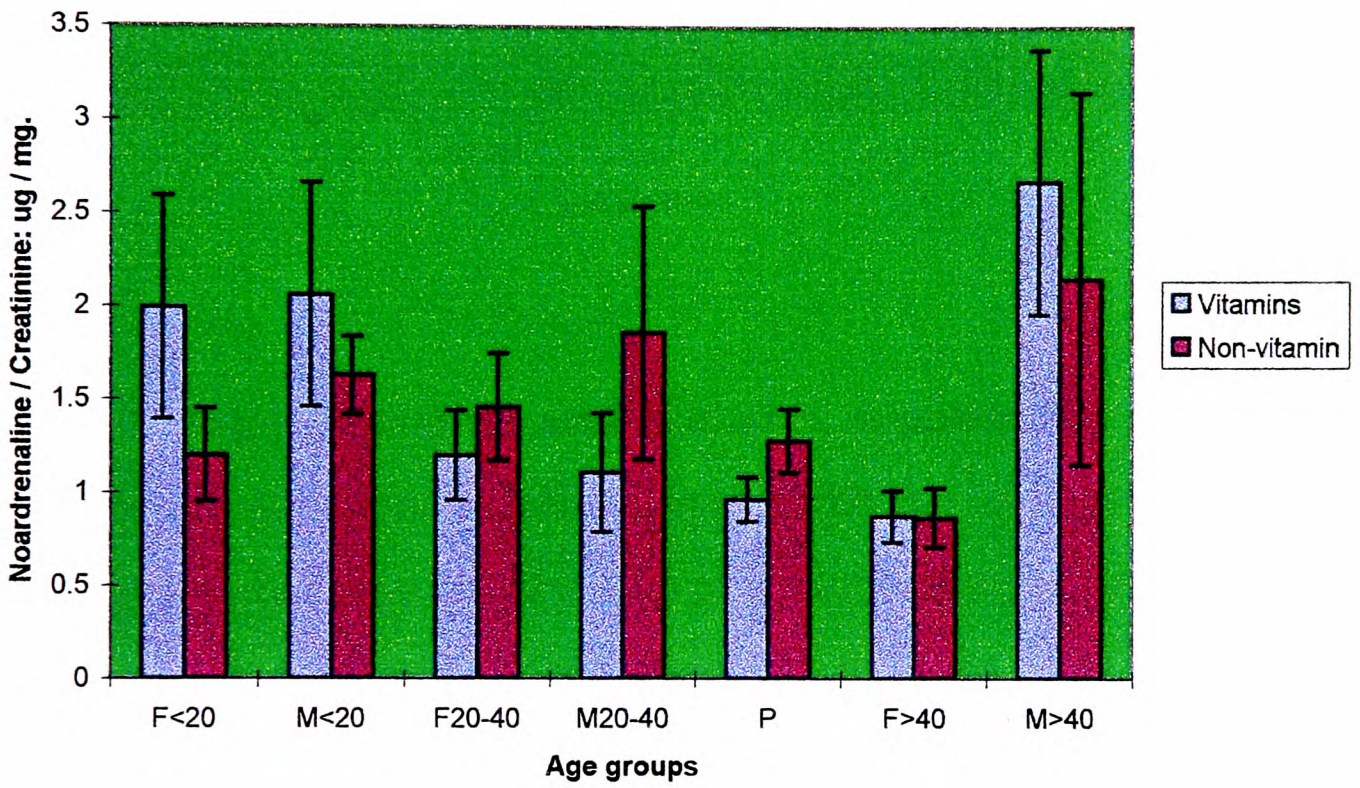
3.7.0(f). Urinary cortisol and vitamins supplements.

The mean cortisol levels in F<20 who took the vitamins were 0.641 ± 0.13; M<20 1.297 ± 0.32; F20-40 1.05 ± 0.22; M20-40 0.806 ± 0.20; P 2.10 ± 0.80; F>40 1.05 ± 0.22; M>40 1.32 ± 0.33 and in those not taking the vitamins F<20 were 1.00 ± 0.18; M<20 0.734 ± 0.12; F20-40 0.698 ± 0.17; M20-40 1.17 ± 0.38; P 1.30 ± 0.36; F>40 2.09 ± 0.96 and in M>40 1.72 ± 0.49 μg / mg creatinine Urinary cortisol levels increased with age in female subjects in both groups but these were not significant ($P>0.05$).

3.7.0(g). Urinary noradrenaline and vitamins supplements.

Male and female subjects in the M<20 and F<20 groups, who took the vitamin supplements, excreted more noradrenaline than their counterparts while in F20-40 and M 20-40, the levels decreased

Fig.3.33. shows urinary noradrenaline levels in subjects taking / not taking vitamin supplements.

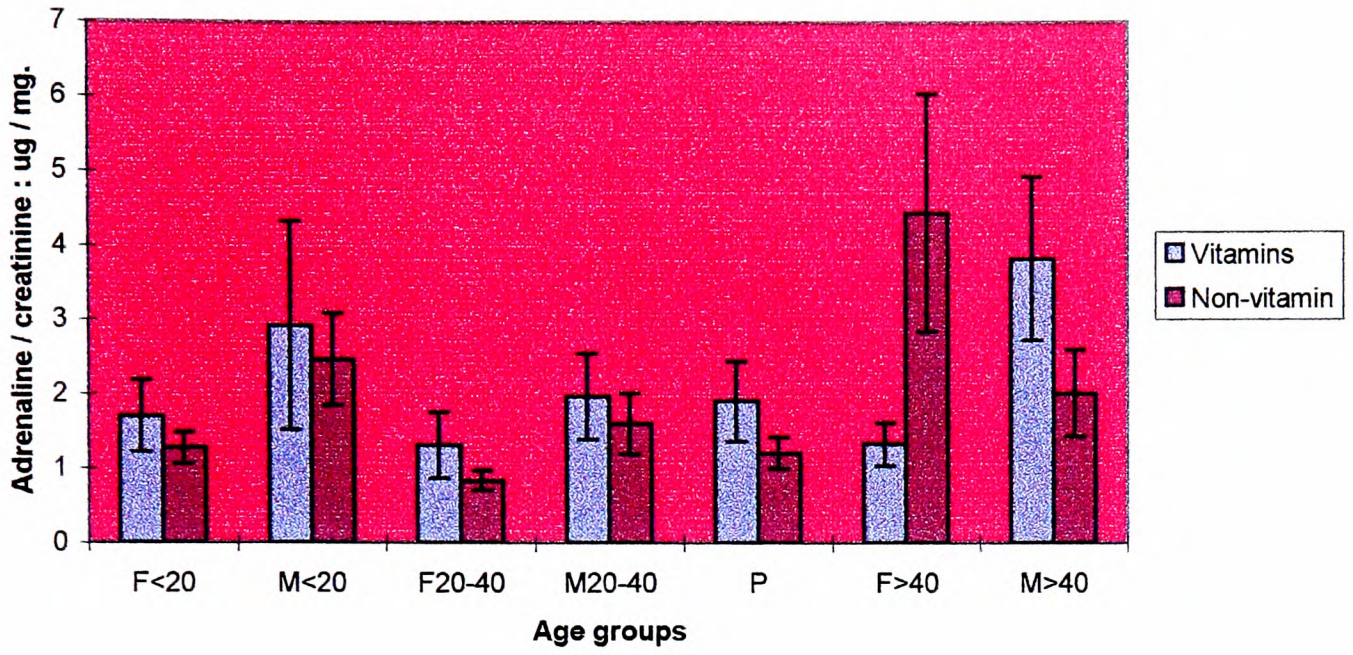


However, only urinary noradrenaline levels in M>40 vitamin takers were significantly higher (P<0.05) when compared to those in F>40 (**fig. 3.33).**

3.7.0(h). Urinary adrenaline and vitamins supplements.

Urinary adrenaline levels were higher in the vitamin supplements takers of all age groups than their respective counterparts except in F>40. In M>40 vitamin takers they were significantly higher (P<0.05) than their F>40 counterparts. These levels were also significant lower in F>40 who took the supplements compared to their counterparts **(fig. 3.34).**

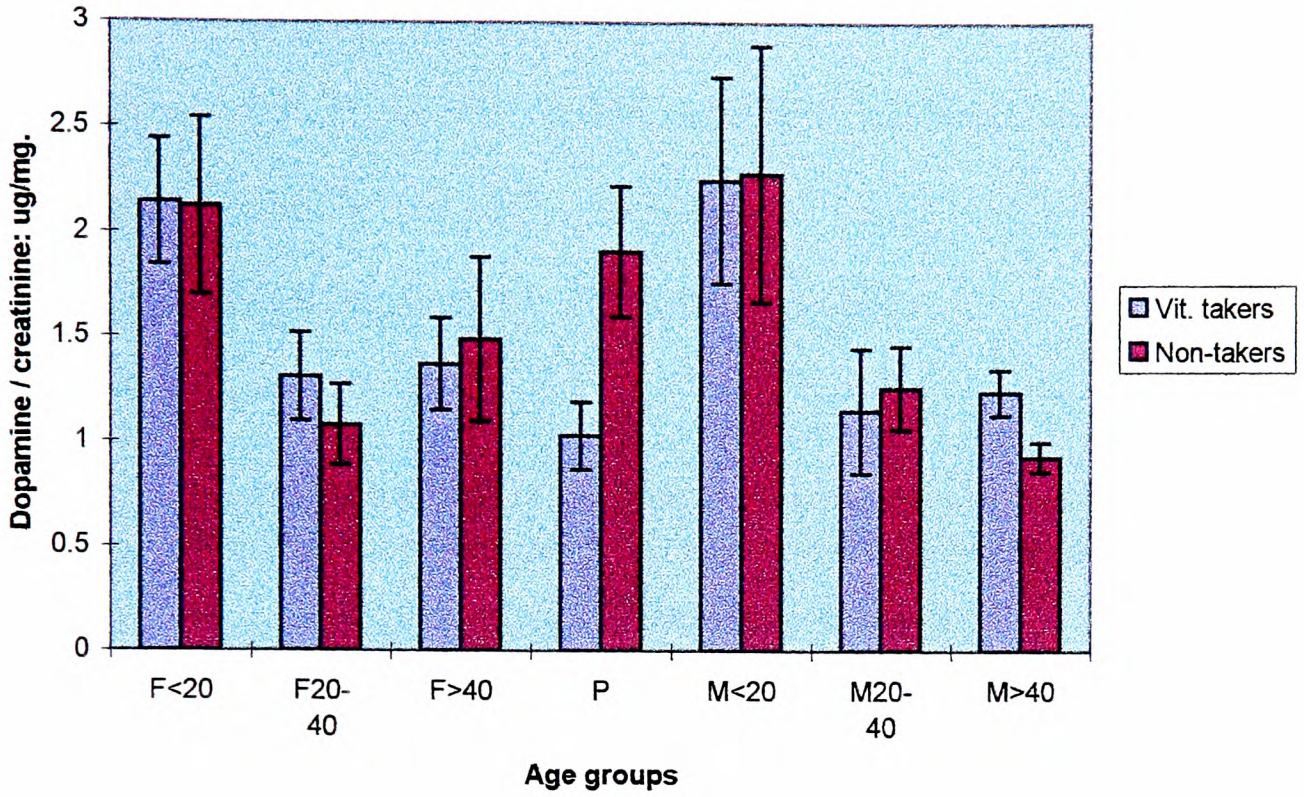
Fig.3.34. shows urinary adrenaline levels in vitamin supplements takers / non-takers.



3.7.0(i). Urinary dopamine and vitamins supplements .

Urinary dopamine levels decreased with age in both genders and in both groups but only the levels in pregnant women and M>40 taking the vitamins were significantly higher ($P<0.05$) than those in their corresponding age groups who did not take the vitamins (**fig.3.35**).

Fig.3.35. shows the levels of dopamine in vitamins / non-vitamins supplements takers



3.7.0(j). Urinary serotonin and vitamins supplements.

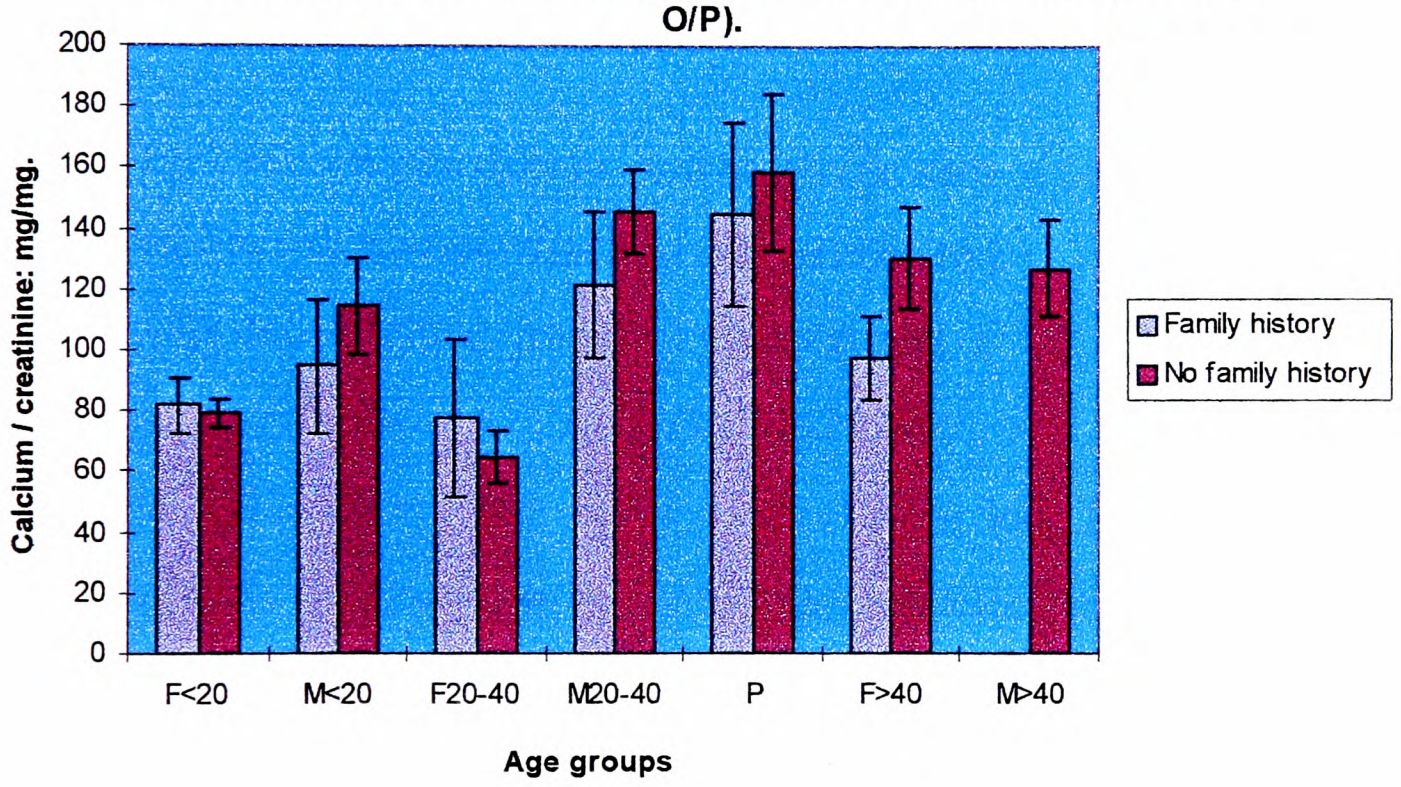
The mean levels of serotonin in F<20 who took the vitamins were 0.080 ± 0.020; M<20 0.062 ± 0.017; F20-40 0.059 ± 0.014; M20-40 0.047 ± 0.013; P 0.032 ± 0.005; F>40 0.042 ± 0.010; M>40 0.060 ± 0.011 and the levels in F<20 who did not take the vitamins were 0.064 ± 0.013; M<20 0.112 ± 0.033; F20-40 0.036 ± 0.008; M20-40 0.035 ± 0.006; P 0.051 ± 0.012 ; F>40 0.031 ± 0.004 and M>40 0.061 ± 0.017 μg / mg creatinine. The levels of urinary serotonin in the vitamins takers in all the age groups were higher than their counterparts but no significantly (P>0.05).

3.8.0(a). Urinary calcium and subjects with a family history of bone diseases (osteoporosis -O/P- and osteoarthritis -O/A).

The mean urinary levels of calcium in F<20 (n=18) with a family history of these diseases were 0.082 ± 0.009; M<20 (n=10) 0.095 ±

0.022; F20-40 (n=11) 0.078 ± 0.026; M20-40 (n=4) 0.094 ± 0.022; P (n=4) 0.145 ± 0.030; F>40 (n=5) 0.097 ± 0.014 and in F<20 (n=18) without a history of these diseases were 0.079 ± 0.005; M<20 (n=14) 0.115 ± 0.016; F20-40 (n=10) 0.065 ± 0.009; M20-40 (n=16) 0.146 ± 0.014; P (n=17) 0.159 ± 0.026; F>40 (n=17) 0.131 ± 0.017 and M>40 (n=20) 0.127 ± 0.016 mg / mg creatinine (**fig.3.36**).

Fig. 3.36 shows levels of urinary calcium in subjects with / without family history of bone diseases (osteoarthritis-O/A- & osteoporosis - O/P).



In F<20 and F20-40 they were higher in subjects with family history compared to those without but in the other age groups they were reversed. Only urinary calcium levels in F20-40, without a family history of bone diseases, were significantly lower ($P<0.05$) than in M20-40 and compared to those in the pregnant group without this history.

3.8.0(b). Urinary magnesium and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

In F<20 with a family history of these diseases these were 0.926 ± 0.082; M<20 0.085± 0.014; F20-40 0.072 ± 0.04; M20-40 0.065 ± 0.013; P 0.076. ± 0.017; F>40 0.088 ± 0.013 and in those without this history they were F<20 0.106 ± 0.012; M<20 0.109 ± 0.011; F20-40 0.063 ± 0.067; M20-40 0.089 ± 0.009; P 0.071 ± 0.005; F>40 0.063 ± 0.007 and M>40 0.065 ± 0.008. mg / mg creatinine. These changes were not significant (P>0.05).

3.8.0(c). Urinary phosphate and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

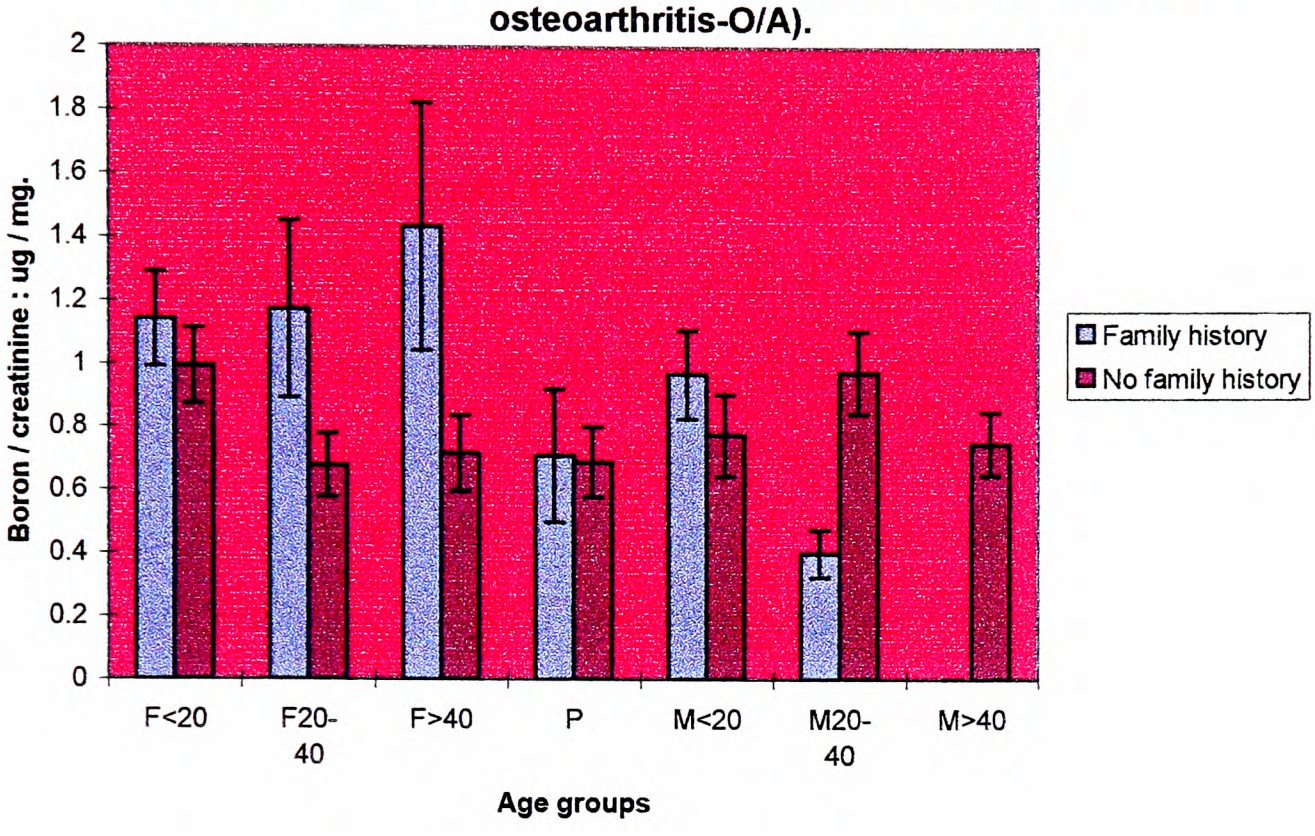
The mean urinary levels of phosphate in F<20 with the history of these diseases were 0.811 ± 0.074; M<20 1.095 ± 0.100 ; F20-40 0.832 ± 0.092; M20-40 0.748 ± 0.170; P 1.112 ± 0.360; F>40 0.761 ± 0.210 and in F<20 without this family history were 0.899 ± 0.069; M<20 0942 ± 0.074; F20-40 0.893 ± 0.093; M20-40 0.969 ± 0.088; of P 0.745 ± 0.055; F>40 0.778 ± 0.590 and M>40 0.913 ± 0.085 mg / mg creatinine. These were not significant (P>0.05).

3.8.0(d). Urinary boron and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

Urinary boron levels increased with age in female subjects with the history but decreased in those without, whilst in male subjects this was reversed. The levels in F20-40 and F>40 with the family history of these diseases were significantly higher (P<0.05) than those without but was significantly lower (P<0.05) in M 20-40 with the history. F20-40 with the history excreted more (P<0.05) boron than their male counterparts

(fig.3.37).

Fig.3.37. shows levels of urinary boron in subjects with / without familly history of bone diseases (osteoporosis -O/P- & osteoarthritis-O/A).



3.8.0 (e). Urinary hydroxyproline and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

The mean levels of hydroxyproline in F<20 with this history were 4.95 ± 0.98; M<20 5.4 ± 0.81; F20-40 6.18 ± 1.6; M20-40 2.98 ± 0.65; P 7.31 ± 2.1; F>40 3.05 ± 0.25 and the corresponding levels in F<20 without were 5.51 ± 0.65; M<20 4.93 ± 0.93; F20-40 5.74 ± 1.4; M20-40 6.49 ± 1.5; P 4.53 ± 0.71; F>40 4.76 ± 0.62 and M>40 4.61 ± 0.57 μg / mg creatinine. The level in M20-40 without this family history was significantly higher (P<0.05) than that in M20-40 with it. The other changes were not significant (P>0.05). In general, those with a family history of these diseases had higher levels of hydroxyproline.

3.8.0(f). Urinary cortisol and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

The mean levels of cortisol in F<20 without this history were 0.82 ± 0.16; M<20 0.785 ± 0.14; F20-40 0.76 ± 0.26; M20-40 1.19 ± 0.34; P 2.00 ± 0.53; F>40 1.92 ± 0.62; M>40 1.41 ± 0.33 and in F<20 with were 0.90 ± 0.18; M<20 1.12 ± 0.27 ; F20-40 1.06 ± 0.17; M20-40 0.54 ± 0.097 ; P 0.52 ± 0.26 and F>40 1.70 ± 0.63 μg / mg creatinine. In general, urinary cortisol levels were higher in subjects with the bone diseases except during pregnancy. The level in F20-40 with this family history was significanty higher (P<0.05) than that in M20-40. Significant findings (P<0.05) were also seen in the pregnant women without than in those with this history as well as between the F20-40 groups (P<0.05)

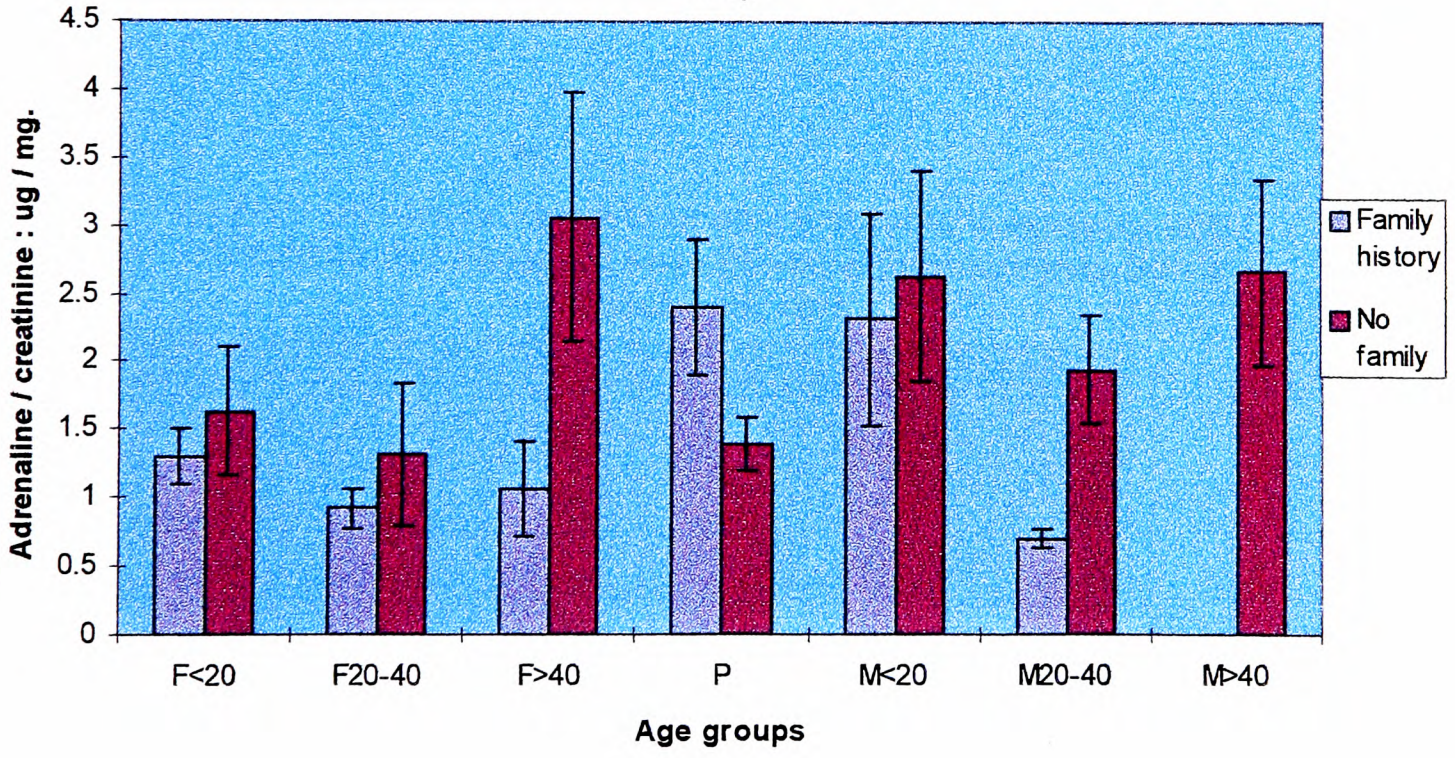
3.8.0(g). Urinary noradrenaline and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

The mean levels of noradrenaline in F<20 with this history were 1.63 ± 0.46; M<20 1.66 ± 0.37; F20-40 1.34 ± 0.24; M20-40 2.29 ± 0.80; P 0.78 ± 0.23; F>40 0.87 ± 0.14 and in F<20 without they were 1.39 ± 0.33; M<20 1.70 ± 0.24; F20-40 1.23 ± 0.28; M20-40 1.49 ± 0.47; P 1.20 ± 0.11; F>40 0.87 ± 0.13 and M>40 2.39 ± 0.80 μg / mg creatinine. In all females age groups with this family history, these were higher than in those without but not significantly (P>0.05).

3.8.0(h). Urinary adrenaline and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

Generally, these levels increased with age in all subjects, except in the pregnant women, without the history. In F>40 and M 20-40 without this family history, the levels were significantly higher ($P<0.05$) than those with while the levels in pregnant women without were lower ($P<0.05$) than those with it. Pregnant women with the history had significnatly higher ($P<0.05$) adrenaline compared to those in F<20, F20-40, F>40 and M20-40 whilst F>40 without the history had significantly ($P<0.05$) higher levels of urinary adrenaline than their counterparts in F20-40 **(fig.3.38).**

Fig.3.38. shows the levels of adrenaline in subjects with / without family history of bone diseases (osteoporosis -O/P- & osteoarthritis - O/A).



