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## **Osteoporosis: a role for lipids**

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### **Author contributions**

A. During: conception of the work, collection of data, analysis of data, and writing of the manuscript.

**Abstract**

An inverse relationship between bone marrow (BM) adiposity and bone mass has been described in different physiological and pathological conditions, including osteoporosis (OP). In osteoporotic patients, lower bone mass density is indeed associated with higher BM fat content, suggesting a potential role for bone lipids in the OP pathogenesis. Nevertheless, some questions remain. Is that BM adiposity a cause or a consequence of the bone loss? What kinds of lipids are involved? Human data are somehow contradictories regarding bone lipid signature related to OP, and animal data are needed to support on one or another way the human observations. Bone lipid signature associated to OP needs to be clarified if we want to understand better their roles in OP.

In that context, by using an ovariectomy-induced OP murine model and looking at lipids in two bone compartments: BM and mineralized tissue (MT), our first challenge was to identify local lipid changes in relation to OP, in view to explore later the mechanisms by which those compounds could alter bone quality, particularly during the mineralization process. As the most striking data, long-term OP resulted in an accumulation of triglycerides, reduced levels of arachidonic and docosahexaenoic acids, an increase of stearoyl-CoA desaturase indices and a reduction of sphingomyelin in the MT, and potential consequences on bone properties and cell activities are discussed. The reader will appreciate that we are at an early stage of understanding the roles of lipids in the OP development and more investigations will be necessary.

**Key words:** lipids; bone marrow; mineralized tissue; long bones; human osteoporosis; ovariectomized murine model

**Abbreviations:**

**AA**, arachidonic acid; **BM**, bone marrow; **BMD**, bone mass density; **DHA**, docosahexaenoic acid; **DXA**, dual X-ray absorptiometry; **CE**, cholesteryl esters; **EPA**, eicosapentaenoic acid, **FA**, fatty acids; **H-MR**, proton magnetic-resonance; **GC**, gas-chromatography; **MT**, mineralized bone tissue; **OP**, osteoporosis; **OVX**, ovariectomized rats; **PC**, phosphatidylcholine; **PGE<sub>2</sub>**, prostaglandin E<sub>2</sub>; **PS**, phosphatidylserine; **SCD1**, stearoyl-CoA desaturase; **SHAM**, sham-operated rats; **SM**, sphingomyelin; **TG**, triglycerides.

## 1. Overview of human data

### 1.1. Osteoporosis (OP) facts & low bone mass

Osteoporosis (OP) is a silent and age-related disease that is often detected too late when a first fracture has occurred, which is concomitant with a 50% increased risk to get a second one. It affects people over 50 year-old and more women (21%) than men (6%). Today, OP management has a high societal cost (€37 billion in Europe for 2010), with 95% going for surgical and long-term cares against only 5% devoted to preventive actions [1]. Because of population ageing and secondary effects of current treatments available, there is a real need of new strategies in terms of OP prevention that goes along with a better understanding of how this pathology takes place.

In the human healthy adult skeleton, there is a balance between bone resorption and bone formation, as results of osteoclast and osteoblast activities, respectively, in order to replace old and/or stressed bone by new bone and to maintain a constant bone mass and quality. This process – named bone remodeling - occurs at multi-sites of the skeleton to repair bone from micro-cracks. By this way, 10% of our skeleton is renewed each year. After 45 years of age, bone resorption takes over bone formation in association with a declined bone turnover; effects that are accentuated in women after menopause and with OP pathogenesis. As such, OP is characterized by a decrease of bone mass and an alteration of bone microarchitecture, leading to a reduction of bone strength and, consequently, in an increased risk of fracture. The clinical diagnosis of OP is made by measuring bone mass density (BMD) via non-invasive techniques, mainly dual X-ray absorptiometry (DXA) and quantitative computed tomography. That allows to segregate individuals into three groups (normal, osteopenic and OP groups) in function of their BMD values (*e.g.*, when DXA T-score  $\geq -1.0$  (normal subjects), between -1.0 to -2.5 (osteopenic subjects), and  $\leq -2.5$  (OP subjects)). These BMD measurements are mostly performed at the lumbar spine and hip sites.

### 1.2. OP: bone marrow fat expansion

In humans, marrow fat content can be assessed by non-invasive magnetic-resonance imaging approaches, such as proton magnetic-resonance (H-MR) spectroscopy. From H-MR spectra, marrow fat content is determined as a percentage relative to water content:  $100 * (I_{\text{fat}} / (I_{\text{fat}} + I_{\text{water}}))$ , where  $I_{\text{fat}}$  corresponds to the saturated lipid peak at 1.3 ppm (smaller unsaturated and

residual lipid peaks at 5.3 and 2.0 ppm being often ignored) [2] (**Figure 1**). By using this approach, it was reported that vertebral marrow fat content followed the increasing order: normal < osteopenic < OP groups, in both a postmenopausal women population [3] and an over 65 year-old men population [4]. Note that changes in marrow fat content are age- and sex- related. In each gender group, vertebral marrow fat content increased with age, however, it was higher (6-10%) in men than women of comparable age between 20 and 60 years [5] and, after 60 year-old, there was a switch, probably in association to the postmenopausal status of women taking over men [6].

