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Is fatty acid composition of human bone marrow significant to bone health?

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ABSTRACT

The bone marrow adipose tissue (BMAT) is a conserved component of the marrow microenvironment, providing storage and release of energy and stabilizing the marrow extent. Also, it is recognized both the amount and quality of BMAT are relevant to preserve the functional relationships between BMAT, bone, and blood cell production. In this article we ponder the information supporting the tenet that the quality of BMAT is relevant to bone health. In the human adult the distribution of BMAT is heterogeneous over the entire skeleton, and both BMAT accumulation and bone loss come about with aging in healthy populations. But some pathological conditions which increase BMAT formation lead to bone impairment and fragility. Analysis *in vivo* of the relative content of saturated and unsaturated fatty acids (FA) in BMAT indicates site-related bone marrow fat composition and an association between increased unsaturation index (UI) and bone health. With aging some impairment ensues in the regulation of bone marrow cells and systemic signals leading to local chronic inflammation. Most of the bone loss diseases which evolve altered BMAT composition have as common factors aging and/or chronic inflammation.
Both saturated and unsaturated FAs originate lipid species which are active mediators in the inflammation process. Increased free saturated FAs may lead to lipotoxicity of bone marrow cells. The pro-inflammatory, anti-inflammatory or resolving actions of compounds derived from long chain poly unsaturated FAs (PUFA) on bone cells is varied, and depending on the metabolism of the parent n:3 or n:6 PUFAs series.
Taking together the evidence substantiate that marrow adipocyte function is fundamental for an efficient link between systemic and marrow fatty acids to accomplish specific energy or regulatory needs of skeletal and marrow cells. Further, they reveal marrow requirements of PUFAs.

1. Introduction

The presence of adipose cells in the adult human bone marrow distinguishes ~~fixes~~ its anatomical feature of two recognized fractions: yellow and red marrow containing different cell types and vascularization. The yellow marrow fraction found primarily in the appendicular skeleton encloses adipocytes showing scarce vasculature, containing only a few capillaries with continuous basement membrane. Red bone marrow is responsible for body blood cell production, it sustains cells of the hematopoietic and osteoblastic lineages, stroma and fat cells intermingled by a rich vasculature composed of a vast network of sinusoids.

Currently, the fat depots enclosed within the skeleton are acknowledged as functionally distinct to peripheral white adipose tissue (WAT), contributing to both systemic and local metabolism although differently, according to their location in the skeleton [1,2]. Evolutional, developmental and genetic evidence support the conclusion that bone marrow adipose tissue (BMAT) is a conserved component of the bone marrow microenvironment [2–4], providing dynamic storage and release of energy and stabilizing the marrow extent. Also, it has been understood that both the amount and quality of BMAT are relevant to preserve the functional relationships between BMAT, bone, and blood cell production. The aim of this piece is to review the information supporting the tenet that the quality of BMAT is relevant to bone health. To this end we first briefly consider the relevance of the recent incorporation of 1H MS and lipid mediators measurements, the significance of normal BMAT accrual, the association between fat composition and skeletal health and finally, contribution of fatty acids as source of regulatory molecules in the bone marrow microenvironment.

2. Methods to evaluate BMAT

Early studies on the relationship among bone and fat tissues in human bone marrow were *ex vivo* histological and histomorphometrics, mainly in pathological samples [5–7]. In addition, gas chromatography

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(GC) and mass spectrometry (MS) were used to determine the fatty acid composition, demanding time for samples processing [8–11].

Measurements of the fat fraction in the bone marrow are based on its water–fat composition. Both magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) can be used for the *in vivo*, noninvasively quantitative assessment of the fat fraction and the characterization of the bone marrow water-fat composition [12–14]. Quantitative MRI measurements of the distinct properties of the fat or water components of the bone marrow require reducing or accounting for the presence of the other component. Early employment of MRI and MRS measured bone marrow signal-weighted fat fraction (SFF), since then much improvements have been made to eliminate the effect of confounding factors and derive the proton density fat fraction (PDFF), which represents a truly standardized biomarker [15,16]. Two main types of techniques have surged for PDFF quantification: single-voxel proton magnetic resonance spectroscopy (MRS) and chemical shift encoding-based water-fat imaging [reviewed in [17]].

Although MRS is a reference method for quantifying fat in a small volume of tissue [18], the heterogeneous distribution of fat in the cavity of one bone or across bones limit its utility. To overcome regional sampling and heterogeneity, for instance in vertebral or hip, MRI is chosen. Actually, MRI appears to be the best method to measure BMAT in human, by measuring adipose tissue across multiple bones [19,20]. In addition, the advantages of both MRS and volumetric MRI have been combined in the improved water-fat imaging methods [21–23]. Also, employing MRI with spectroscopy (MRS) in combination with dual energy x-ray absorptiometry (DXA), allow to evaluate directly the effect of BMAT on skeletal health.