3.8.0(i). Urinary dopamine and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

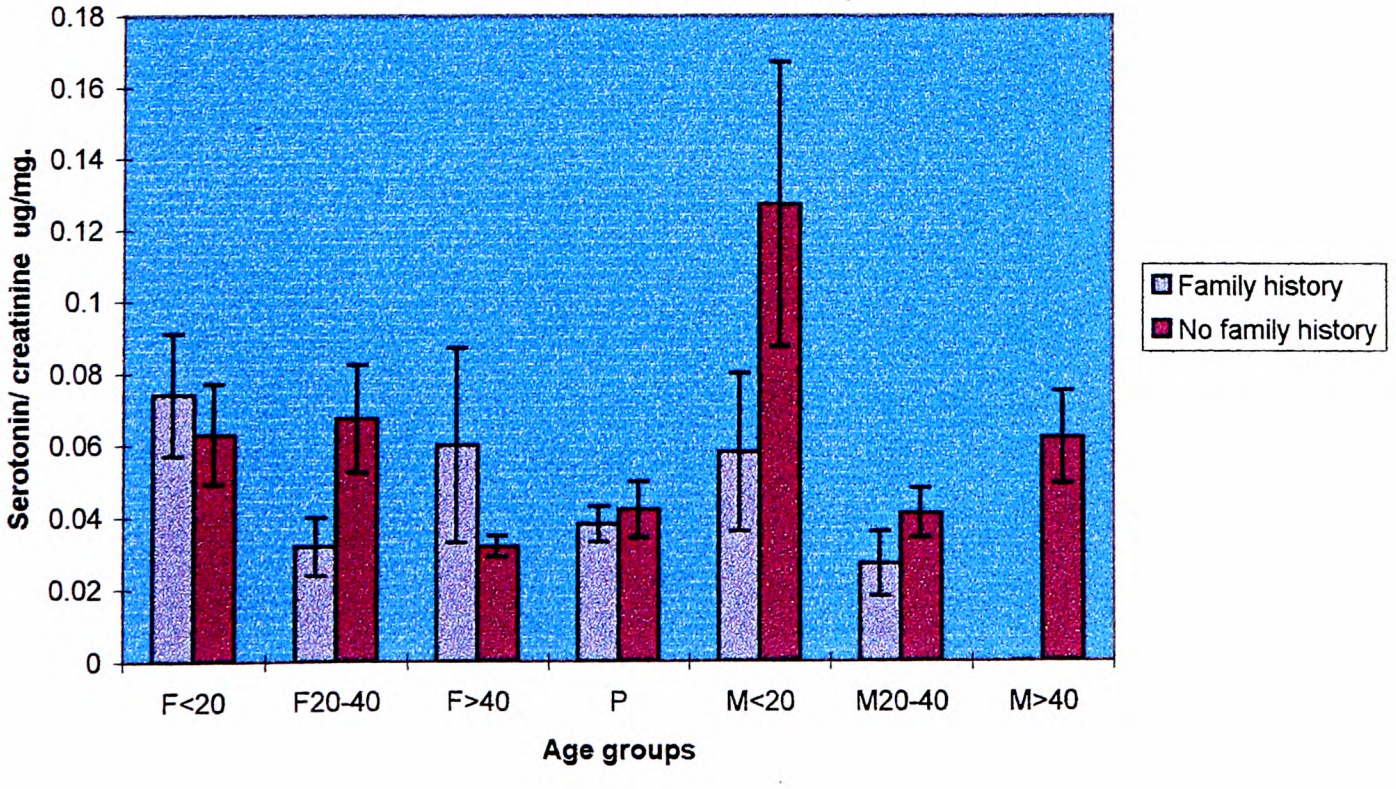
The mean levels of dopamine in F<20 with this history were 2.390 ± 0.430; M<20 1.713 ± 0.250; F20-40 1.239 ± 0.190; M20-40

1.128 ± 0.042; P 1.250 ± 0.200; F>40 2.060 ± 0.550 and in those without they were F<20 1.730 ± 0.290; M<20 2.580 ± 0.780; F20-40 1.222 ± 0.230; M20-40 1.256 ± 0.200; P 1.500 ± 0.230; F>40 1.231 ± 0.190 and M>40 1.300 ± 0.080 μg / mg creatinine. In all age groups, these levels were higher in subjects with this history than their counterparts but not significantly (P>0.05).

3.8.0(j). Urinary serotonin and subjects with a family history of bone diseases (osteoporosis and osteoarthritis).

Mean urinary level of serotonin in F20-40 without the family history was significantly higher (P<0.05) than in F20-40 and M20-40 with this history and also in M<20 without, it was also higher(P<0.05) than those with the history (**fig.3.39**).

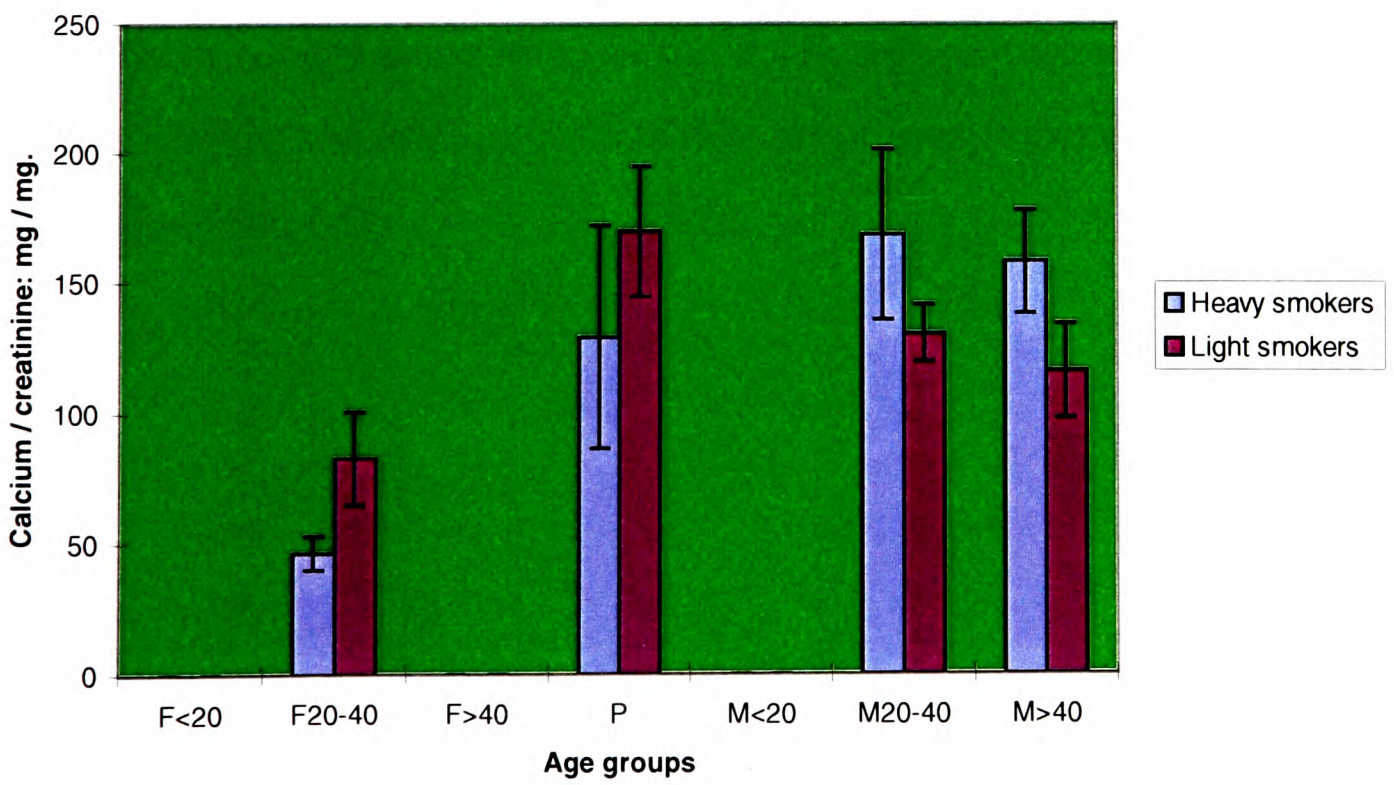
Fig.3.39. shows the levels of urinary serotonin in subjects with / without familly history of bone diseases (osteoporosis-O/P- & osteoarthritis -O/A).



3.9.0(a). Urinary calcium and cigarettes / tobacco smoking.

Subjects who smoke more than 10 cigarettes or 3 ounces of tobacco per day were considered as heavy smokers while light smokers were those smoking less. Most subjects were smokers except those in F<20, M<20 and F>40. The mean levels of urinary calcium in F20-40 (n=7) who smoked heavily were 0.047 ± 0.006; M20-40 (n=6) 0.168 ± 0.033; P(n=6) 0.129 ± 0.043; M>40(n=16) 0.158 ± 0.020 and in those who smoked less were F20-40 (n=7) 0.083 ± 0.018; M20-40 (n=15) 0.130 ± 0.011; P(n=14) 0.170 ± 0.025 and M>40 (n=16) 0.116 ± 0.018 mg / mg creatinine **(fig.3.40).** In F20-40 and P who smoked heavily these were lower compared to those who did not whereas in M20-40 and M>40, these were reversed. In M20-40 who smoked heavily, the levels were significantly higher (P<0.05) than those in F20-40 while in the light smoking subjects in M20-40 and P they were both significantly higher (P<0.05) than in F20-40.

Fig.3.40. shows the levels of urinary calcium in heavy smokers / light smokers.



3.9.0(b). Urinary magnesium and cigarettes / tobacco smoking.

In F20-40 who smoked heavily these levels were 0.070 ± 0.009; M20-40 0.086 ± 0.013; P 0.055 ± 0.006; M>40 0.072 ± 0.011 and in the light smoker they were F20-40 0.066 ± 0.010; M20-40 0.084 ± 0.010; P 0.080 ± 0.00 6; and M>40 0.062 ± 0.009 mg / mg creatinine. In pregnant women who smoked heavily it was significantly lower ($P<0.05$) than their counterparts, however in the other age groups, these levels were increased but not significantly ($P>0.05$).

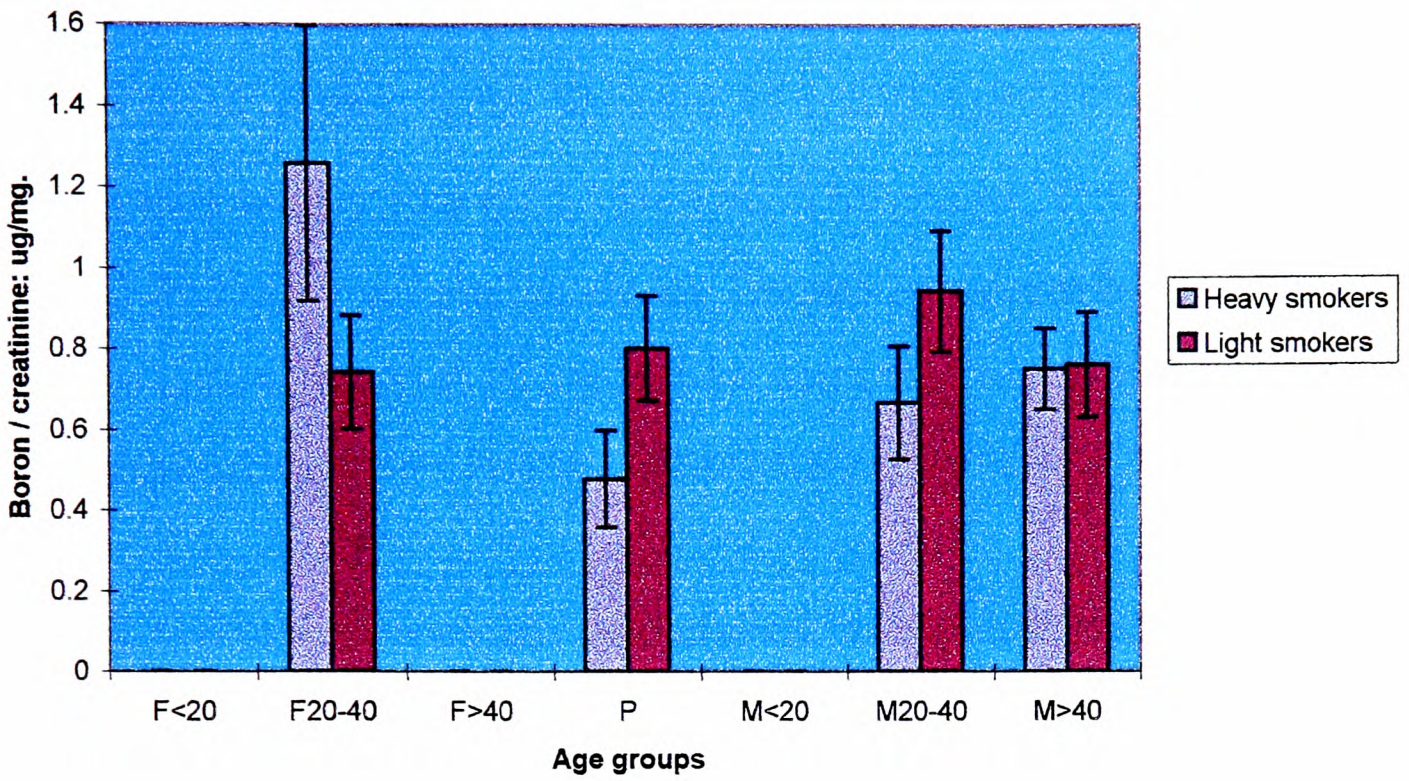
3.9.0(c). Urinary phosphate and cigarettes / tobacco smoking.

The mean levels of phosphate in F20-40 who smoked heavily were 0.771 ± 0.089; M20-40 1.144 ± 0.170; P 1.001 ± 0.230; M>40 1.380 ± 0.130 and in the light smokers they were F20-40 0.911 ± 0.085; M20-40 0.840 ± 0.080; P 0.721 ± 0.041 and M>40 0.837 ± 0.065 mg / mg creatinine. The levels in F20-40, who were light smokers, were significantly higher ($P<0.05$) than those in the pregnant group.

3.9.0(d). Urinary boron and cigarettes / tobacco smoking.

These results are shown in **fig. 3.41** and comparison between heavy and light smokers in the F20-40 and pregnant groups were significant ($P<0.05$).

Fig.3.41. shows the levels of boron in heavy and light smokers



3.9.0(e). Urinary hydroxyproline and cigarettes / tobacco smoking.

The mean levels of hydroxyproline in F20-40 who smoked heavily were 6.32 ± 2.3 ; M20-40 6.13 ± 1.8; P 5.16 ± 1.5; M>40 4.19 ± 0.57 and in the light smoker they were F20-40 5.76 ± 1.0; M20-40 5.7 ± 1.7; P 5.01 ± 0.81 and in M>40 4.69 ± 0.74 µg / mg creatinine. These results were not significant (P>0.05).

3.9.0(f). Urinary cortisol and cigarettes / tobacco smoking.

The mean levels of cortisol in F20-40 who smoked heavily were 0.84 ± 0.19; M20-40 1.69 ± 0.88; P 1.08 ± 0.25; M>40 1.97 ± 0.49 and in the light smokers they were F20-40 0.95 ± 0.21; M20-40 0.82 ± 0.17; P 2.04 ± 0.65 and M>40 1.37 ± 0.41 ug / mg creatinine. The females light smokers and male heavy smokers had higher levels of urinary cortisol than their respective counterparts but these were not significant (P>0.05).

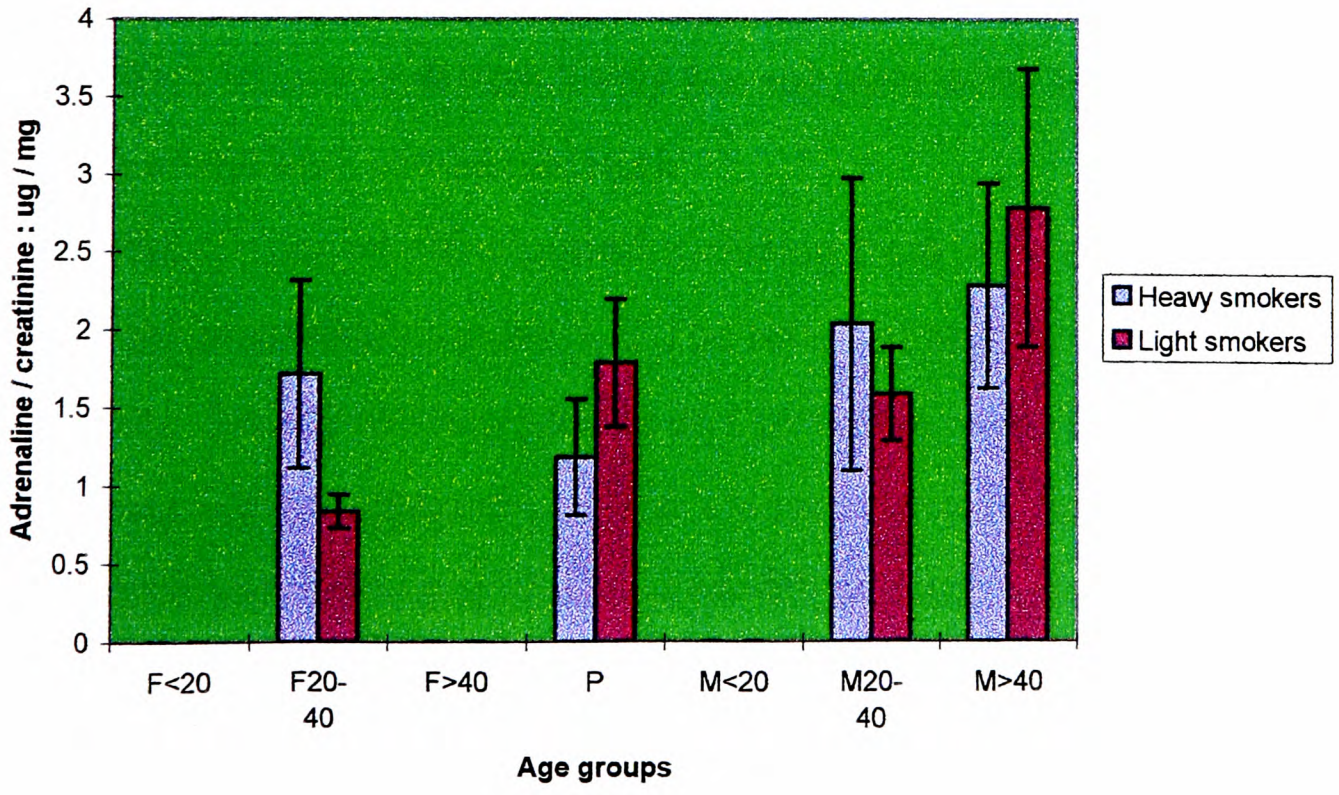
3.9.0(g). Urinary noradrenaline and cigarettes / tobacco smoking.

In F20-40 heavy smokers these levels were 1.08 ± 0.31; M20-40 0.77 ± 0.10; P 0.89 ± 0.20; M>40 0.57 ± 0.23 and in F20-40 light smokers they were 1.38 ± 0.22; M20-40 2.00 ± 0.68; P 0.23 ± 0.11 and M>40 2.75 ± 1.20 ug / mg creatinine Urinary noradrenaline levels were higher in light smokers at all age groups compared to the heavy smokers except during pregnancy but not significantly (P>0.05).

3.9.0(h). Urinary adrenaline and cigarettes / tobacco smoking.

Urinary levels of adrenaline were higher in F20-40 and M20-40 heavy smokers compared to the light smokers but the trend was reversed in M>40 **(fig.3.42).** In F20-40 light smokers, these levels were significantly lower than those in M 20-40 (P<0.05) and in pregnant women (P<0.05).

Fig.3.42 shows urinary adrenaline levels in heavy and light smokers



3.9.0(i). Urinary dopamine and cigarettes / tobacco smoking.

The mean levels of dopamine in F20-40 who smoked heavily were 1.004 ± 0.110 ; M20-40 2.800 ± 0.480; P 0.785 ± 0.140; M>40 0.913 ± 0.079 and in F20-40 light smokers they were 1.343 ± 0.210; M20-40 1.305 ± 0.200; P 1.689 ± 0.260 and M>40 1.102 ± 0.099 μg / mg creatinine. In pregnant women light smokers these levels were significantly higher (P<0.05) than in those who smoked heavily. However in general, the levels were higher in subjects of all ages who smoked heavily compared to those who smoked less (P>0.05).

3.9.0(j). Urinary serotonin and cigarettes / tobacco smoking.

The mean levels of serotonin in F20-40 who smoked heavily were 0.056 ± 0.016; M20-40 0.028 ± 0.005; P 0.030 ± 0.007; M>40 0.061 ± 0.014 and in F20-40 who smoked lightly they were 0.048 ± 0.012; M20-40 0.043 ± 0.008; of P 0.046 ± 0.009 and M>40 0.061 ± 0.016 μg / mg creatinine. These results were not statistically significant (P>0.05).

3.10.0(a). Urinary calcium levels for subjects with bone diseases (osteoporosis and osteoarthritis).

Only F and M>40 reported suffering from bone disorders. The results are presented in **table 3.2.** The levels of urinary magnesium, phosphate, boron, hydroxyproline and serotonin were lower in F>40 with these bone diseases than those without but only the boron levels were significant (P<0.05) while in M>40, calcium, phosphate, boron, hydroxyproline,cortisol, noradenaline, adrenaline and dopamine were

Table 3.2. Shows urinary analytes levels in F>40 and M>40 with / without bone diseases (osteoporosis -O/P- & osteoarthritis- O/A).

Analytes	Groups	bone diseases	no bone diseases
calcium	F>40	0.151± 0.022	0.124± 0.016
	M>40	0.158± 0.037	0.119± 0.015
magnesium	F>40	0.057± 0.006	0.074 ±0.008
	M>40	0.065± 0.021	0.065± 0.007
phosphate	F>40	0.758± 0.044	0.781± 0.086
	M>40	1.990± 0.150	0.821± 0.091
boron	F>40	0.487± 0.16	1.028± 0.16
	M>40	0.942± 0.22	0.673± 0.096
hydroxyproline	F>40	3.41± 0.52	4.73 ±0.68
	M>40	4.9 ±0.86	4.44± 0.72
cortisol	F>40	2.60± 1.4	1.60 ±0.43
	M>40	1.74± 0.48	1.52± 0.49
nordarenaline	F>40	0.91± 0.25	0.86± 0.11
	M>40	2.50 ±1.10	2.23± 1.00
adrenaline	F>40	3.80± 2.30	2.15± 0.54
	M>40	4.76± 1.60	1.59± 0.46
dopamine	F>40	1.191± 0.230	1.500± 0.26
	M>40	1.020 ±0.190	1.39 ±0.770
serotonin	F>40	0.032± 0.007	0.040± 0.008
	M>40	0.040± 0.011	0.071± 0.018

(All values in μg/mg except calcium,magenesium & phosphate in mg/mg creatinine).

higher in those with the diseases but not significantly (P>0.05). However, the levels of phosphate and noradrenaline were significantly higher (P<0.05) in M>40 with the diseases than in F>40. The level of urinary adrenaline was significantly (P<0.05) higher in M>40 with the diseases compared to those without.

3.11.0(a). Urinary analytes levels for women with / without children and with multiple / first pregnancy.

Women (aged 20-40) with children and those without children as well as those with multiple and those pregnant for the first time were compared as pregnancy increases sex hormones and places additional demands on bone metabolism which in turn can affect bone mass. F20-40 (n= 9) with children were compared with F20-40 (n=12) without any children. Pregnant women who already had children and were pregnant again (n= 12) were also compared with those pregnant (n=9) for the first time. The results are shown in **table 3.3.** There were no significant effects except for the followings;

1. calcium and magnesium levels were higher (P<0.05) in women with children and pregnant at the time of the study compared to women with children but not pregnant.
2. boron levels during first pregnancy were significantly lower compared to women without children (P<0.05).

During pregnancy there were increased calcium and magnesium excretion but urinary boron was significantly reduced (P<0.05).

Table 3.3. Shows levels of urinary analytes for women with and without children and pregnant women with children and pregnant for the first time.

Analytes	with children	without children	with children and pregnant	first pregnancy
Calcium	0.066± 0.009	0.074 ±0.022	0.169 ± .031	0.139±0.031
Magnesium	0.054± 0.005	0.077± .012	0.077± 0.007	0.064±0.008
Phosphate	0.835± 0.085	0.886± 0.095	0.745± 0.068	0.907± 0.17
Boron	0.549 ±0.13	1.187 ±0.22	0.748± 0.12	0.622 ±0.17
HP	6.16± 1.5	5.78± 1.4	4.81± 0.81	5.38 ±1.3
Cortisol	1.06 ±0.27	0.81± 0.17	1.90± 0.71	1.49 ±0.50
NA	1.29± 0.31	1.28± 0.23	1.90 ±0.71	1.19± 0.18
AD	0.89 ±0.17	1.31± 0.48	1.80 ±0.50	1.28± 0.24
DA	1.055± 0.290	1.360± 0.130	1.178 ±0.230	1.823±0.300
5 HT	0.052± 0.018	0.049 ±0.010	0.033 ±0.006	0.051±0.013

(All values in μg / mg except calcium, magnesium & phosphate mg / mg creatinine).

3.12.0. Urinary analytes levels for F20-40 pill / non-pill users.

The urinary analytes of those taking the combined (oestrogen and progesterone) contraceptive pill (n = 8) were compared to those not (n =13). The results are presented in **table 3.4.** Those on the contraceptive pill had higher ($P<0.05$) urinary boron levels. Calcium, noadrenaline, adrenaline and serotonin were lower in those taking the pill but these changes were not significant ($P>0.05$).

Table 3.4. shows levels of urinary analytes in F20-40 pill / non contraceptive pill users.

Analytes	Taking pill pill	Not taking
Calcium	0.057 ± 0.008	0.079 ± 0.020
Magnesium	0.066 ± 0.007	0.068 ± 0.011
Phosphate	1.037 ± 0.120	0.758 ± 0.055
Boron	1.314 ± 0.27	0.688 ± 0.14
Hydroxyproline	7.53 ± 1.9	4.97 ± 1.1
Cortisol	1.02± 0.23	0.85 ± 0.20
Noradrenaline	1.02 ± 0.21	1.44 ± 0.26
Adrenaline	0.83 ± 0.13	1.31 ± 0.44
Dopamine	1.400 ± 0.091	1.225 ± 0.23
Serotonin	0.043 ± 0.009	0.055 ± 0.014

(All values μg / mg except calcium, magnesium & phosphate mg /mg creatinine).

3.13.0. Levels of urinary analytes for F>40 with and without hysterectomy.

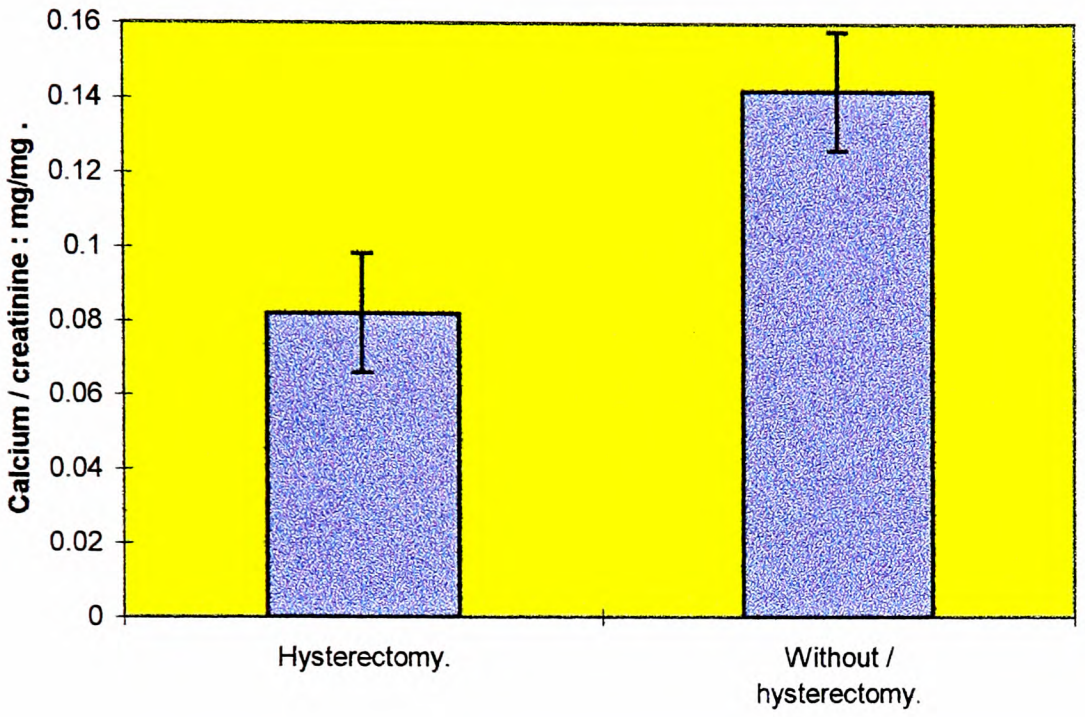
The results are given in **table 3.5.** Those who had hysterectomy (n=7) were compared to those without (n=15). All the bone turnover markers, except magnesium and all the stress hormones, except dopamine, were higher (P>0.05) in women without hysterectomy, but only urinary levels of calcium were significantly higher (P<0.05; **fig.3.43).**

Table 3.5. shows urinary level of analytes for F>40 with / without hysterectomy.

Analytes	with hysterectomy	Without hysterectomy
Calcium	0.082 ± 0.016	0.141 ± 0.016
Magnesium	0.072 ± 0.013	0.067 ± 0.008
Phosphate	0.699 ± 0.068	0.810 ± 0.087
Boron	0.652 ± 0.19	0.987 ± 0.17
Hydroxyproline	4.01 ± 0.57	4.53 ± 0.73
Cortisol	1.083 ± 0.37	2.24 ± 0.69
Noradrenaline	0.80 ± 0.23	0.90 ± 0.11
Adrenaline	2.33 ± 0.98	2.73 ± 0.99
Dopamine	1.650 ± 0.410	1.309 ± 0.230
Serotonin	0.033 ± 0.006	0.040 ± 0.009

(All values μg / mg except calcium, magnesium & phosphate mg / mg creatinine).

Fig.3.43. shows the levels of calcium in F>40 with / without hysterectomy.



3.14.0. Levels of urinary analytes for F>40 on / not on HRT .

The results for those on (n=7) and those not (n=15) on HRTare presented in **table 3.6.** There were no significant changes in any of the analytes however, calcium, phosphate, hydroxyproline, noradrenaline and adrenaline were higher in those not on HRT.

Table 3.6. Shows the levels of urinary analytes for F>40 on / not on HRT.

Analytes	On HRT	Not on HRT
Calcium	0.114 ± 0.016	0.127 ± 0.018
Magnesium	0.069 ± 0.010	0.068 ± 0.008
Phosphate	0.725 ± 0.11	0.798 ± 0.79
Boron	1.08 ± 0.21	0.785 ± 0.17
Hydroxyproline	3.81 ± 0.89	4.65 ± 0.65
Cortisol	1.35 ± 0.45	2.14 ± 0.69
Noradrenaline	0.71 ± 0.16	0.95 ± 0.13
Adrenaline	2.03 ± 0.95	2.85 ± 0.99
Dopamine	1.700 ± 0.470	1.286 ± 0.200
Serotonin	0.054 ± 0.019	0.030 ± 0.003

(All values μg / mg except calcium, magnesium & phosphate mg / mg creatinine).

3.15.0. Levels of urinary analytes for pre-menopause and post-menopause women.

Pre- (n = 12) and post-menopausal (n = 10) women in those over 40 years old were compared. The results are presented in **table 3.7.** Urinary hydroxyproline and serotonin levels were higher in post-menopausal than in pre-menopausal but only calcium, boron (**fig. 3.44**) and dopamine levels were significantly greater ($P<0.05$) whilst phosphate, cortisol, noradrenaline and adrenaline levels were all lower.

