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Physiologic and pathologic effects of dietary free fatty acids on cells of the joint

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Abstract

Fatty acids (FAs) are potent organic compounds that not only can be used as an energy source during nutrient deprivation but are also involved in several essential signaling cascades in cells. Therefore, the balance of intake of different dietary FAs is critical for the maintenance of cellular functions and tissue homeostasis. A diet with an imbalanced fat composition creates a risk for developing metabolic syndrome and various musculoskeletal diseases, including osteoarthritis (OA). In this review, we summarize the current state of knowledge and mechanistic insights regarding the role of dietary FAs such as saturated FAs, omega-6 polyunsaturated FAs (PUFAs), and omega-3 PUFAs on joint inflammation and OA pathogenesis. In particular, we review how different types of dietary FAs and their derivatives distinctly affect a variety of cells within the joint, including chondrocytes, osteoblasts, osteoclasts, and synoviocytes. Understanding the molecular mechanisms underlying the effects of FAs on metabolic behavior, anabolic and catabolic processes, as well as the inflammatory response of joint cells, may help identify therapeutic targets for the prevention of metabolic joint diseases.

Graphical Abstract:

Fatty acids are involved in essential signaling cascades in cells, and a diet with an imbalanced fat composition can cause musculoskeletal diseases such as osteoarthritis. This article reviews the current state of knowledge regarding how dietary fatty acids affect the different cell types of the joint and play a role in joint inflammation and osteoarthritis.

Keywords

obesity; cartilage; bone; synovium; arthritis; diabetes

Introduction

Dietary fatty acids (FAs) provide an important energy source, participate in cell signaling cascades, and function as building blocks for various molecules, but also serve as essential

mediators of inflammation. Therefore, the balance of FA intake is critical for the maintenance of cellular functions and tissue homeostasis. Recent studies suggest that a diet containing an excess of saturated fatty acids (SFAs) or a high ratio of pro-inflammatory omega-6 polyunsaturated FAs (PUFAs) to anti-inflammatory omega-3 PUFAs may lead to diet-induced obesity and several obesity-related comorbidities, such as type 2 diabetes mellitus (T2DM), cardiovascular disease, and musculoskeletal disorders, including joint diseases such as osteoarthritis (OA).^{1–3}

OA is a painful and debilitating joint disease characterized by the degeneration of cartilage, remodeling of the surrounding bone, and pathologic changes in many joint tissues.⁴ The synovial joint comprises a variety of tissues and cell types necessary for overall joint function. Articular cartilage is a thin connective tissue, covering the load-bearing surfaces of long bones in this joint. The unique matrix composition of cartilage provides a nearly frictionless surface during joint movement. Chondrocytes, as the only cells in cartilage, maintain the cartilage matrix through a homeostatic balance of matrix synthesis and degradation. The bone tissue directly underlying cartilage in the joint is termed subchondral bone, which can be further divided into the subchondral bone plate and the trabecular bone in the epiphysis. This trabecular compartment is highly vascularized and provides nutrients to the deep zones of cartilage. Subchondral bone is a dense, specialized structure that responds to the loads applied to the joint.⁵ Osteoblasts and osteoclasts are the main cells responsible for bone formation and resorption, respectively. Another tissue type found in the articular joint is the synovium. Synovial tissue consists of two layers: intima and subintima. The inner layer—the intima—consists of two types of cells: fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes (MLS). FLS are important in the secretion of the proteins hyaluronan and lubricin, which maintain the viscosity of the synovial fluid and are believed to contribute to joint lubrication, while MLS may function as tissue-resident macrophages. Additional tissue types, including menisci, ligaments, tendons, and adipose depots such as the infrapatellar fat pad (IPFP), all contribute to the biomechanical stability and function of the joint.

It has been reported that obesity is the number one preventable risk factor for OA development.⁶ Historically, the link between obesity and OA was primarily attributed to biomechanical factors with the conventional thought that increased body weight due to obesity increases wear and tear on the joints. However, it is now clear that OA development involves active cells processes and has been correlated with an increase in the production of both matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). In particular, MMP-1, -3, -9, and -13 and ADAMTS-4 and -5 have been shown to be elevated in OA.⁷ These enzymes are mainly responsible for extracellular matrix degradation, cartilage and bone damage, and synovial inflammation. Additionally, OA is characterized by the loss of glycosaminoglycans (GAGs) and alterations in collagen ultrastructure.

While biomechanical factors play a critical role in the onset of post-traumatic OA,⁸ growing evidence suggests that the chronic inflammation and dietary imbalance associated with obesity may play a more important role than body weight in metabolic OA.⁹ For example, extreme obesity in mice due to impaired leptin signaling did not increase the incidence of

knee OA when compared with wild-type control mice, suggesting a role for diet-associated inflammation and metabolic dysregulation in OA development.¹⁰ Moreover, obesity-related metabolic changes may directly affect cartilage, meniscus,¹¹ synovium, synovial fluid,¹² and other joint tissues at the molecular and cellular levels. For example, dietary FAs and obesogenic factors may change how the cells within the joint tissue sense and respond to mechanical loading. In particular, signaling of the mechanosensitive ion channel transient receptor vanilloid 4 (TRPV4), which is highly expressed in cartilage and bone, was altered by omega-3 fatty acid¹³ and various factors associated with metabolic syndrome, such as leptin and resistin.¹⁴ Furthermore, TRPV4-deficient mice had increased susceptibility to obesity and obesity-associated OA under a high-fat diet feeding regimen.¹⁵ These results indicate that mechanosensitive pathways in cartilage may be modulated by dietary FAs.

Interestingly, the makeup and concentration of fatty acids may also play a role in the pathogenesis of OA, with various direct and indirect effects on the joint tissues including cartilage, bone, and synovium. However, the precise role of dietary and circulating FA within articular joints remains controversial. Here, we review the literature investigating the role of FAs on a variety of cell types within the synovial joint, including chondrocytes, osteoblasts, osteoclasts, and synoviocytes, in the context of OA as a whole-joint disease.⁴ We focus on cell signaling pathways mediated by FA and their receptors, with an emphasis on dysregulation caused by dietary FA content associated with obesity.