Bone marrow (BM) adiposity can also be assessed *in situ* by a histomorphometry approach, by evaluating the different volumes occupied by adipose tissue, hematopoietic tissue and trabecular bone compartments from an analyzed bone biopsy section. By this approach on iliac crest biopsies issued from a mix-gender patient population, there was an increase of adipose tissue volume concomitant to a decrease of bone trabecular volume, first, with age in the two gender group and, secondly, in the OP female group compared to the age-matched control group [7], in agreement with the observations obtained by non-invasive approaches. In sum, OP-related bone loss is associated with an increased BM adiposity.

### **1.3. Origins of the reciprocal relationship between bone mass and marrow adiposity**

An inverse relationship between BM adiposity and bone mass has been established in different physiological and pathological conditions, including during bone acquisition, aging, diabetes, starvation, anorexia nervosa as well as OP [2, 8-10] (**Figure 2**). One of the reasons is that marrow adipocytes (fat-storing cells) and osteoblasts (bone-forming cells) have a common origin: the mesenchymal stem cells, which are contributing to the osteoblastogenesis/ adipogenesis pathways fate [11]. Moreover, it is now more and more documented that marrow adipocytes and all bone cell types (*i.e.*, osteoblasts, osteoclasts (bone-resorbing cells), and osteocytes (mechano-sensor cells)) participate in a local communication network by secreting factors, which can modulate marrow stem cell lineage decision as well as adipocyte and bone cell differentiation/ maturation [12-14]. In that regard, lipids were purported to play key roles in the bone physiology, by being actors in the mineralization process and regulators of important signaling pathways for bone health (review [15]). As such, several fatty acids (FA) were reported to affect bone cell survival and functions *in vitro* as summarized in **Table 1**, supporting the idea that bone marrow adipocytes *in vivo* could directly influence neighboring bone cells via the release of FA, possibly a triggering factor for OP as will be discussed later. The bone tissue-level microenvironment is

thus determinant and perturbations of its molecular composition, *e.g.*, its lipid content, might contribute to changes in bone microarchitecture via a disequilibrium of the two cellular adipocyte/ osteoblast and osteoblast/ osteoclast balances that condition bone remodeling [16]. Therefore, it is important to look locally at the lipid composition changes in relation to bone quality.

#### **1.4. OP: low marrow unsaturation index or not?**

If marrow adiposity is a now well-recognized fact in human OP, several questions remain unanswered. Among them, what kinds of lipids are increased within OP bones? Regarding the bone lipid signature, the only few human data available in the literature are somehow contradictories. The marrow unsaturation fat index can be determined from H-MR spectra as a relative percentage of the unsaturated lipid signal ( $I_{UL}$  at 5.3 ppm) compared to the sum of all lipid signals ( $I_{UL}$  at 5.3ppm +  $I_{RL}$  at 2.0 ppm +  $I_{SL}$  at 1.3 ppm) (**Figure 1**). By that way, in a postmenopausal women population, the vertebral marrow unsaturation index followed the decreasing order: normal > osteopenic > OP groups [3], suggesting a potential enrichment in saturated lipids during OP development. Moreover, in a postmenopausal women population without OP (DXA T-score > -1.5), low vertebral unsaturation index were reported in women with fragility fractures; an effect accentuated with a diabetic status [17], implying that vertebral marrow fat composition could be a new imaging tool for fracture risk assessment prior OP diagnosis [17].

In contrast, by using the gold standard gas-chromatography (GC) tool, no changes in marrow fatty acid profiles – and, at *fortiori* unsaturated fat levels – were observed between normal, osteopenic and OP groups, neither in a Chinese population over 65 year-old (with several bone sites and both gender combined) [18], nor in BM supernatant fluids of iliac crests from postmenopausal women [19]. Interestingly, in that later study [19], when the OP group was sub-divided into women “without” and “with” a recent hip fracture, higher unsaturated fatty acid levels were observed in BM supernatant fluids from the sub-group “with fracture”. In sum, human data show discrepancies about a potential marrow lipid pattern associated to OP pathogenesis; indeed, OP could be correlated to a low marrow unsaturation index or not, or even opposite. To the best of our knowledge, until recently, there was none animal study to support on one or another way the above human observations. It became clear that more investigations were needed in that direction to understand better the involvement of the marrow fat expansion in the OP pathophysiology.

## 2. Animal investigations

So far, only three studies have investigated *in situ* lipid changes in relation to the OP status independently to any nutritional intervention, by using a postmenopausal OP murine model and looking at lipid composition in long bones (femurs and tibiae) [20-22]. However, these three studies had different strategies making uneasy to compare data. The first one was a targeted approach looking at lipoxygenase-generated lipid metabolites in bone marrow (BM) of femurs/ tibiae [20]. Then, the second one was an integrative untargeted omics approach aiming to identify as many metabolites as feasible in the femoral tissue and providing complex data difficult to comprehend (104 changes, including 93 lipid metabolites for which only 43% were annotated) [21]. Finally, the third one, our global biochemical approach, aimed to characterize and quantify the major neutral lipid and phospholipid classes and fatty acid profiles in the two femoral compartments (BM and mineral tissue) as detailed below [22]. Thus, in this section, it will be mostly discussed our approach/ data and compared them to the two other studies when possible. Although it is clear that more studies are needed, the current data suggest several lipid pathways that may be involved in the pathophysiology of OP as it summarized in **Figure 3**.

