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REVIEWS: CURRENT TOPICS

The endocannabinoid signaling system: a marriage of PUFA and musculoskeletal health

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Abstract

The role of diet in health and diseases related to muscle and bone has been an area of active study. Recently, endocannabinoids (EC), endogenous derivatives of arachidonic acid, an omega-6 (n-6) polyunsaturated fatty acid (PUFA), have been discovered to play regulatory roles in bone mass and muscle energy metabolism. This signaling system consists of the G-protein coupled cannabinoid receptors, CB1 and CB2, expressed in central and peripheral tissues and cells, which are variably activated by the production and on demand release of endogenous and synthetic agonists and antagonists. We propose that the balance between omega-6 and omega-3 (n-3) PUFA is an important modifier for the activation and suppression of endocannabinoid receptors and therefore, downstream signaling actions in cells. The potential of dietary PUFA to regulate this signaling system to influence the metabolic and physiological outcomes favorable to musculoskeletal health is the purpose of this review. The important role of n-3 PUFA in metabolic and physiological processes that attenuate muscle and bone loss under conditions of disease and stress is one aspect described herein. In this review, we first introduce the EC agonists (ligands) and their receptors (CB1 and CB2) and the general actions of EC signaling in various organs and systems. Second, we describe EC signaling in bone and muscle and how dietary PUFA influence the levels of endogenous agonists. Third, we discuss the potential implications of how dietary PUFA impact this system to minimize muscle atrophy and osteopenia and support healthy muscle development and bone modeling.

Keywords: Endocannabinoids; Endocannabinoid signaling; Muscle; Bone; Polyunsaturated fatty acids; Omega-3 fatty acids; CB1; CB2; Rodent; Human

1. Introduction

The medicinal qualities of cannabis were reported as early as 2727 B.C. Delta-9 tetrahydrocannabinol (THC), the major psychotropic component in cannabis, was identified in the 1960s [1], and in the late 1980s, the brain receptor for THC was identified as cannabinoid receptor (CB) 1 [2]. In 1990, the CB1 receptor was cloned [3]. The CB2 receptor was first identified in the immune system and has since been identified in peripheral organs (e.g., bone, muscle, and heart) as well as in the central nervous system, albeit in lower concentrations compared to CB1 expression. CB1 and CB2 are G-protein coupled receptors [4], and are variably acted upon by agonist and antagonist ligands. Several natural and synthetic ligands have been identified that target these receptors. Endogenous agonists for CB1, the central cannabinoid receptor, and CB2, the peripheral cannabinoid receptor, were identified in the early and mid 1990s. The agonist, *N*-arachidonoylethanolamide [anandamide (AEA)], was discovered first, followed by 2-arachidonoylglycerol (2-AG). These compounds are synthesized on demand via phospholipids (PL) derived arachidonic acid (AA) and act as paracrine or autocrine ligands for the CB1 and CB2 receptors (Fig. 1). The active component of cannabis, THC (Dronabinol), is medicinally utilized for the stimulation of food intake (orexigenic) in conditions of nausea associated with chemotherapy, and as an appetite stimulant for AIDS patients. Agonists and antagonists have also served as pharmaceutical agents in controlling or countering different functionalities of the endocannabinoid signaling system. For example, CB1 agonist drugs have been used for treatment of pain in multiple sclerosis patients [1]. The specific CB1 antagonist, SR141716A (Rimonabant), is effective for the treatment of obesity; however, recent reports of depression and suicidal thoughts have led to its discontinuation in clinical trials in Europe and the United States [5].

Obesity and its associated health consequences have been identified with dysregulation of the endocannabinoid (EC) system [6]. The chronic activation of the EC system in obesity, and the elevated blood levels of fatty acids resulting from a high fat diet are believed to contribute to the dysregulation of this system. The consequence of dysregulation on peripheral organs, such as muscle and adipose, aggravates the symptoms of insulin resistance and fat accumulation in support of this theory. The discovery of the CB1 and CB2 and the fact that endogenous cannabinoids can activate these

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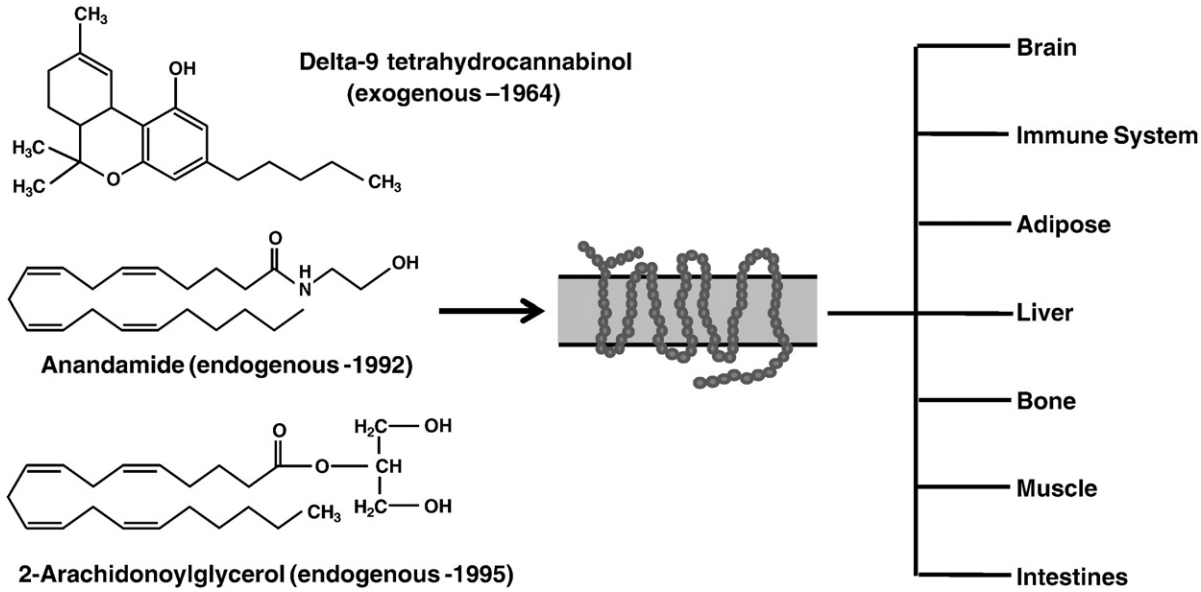

Fig. 1. Illustrated is the structure of the endocannabinoids and the receptors that are present in a variety of tissues. The cannabinoid receptors CB1 and CB2 are activated by the exogenous cannabis agonist THC and the endogenous agonists, anandamide and 2-arachidonoylglycerol, which are derived from arachidonic acid.

receptors started a flurry of investigations as it was theorized that CB1 antagonism would decrease food intake. CB1 is also expressed in peripheral tissues including adipose, liver, pancreas, muscle and bone. The CB1 sites in these tissues have been studied to identify the effects of activation or antagonism of the receptors. Both tissue-specific metabolic impairment and dysregulated organ crosstalk (i.e., adipose to skeletal muscle) have been shown to be involved in the metabolic events of obesity, meaning insulin resistance and lipid synthesis associated with dysregulation of the EC system. In this regard, the EC system appears to be a participant in negative crosstalk for the communications between muscle and adipose. Hence, negative crosstalk, for which the EC system is believed to be involved, is a new approach for understanding obesity. At the opposite end of the spectrum from obesity, e.g., cachexia, efforts are underway to enhance agonistic action of the EC system to promote food intake and preserve muscle mass [7,8]. It is well known that exogenous agonists of the CB1 receptor induce eating, such as the munchies, described by marijuana smokers [9].

The two most studied endogenous cannabinoids, AEA and 2-AG, are synthesized on demand from AA found in glycerolipids (see Fig. 2). The AA in phosphatidylethanolamine is converted to AEA via the actions of transacylase and *N*-acyl phosphatidylethanolamine phospholipase D. The 2-AG is synthesized from PL via phospholipase C and diacylglycerol lipases (DAGL) α and β. As shown in Fig. 2, dietary PUFA from the omega-6 (n-6) and omega-3 (n-3) families will determine the amount of AA in tissue PL, demonstrating that dietary PUFA can determine the potential amounts of the bioactive lipid mediators, AEA and 2-AG, produced in tissues.