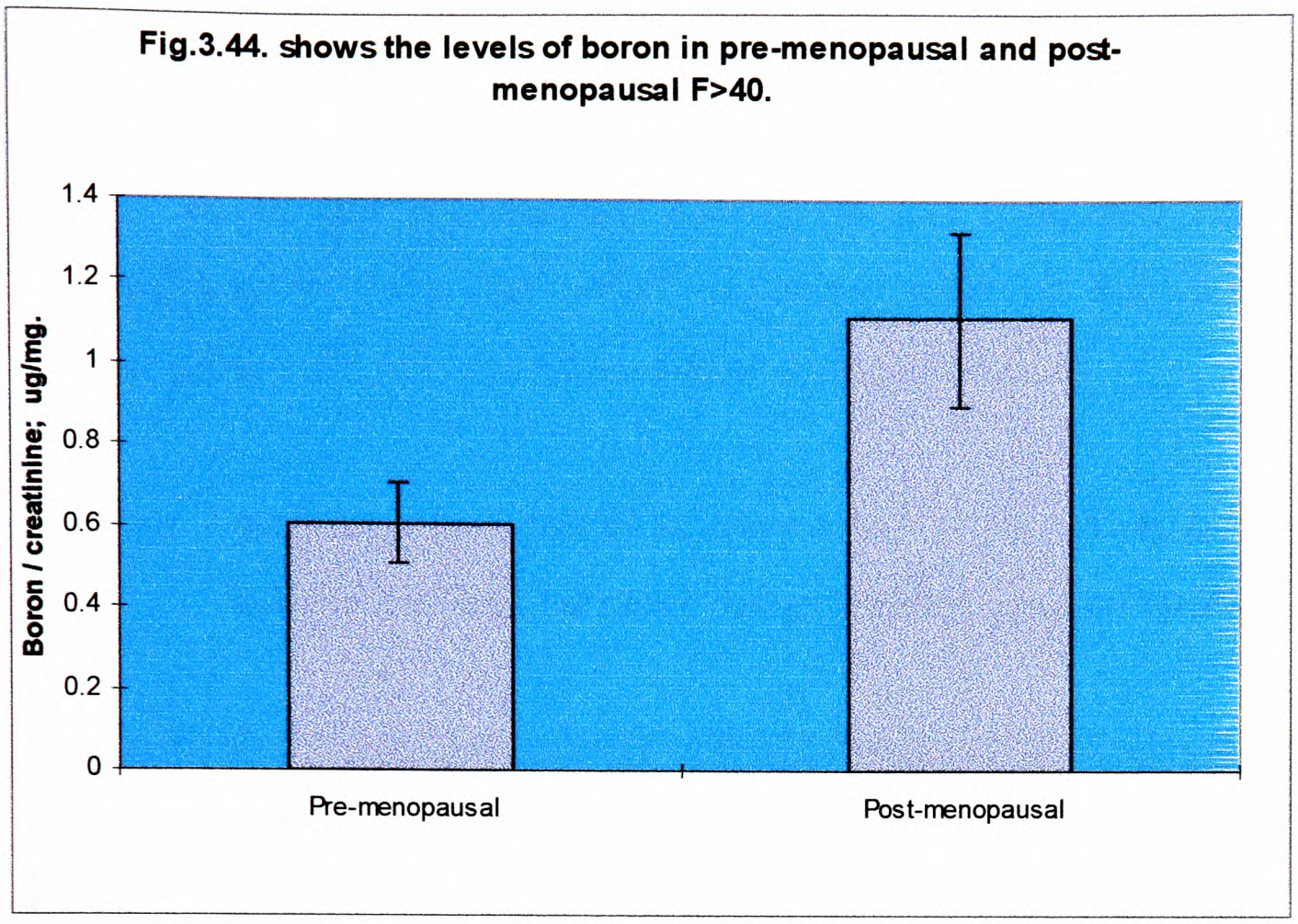


Table 3.7. shows levels of urinary analytes for pre- and post-menopausal women.

Analytes	Pre-menopause	Post-menopause.
Calcium	0.084 ± 0.009	0.170 ± 0.019
Magnesium	0.063 ± 0.009	0.073 ± 0.00
Phosphate	0.871 ± 0.087	0.694 ± 0.087
Boron	0.606 ± 0.1	1.11 ± 0.21
Hydroxyproline	4.1 ± 0.98	4.59 ± 0.53
Cortisol	2.51 ± 1.00	1.34 ± 0.23
Noradrenaline	0.90 ±± 0.17	0.847 0.14
Adrenaline	2.96 ± 1.40	2.30 ± 0.69
Dopamine	0.909 ± 0.140	1.840 ± 0.300
Serotonin	0.031 ± 0.004	0.043 ± 0.011

(All values µg / mg except calcium, mganesium & phosphate mg / mg creatinine).

3.16.0. Levels of urinary analytes for M>40 taking inflammatory drugs (aspirin and voltarel).

M>40 (n = 10) who took these drugs were compared with those who did not (n = 12). The results are given in **table 3.8**. Subjects in the other groups did not take anti-inflammatory medications.

Table 3.8. Shows the levels of urinary analytes for M>40 taking / not taking anti-inflamatory drugs (aspirin and voltarel).

Analytes	On anti-inflammatory drugs	Not on anti-inflammatory drugs
calcium	0.130 ± 0.030	0.132 ± 0.16
magnesium	0.059 ± 0.014	0.071 ± 0.009
phosphate	0.941 ± 0.130	0.884 ± 0.110
boron	0.728 ± 0.17	0.764 ± 0.11
hydroxyproline	5.65 ± 0.97	3.56 ± 0.5
cortisol	1.47 ± 0.50	1.69 ± 0.54
noradrenaline	3.74 ± 1.60	1.13 ± 0.24
adrenaline	3.87 ± 1.20	1.54 ± 0.55
dopamine	1.670 ± 0.130	1.500 ± 0.090
serotonin	0.062 ± 0.017	0.060 ± 0.019

(All values μg / mg except calcium, magnesium & phosphate mg / mg creatinine).

There were no significant changes in the urinary analytes levels between these two groups however, phosphate, hydroxyproline, noradrenaline, adrenaline and dopamine levels were higher in those taking these drugs whilst cortisol was lower (P>0.05).

3.17.0. Levels of urinary analytes during secretory and proliferative phases of menstrual cycle.

Proliferative phase is associated with an increased oestrogen secretion (a higher oestrogen / progesterone ratio) while progesterone level is higher during secretory phase (a lower oestrogen / progesterone ratio). Boron potentiates oestrogen's influence on calcium entry into bone (Neilson, 1992) but its effects on progesterone are not known . **Table 3.9** shows the levels of these analytes.

Table 3.9. Shows the levels of urinary analytes during the secretory and proliferative phases.

Analyte	Secretory phase	Proliferative phase
calcium	0.194 ± 0.028	0.206 ± 0.048
magnesium	0.137 ± 0.017	0.177 ± 0.033
phosphate	0.138 ± 0.19	0.148 ± 0.031
boron	1.263 ± 0.13	1.361 ± 0.140
hydroxyproline	8.01 ± 1.40	8.24 ± 1.100
cortisol	0.71 ± 0.180	0.73 ± 0.160
noradrenaline	0.43 ± 0.150	0.64 ± 0.280
adrenaline	0.97 ± 0.360	1.45 ± 0.660
dopamine	4.700 ± 1.600	2.660 ± 0.520
serotonin	0.110 ± 0.025	0.104 ± 0.025

(all values μg / mg except calcium, magnesium & phosphate mg / mg creatinine).

While there was no significant difference between the levels of these analytes in these two phases, they were all higher except dopamine and serotonin during proliferative phase ($P>0.05$).

3.18.0. Levels of urinary analytes and intake of more / less than 3 types of vegetables.

3.18.0(a). Urinary calcium and intake of more / less than 3 types of vegetables

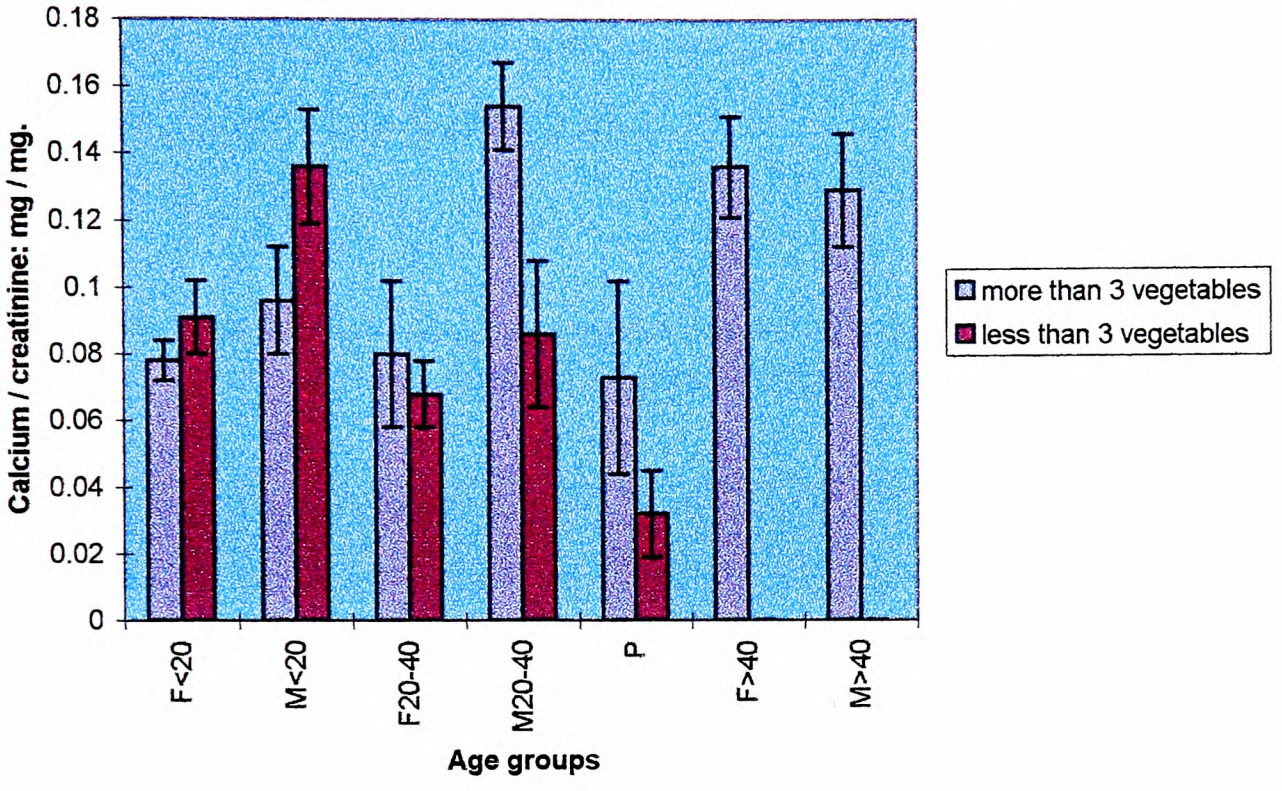
Since several subjects consumed vegetables, those eating more than 3 types formed one group while those consuming 3 and less formed the other. Although the questionnaire requested quantitative data of each vegetable consumed, those provided were unreliable and not comprehensive. Eating a variety of vegetables provides more boron than those eating less (Hunt, 1994). All F>40 and M>40 subjects ate more than 3 vegetables.

The mean levels of calcium in F<20 (n=33) eating more than 3 types of vegetables were 0.078 ± 0.006; M< 20 (n=16) 0.096 ± 0.016; F20-40 (n=12) 0.080 ± 0.022; M20-40 (n=17) 0.154 ± 0.013; P (n=14) 0.073 ± 0.029; F>40 (n=22) 0.136 ± 0.015 and M>40 (n=22) 0.129 ± 0.017 and in F<20 (n=8), eating less than 3 they were 0.091 ± 0.011; M<20 (n=8), 0.136 ± 0.017; F20-40 (n=9), 0.068 ± 0.010; M20-40 (n=4) 0.086 ± 0.022; and P (n=7) 0.032 ± 0.013 mg / mg creatinine respectively **(Fig.3.45).**

F<20 and M<20 who ate more than 3 types of vegetables had lower levels of calcium than those who ate less while the levels in the subjects between 20-40 years who ate more than 3 were higher. Urinary calcium level in M20-40 who ate more than 3 vegetables was significantly higher (P<0.05) than those eating less. Furthermore, the level in M20-40 who ate more than 3 was also significanlty higher (P<0.05) than that in F20-40. In M<20 who ate less than 3 the level was also higher but not significantly (P= 0.06) compared to F<20. The level of calcium in F20-40 eating more

than 3 types of vegetables was significantly lower (P<0.05) than that in the pregnant group.

Fig.3.45. shows the levels of urinary calcium in the subjects eating more / less than 3 types of vegetables.



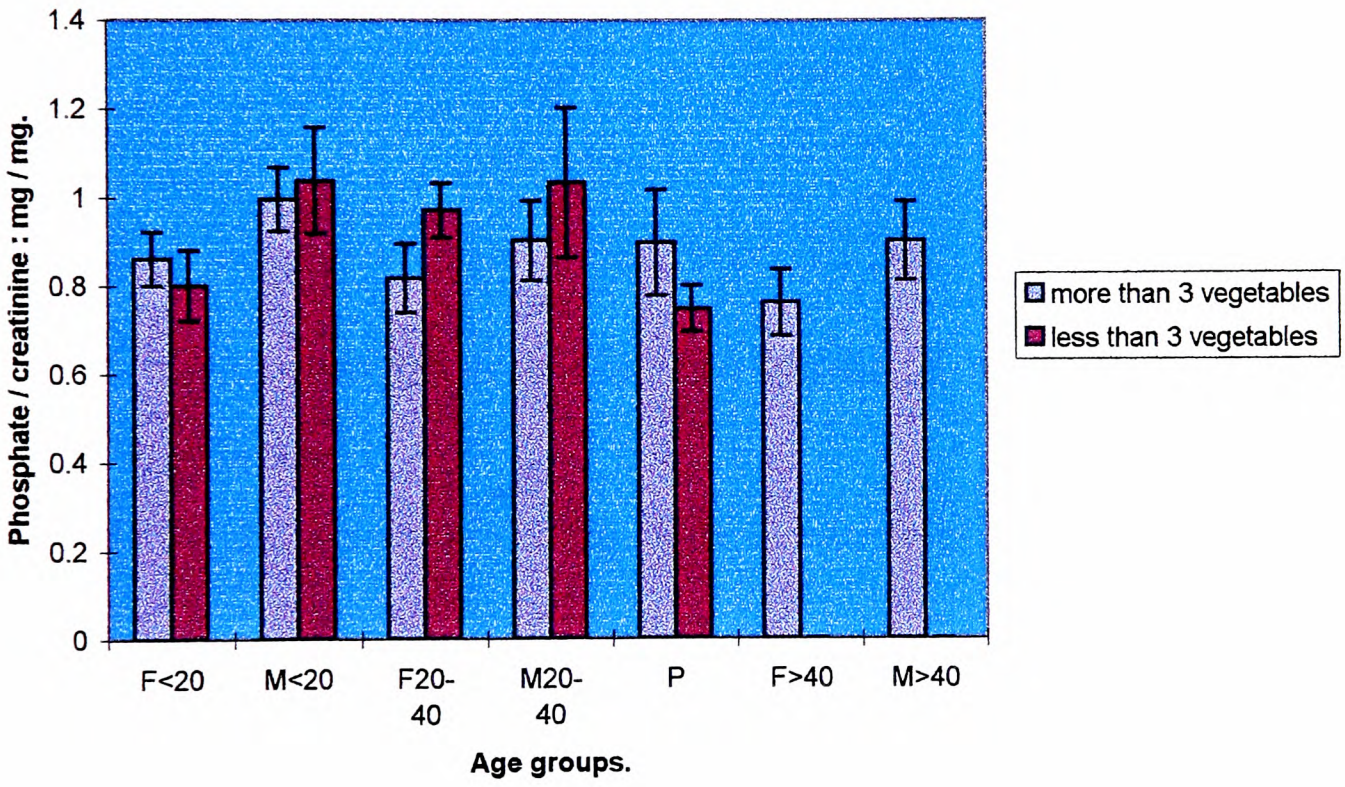
3.18.0(b). Urinary magnesium and intake of more / less than 3 types of vegetables.

The mean levels of magnesium in F<20 eating more than 3 types of vegetables were 0.095 ± 0.008; M<20 0.089 ± 0.010; F20-40 0.077 ± 0.012; M20-40 0.089 ± 0.009; P 0.072 ± 0.006; F>40 0.072 ± 0.007 and M>40 0.064 ± 0.008 while in those eating less than 3 they were F<20 0.092 ± 0.011; M<20 0.126 ± 0.013; F20-40 0.064 ± 0.005; M20-40 0.064 ± 0.013 and P 0.072 ± 0.011 mg / mg creatinine. Female subjects between the age of 11-20 and 20-40 years who ate more than 3 vegetables had higher levels of magnesium than those who ate less (P>0.05).

3.18.0(c) Urinary phosphate and intake of more / less than 3 types of vegetables.

In M<20 eating more than 3 types of vegetables mean urinary phosphate level was significantly (P<0.05) lower than that in M<20 eating less than 3 while, F20-40 who ate less than 3 vegetables had significantly (P<0.05) more phosphate in their urine than the pregnant group who ate more than 3 **(fig. 3.46).**

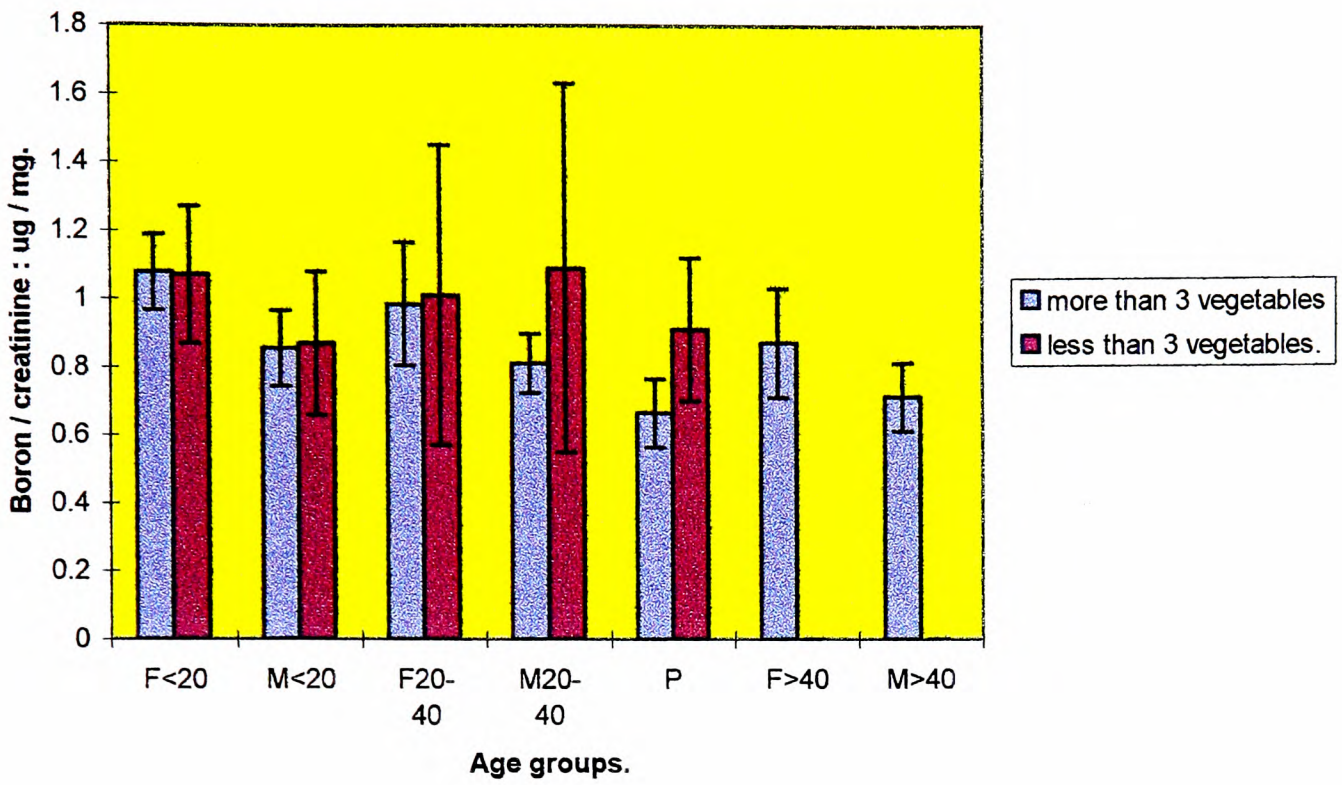
Fig. 3.46. shows urinary phosphate levels for subjects eating more / less than 3 types of vegetables.



3.18.0(d). Urinary boron and intake of more / less than 3 types of vegetables.

The results are shown in **fig. 3.47.** Female (F20-40 and pregnant women) and male (M<20 and M20-40) subjects who ate less than 3 types of vegetables had higher levels of urinary boron than their counterparts except those in F<20 but these changes were not significant (P>0.05).

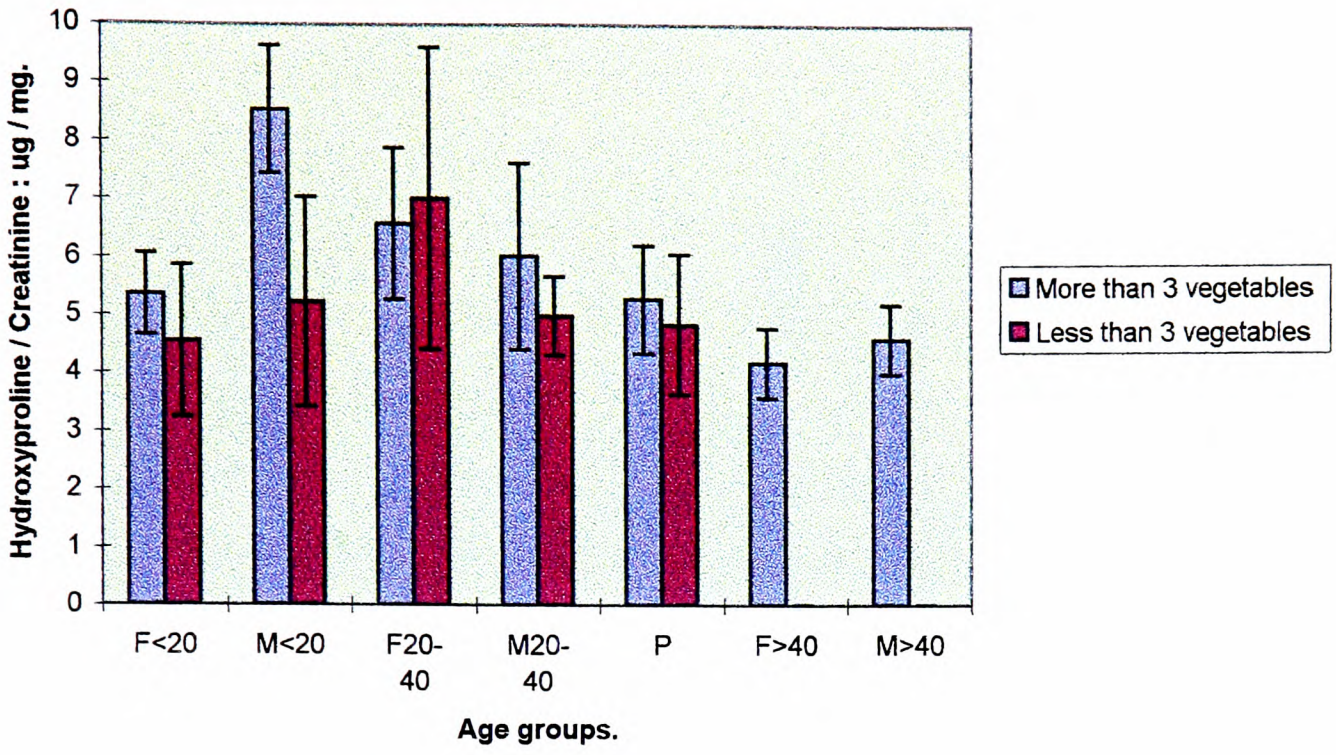
Fig. 3.47. shows urinary boron levels in subjects who ate more / less than 3 types of vegetables.



3.18.0(e). Urinary hydroxyproline and intake of more / less than 3 types of vegetables.

The results are shown in **fig. 3.48**. Subjects in each of these age groups who ate more than 3 vegetables had higher (P>0.05) urinary hydroxyproline levels than their respective counterparts who ate less except those in F20-40.

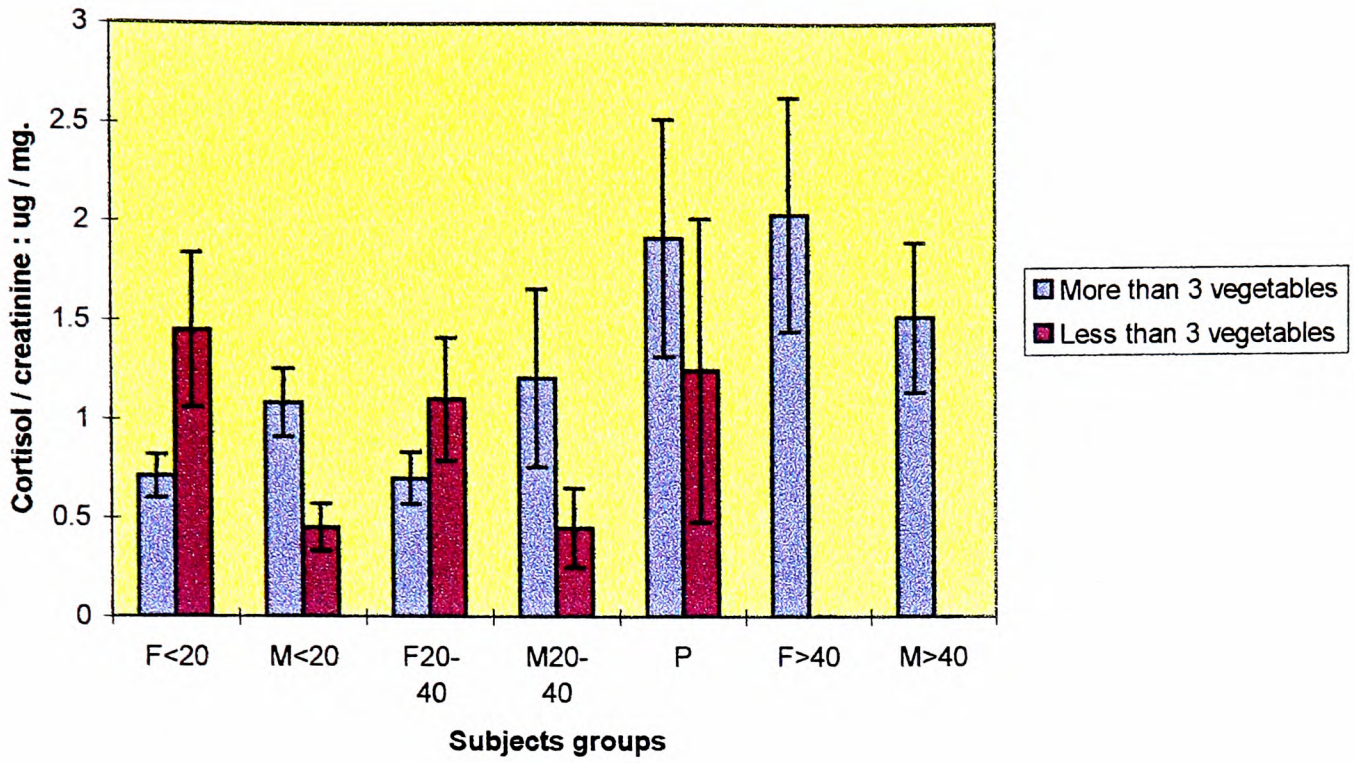
Fig. 3.48. shows urinary hydroxyproline levels in subjects eating more / less than 3 types of vegetables.



3.18.0(f). Urinary cortisol and intake of more / less than 3 types of vegetables.

Female subjects between 11-20 and 20-40 years old, but not pregnant group, who ate more than 3 types of vegetables had lower cortisol levels than those who ate less, but, in male subjects this was reversed **(fig. 3.49).** While urinary cortisol level in M<20 eating more than 3 types of vegetables, was significantly higher ($P<0.05$) than that in M<20 eating less than 3, it was not significant ($P = 0.06$) compared to F<20 who also ate more than 3. M<20 eating less than 3 types of vegetables had significantly ($P<0.05$) lower cortisol level than their counterparts in F<20. Also F20-40 who ate less than 3 had significantly higher ($P<0.05$) cortisol level than their counterparts in M20-40. The level in F20-40 who ate more that 3 was significantly ($P>0.05$) higher than that in the pregnant group.

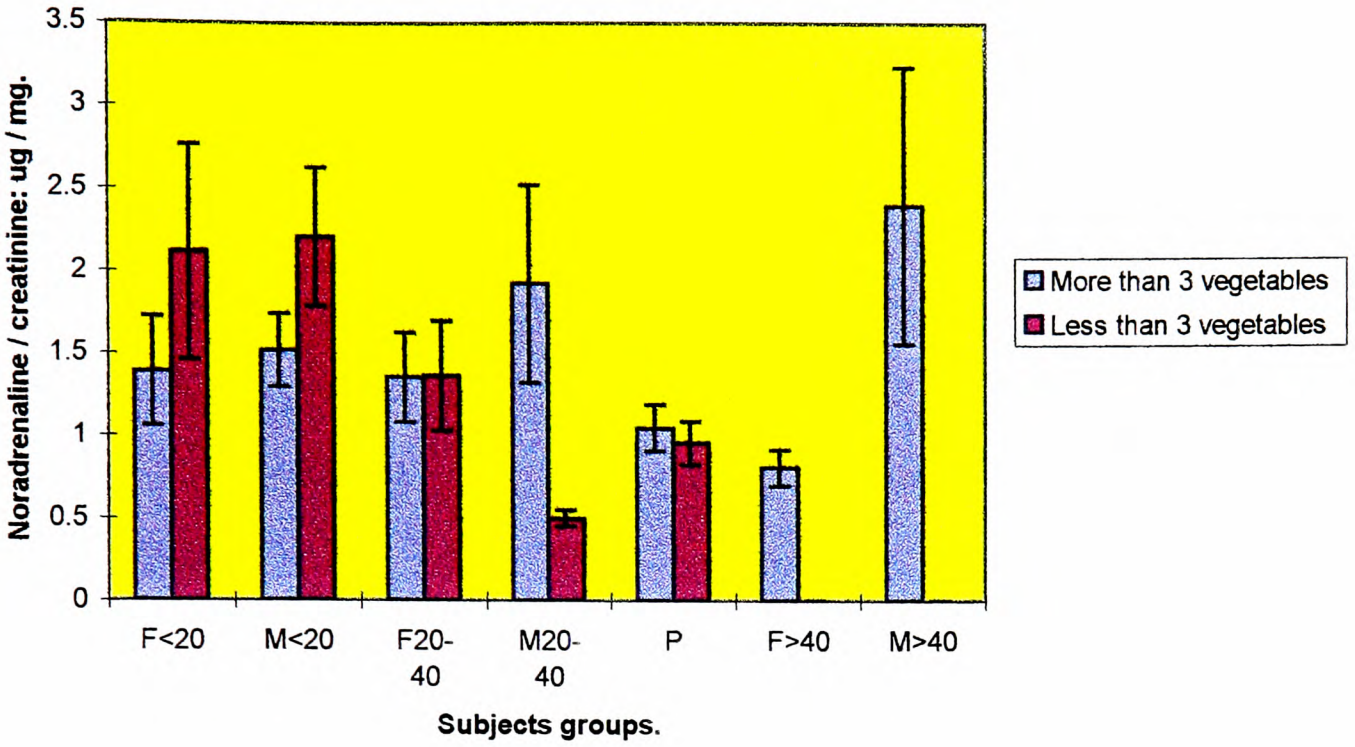
Fig.3.49. shows urinary cortisol levels in subjects eating more / less than 3 vegetables.