### 2.1. The postmenopausal OP murine model

The ovariectomy-induced OP (OVX) murine model is a widely used and suitable preclinical model for postmenopausal OP research. Indeed, estrogen-deprivation induced by ovary removal results in an accelerated bone loss – by 50% at 5 weeks post-surgery in rats [23] – in association to an increased bone fracture risk, like in postmenopausal women. In murine, the bone remodelling steady-state (balance between bone resorption and bone formation) is a site-specific, age-dependent process and the skeletal maturity is reached at 6-7 mo.-old for rats [24] and 4 mo.-old for mice [25]. Thus, for human OP comparison purpose, it is important to take in consideration the skeletal maturity of animals as done in the two OVX rat studies, for surgical procedures were performed at 6 or 7 mo. of age and OP effects studied at 4 mo. or 9 mo. post-surgery, respectively [20, 22]. In the OVX mice study [21], animals had surgery at 2 mo. of age and OP effect was examined 2 mo. later, meaning that skeletal maturity of animals was reached at the end point of the study and, thus, bone growth could interfere with the OP status. Note that “OP” effects were evaluated against a control sham-operated (SHAM) animal group.

## 2.2. An *in situ* approach to study bone lipids

Lipids are present in two major compartments of the bone: the bone marrow (BM; soft tissue) and the mineralized tissue (MT; the hard part). However, bone lipids are usually regarded from the BM side in reason of their better accessibility and higher content where they can reach up to 90% (by marrow weight) and derived in majority from marrow adipocytes and in from hematopoietic cells [15]. Nevertheless, the MT contains also lipids, even though at much lower levels (less than 5%, by weight). They can be considered as loosely-bound/ easy extractable or tightly-associated lipids, depending if they are issued from: a) bone cells (mostly osteocytes representing 90-95% of total bone cells) and blood circulation irrigating the MT, or b) complexes with macromolecules and mineral in the mineralized extracellular matrix [15].

We recently proposed an *in situ* approach to characterize lipids in both BM and MT compartments, by taking into account several analytical issues such as the initial sample size, potential contamination of the MT by BM lipids as well as the poor accessibility/ extractability of lipids present in MT [26]. By applying this original approach to healthy rat femurs, we reported that each bone compartment had its own lipid signature. For instance, the MT was a more metabolically active tissue in terms of long chain fatty acids (*i.e.*, fatty acid profile enriched in arachidonic (AA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids), compared to BM [26], pointing out the rational to examine lipids in the two bone compartments, in particular for a BM adiposity-associated bone disease like OP. For comparison, in the two other studies, lipids were examined either in the BM fraction of femurs plus tibiae combined [20] or in the femoral tissue freed from BM, but without any further washing step, implying a potential contamination of the bone tissue by BM lipids [21].

## 2.3. Lipid changes in OP bones

Unlike humans, in animal studies, invasive tools are commonly used. First, lipids can be extracted from bones, *e.g.*, by using the above mentioned *in situ* approach, and then analysed either by classical chromatography HPLC and HP-TLC methods [22] or by high-throughput liquid chromatography tandem mass spectrometry (LC- or UPLC- MS/MS) tools [20, 21].



### 2.3.1. Triglycerides (TG) accumulation in BM and MT of OP femurs

Like in post-menopausal OP women, a low bone mass was associated with a significant increase of fat content in BM of OVX rat femurs, when compared to control SHAM rats. That increased adiposity affected the MT compartment of OVX femurs as well [22].

In the BM of OVX rat femurs, there was a marked increase in amounts of both energy-storage lipids (*i.e.*, TG) and membrane-associated lipids (*i.e.*, the phospholipids SM, PC and PS), suggesting a hypertrophy and/or increased number of marrow adipocytes to allow a greater TG storage capacity [22]. In support, in healthy tibia of mice fed with a high fat diet for 6 weeks, there was an increased volume of BM adipose tissue - assessed by histology - in association to an increased size of adipocytes and a higher content of TG in BM [27].

In the MT of OVX rat femurs, the increased fat content concerned only neutral lipids (amount of phospholipids was unchanged), and mainly TG [22]. In agreement, all of the 19 identified TG species (15 annotated/ 4 putative) were increased in the femoral tissue of OVX mice [21]. That accumulation of TG in the mineral compartment of OP bones raises questions of whether MT is a passive tissue undergoing an infiltration of medullary TG or a metabolically active tissue in which osteocytes could be capable of TG synthesis. In that regard, in two alcohol-induced rabbit osteonecrosis and rat bone loss models for which histological examinations were performed at the femoral head and tibia diaphysis sites, respectively [28, 29], it was reported that alcohol induced an accumulation of TG droplets within osteocytes, suggesting an intracellular TG synthesis following fatty acid uptake rather than the pinocytosis of large TG vesicles by osteocytes [29]. In these two studies [28, 29], the cytosolic accumulation of TG droplets was associated with a cell apoptosis and an increased number of empty lacuna - spaces in which are lodged osteocytes -. Thus, it is possible that similar events, *i.e.*, changes in osteocyte metabolic activities and survival, occur in OP bones.