Degradation of AEA occurs by fatty acid amide hydrolase (FAAH) to form AA and ethanolamine; monoacylglycerol lipase (MAGL) and FAAH, to a lesser extent, degrades 2-AG to form AA and glycerol (Fig. 3). As expected, both the synthetic and degradative pathways are regulated in the body to control the amounts of endogenous agonists for EC signaling.

Circulating AEA and 2-AG levels are greater in visceral obese humans compared to lean [10] or subcutaneous obese individuals [11] and are evidence of increased EC tone in obesity [6]. Further, the concentrations of 2-AG in blood positively correlate with free fatty acid and triglyceride (TG) levels but are inversely related to high-density lipoprotein cholesterol [11]. Interestingly, lifestyle changes that led to reduced visceral fat in obese men was associated with decreased blood 2-AG levels [12].

2. Endocannabinoid signaling system

2.1. EC actions on energy metabolism and physiology (adipose, liver, muscle, and immune cells)

Cannabinoid receptors are expressed in most peripheral organs, and researchers over the past few years have sought to elucidate the relationship between the central and peripheral cannabinoid system as it relates to obesity and systemic energy regulation [13,14]. Metabolic changes observed with obesity are associated with increased expression of the CB1 receptor in adipose, liver, pancreas and muscle, and altered crosstalk amongst these peripheral tissues and with the central nervous system. Recently, a proposed relationship between muscle and adipose has been described by Watt based on current studies [15]. In this example, the EC system is engaged by the production of endocannabinoids derived from adipocytes that down-regulate insulin action in muscle (glucose uptake), and the use of an antagonist for CB1 restores insulin responses in muscle [15].

Pre-adipocytes and mature adipocytes express cannabinoid receptors and human adipocytes have been shown to synthesize endocannabinoids [16]. However, elevation of blood 2-AG levels, but not AEA, have been positively correlated with increased visceral fat deposition [17]. CB1 expression and binding efficiency are increased during adipocyte differentiation, while CB2 expression is decreased [18]. CB1 activation increases lipid accretion in mature adipocytes. Synthetic CB1 agonists and AEA have been shown to increase adipocyte differentiation, the adipocyte differentiation marker, peroxisome proliferator-activator receptor γ (PPARγ), and lipid droplet accumulation [18]. These effects in adipocytes were reversed by CB1 antagonism with Rimonabant [10].

Leptin, an adipocyte hormone, may also be involved in metabolic regulation associated with the EC system. Leptin treatment has been shown to decrease endocannabinoid concentrations in mature adipocytes [10], and it has been postulated to regulate adipose metabolism and endocannabinoid actions [6]. Leptin is one neuropeptide that controls the levels of AEA and 2-AG in the brain to

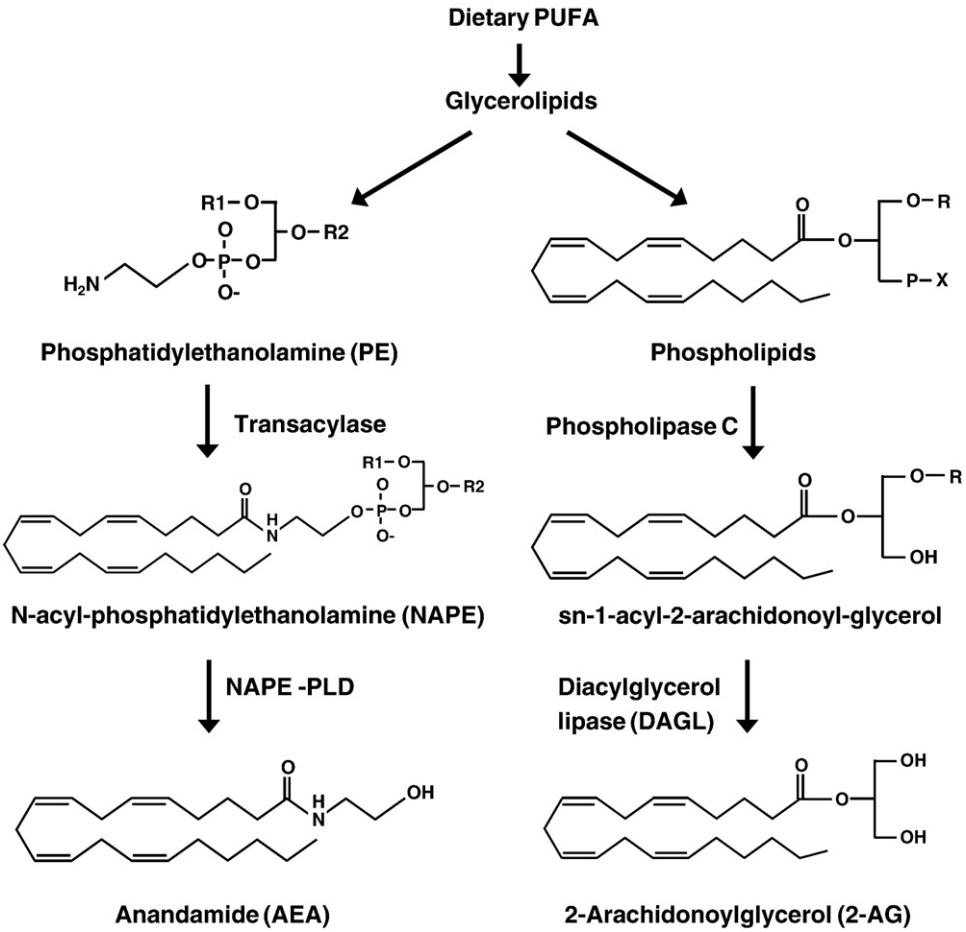

Fig. 2. Endogenous cannabinoids arachidonoylethanolamide (anandamide, AEA) and 2-AG are synthesized on demand from AA and catabolized by related enzymes. The types and amounts of dietary n-6 and n-3 PUFA can influence the concentrations of AA in glycerolipids.

mediate the orexigenic and anorectic effects of the EC signaling system [19].

Studies have been conducted to understand the genetic and dietary factors that influence the EC system and lipid metabolism. Compared to CB1 knockout mice, the wild-type (WT) littermates fed a high fat diet had increased CB1 receptors in hepatocytes, higher circulating leptin concentrations, and developed obesity and liver steatosis [20]. In WT mice fed a regular chow diet and treated with a CB1 agonist, there was glucose intolerance, decreased insulin sensitivity and increased de novo hepatic lipogenesis [21]. The work

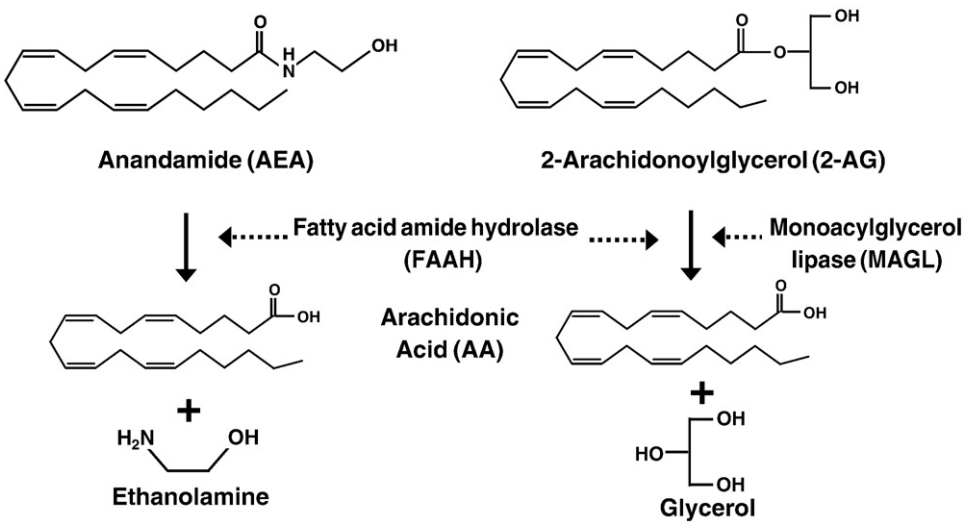

Fig. 3. Endogenous cannabinoids arachidonoylethanolamide (AEA) and 2-AG are catabolized by related enzymes. The degradation of the endocannabinoids is an important regulatory pathway in the function of EC signaling.

by Osei-Hiyaman et al. [20] showed that WT mice with diet-induced obesity had increased liver sterol regulatory element binding protein-1c and elevated fatty acid synthesis as evidenced by increased acetyl-CoA carboxylase and fatty acid synthase expression; however, these effects were abolished with CB1 antagonist treatment. Thus, activation of CB receptors by endogenous agonists promotes energy storage in adipose but blocking receptor activation with antagonists causes weight loss and improves insulin sensitivity in muscle.