3.18.0(g). Urinary noradrenaline and intake of more / less than 3 types of vegetables.

While urinary noradrenaline levels in F<20 and M<20 who ate less than 3 types of vegetables were higher than their counterparts but these were not significant (P>0.05). In contrast, the levels in M20-40 eating more than 3 were significantly greater (P<0.05) compared to those eating less. Similarly, in M20-40, who ate less than 3 types of vegetables, they were greater than their counterparts in the pregnant women, F20-40, M<20 and F<20. The levels in M>40 eating 3 types of vegetables were also significantly higher (P<0.05) than those in the F>40 **(Fig.3.50).**

Fig.3.50. shows urinary noradrenaline levels in subjects eating more / less than 3 vegetables.



3.18.0(h). Urinary adrenaline and intake of more / less than 3 types of vegetables.

The mean levels of adrenaline in F<20 eating more than 3 types of vegetables were 1.61 ± 0.28; M<20 2.55 ± 0.67; F20-40 1.27 ± 0.48; M20-40 1.79 ± 0.38; P 1.60 ± 0.24; F>40 2.28 ± 0.81; M>40 2.69 ± 0.72 and in F<20 eating less than 3 they were 0.77 ± 0.13; M<20 2.34 ± 0.87; F20-40 0.92 ± 0.21; M20-40 1.36 ± 0.72 and P 1.99 ± 0.99 ug / mg creatinine. These were higher in all these age groups in male and female subjects who ate more than 3 vegetables but only the levels in F<20 were significantly higher (P<0.05) than their counterparts.

3.18.0(i). Urinary dopamine and intake of more / less than 3 types of vegetables.

The mean levels of dopamine in F<20 eating more than 3 types of vegetables were 2.010 ± 0.260; M<20 2.330 ± 0.610; F20-40 0.947

± 0.140; M20-40 1.318 ± 0.863; P 1.458 ± 0.220; F>40 1.369 ± 0.210; M>40 1.012 ± 0.080 and in F<20 eating less than 3 they were 2.610 ± 0.900; M<20 1.870 ± 0.450; F20-40 1.497 ± 0. 420; M20-40 0.863 ± 0.140 and in P 1.710 ± 0.420 ug / mg creatinine respectively. F<20 and F 20-40, as well as pregnant women, who ate more than 3 types of vegetables, had lower urinary dopamine levels compared to their respective counterparts with significantly (P<0.05) lower level in F20-40 compared to those in the pregnant women, but the trend was reversed in male subjects.

3.18.0(j). Urinary serotonin and intake of more / less than 3 types of vegetables.

The mean levels of serotonin in F<20 eating more than 3 types of vegetables were 0.069 ± 0.013; M<20 0.083 ± 0.023; F20-40 0.052 ± 0.013; M20-40 0.062 ± 0.039; P 0.037 ± 0.005; F>40 0.040 ± 0.008 and M>40 0.065 ± 0.014 while the levels for those eating less than 3 were F<20 0.071 ± 0.025; M<20 0.145 ± 0.006; F20-40 0.060 ± 0.023; M20-40 0.038 ± 0.018 and in P 0.057 ± 0.020 ug / mg creatinine. The levels were higher in females subjects in F<20 and F20-40 years old, including pregnant women, who ate less than 3 types of vegetables compared to their respective counterparts while in male subjects of similar age, the trend was reversed but none of these were significant (P>0.05).

3.19.0. The combined effects of alcohol, stress and weight bearing exercise on urinary analytes for F<20 and M<20.

The levels of urinary analytes between male and female subjects in F<20 and M<20, were compared to deduce the relationship between alcohol, stress and exercise on bone markers turnover. 4 subjects (n = 4) from M<20 and 5 (n = 5) from F<20 consumed alcohol, were stressed and did not take weight bearing exercises were placed in group A, while group B consisted of 7 subjects (n = 7) from M<20 and 10 (n = 10) from F<20 who did not drink alcohol, were not stressed and took regular weight bearing exercises. The results are shown in **table 3.10.** There were insufficient data in the other groups for this type of analysis.

The levels of the analytes in group B in F<20 were generally higher than those in group A, except for calcium, magnesium and cortisol. The levels of hydroxyproline were significantly ($P<0.05$, **fig 3.51**) lower in group B than in group A in F<20 and M<20. In group B, M<20 had significantly higher adrenaline level ($P<0.05$) than F<20.

While, the levels of calcium, phosphate, hydroxyproline and cortisol were higher in groups A in F<20; in M<20, the levels of phosphate, magnesium, boron, hydroxyproline, cortisol, adrenaline and serotonin were higher. The levels of urinary phosphate, hydroxyproline, cortisol, noradrenaline and dopamine were higher in group A than in group B for both F<20 and M<20.

Table 3.10. Shows urinary levels of analytes for groups A & B in F<20 and M<20.

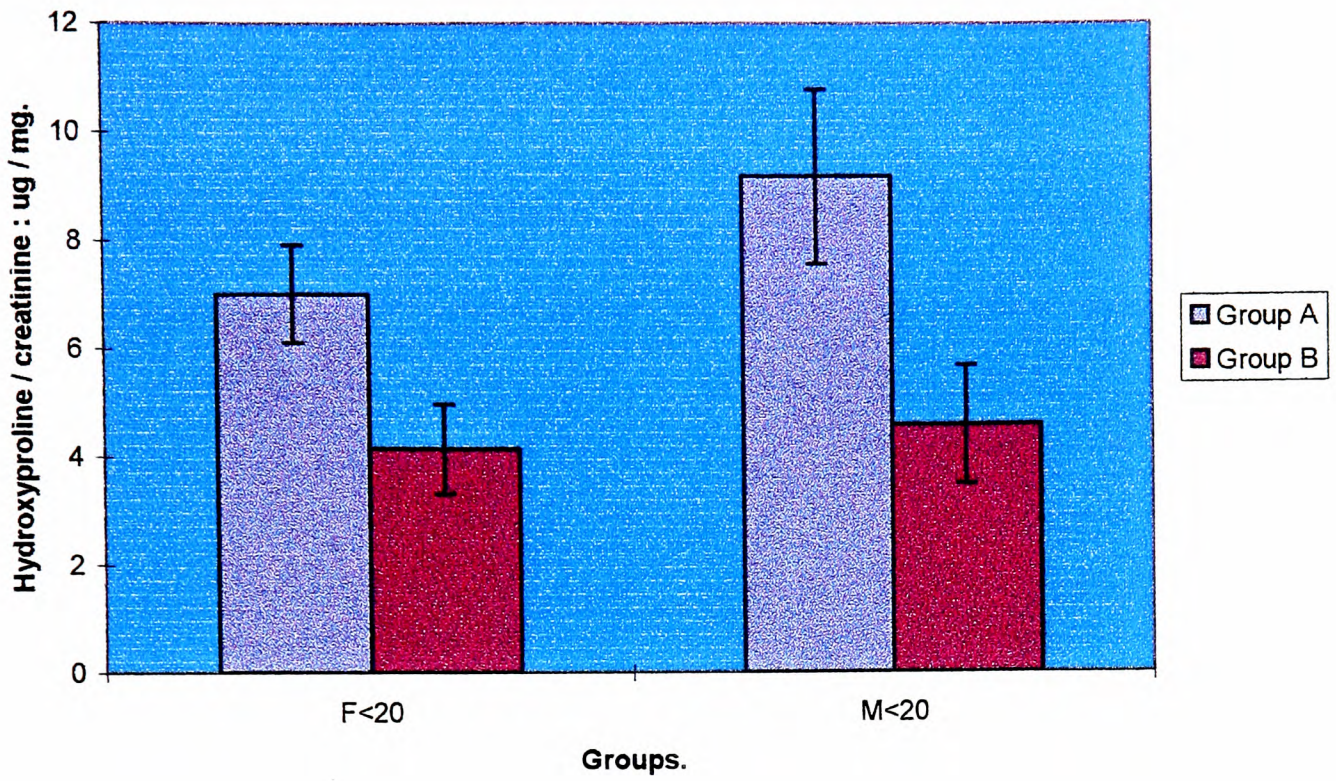
	Group	Calcium	Mg	P'phate	Boron	HP	Cortisol	NA	AD	DA	SHT
F<20	A	0.192 ± 0.110	0.127 ± 0.024	0.820 ± 0.130	1.130 ± 0.230	7.00 ± 0.90	0.479 ± 0.17	0.096 ± 0.015	0.225± 0.086	1.618 ± 0.390	0.054 ± 0.025
	B	0.109 ± 0.020	0.114± 0.017	1.202 ± 0.230	1.56 ± 0.34	4.15 ± 0.83	0.439 ± 0.140	0.203 ± 0.053	0.290 ± 0.120	2.65 ± 0.78	0.074 ± 0.020
M<20	A	0.107± 0.036	0.189 ± 0.087	1.85 ± 0.69	1.53 ± 0.69	9.18 ± 1.60	0.52 ± 0.14	0.210 ± 0.078	0.408 ± 0.180	1.945 ± 0.430	0.141 ± 0.110
	B	0.145 ± 0.027	0.133 ± 0.039	1.34 ± 0.37	0.888 ± 0.240	4.60± 1.10	0.282 ± 0.086	0.233 ± 0.065	0.73 ± 0.46	2.54 ± 0.70	0.058 ± 0.016

All values ug / mg except calcium, magnesium and phospate mg / mg creatinine.

(Group A : consumed alcohol, stressed and no weight-bearing exercise)

(Group B : did not consume alcohol, not stressed and weight-bearing exercises)

Fig. 3.51. shows urinary hydroxyproline levels for F<20 and M<20 for groups A & B.



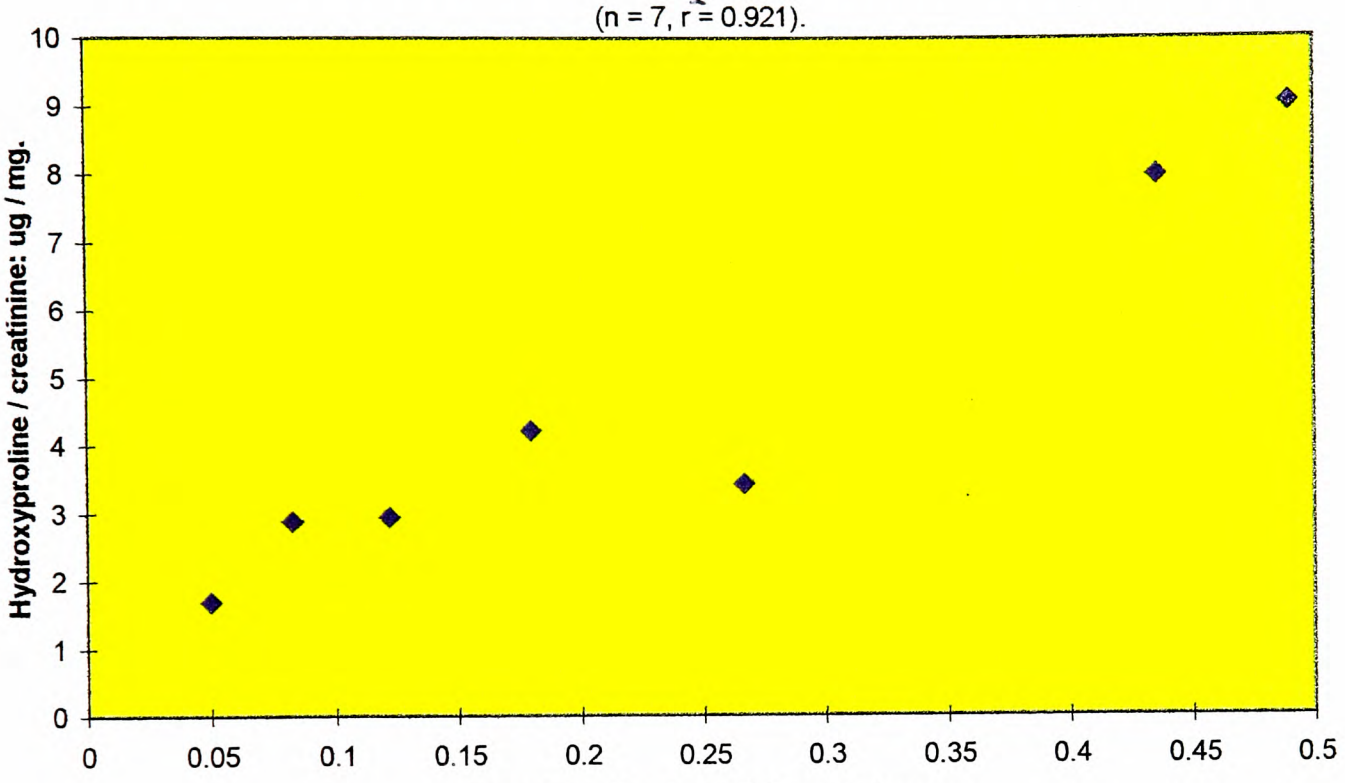
3.20.0. Correlations of urinary boron, stress hormones, serotonin and the bone markers for F<20 and M<20.

Urinary boron, stress hormones and serotonin were correlated with calcium, magnesium phosphate and hydroxyproline and the values are shown in **tables 3.11, a, b, c and d**. There were no positive correlations between boron, magnesium, phosphate and hydroxyproline in groups A or B for F<20. However for M<20 in group A, correlation of boron with hydroxyproline was significant ($P< 0.01$) with positive trends for phosphate and magnesium and in group B, it correlated significantly with magnesium ($P<0.01$), phosphate ($P<0.025$) and hydroxyproline ($P<0.025$).

There was no correlations between urinary cortisol and the bone markers in any of the groups but positive trends for calcium in group A for F<20 and phosphate in group B for M<20 but a negative trend for magnesium in group A for M<20 were seen. There were positive trends

between noradrenaline, calcium and magnesium in group A for F<20, magnesium and phosphate in group B for M<20 and a positive correlation for hydroxyproline in group B for M<20 (P<0.005, **fig. 3.52**).

Fig.3.52. shows the relationship between urinary noradrenaline and hydroxyproline in group B for M<20.
(n = 7, r = 0.921).



There was no positive correlation between urinary adrenaline and the bone turnover markers in any group , however, there was a negative correlation (P<0.005, **fig. 3.53**) with phosphate in group A for F<20.

Tables 3.11 a, b, c & d show the r^2 values for boron, cortisol, noradrenaline, adrenaline , dopamine and serotonin with bone turnover markers in groups A & B for F<20 and M<20.

Table 3.11a

F<20 Group A	Boron	Cortisol	Noradrenaline	Adrenaline	Dopamine	Serotonin
Calcium	0.609	0.726	0.056	0.357	0.261	0.135
Magnesium	0.487	0.001	0.584	0.097	0.91	0.63
Phosphate	0.082	0.321	0.178	0.929	0.013	0.004
Hydroxyproline	0.117	0.632	0.353	0.001	0.011	0.014

Table 3.11b

F<20 Group B	Boron	Cortisol	Noradrenaline	Adrenaline	Dopamine	Serotonin
Calcium	0.086	0.112	0.299	0.037	0.107	0.057
Magnesium	0.388	0.334	0.257	0.123	0.226	0.149
Phosphate	0.004	0.028	0.018	0.092	0.005	0.170
Hydroxyproline	0.351	0.021	0.014.	0.011	0.009	0.162

(Group A : consumed alcohol, stressed and no weight-bearing exercise)

(Group B : did not consume alcohol, not stressed and weight-bearing exercises)

Table 3.11c

M<20 GroupA	Boron	Cortisol	Noradrenaline	Adrenaline	Dopamine	Serotonin
Calcium	0.009	0.580	0.007	0.003	0.100	0.067
Magnesium	0.671	0.723	0.126	0.100	0.070	0.077
Phosphate	0.713	0.456	0.310	0.295	0.104	0.005
Hydroxyproline	0.883	0.400	0.001	0.002	0.027	0.399

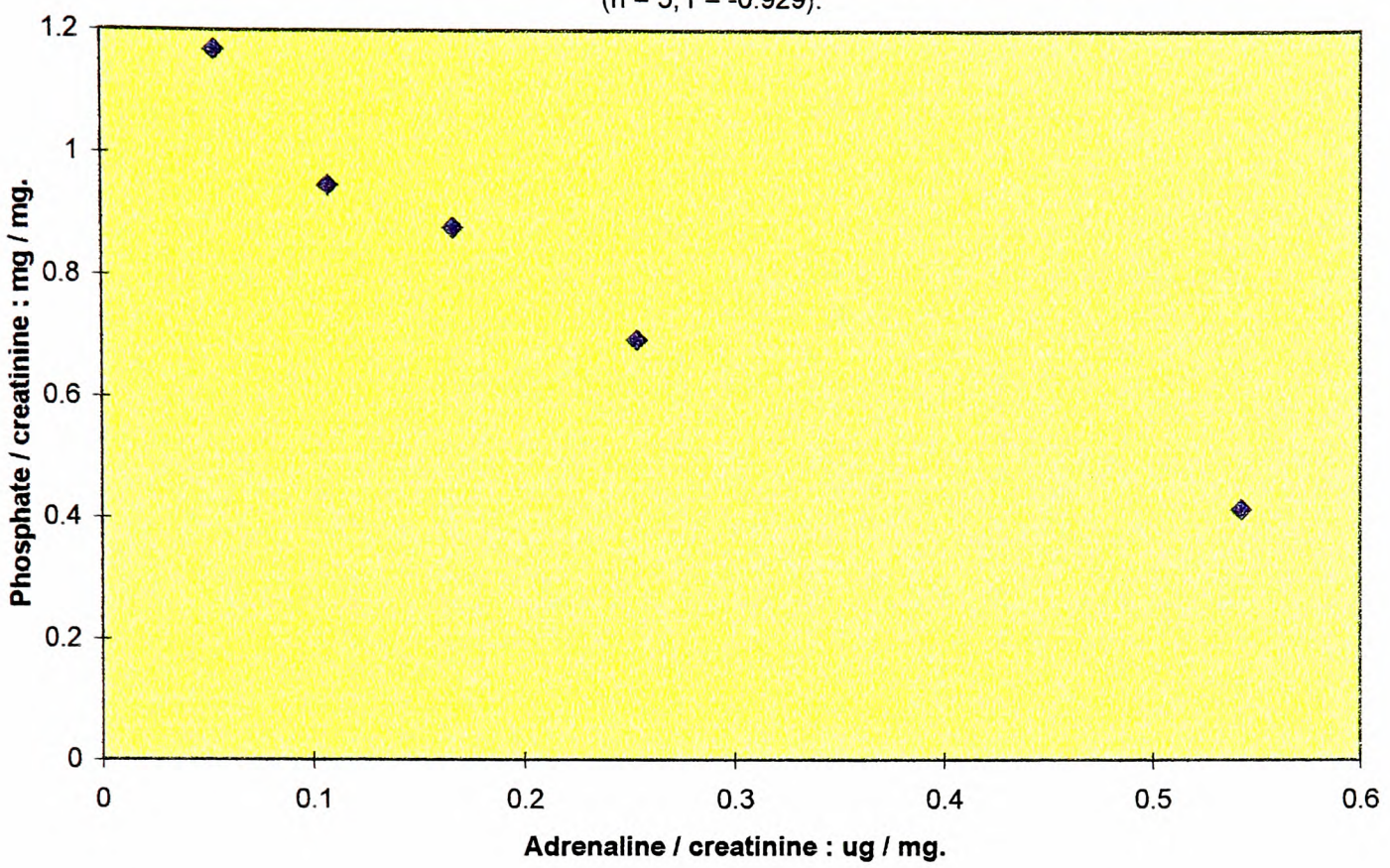
Table 3.11d

M<20 GroupB	Boron	Cortisol	Noradrenaline	Adrenaline	Dopamine	Serotonin
Calcium	0.02	0.336	0.135	0.021	0.680	0.016
Magnesium	0.781	0.304	0.512	0.071	0.128	0.556
Phosphate	0.732	0.535	0.551	0.168	0.414	0.444
Hydroxyproline	0.705	0.182	0.921	0.064	0.144	0.163

(Group A : consumed alcohol, stressed and no weight-bearing exercise)

(Group B : did not consume alcohol, not stressed and weight-bearing exercises)

Fig.3.53. shows the relationship between urinary adrenaline and phosphate in group A for F<20 ($n = 5$, $r = -0.929$).



There was no significant correlation between dopamine and serotonin and the bone turnover markers in any of the groups except between dopamine and magnesium in group A for F<20 (P<0.005) and calcium in group B for M<20 (P<0.025).

CHAPTER 4.

DISCUSSION.

4.0.0. Introduction.

This chapter includes the following:

i) discussion of the results,

ii) conclusions,

iii) suggestions for the promotion of a greater bone mass by changing diet and life style.

iv) recommendations for future work.

This study shows that there was a clear relationship between exercise and stress levels on bone turnover and a strong relationship between lower vegetable intake, excessive smoking and heavy alcohol consumption, menopause and bone resorption. However, other findings such as vitamin supplements, previous pregnancy, contraceptive pill, hysterectomy and HRT showed trends whilst family history of bone disease and phase of menstrual cycle showed no significant effect on bone.

i) DISCUSSION OF RESULTS.

4.1.0. Effects of age, gender, life style, nutrition, reproductive factors, history of bone diseases and anti-inflammatory drugs on urinary bone markers turnover.

4.1.0(a) Age.

There are many changes in diets and lifestyle which are associated

with ageing with implications for osteoporosis. Dietary calcium is less than the recommended requirement for most adolescents (Porthmouth et al, 1994) and for elderly people, especially females (Cumming, 1990). In the elderly, reduction in absorption, due to ageing, may further reduce the bioavailability of calcium and other essential nutrients for bone health. The raised serum calcium levels with increased dietary intake increase bone activity (Birdwood, 1996). Excess plasma calcium results in a corresponding loss in urine through renal filtration and excretion since the normal ranges are controlled by a number of hormones. On the other hand, a lower dietary intake increases the release of calcium from bone and its reabsorption in the kidneys to maintain the normal levels. Calcium absorption from the intestines is enhanced by vitamin D (Cooper & Umbach, 1996). The increased urinary calcium loss with age, in F>40 and M>40 suggests a greater bone resorption as the dietary intake of calcium in these subjects appears low (qualitative analysis of information on the questionnaire and on the trends indicated in the literature - Hallworth, 1998). With a low dietary calcium intake, the secretion of parathyroid hormone will increase the release of calcium from bone to maintain plasma level (Forero et al, 1987). This also suggests a decreased intestinal absorption of calcium as ageing slows down this process (Dawson-Hughes et al, 1995), or the reduced levels of circulating sex hormones at menopause causing a decrease in absorption and utilisation of calcium (Jakob et al, 1997). Reduced levels of sex hormones occur earlier in females than in males (Rozenberg et al, 1995) and as dietary intake of the F>40 is largely comparable to those of M>40 (from questionnaire), the significant reduction in the levels of female sex hormones may have contributed to the changes in urinary calcium.

Urinary magnesium levels will be increased with dietary intake or

with excessive release from bone. Although urinary magnesium excretion increased in F>40 compared to that in M> 40, (not significantly) this may suggests unrecorded dietary differences or perhaps a greater loss from bone. The continued loss of magnesium and calcium over a long time in F>40 could result in severe depletion of these elements from bone and therefore a decrease in bone mass. The reduced levels of urinary magnesium in M>40 level may be due to dietary variations, increased utilisation or reduced urinary excretion.

The increased urinary phosphate levels with age in both genders are consistent with the findings of Comspton, (1996) who suggested that kidneys become less efficient in the F>40 and M>40 and therefore are unable to reabsorb filtrated phosphate. However, it is likely that dietary intake of phosphate is normal and therefore the increased urinary phosphate excretion correlates with that of calcium in these subjects which is consistent with bone resorption in this age group (Nieves et al, 1998).

The levels of urinary boron appear to increase with age in both genders but the contrasting levels in F 20-40 compared to F<20 and F>40 may be due to variations in the type or amount of vegetables (Moseman, 1994), beer and wine (Hunt, 1994) consumed. A more detailed dietary survey is therefore necessary to assess boron level intake. In addition, boron content of foods need to be added to dietary assessment packages to promote this type of analysis. The increased urinary boron excretion with age in the female subjects, may be due to the increased consumption of vegetables in F>40 and vegetables and beer in F<20 (qualitative evidence from the questionnaires) compared to F 20-40 and thus its excretion (Moseman, 1994).

Hydroxyproline is a biochemical marker of bone resorption (Russell,

1997). The increased urinary hydroxyproline levels with age, in women and men are consistent with an increased bone collagen turnover (Kessler & Takahara, 1996). A decrease in muscle mass as a result of changes in body composition, diets and physical activities in the older age groups could reduce bone mass by promoting the loss of bone. It is also considerably higher during pregnancy when maternal bone is known to be under considerable stress due to the increased demands for bone minerals for foetal development (Tojo et al, 1998). In contrast, hydroxyproline levels are lower in the younger age groups and this is consistent with an increased bone collagen formation (Kessler & Takahara, 1996). Furthermore, as dietary intake of hydroxyproline is usually minimal, the increased urinary levels in F>40, M>40 and pregnant subjects may indicate an increased bone collagen turnover (Compston, 1996; Nguyen, 1995).

In addition, poor dietary intake of nutrients, such as ascorbic acid and vitamin K and reduced absorption from the guts in the elderly may contribute to the higher loss of hydroxyproline. Ascorbic acid (vitamin C), a co-factor in the production of hydroxyproline, promotes aggregation of ribosome in the endoplasmic reticulum to facilitate collagen synthesis (Devlin, 1997) while vitamin K is required for the synthesis of osteocalcin, a calcium-binding protein needed for mineralisation. In addition, the lower sex hormones and higher levels of stress hormones in F>40 and M>40 compared to adolescents, may also contribute to the increased urinary excretion as a result of increased bone resorption.

Bone resorption may be influenced by the increased cortisol levels in plasma with age and during stress. The significant increases in urinary cortisol in F>40 and M>40 support the findings of Laroche et al, (1997). The increased bone resorption will cause a greater loss of bone minerals

such as calcium and phosphate. Cortisol may inhibit insulin-like growth factors I and II synthesis and thus promote bone resorption (Bohannon & Kenneth, 1994) by inhibiting incorporation of proline into hydroxyproline (Vitto & Teir, 1972). The elevated cortisol levels reduce sex hormone production by decreasing the hypothalamo-pituitary influence on the gonads and also reduce oestrone production from adipose tissues resulting in the loss of bone markers such as calcium and magnesium as well as hydroxyproline (Lukert and Raisz,1990; Birdwood, 1996). In post-menopausal women, this effect may be potentiated by an increased production of melatonin as a result of the low levels of sex hormones (Cagnacci et al, 1997).

Urinary noradrenaline (NA) increases with age in both male and female subjects (Kent et al, 1993). Stimulation of the hypothalamic-pituitary-adrenal axis may be increased in older people indicating a heightened stress level. The 4 fold increase of urinary adrenaline level in F>40 compared to F<20 and the 3 fold increase in M>40 compared to M<20 may imply that subjects over 40 years old are secreting significantly more adrenaline (Hafez, 1998). From the data, it appears that F>40 and M>40 subjects are more stressed and therefore possibly more prone to bone disorders and other stress related diseases.

The decreased levels of dopamine in both genders, may be due to a general reduction in the number and activity of dopaminergic neurones with age as a result of damage from age-related metabolites of cumulative oxidative stress (Sun et al, 1999). It may also indicate that there is a greater conversion of dopamine to noradrenaline and adrenaline as the adrenal medulla is a source of dopamine in the peripheral system. However, further work is needed to elucidate possible mechanisms.

The increased levels of urinary serotonin with age in both genders

corroborate the findings of Hudgel & Gordon, (1997) that raised serotonin may contribute to the overall increase in the production of cortisol in F>40 and M>40. In addition, Tecott et al, (1995) reported that 5HT3, a multiple receptor subtype, is activated by serotonin during active chondrogenesis in the vertebral column, limbs and craniofacial region during growth and development. As ageing is related to a decrease in the rate of chondrogenesis and increase in cartilage loss (Porth, 1994), the greater levels of serotonin in the elderly, may have other effects such as regulation of catecholamines. However, the relationships between serotonin and bone turnover remain unclear and require further work.

The significant decrease in urinary creatinine in F>40 compared to F<20 and F20-40 is consistent with an age-related reduction in kidney functions and muscle mass (Baraldi et al, 1998). Creatinine levels are related to muscle mass and therefore changes may reflect changes in body composition.

4.1.0(b) Gender.

Male and female have different bone mass due to genetic differences. The higher urinary calcium turnover in M<20 compared to F<20 may be related to the increased demand for greater bone growth associated with male during puberty in whom bone and muscle mass are genetically bigger (Rozenberg et al, 1995). However, the male subjects on the whole, consumed more foods than their female counterparts who often diet to maintain a slim body image to meet aesthetic approval. The increased urinary calcium levels during pregnancy compared to non-pregnant women and men of similar ages may be due to hormones changes and other dietary factors associated with pregnancy (Ortega et al, 1998). The higher levels of sex hormones and calcitonin may protect against

excessive maternal bone breakdown during pregnancy in the advent of reduced dietary intake. In addition, the increased weight gained during this period may promote bone remodelling.

While urinary magnesium levels increased in F>40, they decreased in M>40 which may suggest a gender difference. However, gender differences alone cannot account for these changes since they are not

observed in the other age groups. The earlier onset of menopause may account for some of these changes as in the case for calcium (Goldhill, 1997).

As 17 % of the female subjects were vegetarians, lower phosphate levels are expected as vegetables are weak sources of phosphate (Fenech, 1998). The increased levels of urinary phosphate with age in both genders, may be attributed to dietary changes or to an increased release from bone. Meat diet, with a higher yield of proteins, is a rich source of phosphate (Spencer et al, 1983) and may account for the higher urinary phosphate in these omnivorous male subjects.

The absence of gender difference in the levels of urinary boron in all age groups suggests that the absorption, utilisation and excretion of boron do not vary significantly between male and female (Naghii et al, 1996). The main sources of boron are vegetables, fruits and water and it is very likely that these were consumed by all subjects because the omnivorous males also consumed some vegetables.

There were no gender differences among all the age groups in respect to cortisol. However, the significantly raised urinary cortisol levels during pregnancy compared to non-pregnant subjects and men of similar age may not be due to gender differences but to the pregnancy. The changes in urinary noradrenaline excretion between genders, within the 3

age groups, may be due to changes in sympathetic nervous system activities and therefore the secretion of catecholamines (Hinojosa-Laborde et al, 1999). During stress, the sympathetic nervous system is attenuated in females due to oestrogen (Jeong et al, 1999). Felton, (1998) reported that as nurses work in a highly stressful environment, raised levels of these hormones are to be expected. In addition, the higher adrenaline levels in the male subjects may be due to higher stress levels.
It is possible that male nurses are under more stress than the female or that they are less able to cope with the stressors associated with this type of job.