### 2.3.2. Cholesteryl esters (CE) increase in the MT of OP femurs

Among neutral lipids, cholesteryl esters (CE) amount was also increased in the MT of OVX rat femurs [22], raising similar questions than for TG. Are they issued from an infiltration by blood circulating CE – since BM does not contain CE -, or locally produced? Note that CE, as a tightly associated-to-mineral constituent, might play a role in the mineralization process [26], in regards to what happens during the atherosclerotic vascular plaque formation. Indeed, in that later process, CE accumulates preferentially in the mineral part, making it a potential initiator/ activator of the vascular calcification [30]. Note that both vascular calcification and bone mineralization are similar mechanisms, implying matrix vesicles production by vascular

smooth cells and osteoblasts, respectively. Thus, an unusual accumulation of CE in OP bones might affect the bone mineralization events driven by osteoblasts, resulting in adverse effects on the original properties of the mineral. Note that three sterol metabolites, including one from the “cholesterol and derivatives” category, were enhanced in the femoral tissue of OVX mice [21]. In sum, local cholesterol metabolism should require further attention in OP pathophysiology.

### 2.3.3. *Sphingomyelin (SM) reduction in the MT of OP femurs*

Knowing that local SM catabolism is essential for the mineralization process in healthy bones [15], it was surprising to find a significant reduction of SM in the MT part of OVX rat femurs [22]. Nevertheless, this finding is in agreement with the data reported in OVX mice femurs, indicating a significant reduction of three to five SM species concomitantly to an increase of six metabolites – one glycosphingolipid and five ceramide species -, but only one of them was annotated [21]. Thus, OP could be associated with a local dysregulation of sphingolipid metabolism, such as an exacerbated catabolism of SM that would not allow mineral to be produced properly. In support, patients with rare forms of OP associated with a defective bone mineralization had mutations in the *SGMS2* gene encoding for the SM synthase 2 (or nSMS2) [31]; a membrane-bound enzyme - catalyzing SM hydrolysis to form phosphocholine and ceramides - highly expressed in bones and required for normal mineralization [15]. Overall, more investigations will be needed in the future regarding the potential role of SM metabolism in the development of OP.

### 2.3.4. *Individual fatty acids (FA) changes in OP femurs*

The unsaturation index (% mono- plus poly-unsaturated FA) and, *at fortiori* the saturation index (% saturated FA), were not modified in OVX rat femurs, compared to control SHAM femurs [22]. These data are in agreement with several human reports [18, 19] and, in some extents, with the previous OVX rat study [20]. Nevertheless, several individual FA were affected by the long-term ovariectomy in rats, *i.e.*, an enrichment in myristic (14:0), palmitoleic (16:1n-7) and linolenic (18:3) acids and a reduction in oleic (18:0), arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3) acids with OP [22]. These FA changes were consistently observed in both BM and MT compartments of OVX rat femurs [22], suggesting a more global - rather than a tissue-specific – OP-associated mechanism, possibly via a bone infiltration by a pool of circulating FA according to the hypothesis of a local TG synthesis discussed above. In agreement, a significant reduction of AA and DHA percentages,

but not for eicosapentaenoic acid (EPA, 22:5n-3), was also noticed in BM of OP long bones at 4 mo. post-ovariectomy (other FA were not mentioned) [20]. Note that, independently of our results, all of the 31 fatty acyl metabolites, including six unusual fatty acids (2 annotated/ 6 putative), were enhanced in OVX mice femurs [21].

The local reduction of AA observed in OP rats [20, 22] was consistent with previous OP women data [19] showing a decrease of AA in medullar fluids of the sub-group “with” a recent hip fracture, compared to the “without” fracture sub-group, suggesting that local AA disappearance might be a marker of bone fracture. Furthermore, AA is a precursor of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, a cyclooxygenase (COX)-derived product), locally produced and known as a potent regulator in bone physiology by influencing both bone formation and bone resorption in a concentration-dependent manner [15]. In rabbits, local PGE<sub>2</sub> synthesis was enhanced in response to bone fracture [34] and its inhibition by indomethacin (a COX-inhibitor) impaired bone fracture healing [35], suggesting a PGE<sub>2</sub> requirement during bone trauma conditions. In humans, high dietary intakes of AA were associated with a reduced risk of hip fracture in elderly adults [36] and, inversely, low unsaturated FA n-6 intakes aggravated that risk in old women [37]. Thus, in OP, the local AA disappearance may reflect a higher PGE<sub>2</sub> production in response to an increased demand for bone repair, a point that needs to be checked. In support, ovariectomy resulted in a significant increase in PGE<sub>2</sub> release *ex vivo* from cultured OVX rat parietal bones [38] as well as an increase of an eicosanoid metabolite (the 13,14-dihydro-15-keto PGE<sub>2</sub>; a primary metabolite of PGE<sub>2</sub>) *in situ* in OVX mice femurs [21]. In addition, AA, like DHA and EPA, is a precursor of lipoxygenase (LOX)-derived lipid mediators, such as leukotrienes, lipoxins, and hydroxyl-eicosatetraenoic acids, having potent pro-inflammatory or anti-inflammatory/proresolving activities. Ovariectomy resulted in an altered profile of these compounds in murine BM, with a relative increase in proportion of LOX products derived from AA relative compared to those from DHA [20], thus probably impacting the inflammatory status within the BM compartment of OP bones. In that context, it would be interesting to see if these local LOX-derived mediator changes could be directly associated to some inflammatory cytokine changes in BM [20].