Pleiotropic functions of fatty acids

Fatty acids play multiple crucial roles in cells; most importantly, providing the key energy source under nutrient starvation. During FA degradation (e.g., FA β -oxidation), cells are able to generate adenosine triphosphate (ATP), which is then used as a chemical fuel by the cells.¹⁶ FAs, particularly long-chain FAs, can also be incorporated into phospholipids, maintaining cell integrity by regulating membrane fluidity and permeability.³ Extracellular FAs are also potent signaling molecules; for instance, palmitic acid (PA) is postulated to mediate Toll-like receptor 4 (TLR4) signaling.¹⁷ For these roles, FAs can be introduced into the mammalian cell or be synthesized within the cytoplasm. However, for the scope of this review, we will focus primarily on the influence of extracellular FAs.

Fatty acid categorization

Naturally occurring, biologically functional FAs can be categorized by several parameters indicative of their chemical structure. Based on the length of their carbon chain, they are named short- (5 or fewer), medium- (6–12), or long-chain (13 or more). FAs can also be categorized as saturated FAs (SFAs) or unsaturated FAs based on the absence or presence of a double bond. Depending on the number of double bonds, the unsaturated FAs can be further divided into monounsaturated FAs (MUFA) and polyunsaturated FAs (PUFAs), as summarized in Figure 1. Additionally, the site of the final double bond in the chemical structure, relative to the tail end (e.g., omega) of the molecular chain, is used to further define the FA structure (e.g., omega-3, omega-6).

SFAs such as palmitic acid [16:0] (PA) and stearic acid [18:0] (SA) can be found in mammalian animal fats, including lard. MUFAs such as oleic acid [18:1] are typically found in plant-based oils, including olive and avocado oil. PUFAs can be further categorized into omega-3 or omega-6 PUFAs. Docosahexaenoic [22:6] (DHA) and eicosapentaenoic acid [20:5] (EPA) are omega-3 PUFAs, while arachidonic acid [22:4] (ARA) and linoleic acid [18:2] (LA) belong to omega-6 PUFAs. Most omega-3 PUFAs can be found in flaxseed and fish oil, and omega-6 FAs are enriched in meat, eggs, and soybean oil.

Omega-6 PUFAs, particularly arachidonic acid (ARA), are precursors to several pro-inflammatory eicosanoids and prostaglandins (PGs) through the arachidonic acid cascade (reviewed by Saini and Keum¹⁸). On the other hand, omega-3 PUFAs are precursors for anti-inflammatory molecules called resolvins and protectins, which are believed to mediate the resolution of acute inflammation (reviewed by Kohli and Levy¹⁹). Therefore, omega-6 PUFAs are often referred to as pro-inflammatory, while omega-3 PUFAs are called anti-inflammatory FAs. Nevertheless, it is important to note that omega-3 and omega-6 PUFAs are both required for maintaining human health, and a pro- or anti-inflammatory response to these PUFAs may depend on cell type and context. Although human beings are capable of synthesizing many types of FAs (i.e., non-essential FAs), some FAs (i.e., α -linolenic and linoleic acids) rely on dietary intake. The FAs that must be acquired through diet are termed essential FAs and are critical for maintaining human health.

Fatty acid trafficking and cellular uptake

Dietary FAs are transported primarily in plasma by either fatty acid-albumin complexes or structures called lipoproteins. Lipoproteins include chylomicrons and high-, low-, and very low-density lipoproteins (HDL, LDL, and VLDL, respectively). Lipoproteins primarily consist of triglycerides, phospholipids, cholesterol, and proteins, and play a role in lipid transport from the intestine to all the other cells within the organism.

Two important groups of membrane receptors, LDL receptors (LDLRs) and LDL-related receptors (LRPs), detect lipoprotein structures and are important in mediating the cellular transport and signaling of lipids. LDLR and LRP belong to the endocytic type of receptors, which mediate the internalization and lysosomal delivery of the entire lipoprotein complex to cells. In addition, CD36 (or fatty acid translocase; FAT) belongs to the family of scavenger receptors which also binds lipoproteins but mediates only FA translocation to cells.²⁰ A recent study suggests that cellular uptake of oxidized LDL via CD36 binding is dependent upon specific FA types and doses.^{21, 22} Therefore, extracellular FAs can be introduced into cells by being incorporated into the lipid membrane or through FA transport proteins.

Fatty acid and cellular signaling

Recent studies demonstrated that FAs can directly signal through several types of membrane receptors. The most widely described family of FFA receptors are G protein-coupled receptors: FFAR1-FFAR4, with FFAR1 (GPR40) and FFAR4 (GPR120) being the primary members. GPR40 and GPR120 play a key role in binding long-chain PUFAs. The most

potent ligands for GPR40 are omega-3 PUFAs (e.g., DHA and EPA, as reviewed by Hara *et al.*²³). However, SFAs, such as PA, were also shown to bind to this receptor.²⁴ GRP120 can be stimulated by both omega-3 and omega-6 PUFAs, and is known to mediate anti-inflammatory effects in several cell types.²⁵

In addition, SFAs and oxidized LDL have been shown capable of activating Toll-like receptors (TLRs). These pattern-recognition receptors (PRRs) are known to mediate important inflammatory signaling cascades through pathogen recognition. It was originally hypothesized that, due to the structural similarity between SFAs and lipid A (an endotoxin of lipopolysaccharide), SFAs are direct ligands for TLR4 and thus can elicit inflammation in the absence of pathogens. However, several lines of accumulating evidence argue against SFAs as direct agonists of TLR4.^{26, 27} Using macrophages as a cellular model, Lancaster *et al.* recently demonstrated that TLR4-dependent priming, which alters cellular metabolism and the membrane lipidome, is required for the inflammatory effects of SFAs (i.e., SFAs act as a secondary trigger following TLR4 activation).¹⁷ Despite the fact that mechanisms by which SFAs activate TLR4-dependent inflammation still remain under intensive investigation, there is compelling evidence that TLR4 plays an important role in mediating obesity-induced inflammation.