Generally, there was no gender difference in the levels of urinary adrenaline in F>40 and M>40. The older male nurses may be more experienced and therefore more able to cope with the stressors. However, the increased adrenaline levels in M<20 and M20-40 compared to F<20 and F20-40 respectively indicate that adolescent and middle-age men either have a more stressful life or respond differently to stressors or simply have poor coping strategies. In addition, the regulation of the sympathetic nervous system in the female subjects, is altered such that sympatheto-adrenal activation is attenuated or sympatheto-adrenal inhibition is augmented (Hinojosa-Laborde et al, 1999). Although, female ovarian steroids are raised before ovulation with local increases in noradrenaline level, this is subsequently converted to adrenaline through the increased production and activation of phenylethanolamine-N-methyltransferase (Murray et al, 1990) but may not influence the circulating levels of these hormones (Nguyen et al, 1999).

There was no gender difference in urinary dopamine or serotonin levels between the different age groups indicating that the sex hormones may not influence these hormones.

4.1.0(c) Life style and nutritional factors.

Alcohol.

Chronic alcohol abuse is associated with low bone density and high risk of fractures, while moderate alcohol consumption may help to maintain bone density in post-menopausal women by increasing endogenous oestrogen or by promoting secretion of calcitonin (Feskanich et al, 1999). High levels of alcohol interfere with intestinal absorption of calcium by reducing the synthesis of calcium-carrier proteins in pre-menopausal women and increase catecholamine secretions (Albers, 1990) by acting as a stressor (Horton et al, 1998). The higher calcium turnover in pregnant women who consumed more alcohol may result from a reduction in osteoblast activity (Sampson, 1998) or possible liver damage (Laitinen & Valimaki, 1991). However, the alcohol intake may not be singularly implicated as some bone loss is associated with pregnancy (Dunne et al, 1993). This effect may also be due to the levels of alcohol consumed and the degree of diuresis. A high flow of urine will dilute all analytes but the amount excreted may increase due to the high volume. The loss of analytes in urine in subjects consuming higher level of alcohol may reduce plasma concentration in susceptible individuals. This may trigger a homeostatic mechanism to compensate for the changes.

Alcohol consumption does not appear to affect the levels of urinary magnesium or phosphate.

There is an increase in the number of women between 15-20 years compared to F>40 and M>40 who regularly drink beer (Coleman et al, 1997). As beer is another source of boron (Iyenger et al, 1988), the increased urinary levels of boron in F<20 therefore may reflect an increase boron intake. In addition, the increased levels of sex hormones associated

with puberty may have also contributed to the raised boron level as they promote absorption of this element from the gut (Neilsen et al, I987). Increased consumption of boron with alcohol may be related to the greater loss in urine since excess of this analyte is excreted which may explain the changes seen in the pregnant women and in F<20.

Heavy alcohol consumption is associated with menstrual irregularities, early menopause in pre-menopausal subjects (Sarkota et al, 1999) and exaggeration of oestrogen reduction during menopause (Greendale et al, 1999). However, it is not clear if the increased levels of cortisol in the post-menopausal subjects was due to an increase alcohol consumption or to other factors such as increased stress (Wand et al, (1999).

The raised urinary adrenaline levels observed with age in female heavy alcohol drinkers compared to the male drinkers indicate an age-related gender difference. Alcohol may preferentially increase the damage to the Kupffer cells due to greater free radicals production in female subjects (Thurman et al, 1998). However, the significantly higher levels of adrenaline in M<20 compared to F<20 drinking alcohol may be due to suppression of the gonadal activity since it is unlikely that these adolescents have significant hepatic damage (Ebeling, 1998). It may also be that in females, the response to alcohol is attenuated due to decrease in sympathetic activity. There is a negative relationship between the sex hormones, alcohol and adrenaline since oestrogen decreases IL-1 and IL-2 (Piolo et al, 1992) and catecholamines increase IL-1 and IL-2 (Zima,1993). In F>40, the increased adrenaline level may be due to the lack of oestrogen and subsequent increase in sympathetic nervous system activity leading to an increased production of catecholamines.

The reduced dopamine levels with age in both genders with low

alcohol consumption supports the conclusions of Zima, (1993) that social drinking reduces the activities of the hypothalamic-pituitary-adrenal axis and therefore the levels of catecholamines. Alcohol consumption does not, generally, influence serotonin levels. However, the decreased levels of serotonin with age in both genders, particularly during menopause, is consistent with a period of increased bone resorption but its significance on bone minerals requires further work. Archer, (1999) reported an increase in serotonin level following treatment with oestrogen therapy which shows that the oestrogen may be modulating serotonin level in this age group. If alcohol suppresses gonadal activity and therefore sex hormones, it may explain the decreased serotonin levels in the older subjects.

Stress.

It is well documented that stress increases sympathetic activity and the production and secretion of catecholamines (Clancy & McVicar, 1994) and glucocorticoids (Bugajski et al, 1999). Catecholamines enhance the production of cytokines such as IL-1, IL-2 and TNF, some of which such as IL-6 may decrease bone mineralisation and promote resorption (Mysliwska et al, 1998). In addition, they also increase prostaglandin levels, particularly PGE_2, IL-1 and TNF which promote osteoclast formation resulting in increased bone resorption and calcium excretion in the stressed subjects (Lader & Flanagan, 1998).

Activation of the hypothalamo-pituiary-adrenal axis by stressors increases the secretion of cortisol which also interplay with the cytokines and affect bone. As expected both female and male subjects excrete more cortisol when stressed (Contreras et al, 1985). Stress increases the secretion of corticotropin-releasing hormone (CRH) which stimulates the

release of adenocorticotropin hormone and this acts on the adrenal cortex to release cortisol (Bugajski et al, 1999). In addition, CRH may stimulate the neurons in the sympathetic nervous system centre which may further activate the release of catecholamines from the adrenal medulla (Cahusac et al, 1998). Since cortisol is involved in the resistance phase of the stress response, further secretion of catecholamines during this period may enhance bone resorption. The increased cortisol turnover with age contributes to a higher rate of bone turnover and increases the risk of osteoporosis by causing significant alteration in plasma alkaline phosphatase activity resulting in increased urinary calcium and phosphate excretion (Islam et al, 1998). Although cortisol levels were within normal ranges, prolonged stress may predispose these subjects to bone changes possibly through a consistently higher level of this hormone or together with other causative factors. These subjects should, therefore, be particularly cautious and address any stressor which increases the level of these hormones.

Urinary noradrenaline level generally decreases with age, in both genders which may indicate that the older subjects are more able to cope with stress (Shui, 1998). The higher urinary NA level in the male subjects of all ages, compared to female may suggest that females are better at coping with stress or that there is a gender difference (Gaillard & Spinadi, 1998). The increased urinary NA levels in the non-stressed pregnant women compared to their counterparts is unexpected as stress and anxiety increase during and immediately after partuition (Milad et al, 1998). However, it is possible that the information given on the questionnaire does not reflect the true state of their stress level.

The increased levels of urinary dopamine in all ages, are expected as some dopamine is released with noradrenaline and adrenaline from the

adrenal medulla during stress.

The increased serotonin levels in F<20, M<20 , F20-40 and M20-40 support the findings of Eisman et al, (1995). Stress increases catecholamine production with increases in synthesis of IL-3 and IL-4, which in turn, promote serotonin synthesis. Yong, (1997), noted that IL-3 also stimulates bone marrow haemopoietic cell differentiation into mast cells which then produce serotonin. The decreased serotonin levels in stressed F>40 and M>40 subjects may reflect a reduction in bone morphogenetic protein-1 (BMP-1) which reduces activation of TGF-β and decreases the synthesis of procollagen and consistent with reduced bone formation (Liu et al, 1997). Stressed subjects of both genders excreted more boron than those who were not as raised levels of cytokines and prostaglandins caused by the increased catecholamines during stress might have led to increased turnover of boron which has anti-inflammatory properties (Hall et al, 1994).

Vegetables.

Vegetables contain many vitamins and minerals required for bone formation and good health. However, they also contain chelating agents which may reduce the bioavailability of some minerals such as calcium and magnesium. All subjects consumed vegetables, although the amount and types are not accurately known and this may have contributed to the variations in the results and therefore difficulties in interpretion.

Plant-based diets contain phytoestrogen which may promote bone formation especially in post-menopausal women (Albertazzi et al, 1999). Legumes, soyabeans and broccoli contain high levels of isoflavones and micronutrients essential for good health and bone (Messina, 1999; Anderson, 1999). It has been suggested that a diet containing lettuce,

tomato, cucumber, onion, garlic, parsley and dill slow down calcium loss from bone (Muhlbauer, 1999).

The higher levels of urinary calcium in M<20 and F<20 eating less than 3 types of vegetables may suggest an increase calcium turnover from other dietary sources and is unlikely to be from increased bone resorption as this period is associated with increased bone formation (Kessler & Takahara, 1996). The reduced loss of calcium in F20-40, M20-40 and during pregnancy may be due to changes in intake. However adolescent's need for minerals and vitamins may be greater than during middle age, as growth and development of bone begins to level off soon after the second decade of life (Compston, 1996). An adequate intake of these vitamins and minerals with vegetarians is therefore vital to promote healthy bone.

The increased urinary phosphate in M<20 eating more than 3 types of vegetables compared to those eating less may be due to increased dietary intake from other sources and not necessarily from bone as there were no vegetarian among them. Similarly, the higher level observed in F20-40 compared to that in pregnant women may be responsible for the increase calcium excretion as phosphate normally correlates with calcium (Cooper et al, 1996). Furthermore, as phosphate absorption from the gastro-intestinal tract is facilitated by the presence of calcium (Kaufman, 1995), the reduced dietary calcium may seriously compromise phosphate availability for bone formation.

The lack of significant differences in boron levels between vegetarians and non-vegetarians can be attributed to the vegetables consumed by all subjects as even the non-vegetarians consumed some vegetables. Further work is needed to assess the boron content of individual food.

Urinary cortisol levels of vegetarians in F<20 and F20-40 were fairly similar to those in male and female subjects eating more than 3 types of vegetables. Schmidt et al, (1997) reported that cortisol level increases in lacto-ovo-vegetarians compared to omnivorous subjects. However, their work was based on 3 months of concurrent yoga and meditation training which might have contributed to the reduced stress hormones. The increased cortisol level in F>40 eating more than 3 types of vegetables is consistent with the conclusions of Marsh et al, (1980) who reported a correlation between vegetable intake and osteoporotic bone changes in females over 50 years. However, the relationship between vegetable intake and cortisol in these subjects remains unclear. But as catecholamines levels are also low, it supports the findings of Schmidt et al, (1997). Lacto-ovo-vegetarians have higher calcium levels due to the increased milk consumption compare to omnivores (Woo et al, 1998) and lower phosphorus due to reduced meat intake (Spencer, 1983). Further work is required to identify if the loss of these elements is due to increased intake or bone resorption.

The lower excretion of urinary NA by vegetarians subjects in F<20 and F20-40 compared to their respective non-vegetarian counterparts suggests a possible link between NA and vegetables or meat-free diet as non-vegetarians in this study also ate some vegetables. Spencer, (1983) found that vegetarians have a lower bone mass than omnivores because of the lower calcium and phosphate intakes. As bone damage is increased with higher catecholamines (Carraro and Franceschi, 1997), the significantly lower NA turnover in F<20 vegetarians, suggests a possible reduction in this effect. The reduced NA during pregnancy in those eating less than 3 types of vegetables compared to their counterparts may be due to depleted micro-nutrients for the formation of NA as pregnancy is

generally associated with increased catecholamines (Fenech, 1998). The high intakes of vegetables by subjects in F>40 and M>40 may reduce the uptake of calcium and therefore decrease plasma level and consequently increase bone resorption. The significantly higher NA level in M20-40 and F<20 eating more than 3 types of vegetables compared to their counterparts needs further work as higher vegetable intake reduces anxiety and increases anti-oxidants (Rodriguez-Jimenez et al, 1998). The lower adrenaline levels in F<20 and F20-40 vegetarians may be due to general decrease in resting hormone levels. However, while vegetarians may have the necessary anti-oxidants to prevent oxidative damage, they may be deprived of phosphate necessary for bone mineralisation. Further research is needed to determine whether the lower adrenaline levels are related to vegetables intake or to other factors.

The relationship between the increased dopamine levels in men eating more than 3 types of vegetables contrast with those in females and the reason may be gender differences. Fenech, (1998) reported that male vegetarians may have up to 25% less chromosomal material due to reduced vitamin B_{12} . Low vitamin B_{12} from reduced vegetable intake, is also related to homocysteine and may produce abnormal lymphocytes with altered cytokine production. Therefore, eating more vegetables may increase production of cytokines in male but not in the female subjects, and this may account for the increased dopamine in these subjects.

The significant decrease in the levels of urinary serotonin in subjects comsuming vegetables are similar to the findings of Aja et al, (1999) who reported that amino acid-imbalanced diets of vegetarians increases brain serotonin 5-HTS 3 receptors which when activated increase hunger. This stimulus is subsequently blocked by antagonists such a secretin and cholecytokinin produced during feeding time. Subsequently,

appetite will be suppressed and the serotonin levels would fall. Low brain serotonin levels therefore may be linked to dietary intake and low in substrates needed for the synthesis of 5HT.

Vitamin supplements.

These vitamin supplements are widely used by all ages to improve health, prevent diseases and give energy and vitality. The reduction in calcium loss in M<20 and M 20-40 who took these supplements compared to their respective female counterparts and also in pregnant women who did not confirms that these vitamins contribute to the overall changes in calcium turnover and good health in these subjects (Devlin, 1997; Dawson-Hughes et al, 1997). Collectively, these vitamins, particularly vitamins C and D, increase calcium absorption, promote bone utilisation of calcium, reduce urinary calcium, prevent collagen damage by facilitating the addition of hydroxyl groups to the amino acid proline and promote the formation of cross-linkages between collagen molecules (Zerwekh & Reed, 1998).

Both male and female subjects over 40 years old who were taking these vitamins had decreased urinary magnesium and phosphate. Supplementation of these vitamins in the diet of these subjects may increase bioavailability of minerals and therefore reduce bone damage (Basle et al, 1990).

The lower urinary levels of boron in F>40 compared to F<20 taking vitamins suggests that these supplements may promote more boron utilisation during menopause as they consumed more vegetables than F<20. Boron has oestrogen-like activity which may reduce bone damage (Hunt, 1994).

Increased consumption of vitamins promotes intestinal absorption of several bone minerals such as calcium and phosphate and therefore increases bone turnover. Therefore, it is necessary to ensure that the levels of these vitamins are maintained within normal ranges for healthy bone. Reduced intake may also increase levels of minerals through increased bone resorption by increasing collagen breakdown (Malik & Meek, 1996). The significantly higher urinary hydroxyproline levels observed in M>40 who took these vitamins compared to those who did not suggests that these supplements may increase hydroxyproline turnover consistent with an increased substrates availability or bone collagen breakdown (Devlin, 1997 & Dawson-Hughes et al, 1997).

There is no direct evidence linking vitamins, cortisol turnover and bone damage. However, the works of Meydani and Blumberg, (1991) suggests that vitamin B_6 , one of the vitamins in these supplements, is necessary for a healthier immune system particularly during menopause when maximum protection against diseases such as osteoporosis and osteoarthritis is necessary. Malik and Meek, (1996) suggested that raised serum glucose levels by cortisol may increase non-enzymatic glycation and advanced glycated products which increased collagen cross-links damage and intermolecular spacing consistent with age-related changes. They also showed that higher levels of vitamins C and E significantly reduce the glucose related collagen damage. The lower cortisol levels in F>40 observed in those taking these supplements may therefore provide some protection against cortisol-induced bone diseases as well as collagen breakdown (Hosking, 1993).

Multivitamin supplements are generally associated with reduced NA and AD urinary level. The decreased levels of these hormones in female and increased in males with age in those taking the multivitamins

supplements and may indicate a gender difference. However, vitamin B_6 (Leklem & Hollenbeck, 1990) and E (Meydani & Blumberg, 1991) decrease IL-2 production while vitamin D antagonises parathyroid hormone mediated bone damage (Bunker, 1994). Furthermore as Papanicolaou et al, (1998) suggested that several interleukins, including IL-6 contribute to bone resorption during sex hormone deficiency and hyperparathyroidism. These vitamin supplements therefore may benefit older females, either by antagonising or decreasing sensitivity to PTH, magnifying oestrogen or by reducing the production of cytokines during stress which may promote calcium absorption. However, it is not certain whether the plasma levels of these vitamins were raised in these subjects as many take these vitamins to replenish poor dietary intake. Their roles in M>40 need further work.

The lower levels of urinary dopamine in the subjects not taking these vitamin supplements may be due to a general attenuation of catecholamines (Hinojosa-Laborde et al, 1999). The higher levels of dopamine in pregnant women taking these supplements may not be related to the intake of vitamins but to the pregnancy (Nguyen et al, 1999).

The increased serotonin levels with vitamin supplements in all age groups suggests that these vitamins may be promoting serotonin synthesis but the exact mechanism remains unclear (Thibault and Booth, 1999). The raised levels during pregnancy may be due to the increased production of serotonin by the placenta and may not be related to these supplements (Huang, et al, 1998). The effects of vitamin C on bone minerals during pregnancy are unclear as in many cases this is often taken as supplementation (Preston-Martin et al, 1998). However, as well as being an anti-oxidant, vitamin C is necessary for the production of healthy blood cells from the bone marrow (Ajayi & Fadiran, 1998) and healthy collagen

formation.

Exercise.

While lack of exercise reduces bone mass (Brahm et al, 1997), weight-bearing exercise increases bone mass in all age groups (Mazess & Barden, 1991; Ebrahim et al, 1997). The higher urinary calcium excretions in pubertal and middle aged male compared to female subjects suggests a gender difference which may be attributed to the bigger bone and muscle mass in men (Birdwood, 1996). Furthermore, as the male subjects were involved in more strenuous weight bearing exercises, such as rugby and football for two or three times per week, the present data support the conclusions of Alfredson et al, (1997) that this level of exercise increases calcium turnover.

Drinkwater, (1993) suggested that the positive effects of exercise on bone is dependant upon the presence of endogenous sex hormones to increase availability of calcium and phosphate. Oestrogen and testosterone are known to influence bone formation in females and males respectively (Beck et al, 1992). The higher levels of phosphate excretion in males may be due to an increase dietary intake or to increase turnover (Brahm et al, 1997). In addition, excessively high levels of exercise may reduce bone mass due to suppression of sex hormones (Alfredson et al, 1997).

The overall increased urinary boron levels in those who exercised and during pregnancy suggest that exercise increased boron turnover and may contribute to an increased bone formation as boron enhances the effects of oestrogen on bone formation. Furthermore, boron alters plasma membrane potential to prevent cytosolic pH changes by acting as an intracellular buffer (Blaser-Grill et al, 1989). The higher boron levels associated with exercise may prevent intracellular lowering of pH as the

extracellular fluid will have a lower pH as a result of the exercise. A more alkaline environment enhances osteoblast activity and suppresses bone resorption. The reversed boron levels in M>40 requires more research.

The increased urinary levels of hydroxyproline observed in M 20-40 who exercised (increased weight bearing) compared to those who did not support the findings of Thorsen et al, (1997) that the increased bone collagen and calcium turnovers may be due to mechanical loading on bone or to a combination of mechanical loading and metabolic stress. However, mechanical loading can also induce collagen secretion and therefore promote bone formation (Lanyon, 1992).

High intensity exercise increases cortisol secretion as a result of activation of the hypothalamic-hypophyseal-adrenocortical system (Vorobiev & Grigoriev, 1998) and therefore the excretion of this hormone. Raised cortisol level correlates with an increased bone resorption (Arnaud, 1993).

Exercise generally increases NA turnover in both genders due to a greater sympathetic activity as a result of physical stress (Brenner et al, 1998; Alekel et al, 1995). As bone cells possess NA receptors (Marino et al, 1997), they may be involved in bone metabolism. While cortisol, growth and sex hormones influence bone mass (Kraemer et al, 1998), the roles of catecholamines in bone homeostasis is not clear.

The higher level of adrenaline in pregnant women and M<20 not involved in regular weight bearing exercise may reflect their fitness level. In general, it is also higher in sedentary and in less fit subjects as taking regular exercise lowers the basal secretion of catecholamines due to reduced sympathetic activity (Bernet et al, 1998).

The significance of the lower serotonin levels in all age groups with exercise is unclear and requires further research.

Smoking.

There is no consensus opinion on the damaging effects of smoking on bone but heavy smoking generally affects bone status by reducing the availability of bone minerals. In F>40 and M>40 heavy smokers, there is marked bone loss (Johnston, 1994). The increased loss of calcium associated with heavy smoking in M 20-40 and during pregnancy compared to F 20-40 suggests that heavy smoking increases calciuin turnover in males as well as females and may have significant effect on bone particularly during adulthood when bone formation slows down. During pregnancy when calcium turnover is already significantly increased, this effect is exaggerated. During adolescence when bone formation is greater, regular heavy smoking may compromise bone mass by increasing the sex steroid metabolism especially in females (Law et al, 1997). However, both smoking and increased circulating sex hormones may increase calcium turnover (Hollenbach et al, 1993).

Similarly, the loss of phosphate in heavy smokers F20-40 may be attributed to the low oestrogen and possibly vitamin C levels (Birdwood, 1996).

There were no smokers in F<20, M<20 and F>40 but in general the heavy smokers, particularly male and pregnant subjects, had lower urinary boron. It is unclear why heavy smoking decreases urinary boron excretion in the male subjects and during pregnancy whilst significantly increasing it in F20-40. Further work is needed.

The increased levels of urinary cortisol in the male heavy smokers correlate with total and ionised calcium levels (Leino et al, 1998) and therefore may be responsible for the increased urinary calcium excretion (Ortega et al, 1998).

The raised NA and adrenaline levels in the heavy smokers in all age groups may be due to nicotine action on the brain (Court et al, 1998). It may also cause damage to endothelial cell which then promotes monocyte aggregation and thrombosis formation which increase adrenaline levels (Powell, 1998). In long standing heavy smokers, it may even lead to bone damage from raised levels of inflammatory cytokines (Papanicolaou et al, 1998).

The increased levels of dopamine in heavy smokers is probably due to the high level of nicotine which increases the release of endogenous opioids which in turn increase the levels of dopamine (Ismail & el-Guebaly, 1998). Further work is needed to elucidate the roles of smoking on urinary serotonin levels.

4.1.0(d) Reproductive factors.

Pregnancy.

The higher level of calcium during pregnancy compared to non-pregnant women may be due to the extra demand placed on the mother's calcium stores by the growing foetus (Ortega et al, (1998). This may cause a transient loss of bone mass and if not corrected, increase the risk of osteoporosis.

The raised levels of urinary magnesium observed during pregnancy and in women with repeated pregnancies, are probably associated with the raised urinary calcium levels (Ryan 1991) and therefore increases magnesium loss (Zumkley & Lehnert, 1984). However, it may also be due to increased dietary intake during pregnancy or/and increased sex hormones which promote absorption of magnesium.

The raised level of urinary phosphate seen during pregnancy

compared to non-pregnant women may be due to increase bone mineral turnover as a result of the raised levels of cortisol and catecholamines hormones during pregnancy (Smith, 1998). However, these levels were also raised in M20-40 compared to F20-40 which may suggest a greater dietary intake.

The increased levels of oestrogen with repeated pregnancy promote bone formation provided adequate nutrients are consumed. The increased boron loss in women with no previous pregnancies compared to those pregnant for the first time and in women with no children compared to those with children confirms the positive relationship which exists between boron and oestrogen (Naghii & Samman, 1997).

The higher urinary hydroxyproline level during pregnancy compared to non-pregnant women and men of similar age may be due to the transient increase in collagen turnover associated with pregnancy (Nguyen et al, 1999).

High levels of cortisol seen during late pregnancy compared to non-pregnant women may be due to the additional production by the foetus (Yoon et al, 1998). Cortisol turnover is also higher during pregnancy compared to male subjects of similar age and therefore pregnancy may be the main reason for this increase as the foetal adrenal cortex produces cortisol in the third trimester and is involved in partuition (Mesiano & Jaffe, 1997).

Catecholamine levels also increase during pregnancy (Smith, 1998). However, in this study, NA levels in pregnant and non-pregnant women as well as in men of similar age, do not show any significant differences. Further work is needed particularly as the levels of adrenaline in pregnant women, M20-40 and in non-pregnant females of similar ages, increased significantly. The raised levels may suggest an increased activity of the

autonomic nervous system which stimulate the adrenal medulla is common to all normal pregnancies (Milad et al, 1998). In addition, it would appear from the results that psychological stress may be enhanced as some adrenaline levels were also increased.

The data in this study show no pregnancy-related differences in the levels of urinary dopamine although these are frequently raised in the third trimester of pregnancy to increase uterine contraction in preparation for labour (Moustafa et al, 1999).

It appears that urinary serotonin levels are not affected by pregnancy although Huang et al (1998) reported that the placenta produces serotonin for the development and maintenance of the placenta and regulation of foetal development. However, further work is necessary with a bigger population sample on the roles of dopamine and serotonin in pregnancy.

Menstrual phases.

The proliferative phase of menstruation is associated with higher oestrogen and lower progesterone levels and in the secretory phase these levels are reversed. While this study did not show any significant differences in urinary boron level during these two phases, all urinary bone minerals and boron were higher during the proliferative phase which may be due the increased oestrogen levels and therefore an increase in bone turnover (Meecham et al, 1994). During the proliferative phase adrenaline is increased which may be involved in maturation of the ovarian follicle prior to ovulation (Arimura, 1998). Oestrogen increases the activity of the phenylethanoline-N-methyltransferase which converts NA to AD. In contrast, oestrogen may suppress the activity of the sympathetic nervous system thereby decreasing the levels of adrenaline. This latter effect may alter the overall effect seen for adrenaline.

Contraceptive pill.

Taking the contraceptive pill not only increases the levels of circulating oestrogen but also maintains it at a fairly constant level (Acien et al, 1997). The significantly higher boron levels in these subjects compared to those not taking the pill, confirms earlier findings in this study, that boron levels are related to those of oestrogen and that such a combination promotes greater uptake of bone minerals and increases bone formation (Naghii & Samman, 1993). Synthetic oestrogen lowers the levels of cytokines such as IL-1 and IL-6 (Pioli et al, 1992) as well as inhibiting sympathetic activity (Favier et al, 1997). This may account for the lower levels of noradrenaline and adrenaline as these hormones modulate the production of cytokines. The contraceptive pill therefore may increase bone mass by sustaining a higher level of oestrogen as well as reducing levels of circulating catecholamines.

Menopause.

Menopause is associated with loss of ovarian sex hormones and therefore a reduction in bone minerals and consequently bone mass. This is supported by the significantly higher urinary calcium in post-menopausal women (Compston, 1996). The raised urinary boron levels during menopause compared to pre-menopause subjects may be due to the increased vegetable intakes. The increased boron turnover promotes oestrogen synthesis during the menopause which increases intestinal absorption and decreases renal excretion of calcium so that more calcium is available for bone formation (Bendadour et al, 1998). The reduction of oestrogen level at menopause may increase stress hormones levels which may antagonise the benefit from therapeutic boron level (Kamesaroff et al, 1999). The significantly higher levels of urinary dopamine levels in post-

menopausal compared to pre-menopausal subjects may be related to the reduced oestrogen level or increased loss of calcium but the exact relationship remains to be deduced particularly as NA and AD levels were lower in these subjects.

HRT.

Hormone replacement therapy (HRT) which contains synthetic oestrogen and progesterone reduces cortisol levels via the hypothalamic-pituitary-adrenal axis (Laroche et al, 1997). The slightly higher urinary calcium, magnesium, phosphate and hydroxyproline levels in subjects not on HRT may suggest a greater turnover of these bone markers due to the reduced sex hormones (Hallworth, 1998)

HRT also increases boron turnover which may modulate the effects of oestrogen to increase calcium absorption and therefore increase bone mass in post-menopausal women as seen for pre-menopausal women on the pill (Nielsen et al, 1987).

In contrast to the raised cortisol level in pre-menopausal women taking the contraceptive pill, urinary cortisol levels in post-menopausal women on HRT are lower. The increased supply of oestrogen from the HRT decreases cortisol turnover through the regulation of the hypothalamic-pituitary-adrenal axis, and therefore may delay the onset of bone damage in perimenopausal (Komesaroff et al, 1998) and post-menopausal women (Lees et al, 1995; Stampfer & Grodstein, 1994)

The higher noradrenaline and adrenaline levels in F>40 not on HRT may indicate an increased activation of the sympathetic nervous system as a result of the reduced oestrogen levels. Therefore F>40 not on HRT may be at a greater risk of bone damage from the raised catecholamines (Cagnacci et al, 1997; Lees et al, 1995).

Hysterectomy.

All subjects who had hysterectomy were taking oestrogen and progesterone (HRT) as part of their replacement therapy. The lower urinary calcium levels in F>40 who had hysterectomy may be due to the higher level of these hormones and indicates that HRT improves calcium turnover and benefits bone (Tilyard et al, 1992). This therapy becomes very significant as the raised stress hormones levels in these subjects may promote adverse bone changes.

4.1.0e History of bone diseases.

To date, only vitamin D (Cooper & Umbach, 1996) and oestrogen receptors (Notelovitz, 1997) have been genetically implicated in bone diseases such as osteoporosis and osteoarthritis. If these bone diseases are due to genetic defects, then parents with these diseases will pass them on to their offspring. However, if these disorders are the results of environmental factors, then subjects may display symptoms without a family history. The data in pubertal and middle aged women in this study suggest it may be hereditary because of the increased urinary calcium levels which may also result from reduced or inactive vitamin D receptors. As urinary calcium is increased in the elderly subjects, a reduction of vitamin D receptors may occur from cumulative oxidative damage.

Further work is required to determine the genetic control and utilisation of boron in bone particularly between F20-40 and F>40 with a family history of these bone diseases (Barr et al, 1996).

The significantly higher levels of urinary hydroxyproline in M20-40 without the family history does not indicate genetic involvement alone. However, it may involve some genetic elements interacting with environmental factors.

The contrasting levels of urinary cortisol in F20-40 with the family history compared to those in M20-40 and in pregnant women without the history compared to those in F20-40 suggest a more complex relationship between a genetic basis for these diseases, cortisol and bone.

The increased levels of urinary adrenaline levels in pregnant subjects with the history of bone diseases compared to all other female subjects require further evaluation as pregnancy is normally associated with an increased level of this hormone. Adrenaline stimulates the production of cytokines such as IL-1, IL-6, monocyte colony stimulating factor and tumour necrosis factor which may also enhance the bone resorbing capacity of osteoclast (Roodman, 1993). Therefore, the higher adrenaline levels in F>40 without the history of these diseases cannot be considered as having a genetic basis.

It is not clear how the lower serotonin levels in F20-40 and F>40 as well as in M<20 and M>40 who suffered from these diseases compared to their respective counterparts affect bone status or if these have any genetic significance at all. Further research is needed using a more appropriate method with molecular biological techniques.

4.1.0f. Anti-inflammatory drugs.

