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Review

Potential modulatory mechanisms of action by long-chain polyunsaturated fatty acids on bone cell and chondrocyte metabolism

Maryam Abshirini, Bolaji Lilian Ilesanmi-Oyelere, Marlena C. Kruger *

School of Health Sciences, College of Health, Massey University, Palmerston North, New Zealand

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ABSTRACT

Long-chain polyunsaturated fatty acids (LCPUFAs) and their metabolites are considered essential factors to support bone and joint health. The n-6 PUFAs suppress the osteoblasts differentiation via increasing peroxisome proliferator-activated receptor gamma (PPARγ) expression and promoting adipogenesis while n-3 PUFAs promote osteoblastogenesis by down-regulating PPARγ and enhancing osteoblastic activity. Arachidonic acid (AA) and its metabolite prostaglandin E2 (PGE2) are key regulators of osteoclast differentiation via induction of the receptor activator of nuclear factor kappa-B ligand (RANKL) pathway. Marine-derived n-3 LCPUFAs have been shown to inhibit osteoclastogenesis by decreasing the osteoprotegerin (OPG)/RANKL signalling pathway mediated by a reduction of pro-inflammatory PGE2 derived from AA. Omega-3 PUFAs reduce the expression of cartilage degrading enzyme matrix metalloproteinase-13 (MMP-13) and a disintegrin and metalloprotease with thrombospondin motifs-5 (ADAMTS-5) protein, oxidative stress and thereby apoptosis via nuclear factor kappa-betta (NF-kβ) and inducible nitric oxide synthase (iNOS) pathways. In this review, a diverse range of important effects of LCPUFAs on bone cells and chondrocyte was highlighted through different mechanisms of action established by cell cultures and animal studies. This review allows a better understanding of the possible role of LCPUFAs in bone and chondrocyte metabolism as potential therapeutics in combating the pathological complications such as osteoporosis and osteoarthritis.

1. Introduction

Osteoporosis is a bone disorder characterized by an excessive increase in osteoclast-mediated bone resorption, leading to decreased bone mass and bone structural degradation. This health condition results in osteoporotic fractures and is associated with an elevated risk of mortality. Osteoporosis as well as osteoarthritis imposes a large medical and socioeconomic burden on society [1].

Bone marrow mesenchymal stem cells (MSCs) transform into either adipocytes or osteoblasts, the latter initiate bone formation while osteoclasts originate from haematopoetic stem cells and mediate the bone resorption. Additionally, other cells are involved in bone metabolism including macrophages, surface bone-lining cells, chondrocytes and osteocytes [2]. Osteoblast differentiation involves a complex sequence

Abbreviations: LCPUFAs, Long-chain polyunsaturated fatty acids; PPARγ, peroxisome proliferator-activated receptor gamma; PGE2, prostaglandin E2; RANK, receptor for activation of nuclear factor-κB; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, Osteoprotegerin; MMP-13, matrix metalloproteinase-13; ADAMTS-5, a disintegrin and metalloprotease with thrombospondin motifs-5; NF-kβ, nuclear factor kappa-betta; iNOS, nitric oxide synthase; MSCs, mesenchymal stem cells; Runx2, runt-related transcription factor 2; C/EBPs, CCAAT/enhancer-binding proteins; M-CSF, macrophage colony-stimulating factor; c-Fms, M-CSF receptor; MAPK, mitogen-activated protein kinase; JNK, Jun N-terminal kinase; p38, protein 38; ERK, extracellular signal-regulated kinase; NFATc1, nuclear factor of activated T-cells cytoplasmic 1; TRAP, tartrate-resistant acid phosphatase; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; IL-1β, interleukin-1 beta; sGAG, glycosaminoglycan; TNF-α, tumour necrosis factor alpha; 15d-PGJ2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J2; *alp*, alkaline phosphatase; *oc*, osteocalcin; BMMs, bone marrow-derived monocytes/macrophage; RvE1, Resolvin E1; NFATc1, nuclear factor of activated T-cells 1; PI3K, phosphatidylinositol 3-kinase; OSCAR, osteoclast-associated immunoglobulin-like receptor; LPS, lipopolysaccharide; IGF-1, Insulin-like growth factor-1; IGFBPs, IGF-binding proteins; BMP-2, bone morphogenetic protein-2; CLA, conjugated linoleic acid; FFAR4, free fatty acid receptor 4; GPR120, G-protein coupled receptor 120; βarr2, β-arrestin 2; TRAF6, tumour necrosis factor receptor associated factor 6; TAK1, transforming growth factor-β activated kinase 1; TAB1, TAK1 binding protein; PLA, palmitoleic acid; PGES, Prostaglandin E synthase-1; ROS, reactive oxygen spices; NO, Nitric oxide; IL-1Ra, interleukin 1 receptor antagonist; iNOS, inducible nitric oxide synthase; AIA, adjuvant-induced arthritis; SPM, Specialized pro-resolving mediator.

* Corresponding author at: School of Health Sciences, College of Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.
E-mail address: M.C.Kruger@massey.ac.nz (M.C. Kruger).