FAs can also influence intracellular receptors such as peroxisome proliferator-activated receptors (PPARs). PPARs, consisting of PPAR α , PPAR β/δ , and PPAR γ , are able to bind oleic and linoleic acids as well as their metabolites, including prostaglandins or oxidized phospholipids.²⁸ The PPAR family can also bind to retinoid X receptor (RXR) and control the transcription of a number of genes involved in adipogenesis, inflammation, and lipid metabolism. For example, PPAR α regulates genes involved in FA β -oxidation and is mostly found in liver and skeletal muscles.^{28, 29} PPAR β/δ is also involved in fatty acid oxidation, while PPAR γ is known to be involved in regulation of adipogenesis, energy balance, and lipid biosynthesis.²⁸ It is important to emphasize that many studies investigating the PPAR family and other FA receptors in cells utilize chemical agonists. Although these agonists provide significant insights into the role of receptors in many cellular processes, they may not completely recapitulate the bindings between FAs and their receptors.

Cartilage and fatty acids

Cartilage and energy metabolism

Cartilage homeostasis is maintained through a balance of chondrocyte anabolic and catabolic activities. Although chondrocytes, the main resident cell population in cartilage, rely primarily on glucose as their source of energy, FAs and lipids are also believed to play a role in maintaining homeostasis.³⁰ The chondrocyte cell membrane contains large amounts of cholesterol, while phospholipids play an important role in the maintenance of healthy cartilage and joint lubrication. FAs can either be integrated into the chondrocyte primarily in the form of triacylglycerols and phosphatidylcholine,³¹ or they can mediate signaling through a variety of receptors. The imbalance of energy uptake from a high fat obesogenic diet was shown to affect cartilage metabolism, indicating the potential correlation between diet and OA.³²

Fatty acid receptors in chondrocytes

Chondrocytes express membrane receptors both for FAs and lipoproteins, including GPR40³³ and GPR120,³⁴ TLR4,³⁵ and CD36,³⁶ as well as some members of the LDLR and LRP families.^{37–39} Global knockout of GPR40 has been shown to increase predisposition to injury-induced OA in a mouse model and, when challenged with IL-1 β , the *GPR40*^{−/−} chondrocyte produced more inflammatory molecules, such as IL-6, PGE₂, MMP-2 and MMP-9, compared with wild-type cells.³³ Interestingly, Dehne and co-workers reported that GPR40 is up-regulated in human OA chondrocytes.⁴⁰ The discrepancy between these two studies may result from the differences in investigated species. Nevertheless, the detailed molecular mechanisms by which GPR40 modulates OA development requires further investigation. Global *GPR120* knock-out accelerates OA development in the early phase of OA in mice.³⁴ Similarly, patients with OA have significantly down-regulated GPR120 expression in cartilage compared with healthy individuals,³⁴ although the PUFA receptors GPR40 and GPR120 have not been extensively studied in chondrocytes. The activation of the TLR4 receptor by SFAs in chondrocytes has been investigated and reviewed.⁴¹ In brief, TLR4 mediates pro-catabolic signals in cartilage, decreasing cartilaginous matrix synthesis,⁴² while inhibition of these pro-catabolic signals suppresses inflammatory arthritis.⁴³

The LRP5-mediated Wnt/ β -catenin signaling pathway has been shown to down-regulate type II collagen production, while up-regulating MMP-3 and MMP-13 synthesis.³⁹ Particularly, increased expression of β -catenin in chondrocytes induces cartilage degradation. As shown by Zhu *et al.*, cartilage-specific overexpression of β -catenin induced an OA phenotype by increased expression of MMP-3, MMP-13 and collagen X, which suggests that low density lipoprotein signaling is crucial for chondrocyte homeostasis.⁴⁴ In addition, both LRP5 and CD36, a potential marker of hypertrophic chondrocytes, were up-regulated in human OA cartilage.^{36, 39, 45} In contrast, two other members of the LRP family have protective roles in OA: LRP1 is a potent clearing agent of ADAMTS-4³⁸ while LRP4 induces extracellular matrix production and facilitates chondrocyte differentiation.³⁷

The family of PPAR nuclear receptors has been intensively studied regarding their role in cartilage, and the presence of PPAR δ ,⁴⁶ PPAR α , and PPAR γ ⁴⁷ have been confirmed. Although naturally occurring long-chain PUFAs and their metabolites are known ligands for the PPARs, most of the current knowledge about these receptors is based on the use of synthetic agonists: fenofibrates for PPAR α , thiazolidinediones (TZD) for PPAR γ , and GW501516 for PPAR δ . PPAR α agonists have been shown to inhibit inflammation, catabolism,⁴⁸ and the pro-inflammatory effects of IL-1 β ⁴⁹ in human OA cartilage. Contrarily, PPAR δ has been shown to promote OA⁵⁰ as its agonists increase catabolic gene expression and robust fatty acid oxidation in chondrocytes.⁵¹

PPAR γ has been the most studied member of the PPAR family in this context. Activation of PPAR γ diminishes the pro-inflammatory effect of IL-1 β by downregulating MMP-1 expression in chondrocytes.⁵² Furthermore, this activation can inhibit major pro-inflammatory signaling pathways in cartilage and reduce catabolism (reviewed by Giaginis *et al.*⁵³). In addition, the PPAR γ agonist pioglitazone prevented glucose-mediated overexpression of cyclooxygenase 2 (COX2), PGE₂, MMP-13, and IL-6, as well as loss of type II collagen caused by elevated glucose.⁵⁴ Cartilage-specific deletion of PPAR γ

impaired cartilage growth and led to abnormal endochondral ossification,⁵⁵ increased cartilage degradation, chondrocyte apoptosis, and induced mTOR signaling.⁵⁶ Dysregulation of mTOR signaling is observed in morbidities such as diabetes, obesity, and cancer. Furthermore, cartilage-specific mTOR deletion was shown to inhibit OA progression⁵⁷. In OA, PPAR γ is down-regulated by IL-1 β in cartilage.⁵⁸ Interestingly, advanced-glycation end products (AGEs) also down-regulate PPAR γ expression in human chondrocytes.⁵⁹ AGEs are proteins or lipids that become glycated and are known to increase during aging as well as in OA. Yang *et al.* showed AGEs mediated down-regulation of PPAR γ via TLR-4 signaling in human chondrocytes, which suggests the importance of FA receptors in modulating chondrocytes' response to pro-inflammatory stimuli.⁵⁹