Subjects in F>40 and M>40 were patients from one of the local hospitals and were prescribed anti-inflammatory drugs such as aspirin, indomethacin and voltarel to control pain associated with these bone diseases. These drugs reduce local mediators such as prostaglandin E_2 (PG E_2) cytokines and may influence bone health (Kirkham, 1991). PGE_2 is produced from prostaglandin H_2 , a by-product of fatty acid cylooxygenase action on arachidonic acid from which all prostaglandins are produced. These drugs block the action of fatty acid cylooxygenase and reduce the

levels of prostaglandins. Patients with bone diseases such as osteoporosis may benefit from anti-inflammatory drugs such as voltarel and aspirin as these medications appear to reduce these local mediators.

The higher urinary noradrenaline and adrenaline levels seen in subjects who were taking anti-inflammatory medications may be due to the illness which acts as a stressor. However, increased levels of these hormones, if not controlled, may enhance bone loss through increased production of cytokines.

4.2.0. The relationship between boron, stress hormones, serotonin and bone markers.

4.2.0.(a) Urinary boron, bone markers and hydroxyproline.

Although there was no significant correlation between urinary boron and calcium, the positive trends observed, with age, in the male subjects indicate that perhaps with a larger sample size, significant correlation may be obtained as boron increases calcium absorption from the intestines at therapeutic testosterone levels (Hunt, 1994; Naghii & Samman, (1996). Therefore, as the male sex hormone changes with age perhaps boron may have a role to play in bone turnover.

The protective effects of boron on bone is enhanced in post-menopausal women at low magnesium levels (Neilsen, 1994). The correlation between boron and magnesium in M<20, M20-40 and F20-40 suggest a similar effect in these pre-menopausal subjects as well (Volpe et al, 1993). There was no significant correlation between boron and hydroxyproline.

The significant correlation between urinary boron and phosphate in male subjects, in the 3 age groups, indicates that boron may be

contributing to bone metabolism as the absorption of phosphate and calcium is greater with dietary boron supplement (Hunt & Nielsen, 1987).

4.2.0.(b) Urinary stress hormones, serotonin and bone markers.

The positive correlations between urinary cortisol, calcium, magnesium and phosphate in male but not female subjects, with age, suggest that male subjects of all ages are more susceptible to the influence of cortisol, a hormone which is increased during stress. The stage of the menstrual cycle may have contributed to the lack of correlation in female subjects as oestrogen may have reduced cortisol, stress hormones and serotonin levels (Komesaroff et al, 1999). M>40 subjects have higher cortisol levels and may be more vulnerable than F>40 to changes in bone calcium, magnesium and phosphate turnovers. The significant correlation between cortisol and hydroxyproline during pregnancy indicates an increase collagen turnover.

Urinary noradrenaline correlation with calcium, with age, again suggests that post-menopausal female and male subjects are particularly vulnerable to increased calcium turnover. In post-menopausal women, the lower oestrogen levels may have contributed to the positive correlation between calcium and noradrenaline. During pregnancy when stress level is also high a positive correlation is also found. The effects of the raised steroids in the pregnant subjects may have been compromised due to the stress levels.

The significant correlation between urinary adrenaline, calcium and phosphate in M>40 and F>40 indicate that older people of both genders are highly stressed and susceptible to increased loss of bone minerals.

Male subjects, of all ages, with higher dopamine levels may lose more calcium and phosphate as higher level of dopamine alters plasma

membrane permeability to these minerals (Huet et al, 1999; Baines & Drangova, 1998).

4.2.0.(c) Urinary boron, stress hormones and serotonin.

The significant correlations between urinary boron and cortisol levels in all male subjects, especially in M20-40, catecholamines in M20-20 and in M>40 and serotonin in M20-40 suggest that urinary boron level may be modulated by catecholamines possibly via changes in testosterone levels particularly as magnesium level was also lower in M>40 than in F>40 (Neilsen et al, 1987).

4.3.0. The relationship of alcohol, stress and weight bearing exercises on bone in F<20 and M<20.

The higher urinary levels of cortisol with significantly higher hydroxyproline levels in the male and female subjects in group A (heavy alcohol, stressed & no weight bearing) suggest that these lifestyle contribute to increased turnover of collagen and may compromise bone health. In contrast, the higher levels of NA, AD and DA with lower hydroxyproline levels in group B (low alcohol, not stressed & weight bearing) indicate a lower collagen turnover with better bone health. A larger population sample size is needed to confirm these findings and assess the implications for bone status in these subjects.

4.4.0. CONCLUSIONS.

This research shows that lifestyle such as reduced vegetable intakes, heavy smoking and alcohol consumption and lack of weight bearing exercise increase stress level. As a result, the higher levels of cortisol, catecholamines (NA,AD & DA) and 5HT increase the excretion of calcium, phosphate and magnesium which may compromise bone health. The higher levels of boron observed in these subjects offer some protection against excessive resorption.

The main findings are as follows:

Boron and urinary bone markers:

1. There are significant correlations between boron and phosphate with positive trends between urinary boron and calcium in male subjects of all ages indicating greater turnovers of these bone minerals due to increased bone activity.

Age, gender, life-styles and nutrition, reproductive factors, history of bone diseases and inflammatory drugs on urinary bone markers:

2(a) Age and gender are not correlated with significant changes in urinary boron levels.

2(b) Alcohol decreases urinary boron levels in both genders with age but increases it during pregnancy.

2(c) Stress significantly increases urinary boron levels in male subjects particularly in M>40 with increased calcium turnover to promote greater calcium utilisation by bone.

2(d) Increased vegetables consumption and history of bone diseases do not

significantly alter urinary boron level.

2(e) Multivitamin supplements increase urinary boron in male but decrease in female subjects. Urinary boron levels correlate with those of magnesium and phosphate in male subjects.

2(f) The levels of urinary boron decrease with weight bearing exercises, smoking and during pregnancy but it is increased in subjects taking the contraceptive pill and HRT and also following the menopause.

Stress hormones and serotonin and urinary bone markers:

3(a) An increased urinary cortisol is linked to an elevated calcium, magnesium and phosphate turnover in pregnant and in male subjects of all ages and these suggest an increased risk of osteoporosis in these subjects in later life.

3(b) Raised urinary NA increases calcium turnover in male subjects of all ages and also calcium, magnesium and phosphate in pregnant subjects suggesting a link between NA and these bone minerals.

3(c) A higher urinary adrenaline level increases urinary calcium and phosphate turnovers with age in both genders and also calcium and magnesium during pregnancy but only increases phosphate level in the female subjects. This indicates a strong association between adrenaline, calcium and phosphate with age which may contribute to bone changes through raised cytokines levels.

3(d) The positive trends between urinary dopamine and calcium and urinary serotonin and all the bone markers in the male subjects are not conclusive and require more work.

Boron, stress hormones and serotonin:

4(a) There are significant correlations between boron and phosphate in all

male subjects and to adrenaline and serotonin in M20-40 which may suggest a positive relationship between boron, adrenaline and phosphate in these male subjects.

Implications for nurses:

4(b) Male nurses are more at risks of bone mass changes than females because of the increased levels of cortisol, adrenaline, calcium, phosphate and hydroxyproline.

4.5.0. Promotion of greater bone mass.

Diet.

Dietary boron level must be between 0.8-1.2 mg/day (boron value of commonly consumed vegetables and fruits is given in appendix 2). More foods containing calcium (milk, cheese, fish and butter) and phosphate (meat, eggs, whole grains, raw wheat germ, nuts and seeds) must be added to the diet to replace the increased loss of these elements particularly in the elderly. In addition, ascorbic acid supplement to increase hydroxyapatites and hydroxyproline substrates must be taken to reduce collagen cross-links damage.

Multivitamins supplements.

Males and females of 11-20 and 20-40 years old must consume an adequate supply of vitamins to protect bone and for good health. Taking these vitamins in later life may also benefit bone but require more research. Boron supplement may help in promoting bone mass in all subjects.

Stress.

Subjects of all ages must keep stress level low particularly in F>40 and M>40. Work environments as well as personal circumstances must be changed to reduce stress levels as males nurses are at greater risks of bone damage that female nurses due to higher levels of stress hormones, cortisol and adrenaline. Employers should provide stress management strategies to support workers in the work environment (Shiu, 1998) as presently many NHS Trust fails to debrief staff who experience severe psychological and physical stresses at work such as coping with a client's death and physical

assaults by violent patients (Felton, 1998).

Smoking.

Smoking must be avoided by all especially pregnant women and female nurses, as it may lead to an increase in phosphate excretion. Males nurses must not smoke as it increases urinary calcium levels more than in female nurses. Female nurses who do not smoke are generally less stressed.

Vegetables intake.

All subjects must eat sufficient vegetables as those eating fewer than 3 types have raised urinary phosphate, cortisol, noradrenaline and dopamine levels. Female nurses must eat sufficient vegetables regularly to decrease urinary calcium excretion and protect their bones. Vegetarian diet in F<20 shows a reduction in noradrenaline, adrenaline and serotonin levels but may cause deficiencies in other nutrients.

Weight bearing exercises.

Light exercises, taken regularly by subjects of all ages, benefit bone but prolonged high intensity weight bearing exercises may reduce bone mass especially in subjects under 20 years old.

Menopause and HRT.

The incidence of back injuries is high among nurses (Love, 1996) and early preventative intervention program is recommended to decrease the overall incidence of pain and disability in back-injured nurses (Blue, 1996). Menopausal female nurses who continue to work need to consider HRT as it promotes bone health through increased supply of important

bone minerals.

Alcohol intake.

Moderate alcohol consumption (less than 750 ml of wine , 1500 ml of beer or 150 ml of spirits / week) decreases calcium and magnesium excretion in female but not in male or pregnant subjects. Females nurses who consume moderate amount alcohol must do so with caution as the number of female nurses abusing alcohol is increasing (Coleman & Honeycutt 1997). However, heavy drinkers risk losing bone minerals and may suffer bone damage such as osteoporosis in subsequent years.

4.6.0. Recommendations for future research.

Since a number of trends were identified by this study, a larger population is needed to determine whether these are significant. The following work is suggested:

1. The relationship between boron and hydroxyproline levels to deduce its role in collagen formation and breakdown. Other bone markers may be determined as comparisons.

2. The relationship between the type and amount of beer, wine and stronger alcohol (spirits), on boron and bone markers.

3. The effects of vegetarian diet on production of stress hormones (cortisol, noradrenaline, adrenaline and dopamine) and serotonin. This study found a strong link between stress (adrenaline), boron and bone markers but further study on the relationships between boron, noradrenaline, dopamine, serotonin and bone markers is recommended using both in vitro and in vivo studies.

4. The level of stress hormones during the different phases of menstruation and their relationship with sex hormones needs to be studied further to identify their effects on bone.

5. The effects of multivitamin supplements on bone status in male and female subjects of all ages but particularly in men over 40 years old. An assessment of the levels of vitamins and micro-nutrients are needed.

6. The use of questionnaire to assess psychological and physical stressors among nurses and their effects on bone.

7. A detailed asssessment of boron intake in nurses.

8. Considerable difficulties were experienced in obtaining subjects especially at the lower age group. It might be necessary to increase the catchment areas to obtain a larger population for this type of study or to focus on a particular age group. In any case, other schools, hospitals and universities would need to be considered to increase the number of subjects.

APPENDIX 1

NO:

QUESTIONNAIRE:

ROLE OF BORON ON BONE TURNOVER. (OSTEOPOROSIS)

STRICTLY CONFIDENTIAL

A:PERSONAL DETAILS:

1. Age.....yrs. .....months.

2.Sex.....(M/F).

3.Weight.....kg... .....g.(or .....lbs or .....stones).

4.Height.....m .....cms.(or.....ft.....ins).

5. Country of origin.....

6.Ethnic origin:

Are you(please circle) (a) Afro-Caribbean?.....yes/no

(b)Asian?.....yes/no

(c) Caucasian?.....yes/no

(d) Others?(please state).....

B. OCCUPATION:

7.Are you a student? (please circle).....yes/no.

If so which a)Course:.....

b)Present stage.....

c)Duration of course.....

8.Are you employed/unemployed?(please circle).

9.Please state nature of employment if applicable.....

C: **HEALTH & DIET:**

10.Are you in good health.(please circle).....yes/no.

11.Do you drink alcohol?(please circle).....yes/no

12.On average how often do you drink alcohol?(please circle).

(a) once or twice a year.
(b) once a month.
(c) once a week.
(d) two to four times a week
(e) every day.
(f) other (please specify).....

13.In an average week do you drink any of the following?

(please circle whichever is applicable)

(a) larger/bitter/cider.....how much?...../week.
(b) wine.....how much?...../week.
(c) spirits.....how much?...../week.
(d) any other.....how much?...../week

14.Which type of alcohol do you drink most regularly?
(please circle only one).

(a)beer(including ale, larger, bitter, cider).
(b)wine
(c)spirits.
(d)others .(please specify).....

15.Are you usually under stress?(please circle).....yes/no.

16.If yes, how stressed are you?(please circle).

(a) highly stressed
(b) mildly stressed.
(c) occasionally stressed.
(d) rarely stressed.
(e) not stressed at all.

17.Do you smoke cigarettes/tobacco?(please circle).....yes/no.
18.On average how many cigarettes/tobacco do you smoke per day?.....

19.How long have you been smoking?.....(nearest year).

20.On average how often do you exercise?
(a) never
(b) two or three times ayear
(c) once a month
(d) once a week
(e) two or three times a week
(d) every day
(e) other (please specify).....

21.Which type(s) of exercise(s) do you do?
.....
.....
.....
.....

22.Of these which one(s) do you do most frequently?

23.Is your diet mainly(circle one):
(a) vegan?(no meat or diary products)
(b) vegetarian?(no meat).
(c) meat based but in the form of fish or chicken only?
(d) meat based (any type of meat)?

24.Do you take vitamins/minerals supplements?(please circle) yes/no.

25.If yes, what is it called?.....

26. How often do you take this?.....

27.Which cooking oil do you use in your cooking?(please circle).

(a) vegetable
(b) ghee
(c) olive oil
(d) fish oil
(e) animal oil
(f) others (please specify).....
(g)None

28.Do you consume drinks containing caffeine eg tea,coffee or fizzy drinks?(please circle)

(a) never
(b) occasionally.How much...../week.
(c) regularly. How much...../week.

29.How much of the following vegetables do you consume weekly?(please give weight in grammes if possible)

(a) potatoes.....
(b)sweet potatoes.....
(c)carrots.....
(d)cabbage.....
(e)brocolli.....
(f)lettuce.....
(g)beetroots.....
(h)greens.....
(i)spinach.....

30.How much nuts you consume weekly?(please give weight in grammes if possible)

(a)peanuts.....
(b)peanut butter.....
(c)cashew nuts.....
(d)other type of nuts.....

31.How much/many of the following do you eat weekly?

(a)apple.....
(b)quince.....
(c)pear,.....

(d)grapes.....
(e)egg.....
(d)barley.....
(e)sugar beat.....
(f)dandelion.....
(g)flour.....

32.Are you on a diet? (please circle).....yes/no.

33. If yes, please describe

.....
.....
.....
.....
.....

D:FAMILY HISTORY AND MEDICAL HISTORY:

34.Do you suffer from or have ever sufferred from any of the following(s) (please circle):
(a) osteoporosis?
(b) arthritis?
(c) any other bone disorder?.....
(d) high blood pressure?
(e) kidney disease?
(f) diabetes mellitus?
(g) fits/epilepsy?
(h) have you had any operation or disorder eg:
i) of the testes?
ii) of the ovary?
iii) of the uterus?
iv) of the breast?
v) of the kidney?
vi) others ? (please specify).
(i) cancer?
(j) thyroid disorder?
(k) eating disorder eg anorexia or bulimia?
If so, please give details such as :when, duration etc:

.....

.....
.....
.....

35..Does anyone in your family suffer from any of the above condition?(please give details and relationship to you).

.....
.....
.....

36.Have you ever been hospitalised?.....(please circle).....yes/no.

if yes: when:
how long for:
what for:

37.Are you currently on any medication(s)?(please circle).....yes/no.

38 If yes, please give details:

name	**dose**	**frequency**
.....	.....	.....
.....	.....	.....
.....	.....	.....

How long did you take these for:?.....

<u>E:FEMALES ONLY.</u>

39. Do you take birth control pill?(please circle).....yes/no.

40.If yes ,please specify name.....

41.Is your menstrual cycle regular?(please circle).....yes/no

42.If no ,have you had to seek medical help?(please circle)
.....yes/no

43.What help were you given?(please give details):

.....
.....
.....

44.Have you given birth to any children?(please circle)...yes/no
if yes how many children have you given birth to:.....

45.How old is your first child?.....years old.

46. How old is your last child?.....years old.

47.Are you presently pregnant?(please circle).....yes/no.
if yes please state number of weeks of pregnancy.....weeks

48.Are you on hormone replacement therapy(HRT)

(please circle).....yes/no.
if yes please give the following details:
i) when did your menopause start?.....year.
ii)when did you start HRT?.....year.
iii)which type of HRT are you taking?.....

49.At what age did you start menstruating?.....years old.

50.On which day of your cycle was the urine sample collected?
day.....(please count from the day menstruation started)

Thank you for you assistance and cooperation

Harry Chummun,
School of Health,
University of Greenwich,
ERC (Dartford).
2/96.

APPENDIX 2

Average boron level in humans.

Tissues.	Boron / ppm.
Bone	0.90
Skin	0.12
Muscle	0.07
Nervous System	0.11
Liver	0.11
Heart	0.04
Lung	0.07
Kidneys	0.25
Intestine	0.08
Fat	0.07
Faeces	0.18
Scalp hair	4.30
Finger nails	15.20
Toe nails	17.90

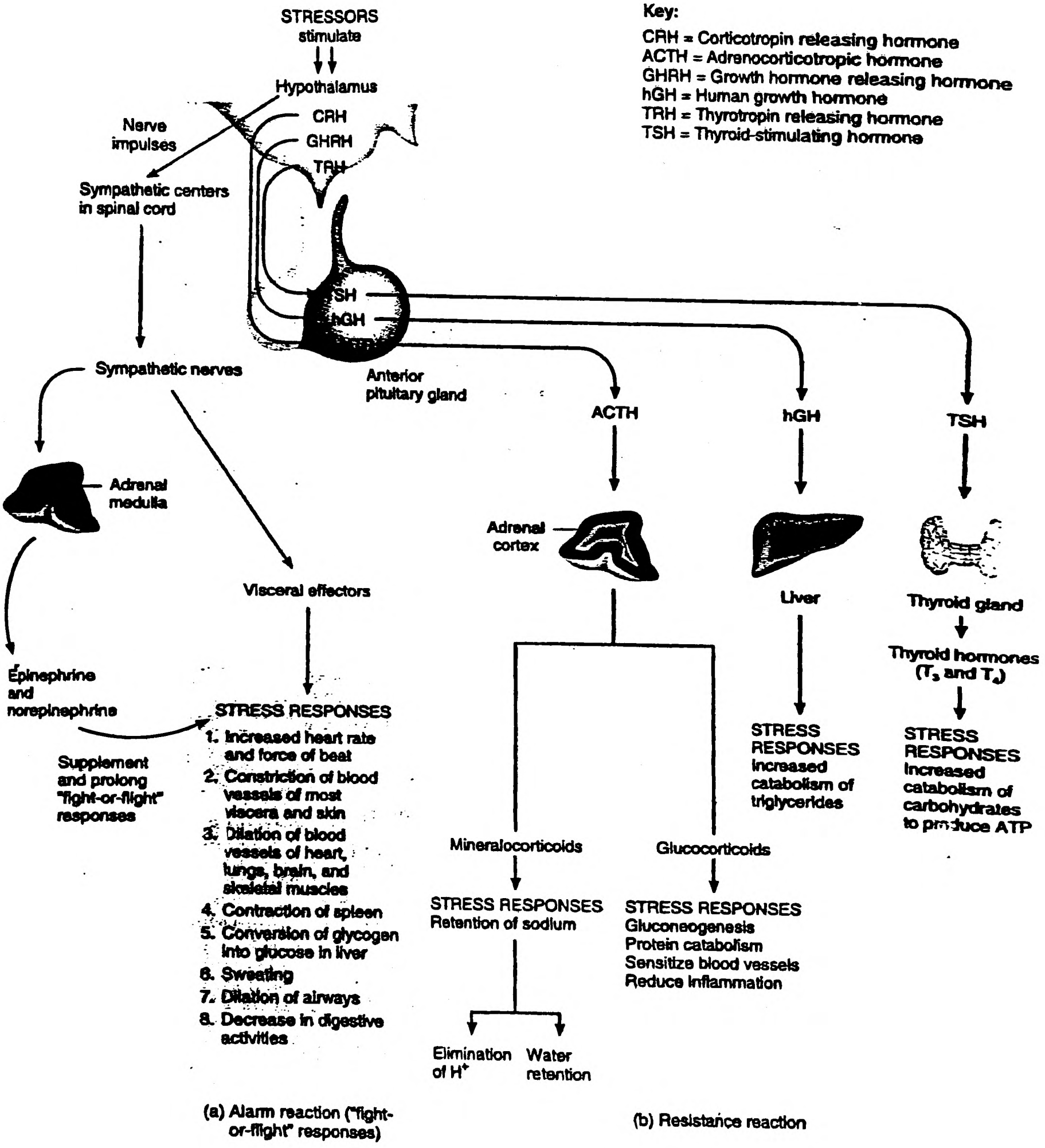
Average boron level in human fluids.

Whole blood (infants)	< 1.25
Whole blood (adults)	0.057
Blood (serum)	0.022
Urine	0.75

Average boron contents of some foods.

Apple	110
Pear	70
Grapes	40
Milk (human)	0.80
Milk (cow)	0.20
Milk (non-fat powder)	2.13
Egg (yoke)	0.0008
Egg (white)	0.14
White flour	0.45
Wheat	0.58

APPENDIX 3.



Stress and the production of noradrenaline, adrenaline, cortisol, growth and thyroid hormones.

(Source: Tortora G.J. & Anagnostakos N.P.(1990). Principles of Anatomy and Physiology. 6th edition. Harper & Row Publication)

Effects of cytokines on bones.

(+ = stimulation; +/- =stimulation or inhibition; - = inhibition).

Cytokines	Cells of origin	Target cells	Effects on bone
IL-1	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoblast Osteoclast	Formation +/– Resorption +/–
IL-3	Macrophage Lymphocyte Mast cell	Osteoclast	Resorption +
IL-6	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoblast Osteoclast	Formation – Resorption +
IL-8	Macrophage Fibroblast Lymphocyte Endothelial cell	Osteoblast Osteoclast	Resorption +
IL-11	Osteoblast Fibroblast Mesenchymal cell lineage	Osteoclast	Formation – Resorption +
TNF-α	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoblast Osteoclast	Resorption +
TNF-β	Macrophage Lymphocyte Mast cell	Osteoblast Osteoclast	Resorption +
M-CSF	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoclast	Resorption +
GM-CSF	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoblast Osteoclast	Resorption +
EGF	Macrophage Fibroblast Endothelial cell	Osteoblast Osteoclast	Formation + Resorption +
TGF-α	Macrophage Fibroblast Mast cell Endothelial cell	Osteoblast Osteoclast	Formation – Resorption +
TGF-β	Osteoblast Macrophage Fibroblast Lymphocyte Mast cell Endothelial cell	Osteoblast Osteoclast	Formation + Resorption +/–
PDGF	Osteoblast Macrophage Fibroblast Mast cell Endothelial cell	Osteoblast Osteoclast	Formation + Resorption +

aFGF	Osteoblast Macrophage Fibroblast Endothelial cell Lymphocyte	Osteoblast	Formation +
bFGF	Osteoblast Macrophage Fibroblast Endothelial cell	Osteoblast	Formation +
IGF	Osteoblast Macrophage Fibroblast Endothelial cell	Osteoblast Osteoclast	Formation + Resorption +
IFN-γ	Macrophage Fibroblast Lymphocyte	Osteoblast Osteoclast	Resorption –
BMP	Osteoblast Fibroblast	Osteoblast Osteoclast	Formation – Resorption +

APPENDIX 5.

NORMAL URINE VALUES OF ANALYTES.

CALCIUM.....100---300 mg/L .

MAGNESIUM.....75---120 mg/L .

PHOSPHATE.....400---1300 mg/L .

BORON.....0.8---1.4 mg/L .

HYDROXYPROLINE.....< 13.11 mg/L.

CREATININE.....790---2800 mg/L.

CORTISOL.....9.8---140 µg/L.

NORADRENALINE.....75----125 µg/L.

ADRENALINE.....75----365 µg/L .

DOPAMINE.....800---4945 µg/L.

SEROTONIN.....23----69 µg/L.

REFERENCES.

Abou-shakra FR, Havercroft JM & Ward NI, (1989). Lithiun and boron in biological tissues and fluids. *Trace Elements Medical*. No.6. P:142 .

Acien P, Mauri M & Gutierrez M, (1997). Clinical and hormonal effects of the combination gonadotrophin-releasing hormone agonist plus oral contraceptive pills containing ethyinyl-oestradiol (EE) and cyproterone acetate (CPA) versus the EE-CPA pill alone on polycystic ovarian disease-related hyperandrogenisms. *Human Reproduction*. March. 12(3). P:423-429.

Aja SM, Barrett JA & Gietzen DW, (1999). Cholecystokinin (CCK-A) and 5 HT-3 receptors interact in anorectic responses to amino acid deficiency. *Pharmacology-Biochemistry and Behaviour*. March, 62(3). P487-491.

Ajayi GO & Fadiran EO, (1998). The effects of 61 days of combined iron (chemiron) and single iron therapy on haemoglobin, packed cells volume, platelets and reticulocytes during pregnancy. *Clinical and Experimental Obstetric and Gynaecology*. 25(3). P:107-111.

Akatsu T, Tukahashi N & Udagawa N, (1991). Role of prostaglandins in IL-1 induced bone resorption in mice in vitro. *Journal of Bone and Minerals Research*. No.6. P:183-190.

Albers MM, (1990). Osteoporosis: a health issue for women. *Health Care for Women International*. Winter. No. 11(1). P: 11-19.

Albertazzi P, Pansini F, Botazzi M, Bonaccorsi G, De-Aloysio D & Morton MS, (1999). Dietary soy supplemention and phytoestrogen levels. *Obstetrics and Gynaecology*. August. 94(2). P:229-231.

Alderman BW, Weiss NS & Daling JR, (1986). Reproductive history and post-menopausal risks of hip and forearms fractures. *American Journal of Epidemiology*. No.124. P:262-267.

Alekel L, Clasey JL, Fehling PC, Weigel RM, Boileau RA, Erdman JW & Stillman R, (1995). Contribution of exercise, body composition, and age to bone mineral density in pre-menopausal women. *Medicine and Science*

in Sports and Exercise. April. P:1477-1485.

Alexander GV & Nusbaum RE, (1951). The boron and lithium contents of human bones. *Journal of Biological Chemistry*. No.192. P:4289.

Alfredson H, Nordostrom P & Lorenzo R, (1997) . Bone mass in females volleyball players : a comparison of total and regional bone mass in female volleyball players and non-active females. *Calcified Tissues International*. No.60. P:338-342.

Allen SA, (1994). Exercise consideration for post-menopausal women with osteoporosis. *Arthritis Care and Research*. December. Vol.7. No.4.

Anderson FH, (1998). Osteoporosis in men. *International Journal of Clinical Practitioner*. April/May. No.52 (3). P:176-180.

Anderson JJ, (1999). Plant-based diets and bone health: nutritional implications. *American Journal of Nutrition*. Sept. 70(3 suppl.). P:539-542.

Anderson JJB, Rondana S & Holmes A, (1996). Nutrition, life styles and quality of life : roles of diet and physical activity in the prevention of osteoporosis. *Scandanavian Journal of Rheumatology*. Supplement 103. P:65-74.

Archer JS, (1998). Relationship between oestrogen, serotonin and depression. *Menopause*. Spring. 6 (1). P71-78.

Arimura A, (1998). Perspective on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine and nervous system. *Japanese Journal of Physiology*. Oct., 48(5). P:301-331.

Arnaud CD, (1993). An integrated view of the role of the endocrine system in the genesis of the osteoporosis associated with ageing. *Osteoporosis Intenational*. Suppl 1. S:37-39.

Baines AD & Drangova R, (1998). Does dopamine use several signal pathways to nhibit Na-Pi transport in OK cells? *Journal of the American Society of Nephrology*. Sept., 9(9). P:1604-1612.

Balliere's *Clinical endocrinology & metabolism*. (1997). No.11(2). P:271-275.

Baraldi A, Ballestri M, Rapana R, Lucchi L, Borella P, Leonelli M, Furci L & Lusvarghi E, (1998). Acute renal failure of medical type in an elderly population. *Nephrology-Dialysis-Transplantation*. 13 suppl. 7. P:25-29.

Barr RD, Barton SA & Schull WJ, (1996). Boron levels in human: preliminary evidence of genetic regulation and some implications for human biology. *Medical Hypothesis*. No.46. P:286-289.

Barr SI & Mckay HA, (1998). Nutrition, exercise and bone status in youth. International Journal of Sport and Nutrition. June. No. 8 (2). P:124-142.

Barran DT, (1991). Thyroid hormone and bone mass (editorial). Journal of *Clinical Endicrinology and Metabolism*. No.72. P:1182-1183.

Barron JA, Barratt J & Malenka D, (1994). Racial differences in fracture risk. *Epidemiology*. No.5. P:42-47.

Barzel US, Massey LK, (1998). Excess dietary protein can adversely affect bone. *Journal of Nutrition*. June. 128(6). P:1051-1053.

Basle M, Mauras Y Audran M Clochon P, Rebel A. & Allain P, (1990). Concentration of bone elements in osteoporosis. *Journal of Bone and Minerals Research*. 5. P:41-47.

Bassey EJ, (1995). Exercise in primary prevention of osteporosis in women. *Annals of the Rheumatic Diseases*. No.54. P: 861-862.

Bataille P & Fardellione P, (1998). Pathophysiology and treatment of idiopathic hypercalciuria. *Current Opinion-Rheumatology*. July. No.10 (4). P:373-388.

Bauer DC, Browner WS & Cauleey JA, (1993). Factors associated with appendicular bone mass in older women. *Annals of Internal Medcines*. No.118. P:157-165.

Beattie JH & Peace HS, (1993). The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroids metabolism in post-menopausal women. *British Journal of Nutrition*. May. 69(3). P:871-884.

Beck TJ, Ruff CB & Scott WWJ, (1992). Sex differences in geometry

of femoral neck with ageing: a structural analysis of bone mineral data. *Calcified Tissue International.* No.50. P:24-29.

Beck TJ, Ruff CB & Bissessur K, (1993). Age-related changes in femoral neck geometry: implication for bone strength. *Calcified Tissue International.* No.53. P:41-46.

Benderdour M, Hess K, Dzonda-Gadet M, Dousset B, Nabet P & Bellerville F, (1997). Effects of boric acid solution on cartilage metabolism. *Biochem.Bio-phys. Res. Comm.* No.234. P:263-268.