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of processes to activate transcription factors required in osteoblastogenesis including Wnt/β-catenin, runt-related transcription factor 2 (Runx2), and Osterix and to inhibit transcription factors involved in adipogenesis including PPARγ and CCAAT/enhancer-binding proteins (C/EBPs) [3,4] (Fig. 1). Differentiation of hematopoietic stem cells to osteoclasts is determined by macrophage colony-stimulating factor (M-CSF) and a receptor for activation of RANKL, mainly synthesised by the osteoblast. M-CSF and RANKL act through their receptors, M-CSF receptor (c-Fms) and receptor for activation of nuclear factor-κB (RANK) located on the osteoclast, respectively [5]. The binding of M-CSF to c-Fms enhances RANK expression in hematopoietic stem cells. RANKL/RANK signal transduction leads to osteoclastogenesis which involves multiple pathways. Binding of RANKL to RANK on the surface of osteoclastic precursors activates nuclear factor kappa B (NF-κB) and three mitogen-activated protein kinase (MAPK) pathways including Jun N-terminal kinase (JNK), protein 38 (p38), extracellular signal-regulated kinase (ERK). Activated NF-κB interacts with nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), transcription factors c-fos and subsequently stimulates the osteoclastogenic genes, tartrate-resistant acid phosphatase (TRAP) and cathepsin K leading to osteoclastogenesis. Osteoblasts regulate osteoclastogenesis via the expression of osteoprotegerin (OPG) which acts as decoy receptor for RANKL [6]. OPG is the endogenous inhibitor of RANKL synthesised by osteoblasts which prevents RANKL binding to RANK. OPG is therefore regarded a protective agent against osteoclastogenesis and bone resorption. The RANKL/RANK/OPG system is key regulator in osteoclastogenesis and disorders in this system are associated with postmenopausal osteoporosis, rheumatoid arthritis (RA), bone tumours and certain bone metastatic tumours [6] (Fig. 1).

Given the importance of bone cell differentiation in the development of osteoporosis and an increase in the evidence of the role of fatty acids as signal transduction molecules in this process, researchers have focused on the key role of different types of fatty acids in bone cell differentiation [7]. Among fatty acids, long chain polyunsaturated fatty acids (LCPUFAs) with a chain length longer than 18 to 20 carbons have been found beneficial and essential for bone health maintenance. LCPUFAs are categorized into two principal families, depending on the location of the first double bond, which is either at the third or the sixth carbon from the methyl end. The PUFAs that have been associated with bone homeostasis include the n-3 series of PUFA α-linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3) and the n-6 PUFAs include linoleic acid (LA,18:2 n-6) and arachidonic acid (AA, 20:4 n-6). Both types of fatty acids play important roles in the structure and function of the cell membrane phospholipid bilayer, acting as precursors of lipid-mediated signalling molecules, which may consequently impact the bone remodelling process [8].

There is accumulating evidence that n-3 LCPUFAs are beneficial for bone health. Animal studies have demonstrated that a high-fat diet is detrimental to bone heath by decreasing bone formation and increasing bone resorption. However, moderate amounts of dietary n-3 LCPUFAs can prevent adiposity and mitigate the detrimental effects of a high fat diet. Growing mice fed with a high-fat diet enriched with a moderate amount of fish oil (3% of energy), had higher bone mass and less adipose tissue compared to mice fed with a similar diet enriched with 0 or 9% of energy from fish oil [9]. However, findings from animal studies with respect to the effect of fish oil on adiposity vary considerably, possibly due to variation in animal obesity models, the fatty acid compositions of high fat and fish oil, and duration of feeding. Feeding a murine model of senile osteoporosis with fish oil prevented the marrow adipose tissue expansion and hematopoietic bone marrow atrophy and ultimately bone loss [10].