Chondrocytes and SFAs

Several studies, including ours, have demonstrated that animals fed a high-fat diet rich in SFAs exhibited accelerated development of spontaneous or injury-induced OA.^{32, 60–63} Long-chain SFAs have been linked to a negative regulation of chondrocyte metabolism and cartilage homeostasis. Our previous study also showed that chondrocytes derived from mesenchymal stem cells (MSCs) and adipose stem cells (ASCs) isolated from high-fat-diet-induced obese mice had decreased cartilaginous matrix production.⁶⁴ In humans, PA has been shown to induce apoptosis in chondrocytes,⁶⁵ while SA induced pro-inflammatory cytokines through lactate dehydrogenase (LDH)-dependent lactate production.⁶⁶ Miao *et al.* proposed that the elevated SFA content induces lactate-mediated hypoxia-induced factor alpha (HIF-1 α) and VEGF production, which will then initiate a pro-inflammatory cascade and OA progression.⁶⁶ SFAs (e.g., PA and SA) and a MUFA (oleic acid) have been shown to increase pro-inflammatory markers, including IL-6, in human chondrocytes.⁶⁷ Furthermore, PA, in synergy with IL-1 β , mediated cartilage damage and chondrocyte apoptosis through TLR4 signaling⁶⁸ (Fig. 2).

Chondrocytes and PUFAs

PUFAs may play a dual role in chondrocyte and cartilage homeostasis. Growing evidence suggests that an increased ratio of omega-6 to omega-3 FAs may be detrimental to cartilage. A high-fat diet rich in omega-6 FA increased the severity of cartilage damage in OA, while a similar diet rich in omega-3 FA ameliorated OA in mice.¹² Furthermore, dietary omega-3 FAs were found to reduce OA in a guinea pig model⁶⁹ and a lower ratio of omega-6 to omega-3 FAs in the diet has been shown to suppress MMP-13 in inflammatory arthritis in rats⁷⁰ (Fig. 2). While the mechanisms of these effects are not fully understood, it has been hypothesized that omega-3 FAs serve as precursors for anti-inflammatory mediators such as resolvins and protectins, while omega-6 FAs are generally metabolized to pro-inflammatory prostanoids.¹

Prostaglandins, derivatives of ARA, have been shown to inhibit chondrocyte differentiation through activation of protein kinases A and C (PKA and PKC).⁷¹ Both PKA and PKC are downstream effectors of PG receptors and are postulated to influence chondrocytes differentiation by activating transcription factors important in collagen X production. Prostaglandin E synthase-1 (PGES), important in mediating PGE₂ synthesis downstream of COX2, has been shown to be up-regulated in OA.⁷² Both PGES and COX2 are known

enzymes converting ARA to PGs and mediating its potential pro-inflammatory role. Conversely, omega-3 PUFAs and their metabolites appear to have a protective effect on chondrocytes and cartilage. These FAs have been shown to decrease mRNA levels of many OA-associated pro-inflammatory enzymes (such as ADAMTS and MMP3)^{73, 74} as well as sGAG loss in mouse⁷⁵ and bovine cartilage.⁷³ EPA inhibited oxidative-stress-induced apoptosis in human chondrocytes, and intra-articular injection of EPA mitigated OA progression.⁷⁶ Additionally, DHA has been shown to ameliorate cartilage damage through a p38 MAPK-dependent mechanism⁷⁷ (Fig. 2). MAPK intracellular signaling is known to mediate pro-catabolic responses in cartilage. Additionally, Wang *et al.* proposed that DHA selectively blocks IL-1 β -induced p38 activation in the MAPK signaling cascade.⁷⁷ These results confirm that DHA ameliorates cartilage degeneration and may be a potent OA-reducing agent.

The *fat-1* transgenic mice (C57BL/6-Tg(CAG-fat-1)1Jxk/J) constitutively express the *C. elegans* gene *fat-1*, which encodes a desaturase that converts omega-6 to omega-3 PUFAs. This mouse model provides a unique opportunity to study the role of omega-3 PUFAs in animals without the confounding factors introduced by diet intervention.⁷⁸ It has been reported that *fat-1* mice do not appear to have reduced onset of spontaneous OA compared to wild-type mice;⁷⁹ however, there was a delayed onset of surgically induced OA with partially downregulated mTOR signaling.⁸⁰ More recent studies have shown that *fat-1* mice fed a high-fat diet rich in omega-6 PUFAs exhibit a significant reduction in OA and synovitis in response to a knee injury in a sex-dependent manner.⁸¹

Bone and fatty acids

Bone and energy metabolism

Bone is reported to be the fourth most metabolically active organ, due to the constant production of ATP by osteoblasts and osteoclasts necessary for their proper function.⁸² Interestingly, bone is also a vital insulin-regulating organ.⁸³ Obesity was initially thought to have a beneficial effect on bone,^{84, 85} but more recent studies indicate that obesity-related chronic inflammation can have a negative effect on bone biology.^{86, 87} This is due in part to the association of obesity with high levels of circulating pro-inflammatory cytokines such as IL-1 β and tumor necrosis factor alpha (TNF- α), which may promote osteoclast activation.^{88, 89} Adipokines and FAs are now also considered to be important factors regulating bone remodeling in obesity.