Expression of the cannabinoid receptors varies in obese and lean individuals and in different kinds of fat tissue (e.g., subcutaneous versus visceral). Bluher et al. [11] reported that visceral adipose tissue (VAT) from lean humans express significantly greater CB1 mRNA compared to both subcutaneously and viscerally obese humans. Matias et al. [10] found a trend towards decreased CB1 receptor levels in obese compared to normal weight humans, and Murdolo et al. [22] found similar mRNA levels for CB1 and CB2 in subcutaneous adipose tissue from lean and obese humans. VAT is known to correlate with metabolic consequences of obesity, and waist circumference is a risk factor for metabolic syndrome and heart disease [23]. These results suggest that differences in CB1 expression in obesity may be specific to VAT and that modulation of CB1 expression is involved in the metabolic dysregulation associated with visceral adiposity. In VAT from both obese patients and diet-induced obese mice [10], 2-AG concentrations increased but not AEA. VAT 2-AG levels were observed to be twofold greater in obese compared to lean individuals; however, there was no difference in DAGLα or MAGL mRNA expression, suggesting that the increase in 2-AG was from an altered availability of biosynthetic precursors [10]. The AEA levels in adipose tissue may be controlled more by degradation than synthesis because FAAH mRNA levels were greater in VAT of lean individuals compared to obese individuals [11]. Gender differences may also be evident as females are reported to have greater circulating concentrations of AEA compared to males in all weight groups [11].

Glucose metabolism can be modified by endocannabinoids in adipose tissue and skeletal muscle. AEA, through activating CB1 receptors in differentiated adipocytes, increased insulin stimulated glucose uptake, but this effect was reduced by the CB1 antagonist, SR141716A [18]. Cannabinoid receptors expressed in skeletal muscle [24] are, in part, responsible for whole body glucose (energy) regulation. As with adipocytes, CB1 protein expression increases with differentiation of myoblasts to myotubes while CB2 expression decreases with differentiation [25]. Human myotubes from lean and obese individuals have been shown to express similar levels of CB1 mRNA [26]. When these myotubes were incubated with media conditioned by human pre-adipocytes from either overweight women or by AEA, there was a reduction in glucose uptake as evidenced by increased insulin receptor substrate-1 (IRS-1, Ser307) phosphorylation and decreased insulin stimulated serine/threonine kinase Akt (Ser 473) phosphorylation. These findings suggest that AEA interferes with insulin signaling and glucose utilization in muscle [25]. The effects of adipocyte-conditioned media were nearly abolished with CB1 antagonist pretreatment [25], indicating that CB1 is likely involved in insulin signaling in skeletal muscle.

Oxidative metabolism in skeletal muscle is altered by EC signaling as well. AEA co-treatment with CB1 antagonism decreased PPARγ coactivator-1α (PGC-1α) mRNA levels in the skeletal muscle of obese subjects, resulting in decreased fatty acid oxidation and glucose uptake [26]. Both antagonist, alone and in combination with AEA treatment, decreased pyruvate dehydrogenase kinase isozyme 4 (PDK4, a downstream target of PGC-1α) expression in skeletal muscle from lean and obese individuals, and while AMP-activated protein kinase (AMPK) mRNA levels increased with CB1 antagonism, the effects were blocked with a combination treatment of AM251 (CB1 antagonist) and AEA, thus, supporting the involvement of CB1 in glucose and fatty acid oxidation in skeletal muscle [26]. Esposito et al. [27] found that CB1 antagonism in myotubes increased glucose uptake, which supports the earlier work by Liu et al. [28] in leptin-deficient obese mice. The pathways for altered glucose uptake and metabolism indicate increased cAMP/PKA and phosphoinositide-3 kinase (PI3K) activity with further induction of downstream signaling molecules [27]. Since the cannabinoid receptors are $G_{i/o}$-protein coupled receptors, their activation decreases adenyl cyclase, reduces cAMP, and hence, impacts downstream signaling cascades in skeletal muscle, leading to reduced glucose uptake. Therefore, based on the information presented, antagonism of the CB1 receptor would reverse the effect on glucose uptake in muscle. Moreover, muscle that is optimally responsive to insulin and not impaired with regard to energy metabolism is likely conditioned to provide better biomechanical signals to support bone formation.

The relationship between cannabinoid receptors and immune regulation is associated with maintenance of immunocompetence. CB2 is the predominant cannabinoid receptor in immune cells and is present in decreasing concentrations in B cells, natural killer cells, monocytes, polymorphonuclear neutrophils, and CD8 and CD4 lymphocytes [29]. CB1 is also expressed in immune cells, although CB2 is expressed at 10- to100-fold higher levels. Both receptors are, however, down-regulated when immune cells are activated, presumably to allow for response to an invading stimulus.

2.2. The EC system is expressed in bone cells and its signaling influences bone modeling and remodeling

Bone is a multifunctional organ that consists of a structural framework of mineralized matrix and heterogeneous populations of chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes and hemopoietic cells. This population of cells produces numerous biological factors in response to biomechanical, hormonal, and dietary influences to regulate local bone metabolism and bone growth in length, diameter, and shape. Osteoblasts produce and mineralize bone matrix, while the specialized multinucleated cells, called osteoclasts, cause bone resorption. The combined and cooperative activities of osteoblasts and osteoclasts result in a bone architecture and bone quality that provides mechanical support and protection for the body. In addition, bone serves as a vital reservoir of minerals, principally calcium and phosphorus, that are necessary for maintaining the normal cellular, neurologic and vascular activities of the body.

Long bone growth is regulated by complex interactions between an individual's genetic potential and multiple environmental factors, including nutrition. Bones grow in length and diameter by a process called modeling. Bone modeling represents an adaptive process of generalized and continuous growth and reshaping which is regulated by the activities of osteoblasts and osteoclasts (Fig. 4) until the adult structure is attained. This growth requires that bone cells function normally and that adequate nutrition is provided. Bone modeling is distinct from bone remodeling. The latter describes the local, coupled process of bone resorption and formation that maintains skeletal mass and morphology in the adult (Fig. 5). The numerous cell-derived growth factors present within skeletal tissues exert local controls on skeletal metabolism. The prostaglandins are major players in bone metabolism as well as in bone and joint diseases. Many of the skeletal pathologies that afflict the adult, e.g., osteoporosis and rheumatoid arthritis, are the consequence of either abnormal bone remodeling and metabolism or an inflammatory process. Recent studies suggest that the onset and severity of some of these pathologies may be delayed and lessened if bone modeling is optimized early in life or if diets are supplemented with nutrients that reduce the tissue concentrations of factors that undermine skeletal health.