Results of BMAT content by all these methods are expressed as percentage, considering the large peak of saturated lipid in the resonance spectrum [19,23,24]. In general, magnetic resonance is technically demanding and requires advanced post processing of resonance spectra; variation in experimental methods together with the heterogeneous nature of BMAT may account of discrepancies in results. However, after correcting all confounding effects, a good agreement has been observed *in vivo* between MRS-based and imaging-based PDFF in both the proximal femur and spine [25,26]. Also, imaging–based and MRS-based bone marrow water and fat fraction measurements have been compared with chemical analysis and histology, with good agreement [27–30].

In addition, employing magnetic resonance techniques enable defining the bone marrow fatty acid composition, since in a normal magnetic resonance spectrum, the unsaturation and polyunsaturation levels can be extracted from the olefinic and diallylic fat peak resonances [31–33]. However, the reliable extraction of the olefinic fat peaks can be challenging because of the overlapping strong water peak, particularly in vertebral bone marrow spectra and younger subjects. Results are expressed as percentage of saturated and unsaturated fatty acid in relation to total lipid content, and are in general stated as the unsaturated/saturated index (UI).

NMRS has also shown as a powerful means for quantifying fatty acid composition in *ex vivo* samples. Compared to GC and MS, NMRS is a nondestructive procedure allowing using the same sample in different subsequent procedures, although, fatty acids are identified at only their saturation and unsaturation levels. Another method employed to evaluate BMAT is dual-energy quantitative computed tomography (QCT), which assesses BMAT density (g/cm^3), however this method requires exposure to relatively high levels of radiation [34], restricting its use.

Recently, NMRS has been improved by using magic angle spinning (MAS) to reduce chemical shift anisotropy and dipole-dipole interactions, obtaining narrow line-widths spectra. Although it has been scarcely used, such high-resolution MAS NMR (HRMAS-NMR) can provide high-resolution spectrum from intact tissue, without the need for extensive extraction steps typically used prior to NMR analyses [35,36].

Lipid mediators (LM) derived from PUFAs have been identified and quantified using liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS)–based LM metabololipidomics, together with authentic biologically derived and synthetic standards. The identification of specific LM and specialized proresolving mediators (SPM) allowed determining endogenous SPM metabolomes, like lipoxins (LX), resolvins (Rv), protectins (PD), and maresins (MaR) derived from arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) [37].

3. Variation of BMAT in healthy population

Adipocytes in the red and yellow marrow fractions surge differently during development, in humans adipocytes accretion within the yellow marrow start around birth, and accelerate between one and two months of age [2,38], and the process continues through aging following a pattern from the peripheral toward the central skeleton. The adult red/yellow marrow condition is reached around the age of 25 years. At that time, red marrow is restricted to the axial skeleton (spine, sternum, ribs, pelvis and skull) and in the proximal metaphysis of the femur and the humerus, showing histologically sparse adipocytes combined with sites of active hematopoiesis. In contrast, adipocytes in the yellow marrow accumulate in thick sheets dislodging almost all hematopoiesis to the proximal ends of the long bones. Consequently, the distribution of BMAT in the human adult is heterogeneous over the entire skeleton [39].

Tavassoli's studies demonstrated two unique populations of fat cells in the bone marrow fractions observing that these fat cells displayed distinct response to hematopoietic demands [5,6]. More recent studies including animal models, histologic, genetic, molecular and ^{1}H-MRS analysis allowed proposing that adipocytes found in the earlier-developed yellow marrow integrate constitutive marrow fat (cBMAT), which is preserved under several metabolic challenges. In contrast, the amount of adipocytes in the red marrow is more variable and responds to hematopoietic and nutritional demands and consequently, it has been named regulated bone marrow fat (rBMAT). In addition, the assessment of qualitative characteristic of adipocytes like size, lipidomic profiles and gene expression, demonstrated divergence among the properties of cBMAT, rBMAT and white adipose tissue (WAT) suggesting that rBMAT is more similar to WAT than to cBMAT [2,3]. These concepts which surged supported mainly on studies in animal models are being cautiously extrapolated toward the heterogeneous spreading of human BMAT, proposing that cBMAT is restricted to the feet and hands, while the rBMAT develops in the proximal femur and lumbar vertebrae [3,40].

Vertebral BMAT content in healthy males increases linearly with age from 20 to 30% at 11 years to 50–60% at 40–50 years old, maintaining that range of fat content until the 9th decade. In women, vertebral fat content at 11 years is similar to values observed in males, increasing subsequently until 50 years old but attaining fat content values 6–10% lowers than men. Then, in women older than 55–60 years the fat content switches to values approximately 10% higher than males [reviewed in [41]].