Osteoblasts and fatty acids

Osteoblasts are responsible for bone formation; thus, they require a constant energy input for their high cellular metabolic activity. In addition, increased ATP production is necessary during the mineralization process. Although glucose is a vital energy source for osteoblasts, FA β -oxidation is reported to cover 40–80% of their energy requirements.⁹⁰

Fatty acid receptors in osteoblasts

Similar to chondrocytes, osteoblasts express several intra- and extracellular FA receptors, including PPARs, GPR40,⁹¹ GPR120,⁹² TLR4,⁹³ and CD36.⁹⁴ PUFA receptors, including

GPR40 and GPR120, are shown to have an important effect on osteoblasts and bone. GPR40 activation has a dual effect on osteoblast differentiation. Initially, GPR40 chemical agonists induce early differentiation markers, including alkaline phosphatase (ALP). However, activation of GPR40 at later stages of differentiation lead to the inhibition of the mineralization process.⁹⁵ Although the knockout of *GPR120* does not change bone parameters in ovariectomized mice when compared to wild-type mice,⁹² the presence of GPR120 in *fat-1* transgenic mice is protective for bone loss, indicating that GPR120 plays an important role in sensing PUFA in osteoblasts. TLR4 stimulation has been shown to both suppress⁹⁶ and promote osteoblastogenesis.⁹⁷

The lipoprotein receptors have been extensively studied in osteoblasts. *CD36* knockout mice exhibited a low bone mass phenotype, as the receptor is mandatory for adequate osteoblast function.⁹⁸ During osteoblast mineralization, there was a significant upregulation of *CD36* mRNA in mice calvarial osteoblasts.⁹⁹ The role of LDL and LRP receptor family members in osteoblasts has also been investigated.^{100, 101} For example, FA oxidation in osteoblasts has been shown to be regulated by Wnt-LRP5 signaling. Upon binding to LRP5/6 and Frizzled proteins, Wnt molecules induce an intracellular cascade to stabilize β -catenin in osteoblasts, promoting cell differentiation. Interestingly, mice lacking LRP5 in osteoblasts and osteocytes had dysregulated FA metabolism and an increased adiposity with reduced bone mass when compared with wild-type. Osteoblasts derived from those mice had reduced lipid oxidation, which could be restored by overexpressing Wnt or LRP5.¹⁰⁰ These studies suggest that lipid metabolism in osteoblasts is an important modulator of bone formation and homeostasis.

All three main members of the PPAR family have been reported to be expressed in mature osteoblasts.¹⁰² Although global knockout of *PPAR α* did not affect bone,¹⁰³ *PPAR α* stimulation, either by FAs or the artificial agonist benzaifibrate, positively influenced osteoblastogenesis and anabolic processes in bone.¹⁰⁴ On the other hand, *PPAR α* agonists may induce bone loss by stimulating adipogenesis at the expense of osteoblast formation, eventually leading to inhibition of bone deposition.¹⁰⁵ Mice with *PPAR δ* global knockout exhibited smaller body size,¹⁰⁶ while *PPAR δ* deletion in all tissues showed a significant reduction in trabecular bone mass compared with wild-type animals.¹⁰⁷ Additionally, treatment with the *PPAR δ* agonist GW501516 increased osteoblast differentiation and prevented bone loss in ovariectomized mice.¹⁰⁷ Furthermore, *PPAR δ* activation enhanced osteoblastogenesis,¹⁰⁴ while *PPAR δ* inhibition resulted in osteopenia. *PPAR γ* , however, is considered pro-catabolic in mature osteoblasts and its activation has been shown to cause bone loss,¹⁰⁸ potentially mediated through GPR40 stimulation.¹⁰⁹ Furthermore, *PPAR γ* overexpression in osteoblasts decreased bone mineral density (BMD) in male mice and accelerated ovariectomy-induced bone loss in female mice,¹¹⁰ while *PPAR γ* inhibition by its chemical antagonist SR1664 promoted osteogenesis.¹¹¹

Osteoblasts and SFAs

The effects of SFAs on osteoblasts have been widely investigated, and it is generally agreed that excess SFAs are detrimental to osteoblasts. Palmitic acid was shown to induce apoptosis through the activation of the c-Jun N-terminal kinase (JNK) apoptotic pathway. JNK

activation mediates cytochrome C release, suggesting that PA induces mitochondrial dysfunction and leads to osteoblast death.¹¹² This FA was also shown to down-regulate SMAD4/5 and RUNX2 signaling, thus inhibiting the mineralization process in osteoblasts.¹¹³ PA also increased TLR4 expression in osteoblasts derived from MSCs, implying SFAs are capable of modulating innate immunity in obesity.¹¹⁴ Furthermore, several studies reported a lipotoxic effect of SFA on osteoblasts^{113, 115} (Fig. 2). Lipotoxicity is a process of lipid accumulation in non-adipose tissue cells. PA-induced lipotoxicity is reported to be the main stimulus for cell apoptosis. Surprisingly, oleic acid was shown to prevent PA-induced lipotoxicity by blocking extracellular signal-regulated kinase (ERK) and nuclear factor kappa B (NF- κ B) activation.¹¹⁶ ERK phosphorylation and NF- κ B activation lead to apoptosis, as well as the release of pro-inflammatory cytokines (i.e., IL-6) by osteoblasts. Additionally, inhibition of intracellular FA synthase in osteoblasts prevents the lipotoxic effect of SFA on those cells.¹¹⁷

Osteoblasts and PUFAs

The role of ARA in osteoblasts is not well understood. ARA has been shown to promote adipogenesis and decrease osteoblastogenesis of MSCs.¹¹⁸ Some studies suggest that ARA inhibits osteoblast differentiation through the cytosolic phospholipase A2 (cPLA₂)-dependent pathway and an increased expression of COX2 and PGE₂.^{119, 120} Interestingly, omega-3 PUFAs, such as EPA and DHA, did not affect osteoblastogenesis.¹¹⁸ However, DHA was shown to induce the viability of mature osteoblasts, as well as ALP and osteocalcin expression.¹²¹ Watkins *et al.* showed that EPA also increased ALP activity and modulated COX2 expression by reducing PGE₂ expression in an osteoblast-like cell line (Fig. 2).¹²¹ The effect of ARA on PGE₂ expression and ALP activity was, in fact, opposite to that of EPA. It is postulated that ARA and EPA may compete with each other for binding to COX2 during prostaglandin production¹²¹. This observation suggests an important role of the omega-6:omega-3 FAs ratio, as COX2 is a rate-limiting enzyme in prostaglandin E2/E3 synthesis. Finally, mice overexpressing the *fat-1* gene have increased trabecular bone volume as well as cortical thickness when compared with wild-type littermates,¹²² indicating the protective effect of omega-3 FAs on bone mass.