An analysis of these processes leads to the recognition that considerable energy is required to accomplish these bone metabolic

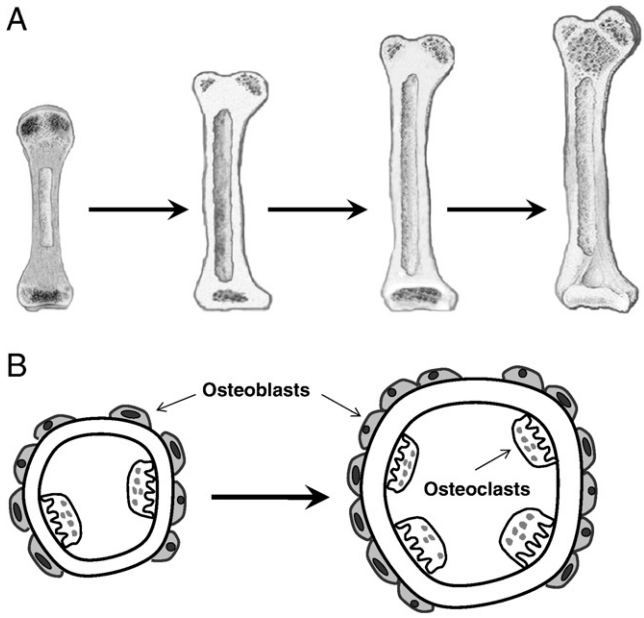

Fig. 4. The bone modeling process includes longitudinal bone growth at the epiphyses of long bone (A) and increases in diameter by endosteal and periosteal bone formation (B). The process is highly influenced by muscle strains on bone to model (in the young) and remodel bone (in the adult) to impact bone growth (mineral accretion) and change the architecture of bone structure throughout life. The mechanostat theory described by Frost [59] is a result of muscle-derived flexural loads orientated symmetrically around the cross sectional circumference of bone causing a uniform increase in bone diameter. Repetitive, similar dynamic flexural straining asymmetrically around the bone activates the flexure-drift feedback system and causes drifts of lamellar bone surfaces in tissue space and contributes to the shaping of bone.

functions [30]. The endocannabinoid system has recently been shown to play an important role in the regulation of bone mass and bone remodeling (see Table 1). Osteoclasts and osteoblasts are responsible for bone resorption and formation, respectively. CB1 and CB2 receptors are both found in osteoclasts. CB2 is abundantly expressed in pre-osteoblasts and mature osteoblasts [35], while CB1 is barely detectable in differentiated osteoblasts [32]. The presence of cannabinoid receptor CB2 and production of AEA and 2-AG are associated with increased bone mass in rodents while CB2-deficient mice demonstrate age-related bone loss [39] (Table 1). When the CB2 receptor is stimulated by agonists in primary osteoblast cultures or the MC3T3-E1 osteoblast-like cell line an increase in cell proliferation, alkaline phosphatase (ALP) activity and mineralization occurred. There was no agonistic effect in osteoblasts cultured from CB2 knockout mice, suggesting a direct effect of CB2 on bone formation [35]. The CB2 agonist, HU-308, has also been shown to decrease osteoclast-like cell formation and their DNA synthesis [35]. In addition, both an endogenous cannabinoid agonist and a synthetic CB1 agonist have been reported to stimulate osteoclast formation [31], while the osteoclast number is reduced via promotion of apoptosis by CB1 and CB2 antagonism [36].

The endogenous CB2 ligand, 2-AG, can be synthesized in osteoblasts and osteoclasts since DAGLα and DAGLβ are expressed in these bone cells [33]. When 2-AG was used as an agonist treatment, however, there was no effect on osteoblast cell proliferation or ALP activity in the MC3T3-E1 osteoblast-like cell line or in primary calvarial osteoblast cultures [33].

Primary bone marrow cultures treated with 2-AG promoted a dose-dependent increase in fibroblast cell colony number and size, as well as calcium and collagen deposition. When these cells were treated with a CB2 antagonist, however, the effects of the natural cannabinoids were abolished [38]. These findings indicate a direct role of CB2 activation on differentiated osteoclasts and osteoblasts, and an indirect action on the proliferation of mesenchymal stem cells.

Genetically deficient $CB2^{-/-}$ mice are healthy and similar in size to WT mice but exhibit significant differences from WT mice in bone metabolism (Table 1). Ofek et al. [35] reported a low bone mass phenotype with high bone turnover which was first observed in 12-week-old females. They had greater osteoclast numbers and mineral appositional rates compared to WT mice. By one year, male mice also showed signs of decreased trabecular bone volume in the $CB2^{-/-}$ mice compared to the WT [35]. However, Idris et al. [36] reported no bone phenotypic differences between the WT and $CB2^{-/-}$ mice at 12 weeks of age. When $CB2^{-/-}$ mice were ovariectomized (OVX) there was trabecular bone loss, decreased trabecular thickness and number compared to the WT OVX mice. The differences observed in these studies highlight the importance of age for bone remodeling to occur and physiological state. The background strain used in both of these studies, C57BL/6J, does not reach peak bone mass until 5–6 months of age [40], and therefore, the lack of bone loss observed by Idris et al. [36] may be an effect of time that was exaggerated in mice that were OVX and then lost bone. Thus, from these two studies, the CB2 receptor appears to be involved in bone turnover but the mechanism of action is poorly understood.

Human genetic studies also support the importance of the CB2 receptor in bone function. Postmenopausal osteoporotic patients were reported to have significant associations of single polymorphisms and haplotypes in the cannabinoid receptor 2 (macrophage) (CNR2) gene compared to matched female controls without such associations [41]. Recently, the same investigators reported several single nucleotide polymorphisms in the CNR2 gene (expresses CB2) which were related to low bone mineral density (BMD) and geometric properties of the bones in the hands in an ethnically homogeneous family sample [42].

OVX is a common procedure performed in rodent models to evaluate anti-osteoporotic drugs. CB2 activation by a synthetic agonist diminished trabecular loss that was attributed to decreased osteoclast numbers in CB2 agonist-treated OVX mice compared to control OVX or sham operated mice [35]. The CB2 agonist promoted an increased cortical thickness above what was observed in the OVX or sham mice which was attributed to an anabolic effect on bone by stimulating endocortical bone formation [35]. Idris et al. [36] reported similar findings with a synthetic CB2 antagonist/inverse agonist in OVX mice. They found that treatment of WT OVX mice with a synthetic CB2 antagonist/inverse agonist prevented bone loss with a normal osteoclast number compared to WT OVX mice. OVX $CB2^{-/-}$ mice also treated with the CB2 antagonist/inverse agonist were not protected from bone loss [36]. How can both antagonist and agonist of the same receptor result in similar findings? One answer could be the doses used. Low doses of the CB2 agonist (HU-308) have been shown to increase the osteoclast number while higher dosages decrease the

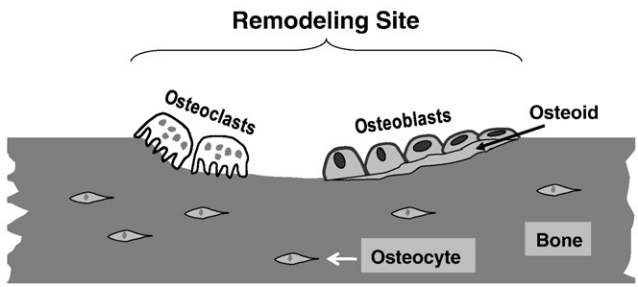

Fig. 5. Bone remodeling is depicted showing the action of osteoclasts in bone resorption followed by osteoblastic bone formation. In bone modeling, the high activities of osteoblasts and osteoclasts can result in rapid changes that alter the shape of bone; however, the osteoblastic activity throughout bone is less during bone remodeling.