Early studies on the relationship between BMAT and bone mineral density (BMD) using magnetic resonance spectroscopy (MRS), showed inconsistent results, partly because BMD determination was restricted to a small volume of bone marrow within a single bone [12,13,42,43], as well as to the heterogeneous distribution of adipose tissue in the cavity of one bone or across bones. Studies using magnetic resonance imaging (MRI) by Shen et al. in healthy adult populations observed a significant negative association between BMAT content and BMD in the hip and spine regions [44], in pelvic, hip, and lumbar spine BMAT with hip and lumbar spine BMD [24] and between pelvic BMAT and BMD (total body) and total body BMAT and BMD in women [19]. Also, measurements in a population composed mainly of premenopausal women and men aging less than 50 years observed a significant negative correlation between the lumbar spine BMD and BMAT, though such association lost significance after adjustment for age, bone mass

index (BMI), fat, and lean mass [45]. Such relationship showed also effective in 5 to 17 years old children but determining pelvic volumetric BMD by whole body MRI and pelvic BMAT using DEXA, emphasizing that the inverse relationship between BMD and BMAT sustains under circumstances of increased bone formation. Thus, this study integrated the inverse relationship between vBMD and BMAT to childhood and adult population [46].

In all, this evidence sustain that both BMAT accumulation and bone loss come about with aging in healthy populations portraying an active relationship in the marrow between bone and adipose tissue, changing along life, adjusting continuously to systemic energy, hematopoietic and growth needs. However, such negative association between BMAT and BMD actually turn into significant bone impairment and fragility in several pathological conditions which strengthen BMAT formation. Early studies performed in women bone's biopsies gave the first evidences that marrow fat increase concomitantly to bone loss in osteoporosis and aging [7]. Later, histomophometric studies performed in human biopsies confirmed increased marrow fat and decreased bone volume in osteoporosis [47,48]. Moreover, independently of aging, in other bone loss conditions like immobilization, microgravity, ovariectomy, diabetes, and glucocorticoid or thiazolidinedione treatments there is increased marrow fat formation [49–51].

Current account on the harmful effect of increased BMAT on bone formation consider at least three aspects: first, osteoblasts and adipocytes originate from a common precursor, the bone marrow mesenchymal stromal cells (MSCs), which under unbalanced osteogenic/adipogenic conditions favor adipose tissue accretion in the bone marrow at expenses of the osteoblastic lineage [49,52]. This issue has been broadly covered in previous reviews [53]. Second, like peripheral adipocytes, bone marrow adipocytes are active secretory cells producing a wide variety of proteins, including hormones, cytokines and adipokines [1,39,54–59], which exert complex systemic effects and/or local actions on their neighboring cells including osteoblasts, osteocytes osteoclasts and hematopoietic cells. The increased or altered activity of marrow adipocytes, or their precursors, may collaborate through their secretion products to impair directly the bone marrow microenvironment, for instance inhibiting osteoblasts activity or promoting osteoclasts differentiation [60,61,62]. Third, lately is argued progressively that also the quality of the lipid products of marrow adipocytes may be significant for skeletal health. The introduction of MRS and MRI analysis, which initially focused in measuring only the amount of bone marrow fat, let to recognize also that the amount of saturated and unsaturated fatty acids changes in the bone marrow under diverse health or disease conditions [63,64]. Such knowledge led to search after the consequence of enhanced or diminished proportion of a specific type of BMAT on its effects on bone density and metabolism.

4. Composition of BMAT in health and disease

In humans, fatty acid composition analyzed by GC in samples of marrow and subcutaneous fat of patients presenting different BMD, demonstrated dissimilar fatty acids patterns in each tissue. In addition, a significant higher content of saturated fatty acid was observed in the proximal femur than in the proximal tibia [63,64]. However, no change in marrow fatty acid composition was evident comparing samples from subjects with different BMD (normal, low bone mass, and osteoporosis) [63,64]. The analysis of intact bone marrow by 1H-MRS in humans, demonstrated significant increased UI in marrow fat of proximal tibia compared to the proximal femur [3]. A recent research using ^{1}H-MRS compared the bone marrow fat UI in the proximal tibial diaphysis in a sample of young adults with varying BMI, observing a relatively strong positive correlation with age but not association with gender, physical activity, basal metabolic rate or glycemic state in the whole sample. Furthermore, the UI did not differ between the obese and control groups based on weight status at the age of 7 years [65]. These observations indicate that despite significant overall consequences of early body adiposity, it has no effect on the UI of tibial diaphysis BMAT in young adult subjects. Remarkably, these measurements in young adults overlap the period in which yellow fat marrow replaces red marrow, when also BMAT content and fat cell size increase [66]. Previous 1H–MRS studies on human BMAT composition conducted on vertebral bone marrow found decreased unsaturated and increased saturated fatty acid associated with impaired skeletal health [32,33,67,68]. Thus, BMAT unsaturation has been reported to decrease with age in women [68] and in subjects with morbid obesity [69], as well as with increasing BMAT content [68]. Studies in postmenopausal diabetic and non-diabetic women, observed decreased UI in diabetic subjects though they had similar lumbar total BMAT content than non-diabetic women [32,33]. Further, women with type 2 diabetes mellitus (T2DM) and prevalent fragility fractures showed the lowest UI [33]. In morbid obese subjects a decreased UI was also observed in T2DM subjects compared with non-diabetics, both at the lumbar spine and at the femur, but in contrast to former observations T2DM subjects had also higher total lumbar and femur BMAT content [69].