To the best of our knowledge, we are first to report a local increase of palmitoleic acid (16:1) in OP rat femurs. This result is not so surprising, knowing that, first, 16:1 is a marker of yellow marrow and its precursor, palmitic acid (16:0), of red marrow [32] and, secondly, there is a red-to-yellow marrow shift occurring with ageing. That shift might be more pronounced during the age-related OP process and thus results in higher 16:1 levels. As mentioned earlier, several FA, including 16:0, were purported to affect bone cells *in vitro* (**Table 1**). It is only

recently that 16:1 was seen as beneficial for bone health, by affecting negatively osteoclasts [33]; however, these anti-resorptive effects would need to be accompanied by pro-osteoblast actions, a point that has not been studied yet (**Table 1**). Nevertheless, we will see right after this increase of 16:1 participates in the elevation of one of the stearoyl-CoA desaturase indexes.

#### 2.3.5. Increase of stearoyl-CoA desaturase indices in OP femurs

Moreover, there was a local elevation of the two stearoyl-CoA desaturase (SCD1) indices (16:1/16:0 and 18:1/18:0 ratios) in both compartments of OP rat femurs [22]. These indexes reflect the  $\Delta^9$  desaturase (SCD1) activity that converts saturated FA (mostly 16:0 and 18:0) into monounsaturated FA (16:1 and 18:1); a key enzyme in *de novo* lipogenesis that has been implicated in a variety of metabolic disorders such as obesity, diabetes, insulin resistance and atherosclerosis [39]. Whether or not these indices are indeed the result of a local increase of SCD1 activity in OP bone remains to be checked. Nevertheless, these observations should be related to two earlier human findings showing, first, higher 18:1/18:0 ratio in BM fluids from OP women with a recent hip fracture [19] and, secondly, a positive association between serum the SCD1 index (assessed as the 16:1/16:0 ratio) and risk of fracture in men (enrolled at 50 years and followed-up up to 35 years) [40]. Taken together, OP could be regarded as a metabolic disorder in which *de novo* lipogenesis pathway would play a key role, however more investigations are needed, first, to see if its products are generated locally or are part of a more global process and, secondly, to understand the consequence of their accumulation in bone.

### 3. Perspectives

The main lipid changes in both compartments (BM and mineralized tissue) of murine OP long bones in association with their potential effects are summarized in **Figure 3**. Long-term OP resulted in an accumulation of TG, a decrease of AA (20:4n-6) and DHA (22:6 n-3), and an increase of SCD1 indices (or 16:1/16:0 and 18:1/18:0 ratios) in the two bone compartments. In addition, in the mineral part, there were an elevation of CE and an increase of SM catabolism, two compounds that may play a role in the mineralization. Thus, it is clear that more data are required, in particular to understand how local fatty acid perturbations could affect functions of 'fatty acid-including' lipids involved in the bone physiology, *e.g.*, CE and phospholipids, regarding to bone cell activities as well as the mineralization process. Finally,

it would be interesting to see if those purported lipid changes in osteoporotic femurs occur also at earlier stages of OP in view to better understand how this disease takes place.

### **Figure legends**

**Figure 1:** Typical proton-magnetic resonance (H-MR) spectrum obtained on one vertebral body (L1 to L4) of women with OP by using a higher resolution (1.5 or 3 Tesla) scanner and positioning the spectroscopy measurement box in the central portion of the lumbar (limited area). On the spectrum, lipids are identifiable as unsaturated lipids (UL, olefinic double bond -CH=CH- protons at 5.3 ppm), residual lipids (RL, methylene -CH=CHCH<sub>2</sub>- protons at 2.0 ppm) and saturated lipids (SL, bulk methylene -(CH<sub>2</sub>)<sub>n</sub>- protons at 1.3 ppm). A spectrum acquired from OP women exhibits a relatively high SL peak compared to the water peak (H<sub>2</sub>O at 4.7 ppm), while it is opposite in non-OP women.

**Figure 2:** OP-related bone loss is inversely associated to an increased marrow fat content.

**Figure 3:** Summary of the current data showing lipid changes in both compartments of murine OP long bones from <sup>a</sup>During et al. [22], <sup>b</sup>Zhao et al. [21], and <sup>c</sup>Poulsen et al. [20] studies.

↑, increasing effect; ↓, decreasing effect

Abbreviations: **AA**, arachidonic acid; **DHA**, docosahexaenoic acid; **CE**, cholesteryl esters; **PC**, phosphatidylcholine; **PGE<sub>2</sub>**, prostaglandin E<sub>2</sub>; **PS**, phosphatidylserine; **SCD1**, stearoyl-CoA desaturase activity; **SM**, sphingomyelin; **TG**, triglycerides.