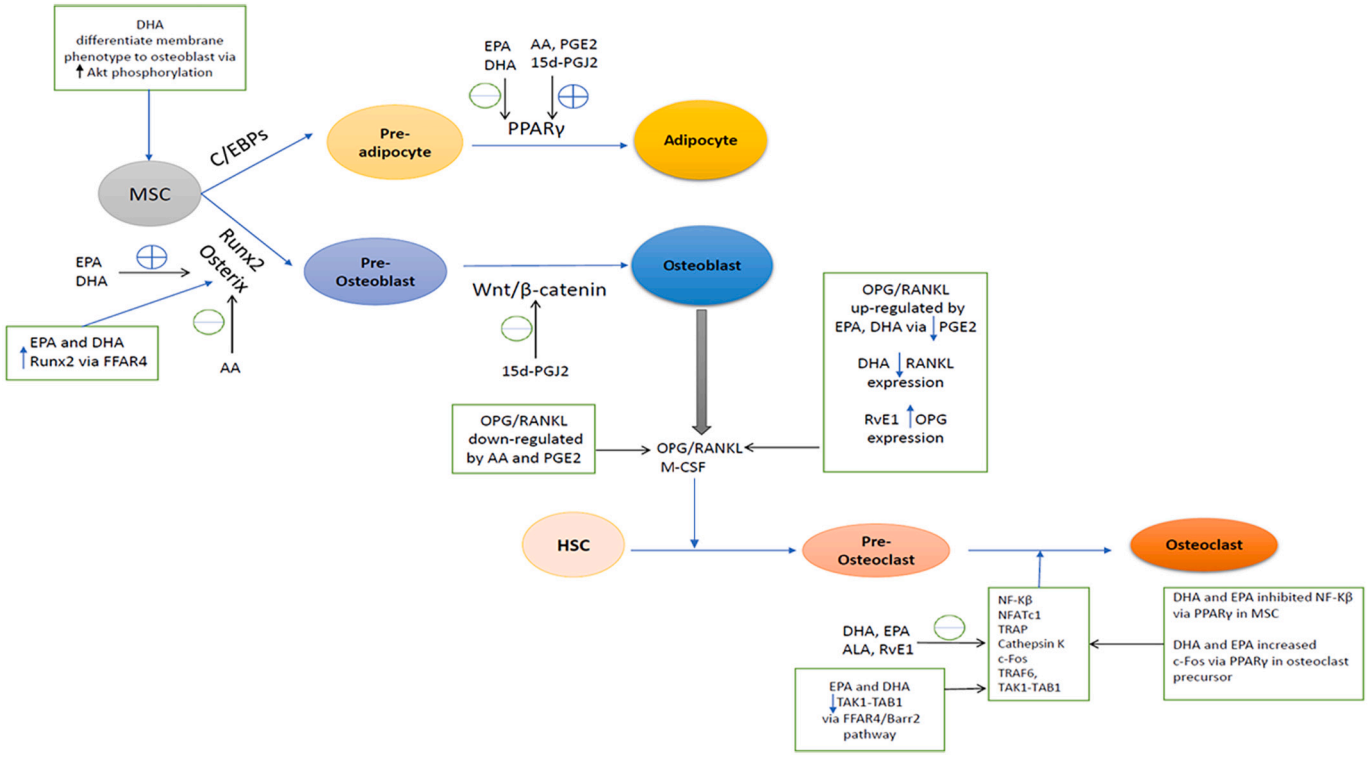

Fig. 1. Proposed schematic implications of PUFAs in bone metabolism. This figure demonstrates that EPA and DHA drive the osteoblastic potential of MSC via increasing osteoblastic transcription factors Runx2 and osterix while inhibiting the adipogenic potential via down-regulation of PPARγ, while AA and PGE2 use the same pathway to enhance adipogenesis and suppress osteoblastogenesis. DHA and EPA have been found to inhibit osteoclastogenesis via increasing the OPG/RANKL ratio as well as supressing NF-KB downstream signalling, whereas AA and PGE2 stimulate osteoclast differentiation by decreasing the OPG/RANKL ratio. DHA and EPA also downregulate osteoclastogenesis via activating the FFAR4/Barr2 pathway while increasing the osteoblastic factor Runx2 via the same pathway. Circled minus shows inhibition and circled plus shows stimulation. MSC: mesenchymal stem cells, HSC: haematopoetic stem cells.

Effects of LCPUFAs, particularly n-3 EPA and DHA have been widely studied in inflammatory joint disease such as rheumatoid arthritis and osteoarthritis. Cytokines such as interleukin-1 beta (IL-1β) enhance the synthesis of matrix metalloproteinases (MMPs), which result in loss of the cartilage extracellular matrix and the degradation of key components, sulphated glycosaminoglycan (sGAG) and collagen II [11]. These fatty acids can inhibit the release of the key regulators of inflammation such as IL-1β, and tumour necrosis factor alpha (TNF-α) by suppressing the NF-κB pathway. The existing evidence from in vitro and in vivo studies suggests that the mechanism of action of n-3 PUFAs seem to be partially mediated by modulating the expression of relevant genes in osteoarthritis pathogenesis. In vitro studies showed that EPA lowered the expression of various catabolic and inflammatory genes such as disintegrin and metalloprotease with thrombospondin motifs-4 (ADAMTS-4), ADAMTS-5, MMP-3, and COX2 [12].

In this review, we summarise the mechanism of action of LCPUFAs, their metabolites and signalling pathways involved in bone and chondrocyte metabolism with the aim to understand the diverse impact of LCPUFAs and their modulatory role as potential therapeutic and nutritional agents in treatment and prevention of bone and joint diseases.

1.1. Effect of PUFAs on bone cell differentiation and role of PPARS

As mentioned earlier, PPARs regulate the differentiation of MSCs to osteoblasts or adipocytes [13]. PPARs belong to a family of nuclear receptors existing in several tissues in the body in three isoforms α, β/σ and γ. All three PPAR isoforms are found in osteoblasts and osteoclasts but PPARγ, particularly PPARγ2 is found to be important in modifying adipose and bone cell function by shifting the differentiation toward adipogenesis [14]. On the other hand, Runx2 elevates expression of osteogenic genes during osteoblast differentiation. PPARγ suppresses the Runx2 transcriptional activity and thereby inhibits osteogenesis [15]. It appears that both transcription factors negatively control each other as overexpression of Runx2 in rat MSC caused a decrease in PPARγ expression [16]. However, induction of PPAR-α or PPAR-β/σ has been shown to promote osteoblastogenesis [17].

The association between PPARγ polymorphisms and the risk of postmenopausal osteoporosis has been reported [18]. The specific fatty acid that acts as a ligand of PPAR-γ determines its dual function of anti-osteoblastic or pro-adipocytic aspects [19]. LCPUFAs and the AA metabolite, PGE2 are regarded as ligands of PPARs [20]. Fatty acids such as AA, LA, ALA, EPA can bind to PPARα and PPARγ [21]. Human MSC that differentiated into osteoblasts when treated with n-6 AA, had increased expression of adipogenic genes PPARγ2 [22]. 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) is an endogenous PG and is a ligand for PPARγ that can induce adipogenesis by binding to PPARγ while supressing osteoblastogenesis via inhibiting the Wnt/β-catenin signalling pathway [23].