Taken together these *in vivo* analysis of human BMAT indicate site-related bone marrow fat composition and an association between increased UI and bone health, but the origin for such heterogeneous fat allocation, its physiological significance or its impact on local or extra marrow tissues remain undefined. Further, knowledge on the lipid composition in the bone marrow microenvironment, that is in the interstitial compartment surrounding bone marrow cells, is scarce. It is also unknown its relationship with systemic lipids, its physiological significance or its variation under a bone stress situation. The *in vivo* evidence supports the notion of a distinctive fatty acid composition in the bone marrow milieu, essentially different from that in plasma, ensuing from the metabolism of adipocytes and other marrow cells.

Some of these issues were addressed by measurements in bone marrow supernatant fluid (BMSF), isolated through low speed centrifugation of iliac crest aspirates [36,70]. The samples were from elder women presenting different BMD, with or without fracture undergoing orthopedic surgery. The lipidomic profiles in both BMSF and serum were compared after analyzing FAs' by GC–MS [70]. A specific pattern of fatty acids characterized BMSF showing higher saturated and decreased unsaturated fatty acids in comparison to blood plasma. The relative levels of unsaturated and saturated fatty acids in BMSF agree well to those observed in the human marrow fat tissue [33,68], implying that the lipid content in BMSF replicate relevant fatty acids derived from the activity of adipocytes and other marrow cells, rather than those provided by blood plasma. Data on the content of individual fatty acids in BMSF suggest an active exchange of lipids between marrow cells supported partly by marrow adipocytes. Analogous to the observations on the composition of BMAT [32,64,68], no consistent difference in the fatty acid composition of BMSF, or plasma, was apparent in relation to women´s BMD [70].

In contrast, the analysis of data in relation to the presence or not of hip fracture in the osteoporosis group, showed an active distribution of unsaturated fatty acid in both BMSF and plasma. In the BMSF of women with fracture there was a switch toward decreased content of total saturated *versus* unsaturated fatty acids, suggesting a dynamic relationship between the composition of fatty acids in the bone marrow milieu and the metabolic requirements of cells. Thus, in BMSF of women with fracture the content of stearic acid decreased significantly, while oleic acid content increased concomitantly, implying a substrate to product relationship conceivably to fulfill specific requirements of unsaturated fatty acids. Moreover, the polyunsaturated eicosatrienoic (EA) and arachidonic (AA) fatty acids decreased importantly, suggesting improved activity of cyclooxygenase-2. These observations highlight the significance of available unsaturated/saturated fatty acids to marrow cells, which appear reacting to increased local demands in the BMSF of women with fracture. Of note, in this study marrow and blood samples were collected after few hours of hip fracture [36,70].

Tracer studies in mice have demonstrated bone as the second most important organ after liver for the clearance of radiolabeled postprandial lipoproteins and glucose [71,72]. Studies in a mouse model presenting impaired delivering of fatty acids to bone marrow adipocytes showed a significant shift from dietary essential fatty acids PUFAs, to *de novo* production of monounsaturated fatty acids, in both femoral and tibial marrow adipocytes [72]. These observations substantiate that marrow adipocyte function is fundamental for an efficient link between systemic and marrow fatty acids to accomplish the specific energy or regulatory needs of skeletal and marrow cells. Further, they reveal a persistent marrow need of essential fatty acids, which if not delivered by blood may derive from local resources.

Taking together, the evidence suggests that preserving bone and marrow health requires upholding in BMAT a definite fatty acid composition comprising increased unsaturated *versus* saturated fatty acids, which demands complex links between systemic and marrow metabolisms.

With aging, some impairment in the complex interplay between the several bone marrow stromal cell types, including immune cells, and systemic signals lead to activation of chronic inflammation. Higher levels of serum inflammatory markers predict bone loss and increased bone resorption in older adults [73–76]. What is more, the sustained basal inflammation in aging may collaborate to the increased content and changed distribution of both body and marrow fat tissue [77,78]. Remarkably, most of the bone loss diseases evolving disrupted BMAT composition, as pointed above, have as common factors aging and/or chronic inflammation.