Table 1
Actions of the endocannabinoids and receptors on bone

Author-Year	Model	Receptor	Outcome	
			Anabolic	Catabolic
Bone, in vivo				
Idris et al. 2005 [31]	$CB1^{-/-}$ mice (CD1 background strain, 9 – 12 weeks)	CB1	Increased total BMD, trabecular bone volume (BV/TV, Tb.Th, Tb.N) at tibial metaphysis, resistant to OVX-induced trabecular bone loss measured by microCT.	
Tam et al. 2006 [32]	$CB1^{-/-}$ mice (C57BL/6J and CD1 background strain, 9-12 weeks)	CB1	$CD1^{CB1-/-}$ males high bone mass; females normal trabecular bone, slight cortical expansion as evidenced by Tb.N, Tb.Th, diaphyseal diameter and medullary cavity diameter by microCT.	$C57^{CB1-/-}$ males and females low bone mass, decreased trabecular bone volume expressed as BV/TV measured by microCT.
Tam et al. 2008 [33]	WT and $CB1^{-/-}$ mice with traumatic brain injury	CB1	WT mice increased 2-AG and decreased NE promoted bone formation measured by BFR, MAR and mineralizing perimeter.	No osteogenic effect observed in $CB1^{-/-}$ mice.
	WT, $CB1^{-/-}$, $CB2^{-/-}$ mice treated with 2-AG or isoproterenol (β androgenic receptor agonist)	CB1, CB2	2-AG enhanced bone formation (BFR, MAR, mineralizing perimeter) in $CB2^{-/-}$ mice. Isoproterenol blocked effects of 2-AG in WT mice.	No effect on bone formation or decreased mineralization with 2-AG in $CB1^{-/-}$.
Idris et al. 2009 [34]	$CB1^{-/-}$ mice (CD1 background strain at 3, 6 and 12 months)	CB1	At 3 months, $CB1^{-/-}$ high bone mass reported as enhanced BV/TV measured by microCT.	At 12 months, lower bone mass (BV/TV); increased adipocytes in bone marrow cavity; increased CB1 expression.
Ofek et al. 2006 [35]	$CB2^{-/-}$ mice (C57BL/6J background at 8-11 weeks)	CB2		$CB2^{-/-}$ mice low bone mass, high bone turnover.
	C3H, OVX mice (51 weeks)	CB2	CB2 agonist (HU-308) attenuated OVX induced bone loss compared to vehicle with greater BV/TV, cortical thickness and MAR and decreased osteoclast number and medullary space.	
Idris et al. 2008 [36]	WT, $CB2^{-/-}$ OVX mice (3 months)	CB2	No difference in trabecular bone volume (BV/TV) before OVX; after OVX $CB2^{-/-}$ partly protected from OVX induced bone loss, CB2 antagonist (AM630) prevented bone loss in WT, but not in $CB2^{-/-}$ mice measured by microCT reported as BV/TV, Tb.Th, Tb.N.	
Bone, in vitro				
Idris et al. 2005 [31]	Osteoclasts cultured from $CB1^{-/-}$ mice	CB1	Resistant to inhibitory effects of AM251 (CB1 antagonist); CB2 antagonist (AM630) inhibited osteoclast formation.	
Idris et al. 2008 [36]	Osteoclasts generated from bone marrow macrophages	CB1, CB2	Antagonists (AM251, AM630) inhibit osteoclast formation and bone resorption; promoted apoptosis.	Agonists (JWH133, HU-308) enhanced osteoclast formation.
Idris et al. 2009 [34]	Primary osteoblast cells, MSC from $CB1^{-/-}$ and WT mice, bone marrow cells	CB1	Antagonist (AM251) decreased bone formation, increased adipocytes. $CB1^{-/-}$ cultures showed increased expression of PPARγ, pCREB.	Non-selective agonist increased bone formation.
Tam et al. 2008 [33]	MC3T3-E1 cell line; NeMCO cells	CB2	Agonist (HU-308) increases cell number and ALP activity; 2-AG had no effect.	
Ofek et al. 2006 [35]	Primary osteoblast and osteoclast cells from bone marrow, NeMCO cells, MC3T3-E1 cells, bone	CB2	Agonist (HU-308) increased osteoblast proliferation, differentiation, colony-forming unit, ALP activity; decreased RANKL mRNA, and formation of osteoclast-like cells.	
Rossi et al. 2009 [37]	Osteoclasts cultured from PBMCs	CB2		Antagonist (AM630) increased TRAP positive osteoclasts.
Scutt and Williamson 2007 [38]	Primary bone marrow cultures, MSC	CB1, CB2	Agonist (CP 55,940, WIN 55212, 2-AG) stimulated proliferation, differentiated colony formation, collagen accumulation in primary cultures, not MSC.	Antagonist BML190, agonists JWH015 and ACEA had no effect on colony growth.

BFR, bone formation rate; BV/TV, bone volume/total volume; MAR, mineral appositional rate; MSC, marrow stromal cells; NE, norepinephrine; NeMCO, primary calvarial osteoblast cells; pCREB, phosphorylated cAMP response element binding factor; PBMCs, peripheral blood mononuclear cells; Tb.N, trabecular number; Tb.Th, trabecular thickness.

osteoclast number [36]. Higher dosages of AM630, the CB2 antagonist, have been suggested to lose selectivity to CB2 and may act via other receptors and/or signaling pathways to prevent bone loss [36]. For example, transient receptor potential vanilloid type 1 (TRPV1) is co-expressed with CB1 and CB2 on osteoclasts and may account for different actions of cannabinoids [37], particularly AEA, which is known to act on the TRPV1 receptor. Undoubtedly, the mouse and human data demonstrate the importance of CB2 in bone remodeling, but the mechanism of action is not fully understood.

The sympathetic nervous system is another important regulator of bone remodeling [43]. It appears that bone protective effects are mediated by innervations through activation of CB1 in the sympathetic nerve fibers within trabecular bone by endocannabinoids released from osteoblasts [33]. Initial reports describing the impact on bone in $CB1^{-/-}$ mice were inconsistent [31,32]. In the background strain CD1 of the CB1 knockout mice, Idris et al. observed a high bone mass phenotype and this phenotype resisted OVX-induced bone loss [31]. A year later, Tam et al. reported the $CB1^{-/-}$ phenotype on CD1 and C57BL/6J backgrounds [32]. The $C57BL/6J^{CB1-/-}$ mice had a low bone mass phenotype in both males and females, which was associated with decreased bone formation rates and increased osteoclast numbers [32]. Gender specific differences, however, were evident in the CD1 background strain. Male $CD1^{CB1-/-}$ mice had a high bone mass while females had a normal bone mass phenotype. The males had no significant alterations in bone remodeling parameters, suggesting that the increase in bone mass occurred at an earlier developmental stage than tested [32].

Studies of traumatic brain injury (TBI) demonstrate heterotropic bone formation in the hip and elbow joints of patients [44] and rats subjected to brain injury were assessed to quantify the osteogenic response [44,45]. The possible association between TBI and increased bone formation has long been recognized but difficult to reproduce

and study. Therefore, the use of TBI may provide better insight into the role of the nervous system and EC signaling in bone, and identify potential systemic soluble mediators that support bone formation. In this regard, Tam et al. used a mouse model of TBI to study the central nervous system effects of endocannabinoids on bone remodeling and found that 2-AG activates CB1 receptors on sympathetic nerves and inhibits norepinephrine release [33]. The suppression of norepinephrine release by 2-AG in TBI removes the inhibitory actions of this catecholamine on bone formation [33]. Thus, it appears that the increase in bone formation observed in the mouse model of TBI is proceeded by an elevation of 2-AG.

2.3. The EC system in bone and the impact of PUFA on agonist levels and EC signaling

Consistent with the findings of endocannabinoid actions in adipose, activation of a central (CB1) and local (CB2) receptors promote bone cell functions. Both cannabinoid receptors have been shown to influence bone modeling and remodeling in rodents [39]. As stated earlier, CB1 and CB2 are found in osteoclasts, and CB2 has been identified in preosteoblasts and osteoblasts; but, CB1 is hardly detectable in osteoblasts [32]. Agonist treatment of the CB2 receptor in primary osteoblast cultures and the MC3T3-E1 cell line promote cell proliferation and activities associated with their mature phenotype [35]. The CB2 agonist also decreased osteoclast-like cell formation [35]. Rather than a direct involvement of CB1 on bone cells, it has been suggested that the bone protective effects of CB1 are meditated by activation of CB1 in the sympathetic nerve from endocannabinoids released by osteoblasts [33]. CB1 activation of the sympathetic nerve inhibits norepinephrine release to reduce its catabolic effect on bone (inhibits bone formation and stimulates bone resorption) [33].