## References

- [1] E. Hernlund, A. Svedbom, M. Ivergard, J. Compston, C. Cooper, J. Stenmark, E.V. McCloskey, B. Jonsson, J.A. Kanis, Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA), *Arch Osteoporos*, 8 (2013) 136.
- [2] A.V. Schwartz, Marrow fat and bone: review of clinical findings, *Front Endocrinol (Lausanne)*, 6 (2015) 40.
- [3] D.K. Yeung, J.F. Griffith, G.E. Antonio, F.K. Lee, J. Woo, P.C. Leung, Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study, *Journal of magnetic resonance imaging : JMRI*, 22 (2005) 279-285.
- [4] J.F. Griffith, D.K. Yeung, G.E. Antonio, F.K. Lee, A.W. Hong, S.Y. Wong, E.M. Lau, P.C. Leung, Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy, *Radiology*, 236 (2005) 945-951.
- [5] H. Kugel, C. Jung, O. Schulte, W. Heindel, Age- and sex-specific differences in the <sup>1</sup>H-spectrum of vertebral bone marrow, *Journal of magnetic resonance imaging : JMRI*, 13 (2001) 263-268.
- [6] J.F. Griffith, D.K. Yeung, H.T. Ma, J.C. Leung, T.C. Kwok, P.C. Leung, Bone marrow fat content in the elderly: a reversal of sex difference seen in younger subjects, *Journal of magnetic resonance imaging : JMRI*, 36 (2012) 225-230.
- [7] S. Verma, J.H. Rajaratnam, J. Denton, J.A. Hoyland, R.J. Byers, Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis, *J Clin Pathol*, 55 (2002) 693-698.
- [8] C.J. Rosen, M.L. Bouxsein, Mechanisms of disease: is osteoporosis the obesity of bone?, *Nat Clin Pract Rheumatol*, 2 (2006) 35-43.
- [9] I.R. Reid, Fat and bone, *Archives of biochemistry and biophysics*, 503 (2010) 20-27.
- [10] J. Paccou, G. Penel, C. Chauveau, B. Cortet, P. Hardouin, Marrow adiposity and bone: Review of clinical implications, *Bone*, 118 (2019) 8-15.
- [11] A. Houghton, B.O. Oyajobi, G.A. Foster, R.G. Russell, B.M. Stringer, Immortalization of human marrow stromal cells by retroviral transduction with a temperature sensitive oncogene: identification of bipotential precursor cells capable of directed differentiation to either an osteoblast or adipocyte phenotype, *Bone*, 22 (1998) 7-16.
- [12] M.E. Nuttall, J.M. Gimble, Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications, *Curr Opin Pharmacol*, 4 (2004) 290-294.
- [13] T. Negishi-Koga, H. Takayanagi, Bone cell communication factors and semaphorins, *NBoneKEy reports*, 1 (2012) 183.
- [14] S. Muruganandan, C.J. Sinal, The impact of bone marrow adipocytes on osteoblast and osteoclast differentiation, *IUBMB Life*, 66 (2014) 147-155.
- [15] A. During, G. Penel, P. Hardouin, Understanding the local actions of lipids in bone physiology, *Progress in lipid research*, 59 (2015) 126-146.
- [16] N.L. Fazzalari, Bone remodeling: A review of the bone microenvironment perspective for fragility fracture (osteoporosis) of the hip, *Seminars in cell & developmental biology*, 19 (2008) 467-472.
- [17] J.M. Patsch, X. Li, T. Baum, S.P. Yap, D.C. Karampinos, A.V. Schwartz, T.M. Link, Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures, *J Bone Miner Res*, 28 (2013) 1721-1728.

- [18] J.F. Griffith, D.K. Yeung, A.T. Ahuja, C.W. Choy, W.Y. Mei, S.S. Lam, T.P. Lam, Z.Y. Chen, P.C. Leung, A study of bone marrow and subcutaneous fatty acid composition in subjects of varying bone mineral density, *Bone*, 44 (2009) 1092-1096.
- [19] M. Miranda, A.M. Pino, K. Fuenzalida, C.J. Rosen, G. Seitz, J.P. Rodriguez, Characterization of Fatty Acid Composition in Bone Marrow Fluid From Postmenopausal Women: Modification After Hip Fracture, *Journal of cellular biochemistry*, 117 (2016) 2370-2376.
- [20] R.C. Poulsen, K.H. Gotlinger, C.N. Serhan, M.C. Kruger, Identification of inflammatory and proresolving lipid mediators in bone marrow and their lipidomic profiles with ovariectomy and omega-3 intake, *American journal of hematology*, 83 (2008) 437-445.
- [21] H. Zhao, X. Li, D. Zhang, H. Chen, Y. Chao, K. Wu, X. Dong, J. Su, Integrative Bone Metabolomics-Lipidomics Strategy for Pathological Mechanism of Postmenopausal Osteoporosis Mouse Model, *Sci Rep*, 8 (2018) 16456.
- [22] A. During, X. Coutel, N. Bertheaume, G. Penel, C. Olejnik, Long Term Ovariectomy-Induced Osteoporosis is Associated with High Stearoyl-CoA Desaturase Indexes in Rat Femur, *Calcified tissue international*, 106 (2020) 315-324.
- [23] T.J. Wronski, P.L. Lowry, C.C. Walsh, L.A. Ignaszewski, Skeletal alterations in ovariectomized rats, *Calcified tissue international*, 37 (1985) 324-328.
- [24] R. Quinn, Comparing rat's to human's age: how old is my rat in people years?, *Nutrition*, 21 (2005) 775-777.
- [25] W.G. Beamer, L.R. Donahue, C.J. Rosen, D.J. Baylink, Genetic variability in adult bone density among inbred strains of mice, *Bone*, 18 (1996) 397-403.
- [26] A. During, Lipid determination in bone marrow and mineralized bone tissue: From sample preparation to improved high-performance thin-layer and liquid chromatographic approaches, *Journal of chromatography. A*, 1515 (2017) 232-244.
- [27] B.J. Adler, D.E. Green, G.M. Pagnotti, M.E. Chan, C.T. Rubin, High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche, *PloS one*, 9 (2014) e90639.
- [28] Y. Wang, Y. Li, K. Mao, J. Li, Q. Cui, G.J. Wang, Alcohol-induced adipogenesis in bone and marrow: a possible mechanism for osteonecrosis, *Clin Orthop Relat Res*, (2003) 213-224.
- [29] D.B. Maurel, S. Pallu, C. Jaffre, N.L. Fazzalari, N. Boisseau, R. Uzbekov, C.L. Benhamou, G.Y. Rochefort, Osteocyte apoptosis and lipid infiltration as mechanisms of alcohol-induced bone loss, *Alcohol Alcohol*, 47 (2012) 413-422.
- [30] D.G. Reid, C.M. Shanahan, M.J. Duer, L.G. Arroyo, M. Schoppet, R.A. Brooks, R.C. Murray, Lipids in biocalcification: contrasts and similarities between intimal and medial vascular calcification and bone by NMR, *Journal of lipid research*, 53 (2012) 1569-1575.
- [31] M. Pekkinen, P.A. Terhal, L.D. Botto, P. Henning, R.E. Makitie, P. Roschger, A. Jain, M. Kol, M.A. Kjellberg, E.P. Paschalis, K. van Gassen, M. Murray, P. Bayrak-Toydemir, M.K. Magnusson, J. Jans, M. Kausar, J.C. Carey, P. Somerharju, U.H. Lerner, V.M. Olkkonen, K. Klaushofer, J.C. Holthuis, O. Makitie, Osteoporosis and skeletal dysplasia caused by pathogenic variants in SGMS2, *JCI Insight*, 4 (2019).
- [32] M. Tavassoli, D.N. Houchin, P. Jacobs, Fatty acid composition of adipose cells in red and yellow marrow: A possible determinant of haematopoietic potential, *Scand J Haematol*, 18 (1977) 47-53.
- [33] B. van Heerden, A. Kasonga, M.C. Kruger, M. Coetzee, Palmitoleic Acid Inhibits RANKL-Induced Osteoclastogenesis and Bone Resorption by Suppressing NF-kappaB and MAPK Signalling Pathways, *Nutrients*, 9 (2017).
- [34] S. Dekel, G. Lenthall, M.J. Francis, Release of prostaglandins from bone and muscle after tibial fracture. An experimental study in rabbits, *J Bone Joint Surg Br*, 63-B (1981) 185-189.