Bendadour M, Bin-Van T, Dicko A & Belleville S, (1998). In vivo and in vitro effects of boron and boronated compounds. *Journal of Trace Elements Medicines and Biology.* Vol.,12. P:2-7.

Bernet F, Dedieu JF, Laborie C Montel V & Dupouy JP, (1998). Circulating neuropeptide Y (NPY) and catecholamines in rats under resting and stress conditions. Arguments for extra-adrenal origin of NPY, adrenal and extra adrenal sources of catecholamines. *Neuroscience* Letters. June 26. 250(1). P:45-48.

Birdwood G, (1996). *Understanding osteoporosis and its treatments : a guide for physicicans and their patients* . Royal College of Physician Publication.

Blaser-Grill J, Knoppik D, Amberger A & Golbach H, (1989). Influence of boron on membrane potential in Eloden densa & Helianthus annus roots and H^+ extrusion of suspention cultured Dancus Carota cells. *Plant Physiology.* 90, P280-284.

Blue C, (1996). Preventing back injury among nurses. *Orthopaedic Nursing.* November/December. 15(16). P:9-20. Quiz 21-22.

Bonde M, Quist P, Fledelium C & Riis BJ, (1994). Immunoassay for quantifying type-1 collagen degradation of products in urine evaluated. *Clinical Chemistry.* No.40. P:2022-2025.

Bohannon AD & Kennett WL, (1994). The ageing skeleton clinics. *Geriatric medicines* . Vol. 10 (4). November.

Bosno C, Tihanyl J, Rivatta C, Parlato G & Viru M, (1996). Hormonal responses in strenuous jumping effort. *Japan Journal of Physiology.*

Brahm H, Mallmin H & Rich-Anin K, (1998). Relationships between bone mass measurements and life time physical activity in Swedish population. *Calcified Tissue International*. May. No.62 (5). P:400-412.

Brahm H, Piehl-Anlin K, Saltin B & Ljunghall S, (1997). Net fluxes over working thigh of hormones, growth factors and biomarkaers of bone metabolism during short lasting dynamic exercise. *Calcified Tissues International*. No.60. P:175-180.

Bray JJ, Cragg PA, Mac Knight ADC, Mills RG & Taylor DW, (1994). *Lecture notes on Human Physiology*. 3rd edition. Blackwell Scientific Publication.

Brenner I, Shek PN, Zameecnik J & Shepherd RJ, (1998). Stress hormones and the immunological responses to heat and exercises. International Journal of Sports Medicine. February. 19(2). P:130-143.

Bruni V, Bigozzi L, Dei M & Brundi ML, (1997). Bone mineral metabolism in diet induced amenorrhoea. *Annals of New York Academy of Science*. June 17. No.816. P:250-262.

Buckwater JA, Glimcher MJ, Cooper RR & Recker R, (1996). Bone biology. *Journal of Bone and Joint Surgery*. August. 77A. 8.

Bunker VW, (1994). The role of nutrition in osteoporosis . *British Journal of Biomedical Science*. P:228-240.

Bugajski J, Gadek-Michalska A, Borycz J & Glod R, (1999). Social stress inhibits the nitric oxide effect on the corticotropin-releasing hormone but not vasopressin-induced pituitary-adrenacortical responsiveness. *Brain-Research*. Jan. 30. 817(1-2). P:220-225.

Cagnacci A, Soldani R & Yen SS, (1997). Melatonin enhances cortisol levels in aged women; reversible by oestrogen. *Journal of Pineal Research*. March. 22 (2). P81-85.

Cahusac PM, Castro MG, Robertson L & Lowenstein PR, (1998). Electrophysiological evidence against a neurotransmitter role of corticotropin-releasing hormone (CRH) in primary somatosensory cortex. *Brain Research*. May 18. 793 (1-2). P:73-78.

Canalis E, (1983). Effects of hormones and growth factors on alkaline phosphatase activity and collagen synthesis in cultured rats calvariae. *Metabolism* 32. P:14-20.

Carraro V & Franceschi C, (1997). Apoptosis of skeletal and cardiac muscles and physical exercise. *Ageing Milano*. Feb-April. 9 (1-2). P19-34.

Cavanagh DJ & Cann CE, (1988). Brisk walking does not stop bone loss in post-menopausal women. *Bone*. 9. P:201-204.

Chan GM, (1991). Dietary calcium and bone minerals status of children and adolescents. *American Journal of Diseased Children.* 145. P:631-634.

Chapin ER & Warren WK, (1994). The reproductive toxicity of boric acid. *Environmental Health Perspectives.* November. Vol. 102. Suppl. 7.

Clancy J & McVicar AJ, (1995). *Physiology and anatomy: a homeostatic approach.* Edward Arnold Publication.

Clarke WB & Webber CE, (1987). Lithium and boron in human blood. *Journal of Laboratory and Clinical Medicines*. 109. P:155-158.

Cohen L & Kitzes R, (1981). Infrared spectroscopy and magnesium contents of bone minerals in osteoporotic women. *Israel Journal of Medical Sciences.* 17. P:1123-1125.

Colager-clifford A, Simon NG, Richter LM, Smokuk SA & Lu S, (1997). Androgen and oestrogen modulate $5HT_{1A}$ and $5\text{-}HT_{1B}$ agonist effects on aggression. *Physiology and Behaviour*. Volume 65. No. 4/5. P:823-829.

Coleman EA, Honeycutt G, Ogden B, McMillan DE, O'Sullivan PS, Light K & Wingfield W, (1997). Assessing substance abuse among health care students and the efficiency of education intervention. *Journal of Professional Nursing*. January/February 13 (1). P:28-37.

Colwell A, Russell RGG & Eastell R, (1993). Factors affecting the assay of 3-hydroxypyridinium cross-links of collagen as marker of bone resorption. *European Journal of Clinical Investigations*. 23. P341-349. Company Publication.

Compston EJ, (1994). HRT for osteoporosis: clinical trials and

pathophysiological aspects of reproduction. *Medical Review.* 3. P:300-324.

Compston EJ, (1996). Osteoporosis : New perspectives on causes , prevention and treatment. *A Royal College of Physician Publcation.* London.

Compston EJ, Judd D & Crawley T, (1987). Osteoporosis in patients with irritable bowels disease. *Gut.* 28. P:410-415.

Concensus Development Conference, (1993). Diagnosis, prophylaxis and treatment of osteoporosis. *American Journal of Medicicne.* 94. P:646-650.

Contreras N, Shantoshi H & Tyrell LJ, (1985). Urinary cortisol in the assessment of pituitary-adrenal function: utility of the 24 hour and spot determination. *Journal of clinical Endicrinology and Metabolism.* Volume 62. No.5.

Cooper C & Atkinson EJ, Heinz WW, O'Fallen WM, Rigg LB & Judd HL, (1992). Is caffeine consumption a risk factor for osteoporosis? *Journal of Bone and Mineral Research.* 7. P:465-471.

Cooper GS & Umbach DM, (1996). Are vitamin D receptors polymorphism associated with bone mineral density?. A meta-analysis. *Journal of Bone and Minerals Research.* 11. P:1841-1849.

Cooper GS, Atkinson EJ & Hensrudd DD, (1996). Dietary protein intake and bone mass in women. *Calcified Tissue International.* 58. P:320-325.

Corali CH & Raisz LG, (1986). Osteoporosis: significance, risk factors and treatment. *Nurse Practiitoner: American Journal of Primary Health Care.* 11(9). P:16-20.

Court JA, Thomas N, Piggott MA, Marshall EF, Morris CM, Lamb RH, Johnson M & Perry EK, (1998). Dopamine and nocotinic receptors binding and the levels of dopamine and homovanillic acid in human brain related to tobacco. *Neuroscience.* Nov. 87(1). P:63-78.

Cualey JA & Cumming SR , (1993). Effects of thiazide dieretic on bone mass, fracture and falls. *Annals of Internal Medicine.* Vol. 73. P:666-673.

Cumming RG, (1990). Calcium intakes and bone mass: a quantitative review of the evidence. *Calcified Tissue International.* 47. P:194-201.

Cummings SR, Kelsey JL, Nevitt MC & O'Dowd KJ, (1985). Epidemiology of osteoporosis and osteoporotic fractures. *Epidemiology Review* . No.7. P:178-208

Cutolo M, (1997). Do sex hormones modulate the synovial macrophages in R.A.? *Annals of Rheumatological Diseases.* May. 56 (5). P:281-284.

Davis GK & Mertz W, (1987). Copper. Vol. 1. P:301-367. In: *Trace Elements in Humans and Animals Nutritions.* Mertz W. (Ed.). Academic Press.

Dawson-Hughes B, Harris SS & Finneman S, (1995). Calcium absorption on high and low calcium intakes in relation to vitamin D receptors genotype. *Journal of Clinical Endocrinology and Metabolism.* 80, P:3657-3661.

Dean JA, (1979). *Lange's Handbook of Chemistry.* 12th edition. McGraw - Hill Publication.

Decker JD & Marshall JJ, (1996). Differentiated cells replication within the periosteum of the pig mandibullar ramus. *Acta-Anat-Basel.* 157 (2). P:144-150.

Delany AM, Gabbitas BY & Cannalis E, (1995). Cortisol downregulates osteoblasts 1(1) protocollagen mRNA by transcriptional and post-transcriptional mechanism. *Journal of Cellular Biochemistry.* 57. P:488-494.

Department of Health, (1994). Report of the advisory group on osteoporosis. *HMSO.* London.

Depsey PA & Dempsey AD, (1996). *Nursing Research: text and workbook.* 4th edition. Little Brown (Inclusive.).

Devlin TM, (1997). *Textbook of biochemistry with clinical correlation.* Fourth edition. John Wiley Publication.

Drinkwater BL, (1993). Exercise in preview of osteoporosis. *Osteoporosis International.* Supplement 1. P:169-171.

Dunne F, Walters B, Marshall T & Heath DA, (1993). Pregnancy associated osteoporosis. *Clinical Endocrinology* (Oxford). 39. P:487-490.

Durlach I & Collery PH, (1984). Magnesium and potassium in Diabetes Mellitus and carbohydrate metabolism : Review of the present status and recent results. *Magnesium.* 3. P:315-323.

Ebeling PR, (1998). Osteoporosis in men: new insights into eatiology, pathogenesis, prevention and management. *Drugs-Ageing*. December. 13(6). P:421-434.

Ebrahim S, Thomson PW, Baskaran V & Evans K, (1997). Randomised placebo controlled trial of brisk walking in the prevention of post-menopausal osteoporosis. *Age and Ageing.* 26. P:253-260.

Eisman JA, Eggar D, Genenich S, Denzlinger C, Schmidt E, Maihammer R, Morisson NA & Kelly PJ, (1995). Genetic of osteoporosis and vitamin D receptor alleles. *Calcified Tissue International.* 56. (Suppl.1). P:48-49.

Elders PJ, Netelenbos JC, Lips P, Van Gintel FC, Khoe E, Leeuwenkamp OR, Hackeng WHL & Van Der Stelt PF, (1991). Calcium supplement reduces vertebral bone loss in perimenopausal women: a controlled trial in 248 women between 46-55 years of age. *Journal of Clinical Endocrinology and Metabolism.* No. 73. P: 533-540.

English J. & Card R, (1994). *Butterworths police law.* 4th edition. Butterworth Publications.

Eriksen EF, Vesterby A & Kassem M, (1993). Bone remodelling and bone structure P:67-101. *In Physiology and Pharmacology of Bone.*

Eyre DR & Kobb TJ, (1984). Quantitation of hydroxypyridinium cross-links in collagen in HPLC. *Analytical Biochemistry.* 137. P:380-390.

Favier R, Spielvogel H, Caceres E, Rodriguez A, Sempore B, Pequiguot J & Pequiguot JM, (1997). Differential effects of ventilation stimulation by sex hormones and almitrime on hypoxic erothrocytosis. *European Journal of Physiology.* May, 434 (1). P97-103.

Feitelberg S, Einstein S & Ismail F & D'Amand C, (1987). Deranged bone mineral metabolism in chronic alcoholism. *Metabolism.* 36. P:322-

326.

Felton JS, (1998). Burntout as a clinical entity: its importance in health care workers. *Occupational Medicines*. Oxford. May. 48 (4), P237-250.

Fenech M, (1998). Chromosomal damage, rate, ageing and diet. *Annals of New York Academy of Science*. Nov. 20. No. 854. P23-33.

Ferero MS, Klein RF & Nelson H, (1987). Effects of age on circulating immunoreactive and bioactive parathyroid hormone level in women. *Journal of Bone Mineral Reserves*. 2. P:363-366.

Feskanich D, Korrick SA, Greenspan SL, Rosen HN & Colditz GA, (1999). Moderate alcohol consumption and bone density among post-menopausal women. *Journal of Women Health.* Jan-Feb. 8(1). P:65-73.

Finkelstein JS, Neer RM & Biller BM, (1987). Osteopenia in men with a history of delayed puberty. *New England Journal of Medcines.* 326. P:600-604.

Forwood MR & Burr DB, (1993). Physical activity and bone mass in futility? *Bone & Mineral.* 21. P:89-112.

Gaillard RC & Spinedi E, (1998). Sex- and sex steroids interactions and the immune systems : evidence for a neuroendicrine-immunological sexual dimorphism. *Domestic-Animal-Endocrinology.* September. 15(5). P;345-352.

Gavenlock AH, (1988). *Practical clinical biochemistry*. 6th edition. Saunders Publications.

Gilganz V, Roe TF & Moras S, (1991). Changes in vertebral bone density in black girls and white girls during childhood and puberty. *New England Journal of Medicine*. 325. P:1597-1600.

Goldhill DR, (1997). Calcium and magneisum. *Care of the critically ill.* Vol. 13. No. 3. P112-115.

Greendale GA, Unger JB, Rowe JW & Seeman TE, (1999). The relationship between cortisol excretion and fractures in healthy older people: results from the MacArthur studeies. *Journal of the American Geriatrics Society*. July. 47(7). P:799-803.

Greenspan FS, (1991). *Basic and clinical andocrinology*. A Lange medical Publication.

Gronowicz GA, Fall PM & Raisz LG, (1994). Prostaglandins E_2 stimulates preosteoblasts replication: an autoradiographis study in cultured rats calvariae. *Experimental Cell Reserves*. 212. P:314-320.

Gumus b, Yigitoglu MR, Lekili M, Uyanik BS, Muezzinoglu T & Buyuksu C, (1998). Effects of long-term alcohol abuse on male sexual function and serum gonadal hormone levels. *International-Urology-and Nephrology*. 30(6). P:755-759.

Gutin B & Kasper MJ, (1992). Can vigorous exercise play a role in osteoporosis prevention? *A Review of Osteoporosis International*. 2. P:55-69.

Guyton A, (1997) . *A textbook of Medical Physiology* . W.B. Saunders

Guanabens N, Pares A & Marinosa L, (1990). Factors influencing the development of metabolic bone diseases in primary biliary cirrhosis. *American Journal of Gastroenterology*. No. 85. P:1356-1362.

Hafez B, (1998). Recent advances in clinical / molecular andrology. *Archives of Andrology*. May-June. 40(3). P:187-210.

Hall HI, Chen SY, Rajendran KG, Sood A, Spielvogel BF & Shih J, (1994). Hypolipidemic, anti-obesity, anti-inflamatory and anti-osteoporotic properties of amines carboxboranes. *Environmental Health Perspectives*. Vol. 102, Suppl. 7. P21-31.

Hallworth RB, (1998). Prevention and treatment of post-menopausal osteoporosis. *Pharmacy World and Science*. October 25. 20(5). P:198-205.

Hansen MA, Overgaad K, Riis BJ & Christiansen C, (1991). Potential risk factors for the development of postmenopausal osteoporosis examined over the last 12 years. *Osteoporosis International*. 1. P:95-102.

Harris Jr. ED, (1990). Rheumatoid Arthritis: Pathophysiology and implications for therapy. *New England Journal of Medicine*. 322. P:277-281.

Harrison JR, Lorenzo JA, Kawaguchi H, Raisz LA & Pilbeam CC, (1994). Stimulation of prostaglandin E_2 production by Interleukin 1 and TGF in osteoblastic MC3T3-E-1 cells. *Journal of Bone Mineral Reserves*. 9. P:817-823.

Harvey RD, McHardy KC & Reid JW, (1991). Measurement of bone degradation in hyperthyroidism and during thyroxine replacement therapy using pyridinium cross-links as specific urinary marker. *Journal of Clinical Endocrinology and Metabolism*. 72. P:1189-1194.

Heaney RR & Recker RR, (1986). Effects of nitrogen, phosphate and caffeine on calcium balance in women. *Journal of Laboratory and Clinical Medicine*. 99. P:46-55.

Hegsted M & Schuette SA, Zemmel MD & Linkwater HM, (1981). Urinary calcium and calcium balance in young men as affected by level of proteins and phosphorus intakes. *Journal of Nutrition*. 111. P:553-562.

Hernandez-Avilla M, Colditz GA, Stampfer MJ, Rosner B, Speizer FE & Willett WC, (1991). Caffeine, moderate alcohol intake and risk of fractures of the hip and forearm in middle-age women. *American Journal of Clinical Nutrition*. 54. P:157-163.

Hillard CJ & Stevenson CJ, (1991). Role of oestrogen in the development of osteoporosis. *Calcified Tissue International*. Suppl., 49. P:55-59.

Hinojosa-Laborde C, Chapa I, Lange D & Haywood JR, (1999). Gender differences in sypathetic nervous system regulation. *Clinical and Experimental Pharmacology and Physiology*. February. 26(2). P:122-126.

Hirsinger C & Jamet E, (1997). Quel roles pour les extensines dans la structures des parois des cellules vegetables? *Regard sur la biologie* 1. P:21-29.

Hollenbach KA, Barrett-Connor E, Edelstein SL & Holbrook T, (1993). Cigarette smoking and bone mineral density in older women and men. *American Journal of Public Health*. Sept., 83(9). P:1265-1270.

Holligberg PW, (1985). Effects of dietary caffeine and aspirin on urinary calcium and hydroxyproline excretion in pre- and postmenopausal women. *Fed. Proc*. 44. P:11-49.

Horowitz M, (1993). Cytokines and oestrogen in bone: anti-osteoporosis effects. *Science*. 260. P:626-628.

Horton TJ, Pagliassoti MJ, Hobbs K & Hill JO, (1998). Fuel metabolism in men and women during and after long-duration exercise. *Journal of Applied Physiology*. Nov., 85(5). P:1823-1832.

Hosking DJ, (1993). Effects of corticosteroids on bone turnover. *Respiratory Medicines*. 87.(Suppl., A.). P:15-21.

Hu G, Duffy P, Swanson C, Ghasemzadeh Mb & Kalivas PW, (1999). The regulation of dopamine transmission by metabotropic glutamate receptors. *Journal of Pharmacology and Experimental Therapeutics*. April. 289(1). P:412-416.

Huang WQ, Zhang CL, Di XY & Zhang RQ, (1998). Studies on the localisation of 5-hydroxytryptamine and its receptors in human placenta. *Placenta*. Nov., 19(8). P:655-661.

Hudgel DW & Gordon EA, (1997). Serotonin induced cortisol release in continuous positive airway pressure (CPAP) treated obstructive sleep apnoea patients. *Chest* 3. P:632-638.

Hunt CD & Nielsen F, (1981). Interactions between boron and cholecalciferol in the chick: In trace elements metabolism in man and animals. *Australian Academy of Science*. Vol. 4. P:597-600.

Hunt CD, (1994). The biochemical effects of physiological amount of dietary boron in animals nutrition model. *Environmental Health Perspectives*. 102. Suppl., 7. P:35-43.

Imbus HR, Cholak J, Miller LH & Sterling T, (1963). Boron, cadmium and nickel in blood and urine. *Archives of Environmental Health*. 6. P:286.

Inoue H, Date Y, Kohashi K, Yoshitomi H & Tsuruta Y, (1996). Determination of total hydroxyproline and proline in human serum and urine by HPLC with fluorescence detection. *Biological and Pharmaceutical Bulletin*.19(20). P:163-166.

Islam N, Chandra S, Ghosh JK & Mitra C, (1998). Cold stress facilitates calcium mobolisation from bone in an ovariectomised rat model of osteoporosis. *Japanese Journal of Physiology*. 48 (1). P49-55.

Ismail Z & el-Guebaly N, (1998). Nicotine and endogenous opoids: towards specific pharmacotherapy. Canadian Journal of Psychiatry. February. 43(1). P:37-42.

Itabashi A, (1998). Calcitonin. *Nippon-Rinsho*. (Japan). June. 56. 6. P:1511-1517.

Iyengar GV, Clark WB, Downing RG & Tanner JT, (1988). Lithium in biological and dietary materilas in: Trace Elements. *Annals of Chemical, Medical and Biological Procedures International Workshop*. 5. P:267-269.

Jakob F, Siggelkow H, Homann D, Kohrle J, Adamski J & Schutze N, (1997). Local oestradiol metabolism in osteoblasts- and osteoclasts-like cells. *Journal of Steroids-Biochem-Molecular-Biology*. April. 61 (3-6). P:167-174.

Jensen JA, Anderson J & Schon JS, (1984). Boric acid single dose pharmacokinetic after intravenous administration to men. *Archives of Toxicology*. 55. P:64-67.

Jeong KH, Jacobson L, Widmaier EP & Majzoub JA, (1999). Normal suppression of the reproductive axis following stress in corticotrophic releasing hormone deficient mice. *Endocrinology*. April. No. 140(4). P:1702-1708.

Jirik FR, Podor T & Hirano T, (1989). Bacterial liposaccharides and inflamaton mediators augment IL-6 secretion by human endothelial cells. *Journal of Immunology*. 142. P:144-147.

Johansen A & Stone M, (1996). Biphosphonates and the treatment of bone diseases in the elderly. *Drug and ageing*. February. Vol. 8. No. 2. P:113-126.

Jones G, Nguyen T & Sambrook PN, (1994). Symptomatic fracture incidence in elderly men and women : the Dibbo Osteoporosis Epidemiology Study (DOES). *Osteoporosis International*. 4. P:277-282.

Johnson Jr. CC, Millar JZ & Slemenda CW, (1992). Supplementation and increases in bone mineral density in children. *New England Journal of Medicine.* 327. P:82-87.

Johnston JD, (1994). Smokers have less dense bone and fewer teeth. *Journal of Research in Sociology of Health.* October. 114(5). P:265-269.

Joosten E, Van den Berg A & Riezler A, (1993). Metabolic evidence that deficiencies of vitamin B_{12} , folate and vitamin B_6 occur commonly in elderly people. *American Journal of Clinical Nutrition.* 58. P:68-476.

Jowsey J & Jonhson KA, (1972). Juvenile osteoporosis: bone findings in 7 patients. *Journals of Paediatrics.* 81. P: 511-517.

Kalu DN, (1995). Evolution of the pathogenesis of post-menopausal bone loss. *Bone.* 17. P135-144.

Kanders B & Demster DW, (1988). Interaction of calcium, nutrition and physical activity on bone mass in young women. *Journal of Bone and Minerals Research.* 3. P:145-149.

Kaplan J, (1983). Regulation and dynamics of transferrin receptors in cultured cells. In: *Structure and function of iron storage and transport proteins*. Editors: urushizaki I, Aisen P, Listowsky & Drysdale. P:283-289.

Kaufman JM, (1995). Role of calcium and vitamin D in the prevention and treatment of post-menopausal osteoporosis: an overview. *Clinical Rheumatology.* 14. Suppl.3. P:6-13.

Kean RW, Harris PA & Thomson PN, (1994). Early postmenopausal bone loss and biochemical markers. *Journal of Bone and Mineral Research.* 9. P:399-402.

Keil DP, Felson DT & Hannan MT, (1990). Caffeine and the risks of hip fracture: the Farningham study. *American Journal of Epidemiology.* 132. P:675-684.

Kelly PJ, Morrison NA & Sambrook PN, (1990). Dietary calcium, sex hormone and bone density in normal men. *British Medical Journal.* 300. P:1361-1364.

Kent GN, Price RJ & Gutteridge, (1993). Effects of pregnancy and lactation on maternal bone mass and calcium metabolism. *Osteoporosis International.* 3 (suppl.1), P44-47.

Kerr DA, Morton A, Dick I & Prince RL, (1996). Exercises effects on bone mass in post-menopausal women are site-specific and load-dependant. Journal of *Bone and mineral Research.* 11. P:218-225.

Kessler E & Takahara K, (1996). Bone morphogenetic protein-1: the type 1 proto-collagen C-proteinase. *Science.* 271. P:360.

Kettner C, Mersinger L & Knabb R, (1990). The selective inhibition of thrombin by peptides of boroginine. *Journal of Biological Chemistry.* 265. P:18289-18297.

Kingsmill VJ & Boyde A, (1998). Mineralisation density of human mandibular bone: quantitative bachscattered electron image analysis. *Journal of Anatomy.* February. 192.(2). P:245-256.

Kirkham B, (1991). Interleukin 1: Immune activation pathways and different mechanisms in osteoarthritis and rheumatic disease. *Annals of the Rheumatic Diseases.* 50. P:395-400.

Klimuik PS, Grennan A, Weinkove C & Jayson MIV, (1989). Platelets serotonin in systemic sclerosis. *Annals of Rheumatic Diseases.* 48. P:586-589.

Kochersberger G, Buckley NJ & Leight GS, (1987). What is the clinical significance of bone loss in primary hyperparathyroidism? *Archives of Internal Medicine.* 147. P:1951-1953.

Konttinen YT, Xu W, Patika H, Imai S, Waris V & Goodman SB, (1997). Cytokines in aseptic loosening of total hip replacement. *Current Orthopaedics.* 11. P:40-47.

Komesaroff PA, Esler MD & Sudhir K, (1999). Oestrogen supplemention attenuates glucocorticoid and catecholamine responses to mental stress in perimenopausal women. *Journal of Clinical Endocrinology and Metabolism.* Feb., 84(2). P:606-610.

Kotake M, Nakai A, Mokuno T, Ooda N, Sawai Y, Shimazaki K, Kato R, Hayakaw N & Wanikawa A, (1996) . Short stature due to growth

hormone deficiency associated with Cushing's disease and Ulcerative Colitis . *Hormone Metabolism Res.* June. 28.

Kraemer WJ, Staron RS, Hagerman FC, Hikida RS, Fry AC, Gordon SE, Nindl BC, Gothshalk LA, Volek JS, Marx JO, Nweton RU & Hakkinen K, (1998). The effects of short-term resistance training on endocrine function in men and women. *European Journal of Applied Physiology*. June. 78(1). P:69-76.

Krall EA & Dawson HB, (1993). Heritable and life-styles determinants of bone mineral density. *Journal of Bone and Minerals Research*. 8. P:1-9.

Kream B, Tetradis S, Lafrancis D, Fall PM, Feyen JHM & Raisz LG, (1997). Modulation of the effects of glucocorticoids on collagen synthesis in foetal rat calvariae by insulin-like growth factor binding protein-2. *Journal of Bone and Mineral Research*. 12(6). P:889-895.

Krolner B & Toft B, (1983). Vertebral bone loss: an unheeded side effect of therapeutic bed-rest. *Clinical Science*. 64. P:537-540.

Kumeda Y, Inaba M & Nishizawa Y, (1998). Secondary osteoporosis and its treatment- diabetes mellitus. *Japanese Journal of Clinical Medicine*. June. 56(60). P:1579-1586.

Kurokouchi K, Kambe F, Yasukawa K, Izumu R, Ishiguro N & Seo H , (1998). TNF-alpha increases expression of IL-6 and ICAM-1 genes through activation of NF-kappa B in osteoblast-like ROS17/2.8 cells. *Journal of Bone and Minerals Reserves*. August. No. 13(8). P: 1290-1299.

Lader CS & Flanagan AM, (1998). Pg E_2 , IL-1 and TNF increase line osteoblast formation and bone resorption in vitro. *Endocrinology*. July, 139 (7). P3157-3164.

Lalor BL, France MW & Powell D, (1986). Bone mineral metabolism in chronic alcoholism. *Metabolism*. 36. P:322-326.

Lanyon LE, (1992). Control of bone architectures by functional loading. *Journal of Bone Mineral Reserves*. 7 (suppl.2). P309-375.

Laroche M, Porteau L, Caron P, Moulinier L, Didier A, Amigues JM, Cantagrel A & Mazieres B, (1997). *Revue Du Rheumatisme - English*

Edition. April. 64 (4). P:267-270).

Lashas L, Masananskaite D, Lashene D & Masanauskiene E, (1996). Rate of characteristics for osteoporosis fractures in the elderly population of kaunas region of Lithuania. *Scandanavian Journal of Rheumatology.* 25. (suppl. 103). P:16-20.

Laitinen L & Valimaki M, (1991). Alcohol and bone. *Calcified Tissue International.* 49 (Suppl.). P:70-33.

Law MR, Cheng R, Hacksaw AK, Allaway S & Hale AK, (1997). Cigarette smoking, sex hormones and bone density in women. *European Journal of Epidemiology.* July. 13(5). P:553-558.

Lees B, Pugh M, Siddle N & Stevenson JG, (1995). Changes in bone density in women starting HRT compared with those in women already established on HRT. *Osteoporosis International.* 5, P344-348.

Leili S & Scanes CG, (1998). The effects of protein restriction on insulin-like growth factor-1 and IGF-binding protein in chicken. Proceedings of the *Society for Experimental Bilogy and Medicine.* September. 218(4). P:322-328.

Leino A, Jarvisalo J, Impivaara O & Kaitsaari M, (1994), Ovarian hormones status, life-style factors and markers of bone resorption in women aged 50 years. *Calcified-Tissue-International.* April, 54 (4), P262-267.

Leklem JE & Hollenbeck H, (1990) Acute ingestion of glucose decreases plasma pyridoxal 5' phosphate and total vitamin B_6 concentration. *American Journal of Clinical Nutriiton.* 51. P:832-836.

Leone FA & Rezende LA, (1998). Allosteric modulation of pyrophosphatase activity of rats osseous plate alkaline phosphatase by magnesium ion. *International Journal of Biochemical and Cellular Biology.* January. 30. 1. P:88-97.

Levy JR, Murray E & Manogas S, (1986). Demonstration of insulin receptors and modulation of alkaline phosphatase activity by insulin in rats osteoblasts. *Endocrinology.* 119. P:1786-1792.

Libanati CR & Baylink DJ, (1992). Prevention and treatment of

glucocorticoids-induced osteoporosis: a pathologenetic perspective. *Chest.* Nov. 103 (5). P:1426-1435.

Litovitz TL, Klein-schwartz W, Oderda GM & Schmitz BF, (1988). Clinical manifestation of toxicity in a series of 784 boric acid ingestion. *American Journal of Emergency Medicines.* May. 6 (3).

Liu QR, Hatter S, Endo S, MacPhee K, Zhang J, Cleary LJ, Byrne JH & Eskin A, (1997). A developmental gene (Tolloid/BMP-1) is regulated in Aplysia neurons by treatments that induce long-term sensitization. *Journal of Neuroscience*. January 5. 17(2). P:755-764.

Loomis DW & Durst RW, (1992). Chemistry and biology of boron. *Biofactor*. 3 (3). P:229-239.