Changes in dietary PUFA result in alterations in the fatty acid composition of tissues, including bone, which have been shown to influence endocannabinoid production and their release. These data suggest that the substrate for endocannabinoids are likely modified in these tissues by dietary lipids, thereby influencing bone and energy metabolism. As an example of this relationship, obese Zucker rats fed fish oil or krill oil had increased eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in VAT and subcutaneous adipose tissue, liver, and heart TG and PL compared to those given control diets. When these rats were fed EPA and DHA, the AA levels decreased in VAT PL [46]. The fatty acid changes observed in the tissues were also reflected in modified endocannabinoid levels as well. In VAT, the AEA levels were significantly decreased in the groups given fish oil and krill oil compared to the control, while 2-AG levels were decreased in the krill oil group [46]. Incubation of mouse adipocytes with AA significantly increased 2-AG levels in adipocytes without a change in AEA. Incubation with DHA significantly decreased AEA and 2-AG in adipocytes [47]. Hence, dietary lipids alter the endogenous cannabinoids and potentially EC signaling.

In rodents, feeding diets containing varying amounts of n-6 and n-3 PUFA resulted in changes in tissue fatty acid composition and the concentrations of endocannabinoids that occurred within a short period of time. Artmann et al. [48] conducted a short-term feeding study in Sprague-Dawley rats where they were fed one of six diets: high palm oil (PO), oleic acid (OA), linoleic acid (LA), AA, fish oil or control, followed by analysis of fatty acids in polar and neutral lipids, endocannabinoids (AEA and 2-AG), and other *N*-acylethanolamines (NAE) in various tissues after one week of feeding. LA and EPA were increased in brain PL in the LA and fish oil groups, respectively, compared to the control. Brain AEA was greater in mice fed the OA and AA diets compared to the control, while 2-AG was increased by all of the diets, except PO and control. The greatest increase in 2-AG was observed in mice in the AA diet group [48]. The small intestine and liver PL and TG were more responsive to dietary fat changes. To summarize, dietary lipid treatments (OA, LA, AA and fish oil) led to fatty acid changes in polar and neutral lipids in the small intestines and liver that reflect the dietary lipid source, as well as shifting the tissue concentrations of AEA and 2-AG [48].

In other experiments, diets deficient in long-chain n-6 PUFA (AA) and n-3 PUFA (EPA and DHA) fed to newborn piglets decreased brain levels of the corresponding NAE, AEA, eicosapentaenoylethanolamide, and docosahexaenoylethanolamide; while a 1% energy intake of AA increased brain AEA levels 5.8-fold [49]. DHA intake seemed to have an inverse relationship with 2-AG levels in the brain of mice fed an n-3 PUFA-deficient diet. Female mice and their male pups fed an n-3 PUFA-deficient diet resulted in significantly decreased DHA levels in PL compared to the adequately fed controls, but increased 2-AG levels in the brain. In contrast, mice fed a high DHA diet had significantly increased DHA in brain PL and decreased 2-AG brain levels [50]. While PUFA intake can alter brain endocannabinoid levels, there is a tighter regulation of brain AA and DHA levels compared to peripheral tissues. These results suggest that similar relationships exist in other tissues where n-3 PUFA can affect the amounts of AA-derived endocannabinoids (2-AG and AEA).

AEA levels in trabecular bone are similar to that in the brain, while trabecular 2-AG levels are approximately one sixth of those in brain [39]. Circulating blood levels are low for both endocannabinoids suggesting that they are formed in bone and then act as autocrine and paracrine ligands. Indeed, the two isoforms of DAGL α and β, the biosynthetic enzymes for 2-AG, have been identified in MC3T3-E1 osteoblast-like cells, primary calvarial osteoblasts and osteoclast-like cells; however, 2-AG has no effect on osteoblast cell number or ALP activity in the MC3T3-E1 cell line or primary calvarial osteoblasts [33].

2.4. Dietary PUFA effects on bone, the muscle and bone relationship, and EC signaling

Several investigations in animals showed that the type and amount of dietary PUFA are associated with changes in bone formation rate and bone mineral content. Dietary modification with n-6 and n-3 PUFA also results in changes in the fatty acid composition of bone compartments. Polar lipids from femur periosteum, marrow and cortical bone reflected dietary modification of the ratio of n-6/n-3 PUFA. As the ratio of n-6/n-3 decreased in the diet with a greater intake of EPA and DHA, less LA and AA were observed in the bone compartments that corresponded with an increase in n-3 PUFA level and a dose responsive decrease in the ratio of n-6/n-3 PUFA in polar lipids [51]. These data suggest that the substrate for endocannabinoids are likely modified in bone by dietary lipids. Watkins et al. reported that a lower dietary ratio of n-6/n-3 PUFA reduced n-6 PUFA and elevated n-3 PUFA in bone, improved bone formation rate in growing rats [51] and conserved bone mineral in OVX rats [52]. Watkins et al. also found that bone-specific ALP activity was higher in growing male rats that were fed a lower dietary ratio of n-6/n-3 PUFA [51] with EPA and DHA, and in OVX rats that were given diets with a ratio of n-6/n-3 PUFA of 5:1 (DHA as the only n-3 PUFA source) with a moderate n-6 PUFA level [52]. In addition, in OVX rats, a high n-6 PUFA intake resulted in greater levels of bone resorption markers (serum pyridinoline and deoxypyridinoline) and lower osteocalcin levels, which would indicate higher bone resorption and lower bone formation [52]. Based on these findings in rats, the dietary balance between n-3 PUFA and n-6 PUFA (linoleic acid) indicate that sources of long chain n-3 PUFA (EPA and DHA) support bone formation activities during bone modeling and remodeling processes.

Prostaglandin E_2 (PGE_2), which is biosynthesized from AA, is a potent biological factor in bone that can activate osteoclasts and bone resorption but also stimulate osteoblast functions [53]. The capacity of the body to produce PGE_2 is dependent upon the amount of AA in

tissue PL which is a function of dietary intakes of n-6 and n-3 PUFA. At moderate levels, PGE_2 supports bone formation, but at high concentrations, it promotes bone resorption [51]. In growing rats, a high dietary ratio of n-6/n-3 PUFA was positively correlated with lower bone formation rates in tibia and a higher capacity for ex vivo PGE_2 production in tibia compared to a lower dietary ratio of n-6/n-3 PUFA [51]. The n-3 PUFA EPA not only reduced the ex vivo production of PGE_2 in both femur and tibia of growing rats [51], but also reduced the protein levels of cyclooxygenase-2 (COX-2), a key enzyme that catalyzes the biosynthesis of PGE_2 from AA in osteoblast-like bone cell cultures [54]. Furthermore, n-3 PUFA feeding helped maintain bone mineral content in OVX rats [52] and mice [55]. Hence, long chain n-3 PUFA (EPA and DHA) can control PGE_2 production in bone via the amount of AA synthesized or the expression of COX-2. The n-3 PUFA is also associated with a higher bone formation rate in growing rats and conservation of BMD in adult OVX female rats. The relatively slow conversion rate of EPA to eicosanoids and the reduced bioactivity of the EPA-derived prostanoids provide an important means for moderating AA prostanoid-mediated physiological and pathological processes [56,57].

In vitro research has shown that markers of osteoblast differentiation and maturation can be modified by PUFA treatments as well. For example, core binding factor alpha-1 (Cbfa1) is a transcription factor involved in initiation of differentiation of osteoblasts and is used as a biomarker of this process. Watkins et al. reported that EPA treatment (1 and 10 μM) of MC3T3-E1 osteoblast-like cells up-regulated Cbfa1 protein expression in culture for 7 d when compared to cells treated with AA and the vehicle control [54]. However, AA treatment at higher levels for 14 d resulted in greater Cbfa1 protein in these cells [54].