- [35] J. Keller, Effects of indomethacin and local prostaglandin E2 on fracture healing in rabbits, *Dan Med Bull*, 43 (1996) 317-329.
- [36] E.K. Farina, D.P. Kiel, R. Roubenoff, E.J. Schaefer, L.A. Cupples, K.L. Tucker, Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults, *The Journal of nutrition*, 141 (2011) 1146-1153.
- [37] J.K. Virtanen, D. Mozaffarian, W.C. Willett, D. Feskanich, Dietary intake of polyunsaturated fatty acids and risk of hip fracture in men and women, *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, 23 (2012) 2615-2624.
- [38] J.H. Feyen, L.G. Raisz, Prostaglandin production by calvariae from sham operated and oophorectomized rats: effect of 17 beta-estradiol in vivo, *Endocrinology*, 121 (1987) 819-821.
- [39] M.T. Flowers, J.M. Ntambi, Role of stearyl-coenzyme A desaturase in regulating lipid metabolism, *Curr Opin Lipidol*, 19 (2008) 248-256.
- [40] H. Melhus, U. Riserus, E. Warensjo, L. Wernroth, K. Jensevik, L. Berglund, B. Vessby, K. Michaelsson, A high activity index of stearyl-CoA desaturase is associated with increased risk of fracture in men, *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, 19 (2008) 929-934.



Table 1. Summary of *in vitro* effects of exogenous fatty acids (FA) on bone cells (adapted from Table 4 of the review [15])

FA	Osteoblasts [15]		Osteoclasts [15, 33]	
	<i>Survival</i>	<i>Main functions<sup>a</sup></i>	<i>Survival</i>	<i>Main functions<sup>b</sup></i>
Palmitic acid (16:0)	↓ <sup>c</sup> viability ↑ apoptosis ↑ autophagy	↓ TNAP activity ↓ RunX2 activity ↓ mineralization capability	↑ viability ↓ apoptosis ↓ osteoclastogenesis	In mature cells: ↑ TRAP positive cells ↑ NF-κB activation
Stearic acid (18:0)	↓ viability ↑ apoptosis	↓ TNAP activity ↓ RunX2 activity ↓ mineralization capability	↓ osteoclastogenesis	↓ TRAP positive cells ↓ resorption capability
Palmitoleic acid (16:1)			→ viability ↑ apoptosis (mature cells)	↓ TRAP positive cells ↓ NF-κB activation ↓ TRAP, MMP-9, CTSK gene expression ↓ resorption capability
Oleic acid (18:1)	→ viability → apoptosis → proliferation		→ osteoclastogenesis	→ TRAP positive cells
Linoleic acid (18:2n-6)	→ viability → apoptosis	↑ TNAP activity ↑ RunX2 expression/activity → PGE2 production → or ↑ mineralization capability		→ TRAP positive cells ↓ resorption capability
Conjugated linoleic acids (CLA, c9t11 and t10c12)	→ viability → apoptosis	↑ TNAP activity ↓ RunX2 expression (t10c12, no data) ↑ mineralization capability (c9t11 only)		For the c9t11/t10c12 mix (1:1): ↓ TRAP positive cells ↓ NF-κB activation ↓ TRAP, MMP-9, CTSK gene expression ↓ resorption capability
Arachidonic acid (AA, 20:4n-6)	→ viability → apoptosis ↓ proliferation	↑ TNAP activity ↑ RunX2 expression/activity ↑ PGE2 production/COX2 expression		→ TRAP positive cells
Eicosapentaenoic acid (EPA, 22:5n-3)		↑ TNAP activity ↑ RunX2 expression		→ TRAP positive cells

