RELATIONSHIP BETWEEN BONE MASS DENSITY AND INSULIN LEVELS IN POSTMENOPAUSAL FEMALE POPULATION

THESIS
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BY

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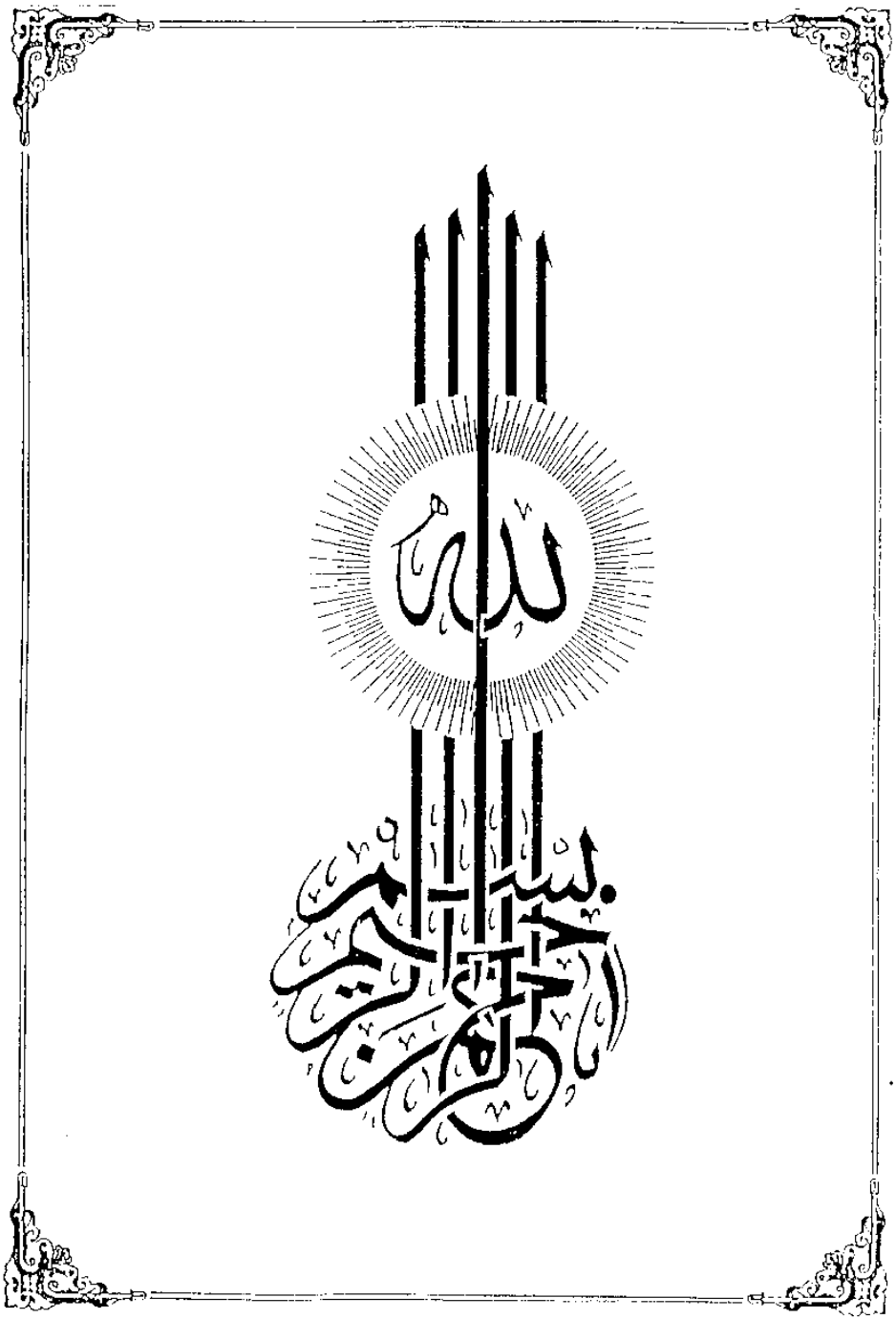

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INTRODUCTION

INTRODUCTION

Bone mass density is closely related to body weight in both men and women **(Reid et al., 1992)**.

The weight-bone mass density relationship in women appears to be substantially mediated by fat-bone mass density interdependence. The association between bone mass density and obesity was attributed to the conversion of androstenedione to oestrogen in fat cells and also to weight bearing **(Barrett and Kriz, 1996)**.