Lopez JM, Gonzalez G, Reyes V, Campino R & Diaz S, (1996). Bone turnover and density in healthy women during breast feeding and after weaning. *Osteoporosis International.* 6. P:153-159.

Love C, (1996). Injury caused by lifting : a study of the nurses viewpoint. *Nursing Times* . August 7. 10(46). P:34-39.

Lukert BP & Raisz LG, (1990). Glucocoids induced osteoporosis : patogenesis and management. *Annals of International Medicines.* 112. P:352-364.

Lutz J, (1986). Bone mineral, serum calcium and dietary intakes of mother and daughter pairs. *American Journal of Clinical Nutrition.* 44, P99-106.

Lynch SE, William RC & Poulson AM, (1989). A combination of platelets-derived and insulin-like growth factors enhances periodontal regenaration. *Journal of Clinical Periodontology.* 16. 9. P:545-548.

Malik NS & Meek KM, (1996). Vitamins and analgesics in the prevention of collagen ageing. *Age and ageing*. 25. P:179-284.

Mamczarz J, Budziszewska B , Antkiewicz-Michaluk L & Vetulani J, (1999). The $Ca2^{+}$ channel blockade changes the behavioural and biochemical effects of immobilisation stress. *Neuropsychopharmacology.* March, 20(3), P:248-254.

Manoglass SC & Jilka RL, (1995). Emerging insight into the

pathophysiology of osteoporosis. *New England Journal of Medicine.* 332. P:305-311.

Mansen D & Palmer G, (1998). Sequences and activity of parathyroid hormone and parathyroid-related protein receptors promoter region in human osteoblasts-like cells. *Gene*. September. 18. 218 (1-2). P:49-56.

Marcus R, (1996). Endogenous and nutritional factors affecting bones. *Bone*. 18. P:11-13.

Marieb E, (1997) . *Human Physiology* . 7th edition. Benjamin/Cummings Publication Company Inc.

Marino F, Cosentino M, Boarbelli R, Ferrari M and Maestrino GJ (1997) . Measurement of catecholamines in mouse bone marrow by means of HPLC with electrochemical detection. *Heamatologica* . July / August. 82(4) . P: 392-394.

Marsh AG, Sanchaz TV & Mickelsen O, (1980). Cortical bone density of adult lacto-ovo-vegetarian and omnivorous women. *Journal of American Dietetics Association*. Feb., P:184-151.

Massey LK & Wise KJ, (1984). The effects of dietary caffeine on urinary excretion of calcium, magnesium, sodium and potassium in healthy young females. *Nutritional Research.* 4. P:43-50.

Massie HR, (1994). Effects of dietary boron on the ageing process. *Environmental Health Prespectives.* 102. Supplement. 71. P:45-48.

Mastromatteo E & Sullivant F, (1994). Summary: International symposium on the health effects of boron and its compounds. *Environmental Health Perspectives.* Vol.102. Suppl.7. P:139-141.

Masuda T & Salu GE, (1997). Cell and matrix reaction to titalium implants in surgically prepared tibiae. *International Journal of Oral-maxillofactory Implants.* July/August. 12. 4. P:472-485.

Mazess RB & Burden HS, (1991). Bone density in pre-menopausal women : effects of age, dietary intakes, physical activity, smoking and birth control pill. *American Journal of Clinical Nutrition*. 53. P132-142.

Mazess RB & Mather W, (1974). Bone mineral contents of North

Alaskan Eskimos. *American Journal of Clinical Nutrition.* 27. P:916-925.

McCoy H, Kenney MA, Montgomery C, Williams L & Orvell R, (1994). Relationship of boron to composition and mechanical properties of bone. (Review). *Environmental Health Perspectives.* Volume 102. (Suppl. 7). P:49-53.

McCully KS, (1993). Chemical pathology of homocysteine atherogenesis. *Annals of Clinical Laboratory Science.* 23. P:477.

Meacham SL, Taper JL & Volpe SL, (1994). Effects of boron supplement on bone mineral density and dietary, blood and urinary calcium, phosphate, magnesium and boron in females athletes. *Environmental Health Perspectives.* November. Volume 102. (Suppl.7). P:79-83.

Messina MJ, (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition.* Sept. 70(3 suppl.). P:439-450.

Mesiano SR & Jaffe RB, (1997). Developmental and functional biology of the primate foetal adrenal cortex. *Endocrinological Reviews.* June. Vol. 18. No.3. P378-408.

Meydani SN & Blumberg JB, (1991). Vitamin E supplement and enhancement of immune responsiveness in the aged: *In micronutrients in health and in disease prevention.* Bendick A & Butterworth CE Jr., editors. 13. P289-306. Marcel Decker. New York.

Milad MP, Klock SC, Moses S & Chatterton R, (1998). Stress and anxiety do not result in pregnancy wastage. *Human Reproduction.* 13 (8). P:2296-2300.

Miller RA & Stutman O, (1981). Decline in ageing mice of anti-TNP cytotoxic T-cell response attributable to loss of lyt-2 IL-2 producing helper cell function. *European Journal of Immunology.* 11. P:751-756.

Molgaard C & Thomson BL, (1997). Whole body mineral contents in healthy children and adolescents. *Archives of Diseases in Children.* January. 76. 1. P:9-15.

Moseman RF, (1994). Chemical disposition of boron in animals and human. *Environmental Health Perspectives.* Vol.102. Suppl. 7. P:113-

117.

Most W, van-der-wee-Pals L, Ederveen A, Papapoulos S & Lowik C, (1997). Ovarictomy and orchidectomy induce a transient increase in the osteoclastogenic potential of bone marrow cells in the mouse. *Bone.* January. No. 20(1). P: 27-30.

Moustafa AM, El-Sayed EM, Badary OA, Mansour AM & Hamada FM, (1999). Effects of bromocriptine on uterine contractility in near-term pregnant rats. *Pharmacologicl Research.* Feb., 39(2). P:89-95.

Muhlbauer RC,(1999). Nutrition- effect of vegetables on bone metabolism. *Nature.* Vol.401. No.6751. P:343-344.

Muessing K & Hunt C, (1991). The effects of dietary boron, vitamin D_3 and their interaction on glycation metabolites in chicks. *Proc. N.Y. Academy of Science.* 45:25.

Murphy S & Khaw KT, (1994). Milk consumption and bone mineral density in middle age and elderly women. *British Medical Journal.* 308. P:939-941.

Murray RK, Granner DK, Mayses PA & Rodwell VW, (1990). *Harper's Biochemistry.* 22nd edition. A Lange Medical Publication.

Mysliwska J, Bryl E, Foerster J & Mysliwka A, (1998). Increase of IL-6 and decrease of IL-2 production during the ageing process are influenced by the health status. *Ageing and Development.* February 16. 100 (3). P313-328.

Naghii MR, Chen SY & Samman S, (1996). The boron contents of selected foods and the estimation of its daily intake among free-living subjects. *American Journal of Clinical Nutrition.* 15. P:614-619.

Naghii MR & Samman S, (1997). The effects of boron supplementation on its urinary excretion and selected cardio-vascular risk factors in healthy male subjects. *Biological Trace Element Research.* Vol. 56. P:273-288.

Naghii MR & Samman S, (1993). The roles of boron in nutrition and metabolism. *Progress in Food and Nutrition Science.* 17(4). P:321-349.

Nakamura K & Kurokawa T, (1998). Stimulation of bone formation by

introsseous injection of basic fibroblasts growth factor in ovariectomised rats. *International Orthopaedics*. 22. 1. P:49-54.

National Osteoporotic Society Annual Report and Accounts, (1994/1995). National Osteoporotic Society Publication.

National Osteoporosis Society Annual Report and Accounts, (1998/1999) National Osteporotic Society Publication.

Need AG & Morris HA, (1991). Effects of salt restrictions on urinary excretion of hydroxyproline excretion in postmenopausal women. *Archives of Internal Medicine.* 151. P:757-759.

Newman RE, (1994). Essentiality of boron for healthy bones and joints. *Environmental Health Perspectives.* November. Vol. 102. Suppl. 7. P:83-85.

Ng KW & Roma E, (1997). Bone biology. Balliere: *Clinical Endocrinology and Metabolism*. April. 11. 1. P:1-22.

Nguyen TT, Tseng YT, McGonnigal B, Stabila JP, Worrel LA & Saha S, (1999). Placental biogenic amine transporter: in vivo formation, regulation and pathobiological significance. *Placenta*. Jan., 20 (1). P3-11.

Nguyen TV, Jones G & Sambrook PN, (1995). Effects of oestrogen exposure and reproductive factors on bone minerals density and osteoporotic fractures. *Journal of Clinical Endocrinology and Metabolism.* 80. P:2709-2714.

Nielsen FH, (1992). Biochemical and physiological consequences of boron deprivation in humans. *Environmental Health Perspectives.* Vol.102. Suppl. 7. November.

Nielsen FH, (1990). Studies on the relationship between boron and magnesium which possibly affects the formation and maintenance of bone. *Magnesium & Trace Elements*. No. 9. P61-69.

Nielsen FH, Hunt C, Mullen LM & Hunt JR, (1987). Effects of dietary boron on mineral, oestrogen and testosterone metabolism in post-menopausal women. *FASEB*. July 1, P394-397.

.

Nielsen ME, Fiatarone MA & Morganti CM, (1994). Effects of high intensity strength training on multiple risk factors for osteoporotic fractures: a randomised controlled trial. *Journal of American Medical Assocation*. No. 272, P: 1909-1914.

Nielsen FH & Mullen LM, (1991). Dietary boron affects blood cells count and haemoglobin concentration in human. *Journal of Trace Elements Experimental Medicines*. 4. P:211-223.

Nielsen FH, (1992). The saga of boron in foods: from a banished food preservative to a beneficial nutrients for humans. *Current topics in plant biochemistry and physiology*. Vol.10. P:274-286.

Nielsen FH, (1998). The justification for providing dietary guidance for the nutritional intake of boron. *Biological Trace elements and Research.* Winter. 66(1-3). P:319-330.

Nieves JW, Komer L, Cosman F & Lindsay R, (1998). Calcium potentiates the effects of oestrogen and calcium on bone mass: reviev and analysis. *American Journal of Clinical Nutrition*. 67. P:18-24.

Norman AW, (1985). The vitamin D endocrine system. *Physiologist.* 28. P:219-232.

Norris RJ, (1992). Medical cost of osteoporosis. *Bone.* 13. P:11-16.

Notelovitz M, (1997). Oestrogen therapy and osteoporosis : principles and practice. *American Journal of Medicine and Science.* January. 313 (1). P2012.

Notelovitz M, (1997). Oestrogen therapy and osteoporosis : principles and practice. American Journal of Medicine and Science. January. 313(1). P:2-12.

O'Neil TW, Felsenberg D, Varlow J & Cooper C, (1996). Influence of sex and geography on the prevalence of vertebral deformity. *Journal of Bone and Mineral Research.* 11. P:1010-1018.

Odell WD & Heath III H, (1993). *Disease-a-month.* Vol.XXXIX. November. No. 11. Mosby Publication.

Ohl F & Fuchs E, (1999). Differential effects of chronic stress on memory

processes in the tree shrew. *Brain Research-Cognitive Brain Research.* January. 7(3). P:379-387.

Ortega RM, Martinez RM, Quintas ME, Lopez-Sobaler AM & Andres P, (1998). Calcium levels in maternal milk: relationship with calcium intake during the third trimester of pregnancy. *British Journal of Nutrition.* June. No. 79(6). P: 501-507.

Orwell ES, (1992). The effects of dietary insufficiency and excess on skeletal health. *Bone.* 13. P:343-350.

Osler M & Schroll M, (1991). A dietary study of the elderly in the city of Roskilde. *Danish Medical Bulletin.* Vol. 38. No.5. P:410-413.

Overgaard K, Hansen MA, Jensen SB & Christain C, (1992). Effects of salcaltinin given intrnasally on bone mass andfracture rates in establish osteoporosis: a dose-response study. *British Medical Journal.* No. 305. P:556-561.

Pandipati S, Driscoll JE & Franceschi RT, (1998). Glucocorticoid stimulation of Na+-dependent ascorbic acid transport in osteoblasts-like cells. *Journal of Cell Physiology.* July. 176. 1. P:85-91.

Papanicolaou DA, Wilder RL, Manoglass SC & Chrousos GP, (1998). The pathophysiologic roles of interleukin-6 in human diseases. *Annals of Internal Medicines.* January 15. 128(2). P:127-137.

Parfitt AM, (1994). The coupling of bone formation to bone resorption : a critical analysis of the concepts and of its relevance to the pathogenesis of osteoporosis. *Metabolic Bone Disease-Related Research.* 4. P:1-6.

Pary T, (1992). Health expenditure in Australia. *Medical Journal of Australia.* 156. P:592-594.

Penland JG & Eberhardt MJ, (1993). Effects of dietary boron and magnesium on brain function of mature male and females Long-Evans rats. *Journal of Trace Element and Experimental Medicines.* 6. P:53-64.

Penland JG, (1994). Dietary boron, brain function and cognitive performance. *Environmental Health Prespectives.* 102 Supplement 7. P:65-72.

Peris P, Pares A, Guanbeus N, Pons F, Martinez de Osaba MJ & Munoz-Gomez J, (1992). Reduced spinal and femoral bone mass and deranged bone minerals metabolism in chronic alcoholism. *Alcohol and Alcoholism.* 27(6). P:619-625.

Perkins SL, Gobbons R, Kling S & Kahn AJ, (1994), Age-related bone loss in mice is associated with an increased osteoclast progenator pool. *Bone.* Vol. 15. No.1. P65-72.

Petridou E & Karpathios T, (1997). The roles of dairy products and non-alcoholic beverages in bone fracture among school age children. *Scandanavian Journal of Social Medicines.* June. 25. 2. P:119-125.

Pfeiffer S, (1998). Variability in osteon size in recent human populations. *American Journal of Physiology and Anthropology.* June. 106. 2. P:219-227.

Phantom C, (1997). *Calcified Tissue International.* 58. P:135-143.

Piolli G, Basini G, Pedrazzoni M, Musetti G & Wieth, (1992). Spontaneous release of IL-1 and IL-6 by peripheral blood mononuclear cells after inflamation. *Clinical Science.* 83. P:503-507.

Pocock NA, Eisman JA & Kelly PN, (1989). Effects of tobacco use on axial and appendicular bone minaerl density. *Bone.* 10. P:329-331.

Portale AA, Holloran BP, Murphy MM & Morris Jr. RL, (1986). Oral intake of phosphorus can determine the serum concentration of 1,25 dihydrovitamin D by determining its production rate in human. *Journal of Clinical Investigation.* 77. P:7-12.

Porth CM, (1994). *Pathophysiology: concepts of altered health status.* J.B. Linpincott company.

Porthmouth K, Henderson K, Graham N, Price P, Cole J & Allen J, (1994). Dietary calcium intakes in 18 years old women : comparison with recommended daily intakes and dietary energy intakes. *Journal of Advanced Nursing* . December 20(6) P:1073-1078.

Powell JT, (1998). Vascular damage from smoking : diseases mechanisms at the arterial wall. *Vascular medicine.* 3(1). P:21-28.

Preston-Martin S, Pogoda JM, Mueller BA, Lubin F, Holly EA, Filipini G, Cordier S, Peris-Bonnet R, Choi W, Little J & Arsan A, (1998). Prenatal vitamin supplementation and risk of childhood brain tumours. *International Journal of Cancer*. 11. P:17-22.

Randell A, Sambrook PN & Ngayen TV, (1995). Direct clinical and welfare costs of osteoporotic fractures in elderly men and women. *Osteoporosis International*. 5. P:427-432.

Reddy KG & Enwemeka CS, (1996). A simplified method for analysis of hydroxyproline in biological tissues. *Chemical Biochemistry*. 29(3). P:225-229.

Reed JA, Anderson JJB, Tylavsky FA & Gallager PN, (1994). Comparative changes of radial bone density in elderly females lacto-ovo-vegetarian and onmivorous. *American Journal of Clinical Nutrition*. 59. (Suppl.) P:1197-1202.

Reid I, Ames R, Evans M & Heaney R, (1993). Effects of calcium supplementation on bone loss in post-menopausal women. *New England Journal of Medicines*. 328. P:460-466.

Reid IR, (1997). *Clinical Endicrinology and Metabolism: International practice and research*. Vol. 11. No.1. April. Balliere Tindall . *Research*. 6. P:507-513.

Rey C & Kim HM, (1996). Characterisation of the apatite crystals of bone and their maturation in osteoblasts cells culture:comparison with native bone crystals. *Connective-Tissue- Reserves*. 35 (14). P:343-349.

Riddle JM, Bluhm GB & Pitchford WG, (1981). A comparative study of platelets reactivity in arthritis. *American New York Academy of Science*. 370. P:22-29.

Rigg BL & Melton LJ, (1987). Dietary calcium intake and rate of bone loss in womem. *Journal of Clinical Investigation*. 80. P:979-982.

Rigg BL, Wahner HW , Dunn WL & Mazess RB, (1981). Differencial changes in bone minerals density of the appendicular and axial skeleton with ageing. *Journal of Clinical Investigation*. 67. P:328-335.

Riggs BL & Melton LJI, (1986). Involutional osteoporosis. *New* England

Journal of Medicine. 314. P:1676-1686.

Rizoli R & Bonjour JP, (1998). Calciotropic hormones and intergrated regulation of calcaemia and calcium balance. *Review Practitioner.* June. Vol. 48 (11). P:1178-1184.

Rodriguez-Jamirez J, Rodriguez JR & Gonzalez MJ, (1998). Indications of anxiety and depression on subjects with different kinds of diet:vegetarians and omnivores. *Bulletin-Association-Medica-de-Puerto Rico.* April / June, 90 (4-6). P:58-68.

Roodman GD, (1993). Roles of cytokines in the regulation of bone resorption : a review. *Calcified Tissue International.* 53(Suppl. 1). P:94-98.

Ross EJ & Linch DC, (1982).Cushing's Syndrome - killing disease: discriminatory value of signs and symptoms aiding early diagnosis. *Lancet* 2. P:646-649.

Rossi V, Breviario F, Ghezzi P, Dejana E & Mantovi A, (1985). A prostaglandin synthesis induced in vascular cells by IL-1. *Science.* 229. P:174-176.

Rozenberg S, Kroll M, Pastijn A & Vandromme J, (1995). Osteoporosis prevention and treatment with sex hormone replacement therapy. *Clinical Rheumatology.* 14. 3. P:14-17.

Russell RGG, (1997). The assessment of bone metabolism In Vivo using Boichemical Approaches. *Hormone Metabolism Research.* 29. P:139-144.

Ryan MF, (1991). The roles of magnesium in clinical biochemistry : an overview. *Annals of clinical biochemistry.* 28. P:19-26.

Saito JK, Davis JW, Wasnick RD & Ross PD, (1995). Users of low-dose glucocorticoids have increased bone loss rates: a longitudinal study. *Calcified Tissue International.* 57. P:115-119.

Saito T, Tazawa K, Yoshinobu Y & Saito M, (1997). Surgical stress inhibits the growth of fibroblasts through elevation of plasma catecholamines and cortisol concentration. *Surgical Today: Japan Journal of Surgical* 27. P:627-631.

Sampson HW, (1998). Effects of alcohol consumption on adult and aged bone: a histopathological study of the rat animal model. *Alcohol-Clinical and Experimental Research*. December. 22 (9). P:2029-2034.

Sarkota T, Makisalo H, Fukunaga T & Erickson CJ, (1999). Acute effect of alcohol on oestrodiol, oestrone, progesterone. prolactin, cortisol and leuteinising hormone in premenopausal women. *Alcoholism: Clinical and Experimental Research*. June. 23(6). P:976-982.

Schmidt T, Wijga A, Von-Zur-Muhlen A, Brabant G & Wagner TD, (1997). Changes in cardiovascular risk factors and hormones during a comprehensive residential 3 months yoga training and vegetarian nutrition. *American Journal of Clinical Nutrition*. 35. P:236-139.

Schneider DL, Barrett-Connor EL & Morton DJ, (1997). Timing of post-menopausal oestrogen for optimal bone density. *Journal of American Medical Association.* No. 277. P: 543-547.

Schwartz F, (1993). Obesity in adult females : the relationship among personality characteristics, dieting and weight. *Association of American Occupational Nursing Journal.* October. No. 41(10). P: 504-509.

Seal BS & Weath HJ, (1980). Effects of boron in drinking water on the male laboratory rats. *Bulletin of Environment Contamination Toxicology.* 25. P:782-789.

Seeman E & Young N, (1993). Risk factors for osteoporosis. *Osteoporosis International*. 3 (Suppl.1). P:40-43.

Sharon M, (1989). Complete nutrition: how to live in total health. *A Priori Publication.*

Shiu AT, (1998). Work and family role juggling and mood states of Hong Kong public health nurses with children. *Journal of Advanced Nursing.* July. 28(1). P:203-211.

Shui AT, (1998). The significance of sense coherence for the perception of task characteristics and stress during interuptions amongst a sample of public health nurses in Hong Kong. *Public Health Nursing*. Aug,. 15(4). P:273-280.

Siobel MJ, Duncan A & Robins SP, (1989).Urinary hydroxypyridinium

cross-links provide indices of cartilage and bone involvement in Arthritic diseases. *Journal of Rheumatology.* 16. P:964-970.

Skodjt H & Russell RDD, (1992). Bone cell biology and the regulation of bone turnover. P:1-70. In: *Cytokines and bone metabolism.* Gowen M. (Ed.) CRC Press.

Slemenda CW, Christian JC, Miller JZ, Reister TK & Johanson CC, (1992). Long term bone loss in men: effects of genetic and environment factors. *Annals of Internal Medicine.* 117. P:286-291.

Smith EL & Gillian C, (1996). Dose-response relationships between physical trading and mechanical competence of bone. *Bone.* 18. P:45-50.

Smith R, (1998). Alterations in the hypothalamus-pituitary-adrenal-axis during pregnancy and the placental clock that determines the length of partuition. *Journal of Reproductive Immunology.* August. 39 (1-2), P215-220.

Smith RA, (1986). Boron in glass and glass-making. *Journal of Non-crystal and Solids.* 84. P:421-432.

Snow-Harter C, Bouxsein ML & Lewis BT, (1992). Effects of resistance and endurance exercise on bone mineral status of young women: a randomised exercise intervention trial. *Journal of Bone and minerals Research.* 7. P:761-769.

Spencer H, (1983). Further studies of the effects of a high protein diet as meat on calcium metabolism. *American Journal of Nutrition.* 37. P:924-929.

Spencer H, Kramer L, De Bartolo M, Norris C & Osis D, (1983). Further studies of the effects of high protein diet on calcium metabolism. *American Journal of Clinical Nurtition.* 37. P:924-929.

Sprague RW, (1992). Boron, metal and minerals annual review. *Metal & Minerals* . Part 2. P:106.

Sproot H & Muller A, (1997). Collagen cross-links in fibromyalgia. *Arthritis and Rhenmatology.* August. 40. 8. P1450-1454.

St. John-Dixon A & Woolf AD, (1988). *Osteoporosis: a clinical guide.*

Martin Dumitz Publication.

Stamper MJ & Grodstein F, (1994). Role of HRT in cardio-vascular diseases: In Lobo RA, (Ed.), *Treatment of post-menopausal women: basic and clinical aspect*. P223-233. New York. Raven Press Ltd.

Stein PC, Torri A & Parsons CL, (1999). Elevated urinary norepinephrine in interstitial cystitis. *Urology*. June ; 53. 6. P:1140-1143.

Sterck JG, Klein-Nulend J, Lips P & Burger EH, (1998). Responses of normal and osteoporotic human bone cells to mechanical stress in vitro. *American Journal of Physiology*. June. 274 (6). P: 1113-1120.

Stevenson JC & Marsh MS, (1992). *An atlas of osteoporosis*. Oxford Publication.

Suda T, Takahashi N & Martin TJ, (1992). Mutation of osteoclasts differentiation. *Endocrinological Review*. 13. P:66-80.

Sun GY, Xia J, Draczynska-Lusiak B, Sununyi A & Sun AY, (1999). Grape polyphenoids protect neurodegenerative changes induced by chronic ethanol administration. *Neuroreport*. Jan. 18, Vol. 10, No. 1, P93-96.

Suta M & Yasin N, (1998). Expression of bone matrix protein mRNA during distraction osteogenesis. *Journal of Bone Mineral Reserves*. 13. 8. P:1221-1231.

Svec F, (1997). Ageing and adrenal-cortical function. Balliere's *Clinical Endocrinology and Metabolism*. 11(2). P:271-275.

Takwshima A & Kitano Y, (1998). Osteogenic potential of cultured periosteal cells in a distracted bone gap in rabbits. *Journal of Surgical Reserves*. July 15. 78(1). P:68-77.

Tecott L, Shtrom S & Julius D, (1995). Expression of a serotonin-gated ion-channel in embryonic neural and non-neural tissues. *Molecular-cellular-Neurosciences*. February. 6 (1). P:43-45.

Thibault L & Booth DA, (1999). Macronutrients-specific dietary selection in rodents and its neural bases. *Neurosciences & Biobehavioural Reviews*. March, 23(4). P:457-528.

Thompson DD & Rodan GA, (1986). Immobilisation products in bone resorption and decrease formation in rats. *Journal of Bone Minerals Reserves.* 1. (Supplement 1). P:96.

Thorsen K, Kristoffersson A, Hultdin J & Lorenteon R, (1997). Effects of moderate endurance on calcium, parathyroid hormone and markers of bone metabolism in young women. *Calcified Tissue International.* 60. P:16-20 .

Thurman RG, Bradford BU, Iimuro Y, Knecht KT, Arteel GE, Yin M, Connor HD, Wall C, Raleigh JA, Frankenberg MV, Adachi Y, Forman DT, Brenner D, Kadiiska M & mason RP, (1998). The role of gut-derived bacterial toxins and free radicals in alcohol-induced liver injury. *Journal of Gastroenterology and Hepatology.* September 13 Supplement. P:39-50.

Tilyard MW, Spears GFS, Thompson J & Dovey S, (1992), Treatment of post-menopausal osteoporosis with calcitrol and calcium. *English Journal of Medicines. 326 . P357-362.*

Tojo Y, Karabayashi T, Honda A, Yamamato Y, Yahati T, Takakura K & Tamaka Y, (1998). Bone structural and metabolic changes at the end of pregnancy and lactation in rats. *American Journal of Obstetric and Gynaecology.* January. 178 (Pt.1). P:180-185.

Tortora GJ & Anagnostakos NR, (1996). *Principles of Anatomy and Physiology.* 6th edition. Harper & Row Publication.

Tran JG, Kovacs SJ, Mc Intosh TS, Davis HM & Martin DE, (1999). Morning spot and 24-hour urinary 6 beta-hydroxycortisol to morning cortisol ratios: intra-individual variability and correlation under basal conditions of CYP 3A4 induction. *Journal of Clinical Pharmacology.* May. 39. 5. P:487-494.

Valimaki MJ, Karkkainen M, Lambert-Allardt C & Laitinen K, (1994). Exercise, smoking and calcium intakes during adolescent and early childhood as determinants of peak bone mass. *British Medical Journal.* 309. P:230-235.

Vitto J & Teir H, (1972). Glucocorticoids-induced inhibition of the biosynthesis of human collagen. *Biochemical Pharmacology.* 21. P:2161-2167.

Volpe SL, Taper LJ & Meacham S, (1993). The relationship between boron and magnesium in the human bone density: a review. *Magnesium Research*. 6. (31). P:291-296.

Vorobiev DV & Grigoriev AI, (1998). Specific glucocorticoids binding at different levels of human motor activity. *Aviation-Space-Environmenta - Medicine*. 69 (8). P:771-776.

Wand GS, mangold D, Ali M & Giggey P, (1999). Adrenocortical responses and family history of alcoholism. *Alcoholism: Clinical and Experimental Research*. July. 23(7). P:1185-1190.

Wang H & Kaizak S, (1998). Serum bone alkaline phosphatase iso-enzyme levels in normal children and children with growth hormone deficiency: a potential marker for bone formation and resorption to growth hormone therapy. *Journal of clinical Endocrinology and Metabolism*. July. 82. 7. P:2056-2061.

Wasnich RD & Davis J, (1980). Effects of thyazide on rate of bone mineral loss: a longitudinal study. *British Medical Journal*. 301. P:1303-1305.

Watson R, (1997). Nutrition. *Elderly Care*. 93. P:27-30.

Weinstein SL & Buckwater JA, (1994). *Turek's Orthopaedics: Principles, Practices and their Applications* . 5th edition . J.B. Lippincott & Co. Publication .

Welch MW, Hammer DW, & Feltman MJ, (1990). Method comparison for calcium determination by flame atomic absorption spectrometer in the presence of phosphate. *Clinical Chemistry*. 36(2). P:351-354.

Wittert G & Nordin BE, (1998), Calcium supplementation suppresses bone resorption in early post-menopausal women. *Calcification Tissue International*. Jan., 62. 1. P:8-12.

Wlodarski KH, (1990). Properties and origin of osteoblasts. *Clinical Orthopaedics*. 252. P:276-293.

Woo J, Kwok T, Ho SC, Sham A & Lau E, (1998). Nutritional status of elderly chinese vegetarians. *Age-Ageing*. July. 27(4). P:455-561.

Wood GW, (1994). An introduction to Boron: History, Sources, Uses and Chemistry. *Environmental Health Perspectives*. November. Vol.2 (suppl. 7). P:5-11.

Yang RS, Fu WM, Wang SM, Lu KS, Liu TK & Lin-Shiau SY, (1998). Morphological changes induced by prostaglandin E in cultured rat osteoblasts. *Bone*. June. 22(6). P:629-636.

Yeap SS, & Hosking DJ, (1995). Glucocorticoid-induced osteoporosis. *Current Opinion in Endocrinological Diabetes*. P: 248-254.

Yong LC, (1997). The mast cell: origin, morphology, distribution and function. *Experimental Toxicology & Pathology*. Dec. 49 (6). P:409-424.

Yoon BH, Romero R, Jun JK, Maymon E, Gomez R, Mazor M & Park JS, (1998). An increase in foetal plasma cortisol but not dehydroepiandrosterone sulfate is followed by onset of pre-term labour in patients with pre-term premature rupture of the membranes. *American Journal of Obstetrics and Gynaecology*. November. 179(5). P:1107-114.

Yoshikuwa H, (1998). Regulatory mechanism of bone remodelling. *Nippon-Ruisho (Japan)*. June, 56 (6), P:1400-1405.

Zerwekh JE & Reed BY, (1998). Normal vitamin D receptor concentration and responsiveness to 1,25 dihydroxyvitamin D_3 in skin fibroblasts from patients with absorptive hypercalcaemia. *Mineral-Electroytes Metabolism*. Vol. 24, No. 5, P:307-313.

Zima T, (1993). Haematological immunologic, endocrine disorders and muscle and bone damage caused by ethanol foetal alcohol syndrome. (Czech). *Sbovnit Lekarsky*. 94 (4). P:303-309.

Zumkley H & Lehnert H, (1984). Magnesium, potassium and hormonal regulation. *Magnesium*. 3. P:329-328.