Treatment of MSC induced to differentiate into osteoblasts with AA suppressed the osteogenic gene *runx2* and alkaline phosphatase (*alp*) osteoblastogenesis early-expression gene, while it had no effect on osteocalcin (*oc*), an osteoblastogenesis late-expression gene. In the same study, a combination of AA with DHA or EPA significantly increased the *ppar*g gene expression in the MSC induced to differentiate into osteoblasts, suggesting that higher fatty acid concentrations induce adipogenesis in MSCs even when they have been induced to form osteoblasts [24]. In an in vivo study, oil from Antarctic Krill (abundant in EPA and DHA) increased Runx2 expression while it reduced PPARγ expression which enhanced the MSCs differentiation into osteoblasts rather than to adipocytes in a mouse model of glucocorticoid-induced osteoporosis [25]. Furthermore, a study by Cugno et al., reported that fish oil rich in EPA and DHA provided protection against the reduction of osteoblast formation and up-regulation of adipocyte differentiation in MSC induced by rosiglitazone, an antidiabetic agent which increases bone resorption and bone marrow adiposity. The in vitro data from the same study found treating human MSC differentiated into osteocytes concurrently with DHA and rosiglitazone, downregulated the adipogenic genes, such as adipsin and FABP4 along the PPARγ/FABP4 axis, and decreased the capability of osteocytes to switch toward adipogenesis. Supporting the in vitro findings, an animal study demonstrated that PPARγ expression was down-regulated by fish oil in mice treated with rosiglitazone [26]. Taken together, these results indicate that the adipogenic or osteogenic fate of MSC depends on the fatty acid source as n-6 AA and PGE2 promote adipogenesis through up-regulating a PPARγ-dependent pathway and down-regulating Runx2 while n-3 EPA and DHA induce osteogenesis via the same pathways.

Activation of PPARγ in osteoclasts is still disputed; although it has been shown that PPARγ enhanced osteoclastogenesis in some studies [27], while other studies reported a decrease in osteoclast formation [28]. There is evidence indicating that different fatty acids have varying effects on PPARs expression in a human osteoclast cell model. Proposing a novel mechanism of PPARs action in osteoclast signalling pathways an in vitro study by Kasonga et al. [29] indicated that DHA and EPA activated PPARα and PPARγ to a larger extent than PPAR β/σ in CD14+ monocytes as a primary osteoclast cell line. Interestingly, PPARs activation by its agonist suppressed osteoclastogenesis via modulation of RANKL signalling. PUFAs as PPAR ligands can mediate osteoclast formation via PPARs. Nakanishi et al. have shown that EPA and DHA inhibited osteoclastogenesis through inhibition of NF-κB transcriptional activity by binding to PPARγ in MSCs. The inhibition of NF-κB transcriptional activity resulted into a reduction in expression of NF-κB regulating genes, TNFα, IL-6 and COX2, leading to decrease in RANKL. However, these fatty acids stimulated the osteoclastogenesis in bone marrow-derived monocytes/macrophage (BMMs) precursor cells as osteoclast precursor cells. This stimulation was associated with enhancement of c-Fos gene expression mediated by PPARγ [30]. This suggests that PPARγ stimulation by n-3 PUFA favouring the osteoclastogenesis or anti-osteoclastogenesis pathway may be unique for different cell types. Further studies using different LCPUFA and cell types are required to confirm the signalling mechanisms used by the PPARs in osteoclastogenesis.

1.2. Effect of PUFAs on osteoclastic differentiation and role of the receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) system

Previous studies have demonstrated the inhibitory effect of PUFA on RANKL and osteoclastogenesis. In a study by Boeyens et al., n-6 AA and n-3 DHA potently inhibited the RANKL-mediated osteoclast formation through down-regulating of the osteoclast specific gene TRAP and cathepsin K in murine macrophages RAW264.7, an osteoclast precursor cell line. DHA was found to have greater inhibitory effect on osteoclastogenesis compared to AA [31]. PGE2, an eicosanoid derivative of AA is a potent regulator of the OPG/RANKL signalling pathway. Treatment of human MSC differentiated into osteoblasts with n-6 AA decreased the expression of osteogenic markers and the OPG/RANKL ratio [22]. Antarctic krill oil containing n-3 PUFA has been shown to inhibit the osteoclastogenesis-related OPG/RANKL//NF-κB signalling pathway via decreasing the secretion of PGE2 and its receptor EP4 in a mouse model of postmenopausal osteoporosis. However, AA as a precursor of PGE2 worsened bone resorption by activating the same pathway [32]. Thus, modifying the ratio of dietary n-6/n-3 PUFAs toward the n-6 results in increased bone resorption via increased production of endogenous PGE2, thereby activation of osteoclastogenesis-related OPG/RANKL/NF-κB pathway [31,33].

