**Skeletal effects of saturated fatty acids**

## Introduction

Bone is a dynamic organ that undergoes constant remodelling by bone-forming osteoblasts and bone-resorbing osteoclasts. The maintenance of healthy bone requires accurate coupling of bone formation and bone resorption. Excess of resorption over formation is a major cause of osteoporosis, a condition characterised by reduced bone mass and changes in bone architecture that lead to fragility and fracture. Osteoporosis affects 50% of people over the age of 60 years and a total of 200 million people worldwide each year (ref). Pharmacological management of osteoporosis aims to reduce fracture risk by attenuating bone resorption or by stimulating bone formation. The most widely used drugs for osteoporosis management are bisphosphonates, which bind to bone mineral and potently suppress osteoclast activity (ref). Another drug that inhibits bone resorption is denosumab, an antibody against receptor activator of nuclear factor kappa B ligand (RANKL). Denosumab attenuates the formation of osteoclasts and inhibits bone turnover (J Mid-life Health 4:147, 13; Nat Rev End 11:418, 15). Osteoporosis remains a major public health issue, and further therapeutics are being sought. In recent years, natural fatty acids have been shown to have favourable effects on bone, creating interest in their development as therapeutics for the management of osteoporosis.

Fatty acids are classified according to the bonds between their carbon atoms - saturated fatty acids contain no double bonds while unsaturated fatty acids contain one or more double bonds. Unsaturated fatty acids are further classified according to the position of the first double bond. Fatty acids with the first double bond between carbons 3 and 4 and carbons 6 and 7 are called ω-3 (omega-3) and ω-6 (omega-6) fatty acids, respectively, regardless of the overall number of double bonds in the carbon chain. Fatty acids in animals and humans are usually found as triglycerides, formed by binding of each of the three hydroxyl groups of a glycerol molecule to the carboxyl group of one fatty acid.

The effects of unsaturated fatty acids on bone have been extensively investigated. *In vivo,* intake of flaxseed oil, rich in α-linolenic acid, provided protection against bone loss induced by ovariectomy in rats (Nutrient 8:597, 16). Daily supplementation of ω-3 fatty acids was significantly beneficial to patients with rheumatoid arthritis (Glob J Health Sci 8:18, 16). *In vitro,* docosahexaenoic acid (an ω-3 fatty acid) inhibited the proliferation and differentiation of osteoclast precursors and enhanced mature osteoclast apoptosis (Cell Signal 29:226, 17). However, negative effects of polyunsaturated fatty acids in bone cells have also been reported. One study showed that ω-6 arachidonic fatty acid, but not ω-3 fatty acids, was inhibitory to osteoblastogenesis and increased osteoclast activity (Osteop Int 24:1647, 13). Another study also reported that arachidonic and docosahexaenoic acids inhibit osteoblast differentiation (Cell Biochem & Funct 27:3, 09). While numerous studies and review articles describe the skeletal effects of unsaturated fatty acids, the actions of saturated fatty acids in bone have been less thoroughly investigated. The current review focuses on the activity of saturated fatty acids in bone.

## Effects of saturated fatty acids on bone cells *in vitro*

(C16:0) followed by stearic acid (C18:0). In one study, plasma concentration of palmitic acid was 97 µM, (Diabetes 52:1641, 03)The same study found that plasma concentration of stearic acid was accounting for 13% of the total circulating fatty acids. Most studies of saturated fatty acid activity in bone and bone cells focused on palmitic acid.

*In vitro,* saturated fatty acids affect both osteoblasts and osteoclasts, regulating their proliferation, differentiation, survival, and function. Investigations of the underlying mechanisms of action of fatty acids in these cells focused on activation of signalling pathways, formation of fatty acid metabolites, and effects on intracellular organelles. Most studies have shown that saturated fatty acids are inhibitory to osteoblasts and stimulatory to osteoclasts, although the opposite effects have also been identified in a small number of studies.

### Effects of saturated fatty acids on osteoblasts

Palmitate (500 µM) reduced viability and induced apoptosis in the osteoblastic cell line MC3T3-E1 (Int J MM 28:535, 11, CHIHTC 94:1101, 14). The apoptotic effect of palmitate was associated with an increase in Fas expression, activation of caspase-3, activation of nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB), and degradation of IκBα, an endogenous inhibitor of NF-κB. Bezafibrate, a synthetic ligand for peroxisome proliferator-activated receptor (PPAR) α and δ, suppressed the apoptotic effect of palmitate by inhibiting the NF-κB signalling pathway (Int J MM 28:535, 11).

Lipotoxicity is an overload of lipids in non-adipose tissues that has negative effects on cell function and viability. In several organs and tissues, autophagy and apoptosis have been identified as the most common mechanisms of lipotoxicity. Another mechanism of lipotoxicity induced by palmitic acid is palmitoylation - the covalent attachment of a 16-carbon fatty acid to cysteine residues of proteins that affects protein hydrophobicity, protein-lipid interaction, and protein trafficking between organelles (Nat Rev Mol Cell Biol 8:74, 07).

Palmitate-induced lipotoxicity in osteoblasts is mediated by autophagy and apoptosis, and is associated with inhibition of protein palmitoylation. Gunaratnam et al. reported that palmitate (100-500 µM) induced lipotoxicity through autophagy and apoptosis in cultured human osteoblasts (Biol Open 2:1382, 13). The emergence of autophagosomes, followed by their fusion with lysosomes to form autolysosomes, indicated the stimulation of autophagy. Apoptosis, determined by analysis of nuclear fragmentation, was induced by palmitate through activation of the Fas/Jun kinase (JNK) pathway. The same group further reported that the palmitate-induced lipotoxicity in osteoblasts decreased the expression of mineralization and differentiation markers and reduced the transcriptional activities of β-catenin and RUNX2. In this experimental system, palmitate reduced the expression of palmitoyltransferase, a gene encoding the enzyme that catalyses palmitoylation (End 155:108, 14). Inhibition of protein palmitoylation by a substrate-analogue inhibitor was also found to reduce osteoblast differentiation without altering proliferation or survival (PLoS ONE 4:e4135, 09). However, the proteins affected by the modified palmitoylation induced in osteoblasts by palmitate have not been identified yet.

Palmitate activity in osteoblasts is regulated by two enzymes: long-chain acyl-CoA synthetase (ACSL) and acetyl-CoA carboxylase. ACSL catalyses the formation of acyl-CoA with fatty acids of 12 to 20 carbon atoms (World J Gastroenterol 21:3492, 15), and acetyl-CoA carboxylase converts acetyl-CoA to malonyl-CoA, a substrate in the fatty acid synthesis pathway (ref). In the human foetal osteoblastic cell line hFOB1.19, apoptosis induced by palmitate (100-500 µM) was blocked by the ACSL inhibitor, triacsin C, indicating that palmitate had to be converted to palmitoyl-CoA to exert the apoptotic effect (Bone 43:394, 08). In cultured rat calvarial osteoblasts, palmitate (100 µM) inhibited mineralization and the expression of the osteoblast differentiation markers Runx2, alkaline phosphatase, osteocalcin, and bone sialoprotein, but had no effect on cell proliferation. The inhibitory effect of palmitate was dependent on acetyl-CoA carboxylase activity, as an inhibitor of the enzyme attenuated palmitate’s effect (JCB 114:1760, 13; BBRC 450:777, 14).