The expression of CB2 in osteoblasts mirrors that of osteoblast gene expression for tissue non-specific ALP, Cbfa1 (Runx2), and parathyroid hormone receptor 1 [35]. In most rodent studies, a lack of cannabinoid receptors or agonist actions on receptors is associated with lower bone formation and bone mass (Table 1).

The collective behavior of skeletal elements is organized to facilitate bone modeling in children during growth and development [53]. Until skeletal maturity is attained, in excess of 90% of the periosteal and endosteal bone surfaces are continuously involved in bone appositional and resorptional activities that result in morphological changes pertinent to growth and reshaping of bone [58,59]. During bone modeling (e.g., long bone appositional growth) surface drifts occur which alter bone cortical thickness, marrow cavity diameter, external diaphyseal and metaphyseal diameters, longitudinal curvatures, total cortical mass, and cross-sectional geometries [59]. These changes in the geometrical character of bone tissue govern the quality of bone, which is not only determined by its material properties, but also by the architectural, physical, and biological factors that influence its mechanical properties [58–60]. Investigators have demonstrated that dietary factors, including lipids, can impact bone morphology and mechanical properties [56,61]. Our laboratory found that when n-3 PUFA-deficient rats were repleted with DHA they experienced compensatory bone formation (increase in mid-diaphyseal diameter of femur and tibia) and improved mechanical properties (increase in second moment) of long bone [62]. Evaluation of bone mechanical properties, both structural and material, can be instrumental in elucidating the quality of bone architecture during bone modeling and the relationship between muscle and bone [63].

Muscle and bone form an operational unit that controls the growth and maintenance, and supports proper functioning of these organs. Muscles cause the largest loads and strains on bone to model and remodel the bone throughout life. These strains inform the physiological mechanisms controlling bone mass and bone strength [64]. This relationship between muscle and bone is best described by the mechanostat theory, which states that increasing muscle mass and force of contraction creates mechanical stimuli that evoke the appropriate cellular activities leading to adaptive changes in bone mass and strength. This can be illustrated as follows. Forearm muscle strength is correlated with BMD [65] and increased forearm strength in dominant forearms of tennis players is associated with a corresponding increase in BMD in that forearm, compared to their non-dominant forearm [66]. Very active humans without exceptionally strong muscles, such as marathon runners, lack the whole bone strength that weight lifters attain [64,67] due to differences in voluntary loads applied to load-bearing bones in the latter athletes. Clinically, it is known that children with cerebral palsy or muscular dystrophy have significantly reduced muscle mass and BMD, compared to their normal peers [68,69]. These adaptive changes and potentialities of bone are also demonstrated by reconstruction surgeries to repair limb defects following bone resection necessitated by tumor. When this involves the tibia, an inserted fibula can hypertrophy and actually replace the weight-bearing tibia [70], thus providing another real-life application of the mechanostat theory.

Long bone modeling in the young (depicted in Fig. 4), following the mechanostat theory described by Frost [59], is a result of muscle-derived flexural loads orientated symmetrically around the cross-sectional circumference of bone causing a uniform increase in bone diameter [71]. Repetitive, similar dynamic flexural straining asymmetrically around the bone activates the flexure-drift feedback system and causes drifts of lamellar bone surfaces in tissue space. Hence, bone surfaces will move towards the flexural concavity arising when the flexural loads are applied, with the convex-tending surfaces activating an osteoclastic drift (osteoclasts digest the basic organic and inorganic constituents of the bone and returns them to the blood facilitating bone resorption) and concave-tending ones activating osteoblastic drift (bone deposition by osteoblasts). Thus, muscle forces have the capacity to control the shape and density of long bones, and therefore its architecture to resist fracture. Consistent with the concept of muscle mass influencing bone mass, the myostatin-null mice exhibit a doubling of muscle mass that resulted in increased femoral and lumbar vertebrae BMD [72,73]. Myostatin is a member of the transforming growth factor-β superfamily of growth and differentiation factors and functions as a negative regulator of muscle growth. Thus, a lack of myostatin in these mice removes the growth limiting action of this factor which controls muscle mass [72,73]. The knockout myostatin mouse provides a unique model to examine the relationships between muscle mass and strain loads to bone explained by the mechanostat theory. The myostatin-null mouse is another example demonstrating that increased muscle mass can directly result in greater BMD which is targeted at optimizing bone mass and musculoskeletal health.

Muscle atrophy and osteopenia are catabolic processes associated with muscle and bone loss which occur during immobilization of limbs, disease and increased age. Lifestyle modifications, including exercise and dietary changes, can benefit bone and muscle. One such dietary attribute is the long chain n-3 PUFA, which has been studied in humans [74–76], animals [51,52,77–80] and bone cell cultures [54,81] in order to understand their actions in bone metabolism and health. More recently, our laboratory [52,80] reported that DHA may be more beneficial to support bone formation and prevent bone loss, while other investigators [82,83] noted that low EPA levels were ineffective in maintaining bone mineral. In human studies, DHA was positively associated with BMD in young men [74] and n-6 PUFA or a higher ratio of n-6/n-3 PUFA was negatively associated with BMD in children and adults [84,85].

Dietary fat modification can alter central and peripheral tissue fatty acid contents which can impact the endocannabinoid system. The endogenous cannabinoids, AEA and 2-AG, are synthesized on demand from AA and concentrations of these agonists are altered by dietary PUFA, with DHA being reported to reduce 2-AG in tissues

Table 2
Actions of the endocannabinoids and receptors on skeletal muscle

Author-Year	Model	Receptor	Outcome	
			Anabolic	Catabolic
Eckardt et al. 2009 [86]	Human skeletal muscle cells	CB1	Antagonist treatments (Rimonabant or AM251) reduced the effects of conditioned media and abolished AEAs effects.	Adipocyte conditioned media and AEA reduced insulin stimulated Akt phosphorylation by 60% and 40%, respectively, and glucose uptake; no change in GLUT1 or GLUT4 levels; AEA increased IRS-1 phosphorylation.
Esposito et al. 2008 [27]	Differentiated L6 myotubes	CB1	Antagonist treatment (Rimonabant) increased glucose uptake via PI3K signaling.	
Cavuoto et al. 2007 [26]	Lean and obese human skeletal muscle cells	CB1	Antagonist treatment (AM251) decreased PDK4 expression (decreases inhibition of glucose flux) without an effect of AEA; PGC-1α decreased with AM251 plus AEA co-treatment in obese myotubes.	Antagonist treatment (AM251) increased expression of AMPKα1 (up-regulation of fat oxidation), this effect was blocked by AEA in myotubes from obese.
Liu et al. 2005 [28]	Female ob/ob mice (8-10 weeks), and soleus muscle isolated from these mice	CB1		Antagonist treatment (Rimonabant, 7 days) reduced food intake, body weight, increased O_2 consumption, and increased glucose uptake.

[46–48]. Although we are only beginning to understand the role of EC signaling in muscle, a major action appears to be mediated via AMPKα1 (Table 2). The potent actions of the EC signaling system in muscle have important implications for insulin resistance and macronutrient metabolism, and have a potential role in muscle and bone communications, as illustrated in Fig. 6.