It has been observed that female patients with non-insulin dependent diabetes mellitus (NIDDM) have increased bone density independent of body weight **(Bauer et al., 1993)**.

This raises the question about the etiology of this association. A causal role for hyperinsulinaemia that frequently accompanies both obesity and NIDDM could be suspected **(Hollenbeck et al., 1987)**.

Aim of the Work:

The aim of this thesis is to study the possible link between insulin levels and bone mass density in post-menopausal female subjects.

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REVIEW OF LITERATURE

soon, the osteoblasts deposit an orderly lamellar arrangement of collagen ***(Lawrence, 1995)***.

Structure of bone:

The basic structural unit of bone is the Haversian system or osteon, which consists of a series of concentric laminations or lamellae surrounding a central canal approximately 20 µm in diameter and 3 to 9 mm in length and contains interlacing reticular tissue. Osteoblasts and osteoclasts in various stages of activity and a neurovascular bundle.

The central canals run parallel to the long axis of the bone and are united by communication with the canalicular canaliculae and by Volkmann's canals which pierce the bone from the outer and inner surfaces.

The bone is surrounded by periosteum which is made up of an outer layer of white fibrous tissue and elastic tissue and an inner cambium layer which has a losser composition, is more vascular and contains cells with osteogenic potency. The periosteum serves as a limiting membrane for bone and is responsible for periosteal osteogenesis, in which the bone substance is increased by a process of accertion. It also forms intimate contact for the attachment of muscles and other structures to establish continuity throughout the musculoskeletal system. Another limiting membrane, the endosteum, lines the surfaces of the cancellous bone ***(Roger, 1996)***.

Bone composition:

Bone connective tissue:

Bone connective tissue is composed of an extracellular collagenous matrix. The ground substance contains glucosaminoglycans, non-collagenous proteins and minerals. The bone mineral is mainly calcium hydroxyapatite crystals deposited

within and on extracellular matrix, collagenous matrix is formed of type I collagen.

Collagen is the main extracellular protein in the body. More than half the body's collagen is in the skeleton. Collagen molecule is composed of three helical polypeptide a-chain, each comprises repetitive triplets to produce the sequence (Glyxy) where Gly is glycine, x is often proline and y is hydroxyproline.

The synthesis of a-chain is controlled by specific chain and the a-chain composition distinguishes at least 14 different types of collagen with different functions and tissue distribution.

Major fibrillar collagen may form covalent link with minor collagen.

During intracellular synthesis, each a-chain precursor goes through an impact series of post-transitional modification (which include hydroxylation ions of proline and lysine residues and glucosylation of hydroxylysine) and is incorporated into a triple helical collagen molecule. When the extensions are removed from both ends of this precursor molecule, collagen is exported from the cell and self-assembly and fiber formation begins. Normal collagen fiber formation depends on exact biosynthesis.

Defective biosynthesis gives rise to several disorders, only some of these disorders affect the skeleton because bone contains only type-I collagen and the main collagen affected is type II collagen ***(Roger, 1994)***.

Non-collagenous proteins:

Comprise 10-15% of bone proteins, their function is incompletely understood. Some are involved in the attachment of osteoclasts to bone matrix, others may play a role in collagen fibrillogenesis, matrix mineralization and regulation of bone remodeling ***(Herring, 1992)***.

Bone cells:

Cell biology of bone:

The lineages of bone-forming cells (osteoblasts) and bone resorbing cells (osteoclasts) probably become separate early in the development. Their function is controlled by a complex system of intracellular signals that involve not only systemic calcium regulating and growth regulating hormones but also local factors.

Bone disease occurs when there is an imbalance between the functions of forming and resorbing cells. Thus accelerated resorption and diminished formation exacerbate decreased bone mass in osteoporosis. Excessive bone mass occurs because bone resorption is impaired as in congenital osteopetrosis, or because formation is excessive as in virally induced Anion osteopetrosis and in Paget's disease of the bone ***(Friedenstein et al., 1992)***.

Types of bone cells:

1. Osteoblasts:

The osteoblast is a highly specialized bone matrix synthesizing cell, it is derived from precursor cells in the periosteum or the stroma of the bone marrow called determined osteoprogenitor cells ***(Friedenstein et al., 1992)***. Osteoblast has a central eccentric nucleus and abundant rough endoplasmic reticulum.