Resolvin E1 (RvE1), a derivative of EPA, is a potent pro-resolving lipid mediator that reduces osteoclast-mediated bone loss. RvE1 exerts its inhibitory effect via suppressing RANKL-induced mRNA expression of osteoclast-specific genes and transcription factors c-fos and nuclear factor of activated T-cells 1 (NFATc1) in the osteoclast precursor cells, RAW264.7. RvE1 also inhibited IL-17-induced RANKL mRNA expression and PGE2 production in osteoblast cells [34]. RvE1 significantly

enhanced the expression of OPG while RANKL remained unchanged in in vitro bone cell cultures. Apparently, RvE1 can modulate osteoclast differentiation through boosting OPG production and maintaining a favourable ratio of OPG/RANKL [35]. This evidence is corroborated by results from a study by Al Kholy et al. that showed RvE1 reduced the synthesis of RANKL and shifted the RANKL/OPG ratio toward bone formation. Downregulation of osteoclast differentiation by RvE1 is mediated by differential regulation of NF-κB and phosphatidylinositol 3-kinase (PI3K) and Akt pathways [36]. DHA also directly inhibited osteoclast formation through reducing the expression of RANKL mRNA in LPS-induced osteoclasts [37]. Furthermore, fish oil suppressed the up-regulation of osteoclast differentiation of RAW264.7 cells treated with RANKL [26].

In a study by Song et al., ALA inhibited RANKL-mediated osteoclastogenesis through downregulation of markers involved in osteoclast differentiation, c-Fos, c-Jun and NFATc1 along with transcription factor proteins tartrate-resistant acid phosphatase (TRAP), osteoclast-associated immunoglobulin-like receptor (OSCAR), and cathepsin K. ALA also suppressed the RANKL-stimulated phosphorylation of JNK, ERK, AKT and NF-κB proteins [38]. A study by Kim et al. showed that DHA suppressed RANKL-induced osteoclast formation in primary murine macrophages through inhibition of NF-kB and MAPKs activation. [39].

1.3. The role of PUFA in cytokine secretion and expression in bone

Previous human studies have shown that inflammatory cytokines are actively involved in the pathogenesis of osteoporosis working as a stimulant of bone resorption and thereby inhibiting bone formation [40]. Inhibitory effects of TNF-α on osteoblast differentiation have been shown in previous studies [41]. Oestrogen deficiency in postmenopausal osteoporosis, stimulates T-cells to produce an excessive amount of TNF-α which suppresses osteoblast activity and bone formation [42]. The combination of TNF-α and IL-6 stimulated osteoclast-like cell differentiated from BMMs which were associated with bone resorption activity both in vitro and in vivo [43]. The n-6/n-3 PUFAs ratios of dietary fatty acid determines the capacity of bone for biosynthesis of PGE2 and therefore by modulating PGE2 synthesis there can be a decrease in bone resorption and an increase in bone formation. In a study by Alnouri et al. feeding growing male rabbits with fish oil and algae oil rich in n-3 PUFA DHA, significantly lowered ex vivo PGE2 levels released from the tibia compared to groups fed with sesame oil or soy bean oil rich in n-6 AA [44]. This result suggests that the inhibitory effect of n-3 PUFA on PGE2 biosynthesis might partially account for the decrease in bone resorption. In a recent study by Cungo et al. supplementation of rosiglitazone-treated mice with fish oil (EPA and DHA) resulted in a higher bone density along with reduced COX-2 activity and pro-inflammatory cytokines IL-6 and TNF-α, as well as higher levels of anti-inflammatory cytokines and the anti-osteoclastogenic cytokine IL-10 [26]. Furthermore, therapeutic doses (200 and 400 mg/kg) of turnip oil rich in the mono-unsaturated fatty acid, oleic acid (29.2%) and PUFAs linoleic acid (25.5%) significantly decreased Cathepsin K and TNF-α mRNA but increased Osx mRNA expression levels in osteoporotic rats. This was accompanied by an enhancement of the thickness of the femur bones [45]. Maternal n-3 PUFA supplementation lowered expression of osteoclastogenic cytokines RANKL and TNF-α, and IL-6 along with increased bone volume, an increase in osteoblasts and a decrease in the number of osteoclasts in male rat offspring [46].

DHA has been shown to decrease IL-1β expression in bone marrow-derived macrophages [47]. DHA inhibited lipopolysaccharide (LPS)-induced osteoclast formation in a murine experimental model through supressing the production of osteoclast-associated cytokines TNF-α in macrophages, mediated by binding to the G-protein-coupled receptor 120 (GPR120) [48]. In a study by Nakanishi et al., fish oil diet containing 4.5% menhaden oil with 0.5% corn oil suppressed ovariectomy-stimulated osteoclastogenesis through inhibition of NF-κB signalling and subsequently a reduction in TNF-α levels, followed by suppression of M-CSF and RANKL [49]. Providing ALA to ovariectomized mice resulted in a decrease in the levels of inflammatory cytokines such as IL-1β, IL-2, IL-6, IL10, TNF-α and MCP1 and iNOS and COX-2 [38].