5. Effects of saturated and poly unsaturated fatty acids (PUFA)

In addition to serving as fuel substrates for marrow cells, certain lipids may originate oxidized lipid mediators serving as small signaling molecule in both physiological and pathological settings [79–81]. These reactive lipids species result mainly by enzymatic action or formed by nonspecific peroxidation. Such lipid mediators are endogenously produced in response to a stimulus and their synthesis is controlled by metabolic or signal transduction proteins [80,82]. The bone marrow microenvironment display appropriate conditions for producing such reactive lipid species because of its lipid rich content and the range of metabolic activity among its diverse cell types, many of which are responsive to autocrine/paracrine signaling.

Both saturated and unsaturated FAs originate such lipid species. Saturated FAs by undergoing nonspecific peroxidation reactions form reactive oxygen species (ROS), which at low level adjust the cells´ peroxide tone, while ROS accumulation lead to cells lipotoxicity [83,84]. Increased free saturated FAs may enhance ROS production through mitochondrial FA beta oxidation [85], and these products could induce signal transduction pathways involved in apoptosis [86,87].

Several studies have observed deleterious effects of saturated FA on bone cells. For instance, osteoblasts in co-culture with adipocytes showed decreased proliferation, differentiation and function [60,61], and the effects resulted from lipolysis [86,88] and increased free FAs in medium [88,89]. Inhibition of FA biosynthesis suppresses the *in vitro* lipotoxic effect [89], while addition of dexamethasone enhanced such response [86,88]. Further, direct treatment of osteoblasts with saturated FA *in vitro* replicated the lipotoxic effect, which results from oxidative stress of cells. Under such condition, cells increased the generation of ROS and lipid peroxidation products, while decreased their content of antioxidant enzymes. The presence of dexametasone enhanced the oxidative stress induced by adipocytes, while the presence of antioxidants ameliorated such effect [88].

Palmitate exerts a cytotoxic action on the function and survival of human mesenchymal stem cells (MSC) and MSC-derived osteoblastic cells [90,91]. Both cell types exposed to physiological increasing concentrations of palmitic acid for varying time showed cell mortality in a time- and dose-dependent manner. Palmitate induced endoplasmic reticulum stress and activation of the nuclear factor κB (NF-κB) and ERK pathways [90,91]. Palmitic acid treatment of cells induced a pro inflammatory response, characterized by the up-regulation of Toll-like receptor 4 expression and increased expression and secretion of IL-6 and IL-8 [90]. MSC-derived osteoblastic cells demonstrated more sensitive to lipotoxicity than undifferentiated MSC. Remarkably, the presence of oleic acid did not affect cell viability, but completely prevented the harmful effect of palmitic acid in cells incubated with both fatty acids [90]. The presence of oleic acid counteracts the activation of all unfavorable pathways produced by palmitic acid. The detoxifying effect of oleic acid might result from stimulating palmitic acid esterification into triglycerides and storage in lipid droplets [90]. Additionally, studies in MSCs obtained from patients suffering osteonecrosis of the femoral head showed enhanced adipogenesis, higher sensitivity to palmitic acid toxicity and subsequent increased production of interleukines-6- and -8, compared to MSCs from control subjects [92]. Likewise, studies on the lipotoxic effect of palmitate on osteogenic differentiation of human cells showed reduced transcriptional activities of both beta-catenin and Runx 2 [93].

Saturated FAs promote *in vitro* osteoclastic differentiation of bone marrow macrophagues or cell-lines by enhancing NF-kB activation and subsequent activation of the ERK pathway and of differentiation markers [94].

In addition, the opposing effects of palmitic and oleic acids display also on the osteoclastogenic differentiation of RAW 264.7 cell cultures. Palmitate enhanced receptor activator of NF-κB ligand (RANKL)-osteoclastogenic differentiation. Such palmitic-induced osteoclastogenesis ensues even in the absence of RANKL and it may result from increased TNFα production. In contrast, oleic acid does not enhance osteoclast differentiation, producing high intracellular triglyceride accumulation, and inhibits palmitic-dependent osteoclastogenesis [94]. Studies in mice models showed that animals on high palmitic-enriched diet presented greater reduction in bone mass and structure than mice on a high oleic-enriched diet, while mice with hampered triglyceride formation because of diacylglycerol acyl transferase 1 knockout, presented larger bone marrow derived osteoclasts and decreased bone mass indices [94].