There is also a high level of alkaline phosphatase, where vesicles containing amorphous calcium phosphate may also be present. The cells are connected together and to the process of subjacent osteocytes by gap junctions. Inactive bone surfaces are lined by a monolayer of flattened cells which histologically are resting osteoblasts. The osteoblasts are responsible for the synthesis of the major proteins of bone including type-I collagen and the non-collagenous proteins of bone such as osteocalcin (bone Gla protein) and osteonectin. The cells are also involved in the

mineralization of bone and produce the enzyme alkaline phosphatase which may be important for this process.

Recent evidence suggests that osteoblast plays a central role in controlling osteoclastic function. The osteoblasts and not the osteoclasts have a specific surface receptors for agents which stimulate bone resorption such as 1,25 dihydroxy vitamin D3 and parathyroid hormone ***(Roger, 1996)***.

Osteoblasts may trigger bone resorption in the remodeling process. This activation step may involve shape changes or secretion of collagenase and related metaloproteins and plasminogen activator which is a proteolytic enzyme. They also secrete autocrine factors as prostaglandins and bone derived growth factors ***(Allan et al., 1991)***.

2. Osteoclasts:

Osteoclasts resorb bone and calcified cartilage. These large multinucleated cells are formed by the fusion of mononuclear precursors. Osteoclasts presumbly are derived from a hematopoietic stem cells rather than from the mesenchymal precursors of the osteoblast. The osteoclast cell line is related to the monocyte-macrophage lineage ***(Suda, 1992)***.

Osteoclasts are large usually multinucleated cells which contain abundant mitochondria, many lysosomes and little rough endoplasmic reticulum. The unique feature of the osteoclast is the ruffled border that is surrounded by clear zone which functions to attach the osteoclast to the bone and isolate the ruffled border from the extracellular fluid ***(Zaidi et al., 1993)***.

Osteoclasts are rich in acid phosphatase as well as other lysosomal enzymes and in carbonic anhydrase that facilitates hydrogen ion secretion. Hydrogen ions are important not only in mobilizing minerals but also in activating lysosomal enzymes that can degrade all components of bone matrix ***(Chatterjee et al., 1993)***.

The other important features of osteoclast are the attachment apparatus which involves vitronectin receptors that can bind a variety of proteins containing Arg-Gly-Asp sequences ***(Helfrich, 1992)***.

3. Osteocytes:

Osteocytes are the osteoblasts that are incarcerated in the matrix which they secrete and become buried deep within the bone. These osteocytes have long processes through which they maintain contact with each other and with the superficial bone lining cells ***(Roger, 1996)***.

4. Other cell types in bone:

1. Macrophages: may be found at resorption sites after the initial removal of bone osteoclasts. They may remove residual matrix that has not been completely digested because they can secrete collagenous as well as lysosomal enzymes. Macrophages also may be source of interleukin-I (IL-1) and prostaglandin E2 (PGE2) ***(Chatterjee et al., 1993)***.

2. Lymphocytes: may play a role by secreting local regulators, somatomedin or insulin like growth factor I (IGF-I) which is a potent stimulator for bone growth.

3. Mast cells: are found adjacent to resorbing bone and can produce heparin ***(Chatterjee et al., 1993)***.

Modeling and remodeling of the bone:

The term modeling refers to the process by which bone grows and alters its shape through resorption and formation at different sites. For example, the long bones enlarge by periosteal formation and endosteal resorption. As they lengthen, the large amount of

bone formed at the growth plate is resorbed to maintain a hollow tubular structure.

The term remodeling refers to the process in which resorption is followed by formation at the same site, hence the two processes are "coupled". This process is important for the overall health and functional integrity of skeletal tissues as well as for the metabolic responses of the bone mineral reservoir. In large mammals, the cortical bone is remodeled by the development of the Haversian system of osteons.

These structures are formed by osteoclastic removal of a cylinder of bone. Behind these osteoclasts, are a vascular loop and mesenchymal cells that differentiate into osteoblasts and form concentric lamellae of new bone around the central vascular canal ***(Martin, 1992)***.

Remodeling of trabecular bone surface occurs as follows:
Osteoclasts excavate scalloped areas called Howship lacunae which are then replaced by packets of new lamellar bone laid down by osteoblasts ***(Raize, 1992)***.

Chemistry of bone:

Bone is made up of organic and inorganic materials and water (fig. 1).