1.4. Implication of PUFA as regulators of growth factors

Bone resorption by osteoclasts secretes various growth factors within the bone matrix which stimulate bone formation through paracrine or autocrine activities. Insulin-like growth factor-1 (IGF-1) is key growth factor that modulates bone mass, enhances osteoblast differentiation, and promotes bone formation. The IGF-1 action is mediated by IGF-binding proteins (IGFBPs) [50,51]. IGFBP-3 is the most abundant form of IGFBPs that regulates the IGF-1 activity by controlling its distribution and interacting with the IGF-1 receptor [52]. Moreover, IGFBP-3 binds to type I collagen and is involved in accumulation of IGF-1 in the skeletal matrix. IGFBP-3 is believed to be necessary for maintaining the stability, transport, and release of IGF-1 [53,54]. In vitro evidence showed an independent function of IGFBP-3 on bone metabolism. In a study by Eguchi et al., IGFBP-3 decreased the expression of osteoblast differentiation markers which are enhanced by bone morphogenetic protein-2 (BMP-2), suggesting that IGFBP-3 suppresses bone formation via the BMP-2 downstream signalling [55]. Using an in vivo rat model of sciatic neurectomy as model for osteoporosis, Hai-Biao et al. demonstrated that administration of IGF-1 increased bone density, decreased RANKL but increased OPG levels in osteoblasts. Further, IGF-1 upregulated the expression of Runx2 and Osterix, two transcription factors that promote osteoblast differentiation [56].

The AA metabolite, PGE2 has been shown to stimulate IGF-1 and its binding protein. In vitro studies have reported that PUFAs may have effects on bone formation and resorption through regulating the synthesis and action of IGF-1 and IGFBP via regulating PGE2 production [57–59].

Dietary conjugated linoleic acid (CLA) are isomers of linoleic acid, mainly found in milk and meat of ruminants as rumen micro-organisms play an important role in CLA production and its precursor. Numerous studies have revealed that CLA has anticancerogenic, antidiabetic, and antiatherogenic effects, as well as effects on the immune system, bone remodelling, and body composition [60]. It has been shown that CLA down-regulates the IGF-1 levels in growing rats and IGFBP-3 levels are altered by dietary CLA supplementation depending on the type of dietary PUFA. IGFBP-3 levels were elevated by CLA in rats given a high n-6 PUFA diet but were lowered by CLA in rats fed a high n-3 PUFA diet. This study also showed that n-3 PUFA and CLA reduced ex vivo production of PGE2 in bone culture and elevated bone formation rate in tibia [61].

There are limited and contradictory data from in vivo studies on the effects of dietary PUFA on IGF-I levels regarding bone metabolism. Green et al. showed that fish oil had no effect on IGF-1 levels, although femur BMD increased with fish oil in growing rats [62]. A clinical trial conducted in adolescent boys indicated that supplementation with fish oil increased DHA status along with IGF-1 levels, however it was not associated with changes in bone mass or bone formation markers [63].

2. Further potential mechanisms of PUFA in bone metabolism

Differentiation of MSCs into osteoblasts or adipocytes involves substantial remodelling of the plasma membrane which involves producing membranes with distinct compositions and biophysical properties. Supplementation with DHA as a lipid component of osteoblast membranes, has been shown to induce extensive remodelling in MSCs which determines the cell-specific properties of osteoblasts. Plasma membrane alteration produced by DHA potentiated osteogenic differentiation of MSCs mediated by up-regulation in phosphorylation of protein kinase B known as Akt at the plasma membrane. This proposes a novel mechanism by which dietary fatty acids can modulate osteogenic differentiation [64].

The free (non-esterified) fatty acid receptor 4 (FFAR4) also known as G-protein coupled receptor 120 (GPR120) is expressed in several tissues including in osteoclasts and osteoblasts. FFAR4 activates signalling pathways, β-arrestin 2 (βarr2), which is offered as a promising mediator for the osteogenic and anti-osteoclastogenic effects of unsaturated fatty acids. As mentioned previously, osteoclastogenesis involves binding of RANKL to its receptor RANK on osteoclast precursors which results in recruitment of tumour necrosis factor receptor associated factor 6 (TRAF6) and formation of transforming growth factor-β activated kinase 1 (TAK1)-TAK1 binding protein (TAB1) complex. FFAR4 can lead to βarr2 signalling which has inhibitory effects on the formation of the TAK1-TAB1 complex. In this way the FFAR4/βarr2 signalling pathway can control osteoclastogenic resorption. It has been shown that DHA, EPA and palmitoleic acid (PLA) exert their effects through activation of the FFAR4/βarr2 pathway to suppress osteoclast differentiation in pre-osteoclasts RAW264.7. The n-6 PUFA AA and n-9 MUFA oleic acid inhibited osteoclastogenesis but did require FFAR4. Interestingly, DHA, EPA, and oleic acid were shown to promote expression of Runx2 in MC3T3-E1 pre-osteoblasts through FFAR4 [65]. Further studies are warranted to elucidate the importance of FFAR4 in mediating the bone protective effects of PUFA. Implications of PUFAs in bone metabolism are illustrated in Fig. 1.