Osteoclasts and fatty acids

Osteoclast origin and function

Osteoclasts are multinucleated cells originating from hematopoietic progenitors and are important in the process of bone resorption. Osteoclasts share a common origin with macrophages and are formed through the fusion of progenitor cells. This process requires high energy consumption and an active cell metabolism. Therefore, FA utilization and signaling play crucial roles in osteoclast homeostasis.

Osteoclast activity is precisely regulated by the molecular triad of receptor activator of nuclear factor kappa-B ligand (RANKL), the signaling receptor for RANKL (RANK), and osteoprotegerin (OPG). RANKL belongs to the TNF superfamily and is a pro-inflammatory cytokine produced by osteoblasts and stromal and immune cells. RANKL can bind to its receptor RANK, located at the cellular membrane of pre-osteoclasts, inducing a cascade of

osteoclastogenesis and bone resorption. In contrast, binding of RANKL to its soluble decoy receptor OPG inhibits bone resorption¹²³.

Fatty acid receptors in osteoclasts

Osteoclasts express FA receptors such as GPR40, -41, -43, and -120,^{124, 125} TLR4,¹²⁶ and CD36.¹²⁷ It was hypothesized that the GPR40 receptor may regulate osteoclast differentiation, after observing bone loss in *GPR40* knockout mice.¹²⁸ This hypothesis was further validated by showing that a GPR40 agonist (GW9508) inhibited RANKL-induced osteoclastogenesis.¹²⁹ GPR120 activation negatively regulated osteoclast maturation¹²⁴ and blocked resorption activity. The SFA receptor TLR4, however, was postulated to positively mediate osteoclastogenesis, PGE₂ production, and bone resorption.¹³⁰

The role of lipoprotein and scavenger receptors in osteoclasts has been widely studied. CD36 and other types of scavenger receptors have been hypothesized to play an important role in the transport and metabolism of cholesterol and estradiol in osteoclasts.⁹⁴ Furthermore, CD36 has also been shown to facilitate progenitor cell fusion,¹³¹ thus mediating the formation of osteoclasts themselves. There is conflicting evidence as to whether a deficiency of *LDLR* in mice inhibits¹³² or promotes osteoclastogenesis.¹³³

Osteoclasts also express all three members of the PPAR family: PPAR α , PPAR δ and PPAR γ .¹⁰² While the role of PPAR α in osteoclasts is not fully understood, *in vivo* treatment with a PPAR α agonist, fenofibrate, as well as synthetic selective PPAR α activator, GW9578, decreased osteoclast number.¹⁰⁵ Expression of PPAR δ is considered to be protective from bone loss, as a PPAR δ global knockout exhibited an increase in a number of osteoclasts and bone resorption;¹⁰⁷ however, the specific role of PPAR δ in those cells has not been well established. Chan *et al.* activated PPAR δ using the specific activator L165041 and showed inhibition of osteoclast formation and bone resorption.¹³⁴ Finally, PPAR γ has been shown to positively regulate osteoclastogenesis *in vivo*. Mice lacking PPAR γ in osteoclasts experienced severe osteopetrosis.¹³⁵ Furthermore, treatment with thiazolidinediones (TZD) increased osteoclastogenesis as well as the number of circulating osteoclast precursors.¹³⁶ On the other hand, Chan *et al.* showed that the PPAR γ -specific activator ciglitazone inhibited osteoclast formation and bone resorption.¹³⁴ These results suggest the importance of PPAR family in osteoclastogenesis and modulation of bone resorption.

Osteoclasts and SFAs and MUFAs

It has been proposed that PA stimulates osteoclastogenesis via the TLR4/MyD88 and NF- κ B pathways, eventually leading to the increased production of macrophage inflammatory protein-1 α (MIP-1 α) and osteoclast survival.¹³⁷ Additionally, another study showed that SFA-enriched, but not MUFA-enriched, meals increased RANKL serum concentration in the postprandial state, implying a potential mechanism by which SFA regulates osteoclast activity.¹³⁸ It has been demonstrated that PA-induced osteoclastogenesis can be prevented by oleic acid¹³⁹ through increased FA oxidation and induced expression of CD36 and the diacylglycerol O-acyltransferase (DGAT1) enzyme.¹³⁹ The loss of DGAT1, an enzyme facilitating formation of triglycerides, has been shown to induce osteoclastogenesis, suggesting that SFAs can enhance this process.¹³⁹ On the contrary, long-chain SFAs have

been shown to inhibit osteoclastogenesis of bone marrow-derived and RAW 264.7 macrophages¹²⁵ (Fig. 2). Furthermore, a recent study showed that palmitoleic acid [16:1] (PLA) induced osteoclast apoptosis, decreased resorption markers (i.e., TRAP and MMP-9) and NF- κ B activity, and down-regulated MAPK, JNK, and ERK signaling in the RAW 264.7 cell line.¹⁴⁰

Osteoclasts and PUFAs

The role of omega-6 FAs and metabolites in osteoclasts remains controversial. Some studies demonstrated that ARA suppressed osteoclastogenesis in human CD14⁺ monocytes¹⁴¹ and the RAW 264.7 cell line,¹⁴² as well as inhibited formation of osteoclast-like cell lines. However, others reported that ARA and its metabolites, including leukotriene B, appeared to enhance osteoclastogenesis¹⁴³ (Fig. 2).

The omega-3 PUFAs seem to have an inhibitory effect on osteoclast function. In ovariectomized mice, supplementation with omega-3 PUFAs increased femoral neck bone mineral density (BMD) and decreased osteoclast number.¹⁴⁴ Furthermore, a diet supplemented with fish oil or omega-3 FAs prevented bone loss during chemotherapy,¹⁴⁵ reduced bone resorption markers, and increased bone density in periodontitis.¹⁴⁶ Additionally, a maternal diet rich in omega-3 FAs was correlated with a down-regulation of osteoclastogenesis in offspring¹⁴⁷ (Fig. 2).