PUFAs may display a range of effects on bone cells, depending on the potential of the two series of PUFAs n-3 and n-6. The 18-carbon fatty acids alfa-linolenic (ALA, 18:3n-3) and linoleic acids (LA, 18:2n-6) respectively, are the precursors for each series. These FAs are considered essentials, because they are not synthetized by human tissues and must be obtained by the diet [95]; the relative abundance of LA in the western diet favors forming products of the n-6 series, in detriment of those derived from the n-3 precursor [96]. The essential FAs through both desaturation and elongation processes form the long chain fatty acids (LCFA) arachidonic acid (AA) (20:4n-6), eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3), (Fig. 1). In humans, these PUFAs fulfill two main physiological functions: First, forming phospholipids they take part in the lipid bilayer structure of membranes, participating in this manner in intercellular communications and highly differentiated membrane functions; second, they are primary precursors for the enzymatic local production of oxidized lipid mediators, which have autocrine and paracrine actions in cells.

In its turn AA, EPA and DHA can originate a variety of oxidation products by the activities of cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450- like epoxygenases as well as from non-enzymatic oxidation (Fig. 1). These LCPUFAs products are classified in two broad categories eicosanoids and docosanoids [81]. The former result from the 20-carbon n-3 and n-6 LCPUFAs and include the prostaglandins, leukotrienes, thromboxanes, lipoxins and *E*-series resolvins [97,98,99]. These compounds are mainly pro inflammatory.

Docosanoids result from oxidation of the 22-carbon n-3 DHA, mono-, di- and tri-hydroxylated derivatives of DHA and include the docosatrienes, protectins (also known as neuroprotectins), the D-series resolvins, and maresins (Fig. 1) [97,98,100]. These families of

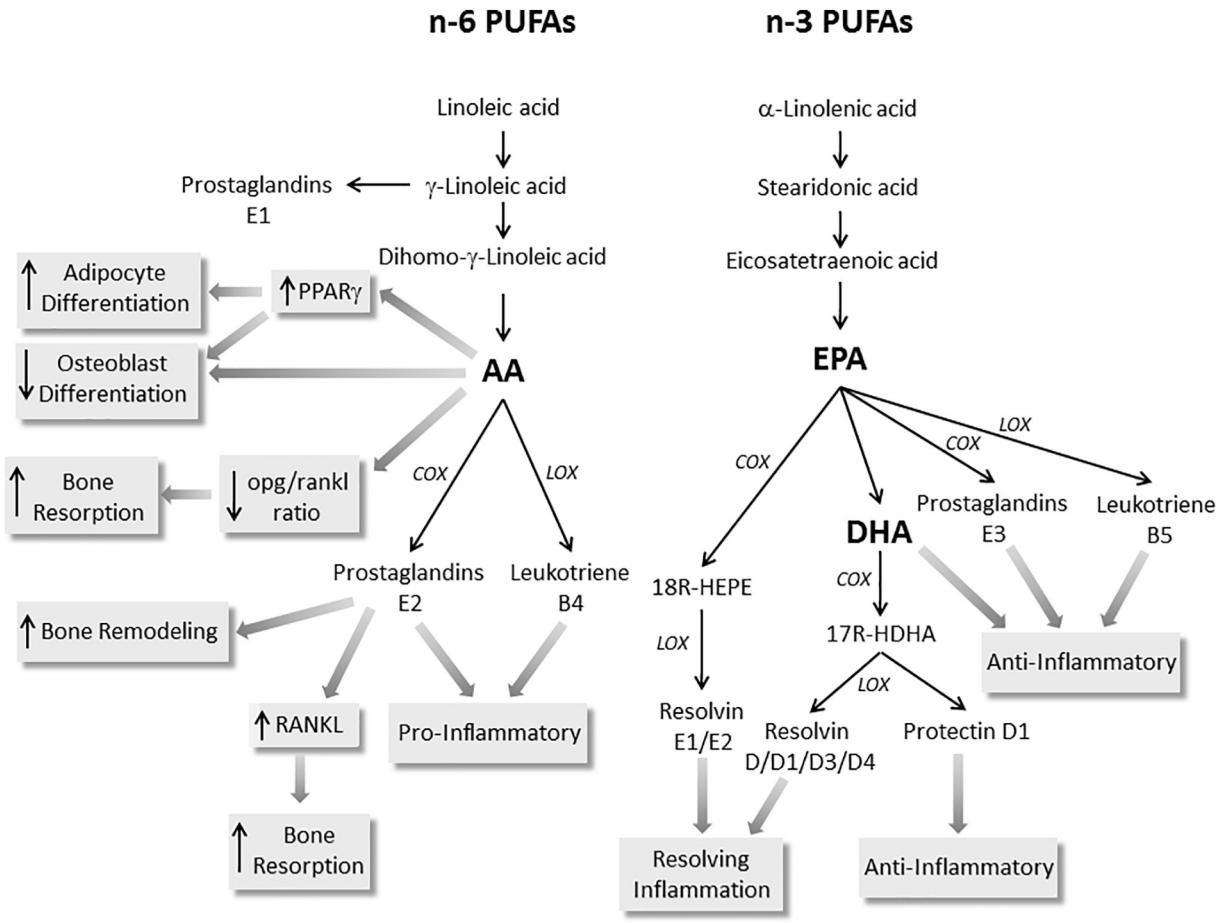

Fig. 1. Metabolism of polyunsaturated fatty acids: linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are successively desaturated and elongated by a common desaturase/elongase enzyme system originating long chain PUFAs. The figure summarizes some of the lipid mediators formed from AA, EPA and DHA. PUFAs, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3); COX, cyclooxygenase; LOX, lipooxygenase; 17R-HDHA, 17R hydroxylated DHA; 18R-HEPE, 18-hydroxyeicosapentaenoic acid; RANKL, receptor activator of nuclear factor kB ligand; OPG, osteoprotegerina.