3. Effect of LCPUFAs on chondrocyte metabolism and osteoarthritis

Chondrocytes are the sole cell resident within cartilage originating from mesenchymal progenitor cells and producing extracellular matrix that mainly consists of proteoglycans and collagen [66]. PUFAs play an important role in chondrocyte and cartilage homeostasis. In vitro studies have revealed the effect of different PUFAs on secretion or expression of inflammatory cytokines, prostaglandins and enzymes involved in cartilage degradation: MMPs and A Disintegrin and Metalloproteinase with Thrombo Spondin motifs (ADAMTS). Fatty acids are integrated into chondrocytes mainly in the form of triacylglycerols and phosphatidylcholine, and mediate signalling pathways. In vitro and in vivo evidence suggested different fatty acids exert different effects on osteoarthritis; saturated fatty acids and n-6 PUFAs resulted in an increase in pro-inflammatory and pro-apoptotic markers, while n-3 PUFAs were associated with a reduction in inflammatory and degradation markers in chondrocytes and synoviocytes [67–69]. In this regard, in vitro experiments using bovine and human chondrocytes showed EPA reduced mRNA and the protein level of COX-2 in a dose dependent manner whereas AA had the opposite effect [70]. An in vitro model of osteoarthritis has shown that EPA reduced the expression of ADAMTS-4, ADAMTS-5, MMP-3, MMP-13, COX-2, IL-1α, IL-1β, TNF-α more effectively than DHA or ALA, while AA had no effect [71]. Prostaglandins have been found to supress chondrocyte differentiation via activation of protein kinases A and C [72]. These protein kinases have been shown to control differentiation through activating the transcription factors involved in collagen X production. Prostaglandin E synthase-1 (PGES) that mediates PGE2 synthesis downstream of COX2, is up-regulated in osteoarthritis [73].

Oxidative stress and reactive oxygen spices (ROS) activate several signalling pathways and play a major role in the pathophysiology of osteoarthritis. Nitric oxide (NO) is a major factor in OA progression through promoting the synthesis of inflammatory and proinflammatory cytokines while PGE2 decreases the synthesis of endogenous interleukin 1 receptor antagonist (IL-1Ra) [74]. NO induces apoptosis of chondrocytes via interaction between inducible nitric oxide synthase (iNOS) and COX-2 systems, which is mediated by the activation of MEK1/2 and p38 pathways [75]. NO regulates the extracellular matrix degradation by increasing the activation of MMPs [76]. In dogs diagnosed with osteoarthritis, supplementation with fish oil improved diverse markers of oxidative stress [77]. Treatment of IL-induced canine chondrocytes with EPA resulted in cartilage protection via reduction in the expression of iNOS and corresponding production of NO. Stimulation with AA also reduced iNOS and MMP-3 but it increased expression of ADAMTS-5 and PGE2 secretion [78]. EPA suppressed chondrocyte apoptosis induced by oxidative stress via decreasing caspase 3 and poly (ADP-ribose) polymerase cleavage, phosphorylation of p38 MAPK, and expression of MMP-3 and MMP-13 [79].

The inhibitory effects of DHA on MMP-13 mRNA and protein expression documented in in vitro and in an in vivo rat adjuvant-induced arthritis (AIA) model are mediated by a p38 MAPK-dependent mechanism [80]. An in vitro study using cytokine-induced canine chondrocytes found that EPA and DHA from a marine source reduced s-GAGs and maintained the level of degradation products of proteoglycan and collagen: uronic acid and hydroxyproline. Furthermore, the expression of the catabolic genes MMP-1, MMP-3, and MMP-13 were down-regulated while anabolic genes AGG and COL2A1 were up-regulated [81]. A ratio of n-6/n-3 PUFA of 1:1 were found to strongly suppress expression of MMP-13 both in in vitro and in vivo models [82]. In line with these findings, a study by Zhang et al. showed edible linseed or soybean oil with a low ratio of n-6/n-3 PUFA induced GPR120 and attenuated EP4, which resulted in suppression of the NF-κB pathway and its downstream cartilage degrading enzymes MMP-13 and ADAMTS-5 in mice with destabilized medial meniscus [83] (Fig. 2).

Specialized pro-resolving mediator (SPM) and bioactive lipid mediators such as resolvins have shown cartilage protective effects. Articular chondrocytes express 15-LO, an enzyme that mediates the synthesis of these lipid mediators which have more potent anti-inflammatory effects than their precursors in protecting chondrocytes and cartilage [84,85]. Resolvin D1 significantly reduced the arthritic score and hind paw oedema and reduced leucocyte infiltration within the paw in a rat model of arthritis. Mechanistically, 17R-RvD1 increased the expression of *Alox15* (15-LOX) transcript, main gene in SPM biosynthesis, while down-regulating *Ptsg2* (COX-2). The 17R-RvD1 receptor ALX/FPR2 is expressed in chondrocytes and 17R-RvD1 exerts its antiarthritic action via ligation with ALX/FPR2 [86]. New evidence shows the protective effect of Antarctic krill oil on osteoarthritis via regulation of autophagy and apoptosis. An in vivo study by Wang et al. reported that Antarctic krill oil treatment significantly increased the expression of key genes involved in autophagy, including LC-3B, Beclin-1, ATG-5, and BNIP-3 and promoted the expression of anti-apoptotic genes, including Bcl-2 and Bcl-xl via the p53-dependent mitochondrial apoptotic pathway [87].

In summary, PUFAs exert their effects via multiple distinct pathways in chondrocytes, thereby a better understanding of mechanism of PUFA is required to assess and support their utilisation in the management of osteoarthritis and related joint disorders.