It has been reported that DHA attenuated proliferation and differentiation of bone marrow-derived osteoclasts and promoted apoptosis in mature osteoclasts.¹⁴⁸ DHA has also been shown to decrease the production of macrophage colony-stimulating factor (M-CSF), an important osteoclast survival factor, through the down-regulation of protein kinase B (Akt) activation, which leads to the inhibition of cyclin-1 production,¹⁴⁸ as well as inhibition of the NF- κ B/RANKL and ERK-JNK-p38 pathways in osteoclasts.^{141, 148} DHA also has the capacity of inhibiting osteoclastogenesis by the stimulation of lipoxygenase production.¹⁴⁹ Furthermore, compared with ARA and LA, DHA and EPA significantly down-regulated osteoclastogenesis and NF- κ B signaling in osteoclasts. In another study, DHA was more potent in down-regulating osteoclast differentiation than EPA.¹⁵⁰ However, Yuan and colleagues showed that DHA inhibited osteoclastogenesis, while EPA and omega-6 PUFA actually promoted osteoclast differentiation.¹⁴⁹ In other studies, Poulsen *et al.* showed that both DHA and ARA inhibited RANKL-mediated osteoclast formation.¹⁵¹ This finding suggests that PUFA metabolism plays a crucial role in osteoclast development. Interestingly, Oh *et al.* recently reported that GPR120/ β arr2 signaling mediates the anti-inflammatory effects of omega-3 FAs in LPS-stimulated macrophages.²⁵ In this regard, it is possible that macrophages and osteoclasts share similar mechanisms by which omega-3 FAs modulate inflammation, although this hypothesis requires further investigation.

Furthermore, *fat-1* transgenic mice were shown to have an attenuated predisposition to rheumatoid arthritis through inhibition of the downstream signaling of the p38 and Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway (JAK-STAT3).¹⁵² Pro-inflammatory cytokines, including IL-1 β and IL-6, are known stimulators of JAK/STAT3 signaling that triggers osteoclast activity. The *fat-1* mice were also reported to have a lower concentration of circulating bone resorption markers.¹⁵³ These

two studies indicate the beneficial role of omega-3 PUFAs in reducing inflammation and maintaining bone quality.

Synovium and fatty acids

Energy metabolism in synovium

The synovium, a heterogeneous tissue encapsulating the articular joints, consists of the synovial membrane with surrounding fibrous and adipose tissue. The synovium plays an important role in maintaining the homeostatic balance in healthy joints by producing synovial fluid, which is necessary for lubrication. In chronic inflammatory synovium, such as in OA and RA, the synovial tissue is highly infiltrated by a variety of immune cells and proliferating synoviocytes. The accumulation of cells greatly increases oxygen consumption, resulting in synovium hypoxia. It has been proposed that oxidative stress due to hypoxic conditions promotes a bioenergetic switch in cell metabolism from oxidative phosphorylation towards aerobic glycolysis (known as the Warburg effect), leading to impaired mitochondria and dysfunctional angiogenesis.^{154, 155} This hypothesis is also supported by the observation that the synovial fluid of RA patients exhibited up-regulated enzymes of anaerobic catabolism and down-regulated enzymes associated with aerobic oxidation and FA oxidation when compared with healthy individuals.¹⁵⁶ Interestingly, 4-hydroxy-2-nonenal (4-HNE), an oxidation product of PUFAs by reactive oxygen species in hypoxic synovium, may inactivate mitochondrial proteins.¹⁵⁷ This result suggests that despite not being used as a primary energy source, FA is an essential contributing factor in inflammatory joint disorders.

Synovium and FA receptors

Although the presence of GPR40 and GPR120 has not been widely investigated in synovial membranes, these FA receptors are expressed in macrophages and adipocytes.¹⁵⁸ Furthermore, CD36,^{159, 160} TLR4,^{161, 162} and a variety of lipoprotein receptors^{163, 164} were found to be expressed in the synovial membrane and surrounding tissues. Inhibition of CD36 abrogated PA-induced inflammation⁶⁷ and TLR4-induced PGE₂ and COX2 synthesis in FLS.¹⁶⁵ Increased accumulation of LDLs led to synovial activation and osteophyte formation.¹⁶⁶

While it is known that all three PPAR receptors are expressed in synovial fibroblasts,¹⁶⁷ their role in synovial cells are not completely understood. PPAR α activation down-regulated IL-1 β -induced inflammatory gene expression in the synovium.¹⁶⁷ In accordance, PPAR δ stimulation by its agonists increased the expression of IL-1 receptor antagonist (IL1-Ra), a potent inhibitor of IL-1, in FLS.¹⁶⁷ Furthermore, PPAR γ is also postulated to have anti-inflammatory effects in the synovium: PPAR γ agonists inhibited MMP-1¹⁶⁸ as well as TNF- α -mediated MMP-13 expression¹⁶⁹ in FLS.

Synovium and FA—It is well-recognized that high-fat feeding promotes the invasion of a variety of immune cells, including macrophages, into the joint synovium. Dietary FAs, particularly SFAs, lead to a phenotypic switch of macrophages toward the M1 pro-inflammatory pathway. Additionally, TNF- α secreted by M1 macrophages can further

induce insulin resistance and catabolic processes in chondrocytes, implying a detrimental role of M1 macrophages in OA development.^{170, 171} Interestingly, we found that conditional depletion of macrophages does not inhibit OA progression in obesity. Rather, this depletion exacerbated synovitis by increasing infiltration of neutrophils and CD3⁺ T cells.¹⁷² Our results emphasize the essential regulatory role of MLS in modulating homeostasis of immune cells in the setting of obesity. A more detailed role of FAs in MLS has been described elsewhere.^{173, 174}

SFAs have also been shown to induce the expression of pro-inflammatory markers such as IL-6, IL-8, MCP-1, and MMPs in human FLS cell culture.⁶⁷ Furthermore, FLSs were able to produce both inflammatory and pro-resolving mediators when stimulated with PA. There was a positive association between synovitis and the plasma level of omega-6 PUFAs in patients with OA. In contrast, the plasma level of omega-3 PUFAs was negatively associated with synovitis.¹⁷⁵ Mediators derived from omega-6 FAs, including PGE₂, have been shown to increase synovial inflammation, while those derived from omega-3 FAs, such as resolvins, were shown to decrease synovial inflammation^{176, 177} (Fig. 2).