lipids mediators actively command the resolution phase of inflammation [101]. The action of prostaglandins and leukotrienes, in the initiation phase of inflammation activate LOX enzymes genes [102], needed for synthetizing in the resolution phase the specialized pro-resolving mediators (SPM) [[101], Reviewed in [103]].

COX converts dihomogammalinolenic acid (DGLA; 20:3n-6), AA and EPA into prostaglandins of the 1-, 2- and 3-series, respectively. COX also catalyzes the conversion of AA to thromboxane A2 (TXA2) [104] (Fig. 1). Human cells have two distinct cox genes known as cox-1 and cox-2 [105]. Cox-1 expresses constitutively in most tissues, but cox-2 is inducible and is normally only expressed in tissues with active inflammation [106]. Since COX-1 and 2 have more specificity for AA than EPA, the synthesis of prostaglandins of the 1- and 2-series is favored rather than prostaglandins of the 3-series [107]. Several transcription factors relevant to inflammation like nuclear factor κB (NF-κB), nuclear factor for interleukin-6 expression (NF-IL-6) and cAMP-response-element-binding protein (CREB) regulate the expression of COX-2 protein [108, 109]. In addition, the activity of COX-2 is regulated by several reactive oxygen species (ROS) necessary to adjust the lipid peroxide tone required for inflammation activation [110–112].

The physiological effects of PUFAs result from their structure or that of their metabolic products which at very low concentration attune various cellular signaling pathways. For example, AA, ALA and LA as well as the prostaglandin 15d–PGJ2 are natural ligands of PPARgamma transcription factor [113,114]. In addition, compounds derived from the metabolism of n-6 fatty acids (such as 13S-HODE, 12S-HETE, and 15S-HETE) also bind to PPARγ2 transcription factor [115,116]. In the bone marrow, activation of PPARγ2 transcription factor by such compounds promotes MSCs' differentiation into adipocytes and consequently leads to a drop in the production of osteoblasts [117,118]. Although products derived from n-3 fatty acids can also potentially induce the pparγ2 gene expression [119], the effect of omega-6 is stronger and enhances the MSC differentiation into adipocytes to a higher degree than omega-3.

Another important group of lipid mediators originated from PUFAs are prostaglandins (PGs), which act *via* G-protein coupled receptors (GPCRs). (PG) E2 and other prostanoids are produced in response to different factors regulating bone metabolism mainly by inducing COX-2 [120]. The effects of PGs on bone are complex, in part because of transient cell-dependent local regulation of COX-2, as well as cell-specific expression of PGs' receptor types [120,121].

Both bone resorption and formation can be regulated by PGE2. Endogenous PGs increase the stimulatory effect of several resorption agonists like cytokines, hormones and growth factors on osteoclast differentiation [122]. The major effect of PGE2 on resorption is indirect by acting on osteoblastic cells enhancing their expression of RANKL and by inhibiting the expression of osteoprotegerin (OPG). The anabolic response to PGE2 shows more reliable in cultures of cells under osteoblastic differentiation. PGE2 stimulates osteoblastic differentiation in MSCs and primary calvarial cell cultures [122], while osteoblastic differentiation is decreased in MSCs from mice deficient in endogenous PGs because of gene deletion for COX-2 [123,124]. Thus, the local production

of PGE2 might sustain cAMP-activated signaling pathways necessary for early proliferation or differentiation of osteoblastic precursors. In addition, PGs play a role in accelerating fracture healing [123].

Some PUFAs have shown protective effects on bone by inhibiting osteoclast differentiation and osteolytic activity. For instance, the effects of the n 3-PUFAs EPA and DHA and the n 6-PUFAs AA and DGLA were compared on murine RAW264.7 cells differentiation model. All PUFAs significantly inhibited RANKL-induced osteoclast formation, in a dose-dependent manner. Accordingly, these PUFAs decreased the expression of marker osteoclastogenic mRNAs and proteins, as well as they decreased bone resorptive activity of osteoclasts. However, AA and DHA showed the most effective inhibitors [125]. Recently, the specific inhibitory action of ALA on RANKL-stimulated osteoclast differentiation was observed *in vivo* in mice models in both LPS-induced and ovariectomized bone loss. RANKL-induced osteoclasts proliferation and differentiation *in vitro* was inhibited by ALA treatment. The PUFA repressed RANKL-induced osteoclast markers, as well as phosphorylation of ERK pathway proteins. LPS-challenged mice receiving ALA (100 and 300 mg/kg) improved the morphometric changes induced by LPS and showed diminished levels of proinflammatory cytokines and chemokines. In addition, in mice showing bone loss by ovariectomy ALA (100 and 300 mg/kg) treatment led to reductions in osteoclasts number, decreased levels of inflammatory interleukines, TNF-α and reduced iNOS and COX-2 enzymes [126].