4. Summary and conclusion

In this present review, we have described the molecular mechanisms involved in the actions of LCPUFAs in bone cells and chondrocytes. In vitro studies have shown treatment of MSC with n-6 AA could suppress the differentiation of osteoblasts via increasing the expression PPARγ and decreasing Runx2 and promoting adipogenesis while EPA and DHA induced the osteogenesis via increasing Runx2 expression and down-regulating PPARγ. EPA and DHA inhibited osteoclastogenesis through inhibition of NF-κB pathway via binding to PPARγ in MSCs, while stimulated osteoclastogenesis in osteoclast precursor cells by promoting c-Fos gene expression via a PPARγ-dependent pathway. The OPG/RANKL/RANK system is the main mediator of osteoclastogenesis. AA and its metabolite PGE2 as a potent stimulator of RANKL expression enhance bone resorption, whereas n-3 EPA and DHA inhibit the osteoclastogenesis-related OPG/RANKL/NF-κB signalling pathway via decreasing the secretion of PGE2 and its receptor EP4. Prostaglandin 15d-PGJ2 induced adipogenesis via acting as ligand for PPARγ while supressing osteoblastogenesis via inhibition of the Wnt/β-catenin signalling pathway. RvE1, a derivative of EPA modulates osteoclast

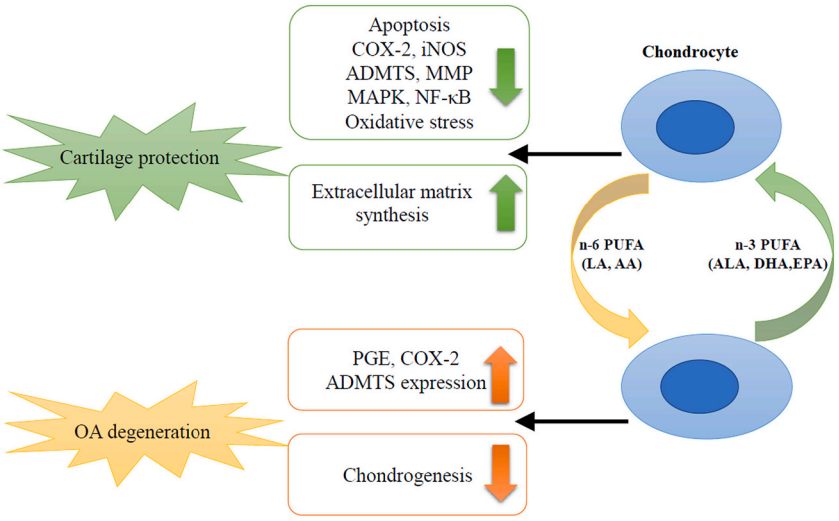

Fig. 2. Implications of LCPUFAs in chondrocyte metabolism. LCPUFAs exert dual effects on chondrocytes leading to mitigating or initiating of cartilage degeneration. The n-6 LCPUFAs, predominantly AA promote the catabolic process by facilitating a pro-inflammatory response, up-regulating the expression of the catabolic genes ADMTS, and supressing chondrocyte differentiation that leads to cartilage degradation. On other hand, n-3 LCPUFAs is considered a therapeutic agent in blocking cartilage degeneration. Their targets include suppressing expression of inflammatory markers, down-regulating the expression of the catabolic genes ADMTS, MMP, and inhibition of various downstream signalling pathways including NF-κB, MAPK, and oxidative stress. LCPUFAs: long-chain polyunsaturated fatty acids; AA: arachidonic acid, EPA; Eicosapentaenoic acid, DHA: Docosahexaenoic acid, LA: Linoleic acid, ALA: alpha-Linolenic acid, ADMTS: a disintegrin and metalloproteinase with thrombospondin motifs, MMP: Matrix Metalloproteinase, NF-κB: nuclear factor-κB, MAPK: Mitogen-activated protein kinase, COX-2: cyclooxygenase-2, OA: Osteoarthritis, PGE: Prostaglandin E2, iNOS: Inducible nitric oxide synthase.

differentiation through enhancing OPG production and maintaining a favourable ratio of RANKL/OPG. Accumulating evidence has shown that n-3 PUFA inhibit osteoclast-mediated pathological inflammation-induced bone resorption via modulating the production of PGE2 and the pro-inflammatory cytokine TNF-α. ALA inhibited RANKL-mediated osteoclastogenesis via down-regulation of markers involved in osteoclast differentiation, c-Fos, c-Jun and NFATc1, TRAP, and cathepsin K. In vitro studies have shown that PGE2 stimulated IGF-1 in osteoblast enriched culture and, thereby enhancing bone formation, which is contradictory to its influence on the osteoclast and bone resorption. Recent evidence offered new mechanisms by n-3 PUFA can suppress osteoclastogenesis via activation of the FFAR4/βarr2 pathway, followed by inhibition of RANKL signalling downstream. EPA is protective for cartilage via the reduction in expression of iNOS and NO synthesis, which is responsible for chondrocyte apoptosis and cartilage degradation via increased MMP-13. Furthermore, DHA inhibited MMP-13 expression via a p38 MAPK-dependent pathway.

In summary, PUFAs exert multiple effects on bone and chondrocyte metabolism via distinct mechanisms of action at cellular and molecular levels which should be considered when employing dietary LCPUFA as therapy for bone and joint diseases.

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