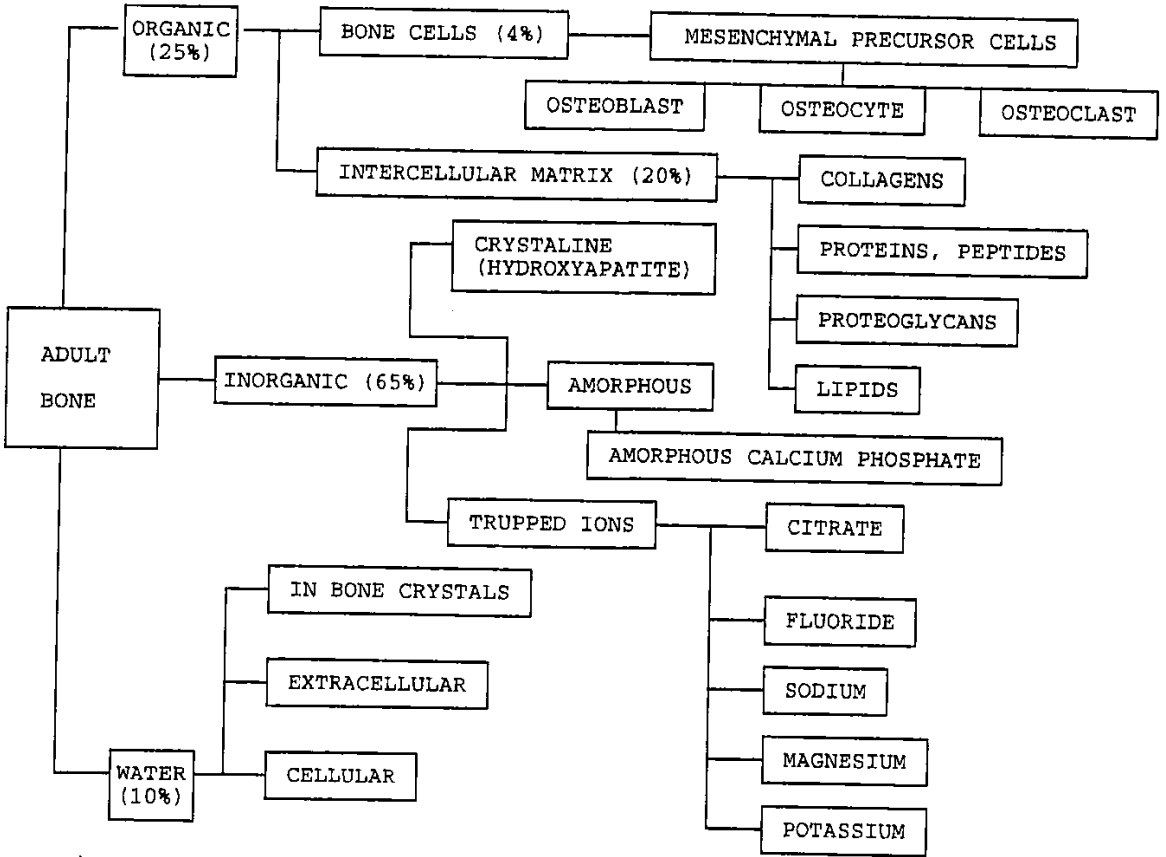

Fig. (1): The composition of bone showing the relative proportions of organic to inorganic to water making up adult bone ***(Roger, 1996)***

1. The inorganic components of bone:

The inorganic or mineral component of bone serves two basic functions:

First, it determines the mechanical properties of bone. The strength of bone depends on the exact chemical composition, nature and three dimensional deposition of mineral, while its ability to resist cyclical loading and to regenerate is due to continuous turnover of skeletal elements.

Second, bone mineral functions as a reservoir of ions, particularly calcium and phosphate. In order to perform this function, ions must be able to be sequestered or removed from the bone mineral by physiological processes, which implies that the energy changes in these processes are within the narrow range available to biological processes ***(Roger, 1996)***.

Bone minerals consist largely of hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) together with transition forms and other minerals absorbed on the surface ***(Johansen et al., 1992)***.

The skeleton contain 99% of body calcium, 90% of phosphorous, 80% of carbonate, 60% of magnesium and 35% of sodium. Hydrogen ion is generated when hydroxyapatite is formed from circulating Ca^{2+} and HPO_4^{2-} when this hydrogen ion is in excess, this can be buffered by demineralization of bone and releasing carbonate and phosphate ***(Johansen et al., 1992)***. There are many bone seeking elements such as aluminum, fluoride, lead and strontium. Deposition of such elements on the bone protect other soft tissues from their lethal effect, but is likely to alter bone cell functions ***(Young et al., 1992)***.

2. The organic components of bone:

The organic components is made up of osteogenic cells as described above and the intercellular matrix of bone which consists