Interestingly, the PUFA's effects on osteoclastogenesis could result from the action of lipoxin A4 (LXA4), a metabolic product of AA resulting from the action of lipoxidase (LOX). LXA4 significantly inhibits osteoclasts differentiation and function, while Boc-2, the specific inhibitor of the receptor of LXA4 (FPR2/ALX) abolished these effects. In addition, LXA4 decreases ovariectomy-induced bone loss. These protective effects were associated to inhibition of several proteins markers for osteoclasts differentiation. LXA4 also diminished the expression of the RANKL:OPG ratio and serum levels of TNF-α, IL-1β, and IL-6. Moreover, LXA4 prevented the production of ROS and the expression of mature osteoclast-specific genes [127].

In relation to pro resolving compounds resulting from DHA, studies in immunological unchallenged mice demonstrated that the bone marrow has a particular pattern of SPM, characterized by higher RvD2, RvE1, and LXB4 levels than in spleen [128,129]. Such increased level of resolvins derived from DHA indicates that bone marrow microenvironment is primed to facilitate timely resolution of inflammation production, restraining impairment risk in this tissue. Moreover, *in vivo* PTH treatment produced a rapid and selective increase in marrow levels of RvD1 and RvD2, but not in the spleen [129]. Further studies *in vitro* demonstrated the ability of macrophages at physiologically relevant concentrations, to engulf apoptotic osteoblasts, a process facilitated by Rvs [129]. Other studies identified that RvD1 reduced macrophage-derived TNF-α, promoting the resolution of inflammation [130]. From these observations the researchers propose to include the involvement of bone resident macrophages or osteomacs in the bone remodeling process [129]. Macrophages efferocytose apoptotic osteoblasts, a process facilitated by RvDs. In addition, macrophages produce TGFβ during efferocytosis and this factor recruits mesenchymal stem cells [131], resulting in renewal of osteoblasts.

In addition, MSCs may collaborate in producing such defined pattern of SPM in the marrow. MSCs obtained from the bone marrow of both human and mice incubated with DHA as substrate, synthetized Rvs mainly from the D series while in the presence of AA cells produced eicosanoids. MSCs showed higher capacity of forming SPM than lung fibroblasts [132].

Findings from *in vivo* studies in human, identify beneficial effects of dietary PUFAs on bone health in some [133–138], but not in other works [139–141]. Several factors collaborate to divergent observations, among others differences in the population baseline characteristics, dietary patterns, source of FAs, as well as from the primary end point measure [142–144]. Furthermore, bone health most likely is influenced by the functional effects of mixed nutrients in human diets rather than by that of a single food or nutrient type.

In summary, marrow adipocyte function appears fundamental for achieving local energy and regulation required by skeletal and marrow cells, though the actual metabolic activity of BMAT in humans is essentially unknown. Diverse and modifying metabolic needs among marrow cells may collaborate in determining the heterogeneous distribution of BMAT in the human skeleton.

Several observations substantiate the relevance of fatty acid composition in bone health. *In vitro* studies identify potent regulatory effects on bone cells of fatty acids derived compounds, highlighting that both saturated and PUFAs may play a part in the inflammatory process. As pointed above, chronic inflammation is associated with increased risk of several age-related diseases including osteoporosis and fragility fractures [73–76].

Fatty acids available to cells encompasses in their structure a spectrum of metabolic and regulatory actions, which if well balanced avoid chronic inflammation, ensuring tissue homeostasis. Notwithstanding, at least two types of factors may collaborate in disrupting the metabolic balance: First, distorted proportion of FAs provided by diet, thus, excessive amount of saturated FA and/or biased availability of essential fatty acids sustain pro-inflammatory conditions. In this respect, the western diet provides an excess of n-6 PUFAs, which has been linked to chronic diseases. Second, several altered metabolic conditions may lead to disruption of the complex cell-dependent regulation of the COX and LOX enzymes. Further work is necessary to precise the actual local necessities of both n-3 and n-6 PUFAs in marrow, and its relation with aging and or chronic inflammation. This knowledge is necessary for improving the dietary essential FAs recommendations for healthier bone preservation.

Conflicts of interest

None.

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