In skeletal muscle, aerobic exercise causes an increase in mitochondrial density and mitochondrial oxidative enzymes [87], a decrease in glycolytic enzyme activity [88] and in Type II (fast glycolytic) fibers [87], and an increase in the number of muscle capillaries [87]. These changes improve the oxidative capacity of muscle resulting in increased use of fatty acids as an energy source, decreased amount of stored fat, and improved insulin sensitivity of muscle [89]. Exercise reverses some of the effects exerted by the EC signaling system in muscle, such as, reduced glucose uptake and macronutrient oxidation (Fig. 6) [90,91]. Furthermore, exercise elevates glucose transporter 4 (GLUT4) mRNA in human muscle [87,92]. Although the GLUT4 transporter is necessary for glucose uptake in muscle, it is regulated by other events including insulin receptor signaling and AMPK pathway to facilitate the metabolism of glucose [91]. Moreover, dietary PUFA appear to influence GLUT4 and glucose utilization. For example, in contrast to a diet rich in n-6 PUFA,

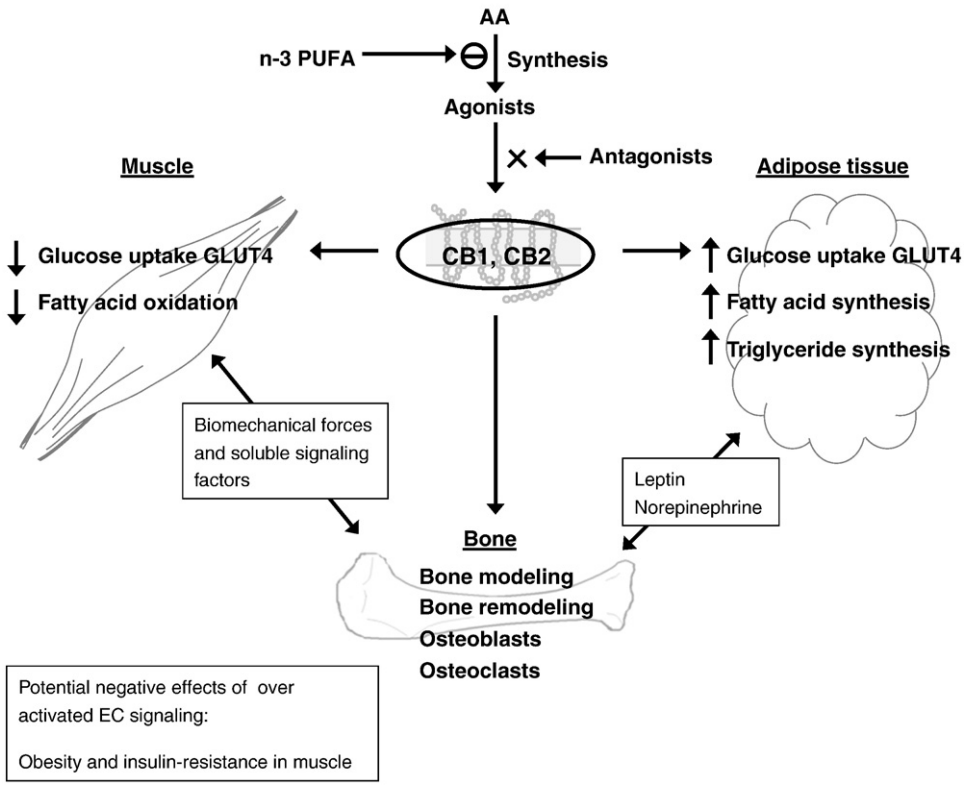

Fig. 6. The EC signaling system and actions on adipose, muscle and bone are shown with emphasis on bone. Over activated EC signaling has implications that lead to insulin-resistance in muscle and weight gain contributing to obesity. Exercise is one physiological factor that prevents the negative effects of over activated EC signaling on muscle and adipose. The intake of dietary n-3 PUFA can reduce the synthesis of endogenous agonists from AA by lowering the concentrations of AA in tissue phospholipids. Biomechanical forces and soluble factors influence bone metabolism as described in this review. It is hypothesized that healthy muscle is achieved by homeostatic regulation of EC signaling when n-3 PUFA are supplied in the diet to optimize the signaling between muscle and bone. Within muscle and bone, the n-3 PUFA facilitate a balance for EC signaling via phosphorylation of AMPK and directing cellular adenosine-5'-triphosphate (ATP) use or ATP production for cellular activities and cell-to-cell communications in these tissues. Moreover, the EC signaling system appears to be affected by leptin, a peptide hormone that influences bone formation and bone mass, and CB1 is proposed to inhibit the catabolic actions of norepinephrine in bone.

skeletal muscle of mice consuming a diet containing n-3 PUFA had elevated GLUT4 expression [93], again mimicking exercise. Exercise also increases PPARγ in skeletal muscle [94] and muscle-specific deletion of PPARγ in mice showed insulin resistance [95]. Specific n-3 PUFA have been shown to activate PPARγ expression in adipocytes. EPA specifically activated PPARγ1 mRNA, while LA, DHA, and n-6 PUFA had no effect [96]. At present, it is not clear how n-3 PUFA affect PPARγ expression in skeletal muscle or in the activation of AMPK and the signaling cascades triggered by this group of kinases. Again, it appears that the effects of exercise reverse the actions of EC signaling on muscle to improve glucose uptake and energy expenditure [90,91].

To summarize, it is advantageous for skeletal muscle to be a more oxidative phenotype, similar to aerobic exercise. There are emerging data to support our hypothesis that consuming n-3 PUFA can mimic the effects exercise has on skeletal muscle [97]; however, the mechanism for this has yet to be determined. Several questions must be answered, such as, what specific aspects of oxidative metabolism are activated in the skeletal muscle of animals consuming n-3 PUFA? Are these actions due to specific n-3 PUFA, or combinations of n-3 PUFA, and what is the most effective n-3 PUFA for activating the oxidative phenotype in skeletal muscle? Does oxidative, trained muscle respond better or more poorly to a disuse challenge, and would it be more protective or less protective to changes in bone modeling/remodeling? Finally, are dietary n-3 PUFA an important nutritional factor for homeostatic control of the EC signaling process for sustaining muscle and bone health? Answers to these questions likely reside in the EC system, which promises to be an area of exciting research that will lead to a better understanding of muscle and bone interrelations.

3. Conclusions

The recent findings for activation or inhibition of cannabinoid receptors in bone require further study to fully understand the impact these receptors and their actions have on bone. Potential effects of this system include actions on genes involved in differentiation of progenitor cells, osteoblast and osteoclast functions, and communications between muscle and bone. The Cbfa1 signaling, for instance, promotes osteoblast differentiation early on but inhibits full maturation of osteoblasts; whereas β-catenin from the Wnt signaling pathway inhibits mesenchymal stromal cells from differentiating into osteoblast precursors, yet enhances the process of osteoblast maturation. The coupling of osteoblasts to osteoclastogenesis requires multiple signals that can be enhanced or inhibited by several cellular messengers, including EC signaling. These and other factors involving leptin or norepinephrine actions are also likely candidates for the bone effects observed with endocannabinoid genetic, pharmacological, and nutritional modulation by different families of dietary PUFA. In addition, possible actions of EC signaling may include organ crosstalk, e.g., between muscle and bone.

The manner of "communication" between muscle and bone is still an area of active research and EC signaling is but one, perhaps novel and unexplored example of organ crosstalk. The mechanism behind this remains unknown and may be indirect. That is, EC signaling that positively influences muscle health and function may translate into enhanced loading activities on bone that in turn stimulate the mechano-sensitive osteocytes to effect downstream cellular activity. In this scenario, EC effects would not be understood as having occurred in a limited local paracrine/autocrine interaction. However, the principal EC, namely, AEA and 2-AG, are measurable in the plasma and therefore would be presumed to be directly "available" to all surfaces of bone. Thus, circulating endogenous AEA could trigger bone events associated with CB2 receptors on osteoblasts.

As described herein, muscle health and strength is a principle factor that controls bone quality throughout the life cycle. The importance of increasing muscle mass and strength during growth and regular exercise are major drivers of bone modeling in the young and bone remodeling in the adult. These relationships between muscle and bone are maintained and sustained by both mechanical and non-mechanical factors. The maintenance of healthy muscle with proper insulin sensitivity is vital for sustaining the biomechanical functions that drive bone formation. While the n-3 PUFA appear to have an effect that is similar to exercise in muscle, this supposition requires further investigation. Important issues presented in this review are the need to understand the role that EC signaling plays in the homeostatic regulation of muscle and bone throughout life and how PUFA influence the amount of endogenous agonists and their actions on receptors. A final thought for future research is "How do n-3 PUFA influence EC signaling in muscle and does EC signaling in muscle lead to changes in bone metabolism that benefit skeletal health?"

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