The *fat-1* transgenic mice have been shown to have a lower predisposition to RA in a KRN serum transfer mouse model.¹⁵² However, metabolic enrichment of omega-3 FAs did not mitigate the joint synovitis of spontaneous OA in *fat-1* mice.⁷⁹

Ligament, meniscus, and fat pads

While this review is focused primarily on cartilage, bone, and synovium, it is important to note that cells in other joint tissues, such as ligaments, meniscus, and infrapatellar fat pad (IPFP), may also be influenced by dietary FAs. There are currently a few studies examining the role of FA metabolism in these tissues. In brief, FA receptors, including TLR4, PPAR γ ,¹⁶² and CD36,¹⁶⁰ have been found in IPFP. The presence of FA receptors has not been reported in ligaments or meniscus. However, SFAs have been shown to promote apoptosis in meniscal cells¹⁷⁸ by inducing ER stress,¹⁷⁹ while omega-3 PUFAs have been postulated to enhance ligament healing.¹⁸⁰

Conclusions

Obesity-related complications in the musculoskeletal system have been extensively studied in the past decade. Due to current trends toward increasing obesity caused by unhealthy diets and lifestyles, it is likely that the prevalence of musculoskeletal disorders will continue to increase. The studies summarized in this review indicate that dietary FA content can significantly influence tissues and cells within the articular joint. Especially in the context of OA pathogenesis, FAs have been shown to mediate both anabolic and catabolic processes of many joint tissues. In summary, there is strong evidence for the presence and function of both intra- and extracellular receptors for FAs in joint cells, as well as the importance of FA-triggered signals. Although there are still missing links in our understanding of the role of dietary FA content, the current evidence supports a protective role of omega-3 FAs and the pro-inflammatory role of SFAs. Additionally, the literature suggests that a balanced ratio of omega-3 to omega-6 FAs is an important factor in modulating joint homeostasis. The

knowledge summarized in this review may trigger new areas of research and future interventions for metabolic joint diseases.

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Figure 1.

Categorization of fatty acids. Fatty acids are characterized based on the number of carbons (C represents number of carbon atoms present) and the bonds between those carbons (D represents the number of double bonds). Short chain FAs have 5 or less carbons, medium chain have between 6 and 12 carbons, and long chain have 13 or more. Fatty acids that contain no double bonds between carbons are considered to be saturated. Butyric acid (found in milk), caprylic and capric acids (found in coconut oil), and palmitic and stearic acids (found in peanut oil) are examples of saturated short, medium, and long chain fatty acids, respectively. Monounsaturated have one double bond (e.g., oleic acid in avocado oil), while polyunsaturated have two or more (e.g., eicosapentaenoic acid in fish oil). In the fatty acid schematic, blue circles represent carbon while red circles represent the oxygen of the carboxylic acid group.

Fatty Acids				
	Saturated	Polyunsaturated Omega - 3	Polyunsaturated Omega - 6	
Cell Types	Chondrocytes	↑ OA-like symptoms ^{32, 60-63} Apoptosis ^{65, 68} IL-6 ⁶⁷	↓ ADAMTSs and MMP3 ^{70, 73, 74} sGAG loss ^{73, 75} Oxidative stress ⁷⁶	↑ OA degeneration ⁷² ↓ Chondrogenesis ⁶⁴
	Osteoblasts	↑ Lipotoxicity ^{113, 115, 117} Apoptosis ¹¹² TLR4 / NfκB ^{114, 116} ↓ RUNX2 / SMAD4/5 ¹¹³	↑ ALP / Osteocalcin ¹²¹ ↓ COX2 / PGE ₂ ¹²¹	↑ COX2 / PGE ₂ ^{119, 120} ↓ Osteoblastogenesis ¹¹⁸
	Osteoclasts	↑ MIP-1a ¹³⁷ RANKL ¹³⁸ ? Osteoclastogenesis ^{137, 139}	↑ Bone density ¹⁴⁴⁻¹⁴⁶ Cell number ^{144, 148} Osteoclastogenesis ^{147, 149} ↓ Bone resorption markers ¹⁴⁶	? Osteoclastogenesis ^{141-143, 149}
	Synoviocytes	↑ IL-6 / IL-8 ⁶⁷ MCP-1 ⁶⁷ MMPs ⁶⁷	↓ Synovitis ¹⁷⁵⁻¹⁷⁷	↑ Synovitis ¹⁷⁵⁻¹⁷⁷

Figure 2.

The effects of fatty acids on joint cells. Saturated, omega-3 polyunsaturated, and omega-6 polyunsaturated fatty acids can have positive and negative effects on the various cells of the joint. In chondrocytes, SFAs and omega-6 PUFAs have more negative effects on cartilage, increasing OA, degeneration, and cell death, while omega-3 PUFAs have a more beneficial effect. Inflammation appears to be positively regulated by SFAs and omega-6 PUFAs in osteoblasts, while mineralization and osteoblastogenesis are negatively regulated. Omega-3 PUFAs have been shown to have the opposite effect. In osteoclasts, there is conflicting

evidence as to whether SFAs and omega-6 PUFAs up-regulate or down-regulate osteoclastogenesis. Omega-3 PUFAs were shown to increase bone density as opposed to omega-6 PUFAs, which increased bone resorption. Synovitis was decreased in response to omega-3 PUFAs, while it was increased by omega-6 PUFAs. In addition, synoviocytes expressed higher inflammatory and degenerative markers in the presence of SFAs.

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