CHAPTER 1

General Introduction

Motivation for the study

Osteoporosis, the most commonly occurring bone disease, is the leading cause of serious morbidity and functional loss in the elderly.[1] This disease is considered a major public health problem in the Western World and the prevalence thereof is increasing in the developing world. Statistics for the prevalence of osteoporosis in South Africa are not available. However, it is predicted that in the developed world one in three women and one in eight men over the age of 50 will suffer an osteoporotic fracture during their lifetime.[2] Although osteoporosis is generally considered a modern disease, forensic evidence shows that bone loss with age already occurred in ancient communities such as the Egyptians dating back to the XIIth Dynasty (1990-1786 B.C.).[3] Various factors have been identified as contributing to osteoporosis, including genetic factors; aging; oestrogen deficiency; low body mass; smoking; physical inactivity; medications such as glucocorticoids; and malnutrition.[4] Among dietary factors, heavy alcohol consumption, low energy intake, low intake of calcium and vitamin D and high protein consumption have been listed.[2,5]

The mature human skeleton is a metabolically active organ that is continuously resorbed and rebuilt by osteoclasts and osteoblasts. Osteoclasts and osteoblasts work together in a synchronised manner, such that bone resorption and formation are closely coupled, this results in the maintenance of a constant level of bone mass. Disruption of this coordination underlies many bone diseases, including osteoporosis.[6] Differentiation of osteoclasts is closely coupled with the function of osteoblasts through a variety of cytokines.[7] Some of the proteins involved in the interaction between cells of osteoblastic and osteoclastic lineage have been identified. These proteins belong to the families of tumor necrosis factors and receptors[8-12] and include *RANKL* (Receptor activator of nuclear factor-κB ligand) and its cognate receptor *RANK* (Receptor activator of nuclear factor-κB), as well as a

decoy receptor *osteoprotegerin* (OPG). When RANKL, expressed on the osteoblast cell membrane, binds to its natural receptor RANK, present on the osteoclast progenitor membrane, osteoclastic differentiation and activation is initiated.[13] OPG is a soluble decoy receptor secreted by osteoblasts that competes with RANKL for binding to RANK, thereby preventing its osteoclastogenic effect.[10-11] The presence of OPG in the bone microenvironment thus limits the number of mature osteoclasts and therefore could have a determining effect on resorption rate and bone mass. The discovery of the OPG/RANKL/RANK system provides a completely new perspective on bone biology. A large number of stimulators and inhibitors of bone resorption, such as cytokines and hormones, have been shown to converge on the RANKL/RANK/OPG pathway, making this an appropriate target for therapeutic intervention.[14]

It is preferable to prevent osteoporosis rather than having to treat it. The bone mass attained early in life may be the most important determinant of skeletal health in later life.[15-16] A balanced diet, amongst others, is considered to be of utmost importance in the prevention of osteoporosis. Adequate consumption of specific nutrients, especially calcium and vitamin D in early life will optimise peak bone mass, and adequate intakes of these nutrients should continue through the remainder of life to maintain bone mass.[15,17]

Polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series are essential for life and health, they cannot be produced by animals and they (or some suitable precursor) must be obtained from plant or animal sources as part of the diet. PUFAs, especially the omega-3 (n-3) PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in fish oil, are of paramount importance for health and disease prevention. The n-3 PUFAs have been shown to be beneficial in the prevention and treatment of a variety of medical conditions such as cardiovascular diseases, neurological disorders, inflammatory diseases, some cancers and rheumatoid arthritis.[18-20] During the past two decades, the effects of dietary long chain PUFAs on bone health received considerable attention.[16,21,22]

There is increasing evidence that lack of certain dietary PUFAs contribute to bone loss.[21,23-24] On the other hand, dietary supplementation of some PUFAs has been shown to be beneficial for bone.[25-28] Animal studies suggest that the n-3 PUFAs

EPA and DHA could reduce the risk of osteoporosis and fractures.[25,29] Human studies confirmed the beneficial effects of dietary PUFA supplementation. A controlled clinical study, for instance, found that supplementation of calcium, γ-linolenic acid and EPA in the diet of elderly women enhance calcium absorption, reduce calcium excretion, and have overall positive effects on bone mineral density.[27] It has also been shown that a reduction of the n-6/n-3 PUFA ratio could result in increased bone strength in animals[30-31] and in humans.[32] Considering these results, it may be possible that the consumption of diets rich in n-3 fatty acids could help to build and maintain a healthy skeleton in the human. As these products are part of the diet, they could be conveniently delivered as dietary supplements to a large population at an affordable cost.

Although results from clinical trials and *in vivo* animal studies suggest that specific PUFAs might benefit bone health, the cellular mechanisms of different PUFAs have not been clarified and need to be investigated. Changes in dietary PUFAs are reflected in the composition of various tissues, including bone cells such as the osteoblasts.[20,33] The cellular presence of specific PUFAs, therefore, could affect osteoblastic functioning via modulation of the synthesis of fatty acid products (e.g. prostaglandins), proliferation, differentiation and synthesis of proteins e.g. RANKL and OPG.

Purpose of the study

To elucidate the mechanisms by which PUFAs prevent bone loss by comparing the mechanism of action on osteoblasts of oestrogen, an anti-resorptive hormone and the action of parathyroid hormone, a pro-formation hormone in low concentrations, with PUFAs representative of the n-3 and n-6 polyunsaturated fatty acid families.

Method of investigation

An experimental study was conducted in which osteoblast-like cells (MG-63 human osteosarcoma cell line and MC3T3-E1 murine osteoblasts) in culture were exposed to PUFAs representative of the n-3 and n-6 polyunsaturated fatty acid families, and bone active hormones. The effects of these agents were tested on a variety of biological parameters characteristic of osteoblasts, e.g., prostaglandin E_2 (PGE_2) synthesis, proliferation, differentiation to mature mineralising osteoblasts as well as OPG and RANKL secretion.

Objectives

The objective of the study was to investigate whether arachidonic acid (AA) (representative of the n-6 PUFAs) and docosahexaenoic acid (DHA) (representative of the n-3 PUFAs), both of which have been shown to have *in vivo* effects on bone,[25,29,30,32] have differential effects on osteoblast-like cells *in vitro*. Experiments were conducted to determine how these PUFAs affected the following osteoblastic functions:

1. ***PGE_2 secretion***

   AA is the natural substrate for PGE_2 synthesis in many cell types including osteoblasts. DHA is not a substrate for prostaglandin synthesis but could interfere with PGE_2 synthesis.

2. ***Proliferation***

   PUFAs as well as their metabolites e.g. prostaglandins and oxidation products may be implicated in osteoblastic cell proliferation.

3. ***Differentiation to mature mineralising osteoblasts***

   Osteoblasts differentiate to mature mineralising osteoblasts when stimulated by osteogenic agents. PUFAs and their metabolites e.g. prostaglandins may affect mineralising properties of osteoblasts.

4. OPG and RANKL synthesis

Most pro-and anti-osteoclastogenic cytokines act primarily through the osteoblast to alter levels of RANKL and OPG; the relative balance of the latter determines overall osteoclast formation. It has been shown that PGE_2 inhibits OPG synthesis and stimulates expression of mRNA for RANKL. PUFAs may thus affect the OPG/RANKL ratio via manipulation of PGE_2.