	→ PGE2 production	
Docosahexaenoic acid (DHA, 22:6n-3)	↓ viability ↑ apoptosis ↓ proliferation	→ TRAP positive cells

<sup>a</sup> Osteoblast-specific bone formation markers: TNAP (tissue non-specific alkaline phosphatase) is a key enzyme in the mineralization process and RunX2 (Runt related transcription factor 2) is a transcription factor that controls osteoblastic differentiation by targeting osteoblast-specific genes, including the one for TNAP.

<sup>b</sup> Osteoclast-specific bone resorption markers: TRAP (tartrate resistant acid phosphatase), MMP-9 (matrix metalloproteinase) and CTSK (cathepsin K) are involved in the bone matrix organic degradation and the transcription factor NF- $\kappa$ B (nuclear factor  $\kappa$ B) targets osteoclast-specific genes, including the TRAP, MMP-9 and CTSK genes.

<sup>c</sup> ↑, increasing effect; ↓, decreasing effect; → no effect.

Figure 1

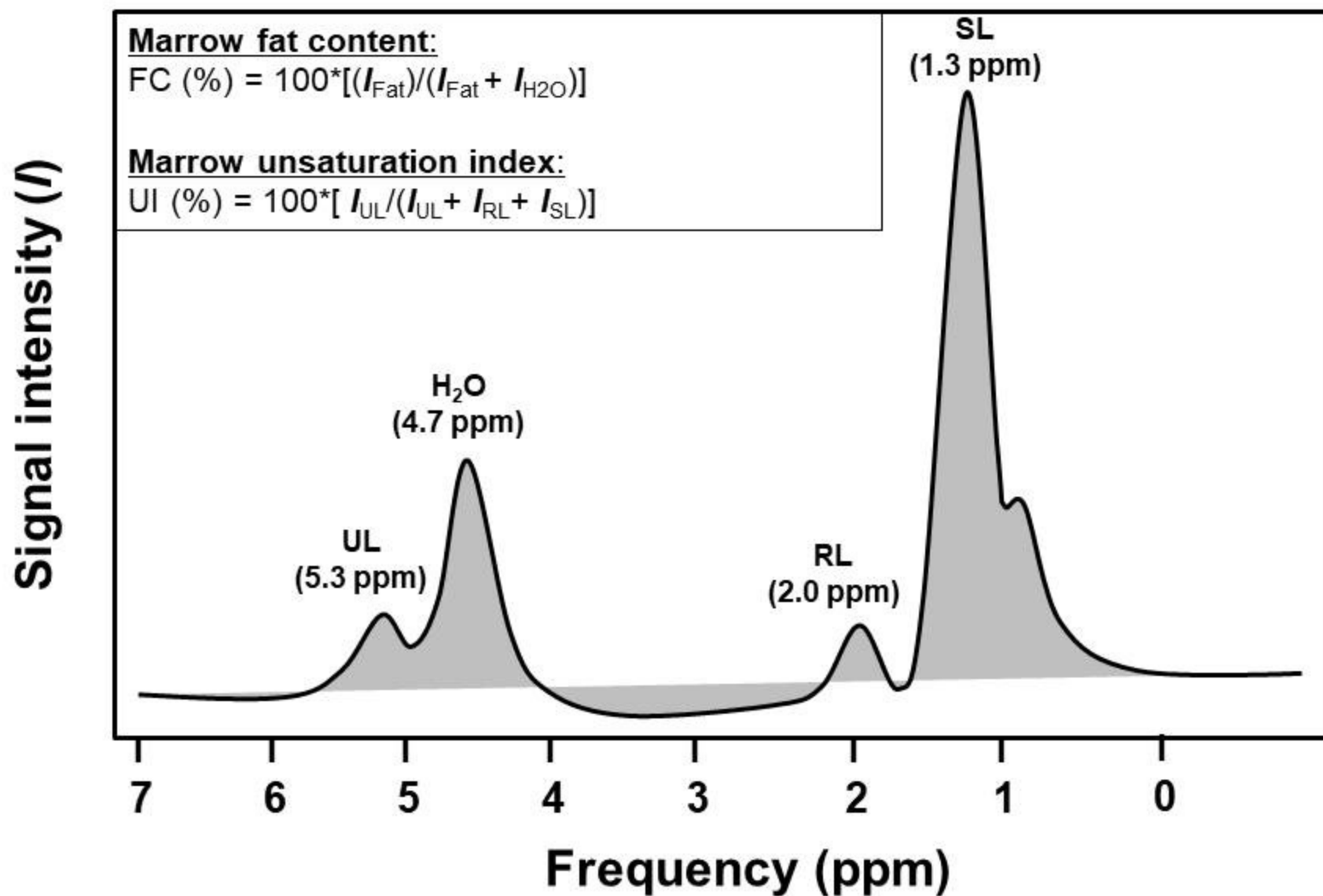


Figure 2

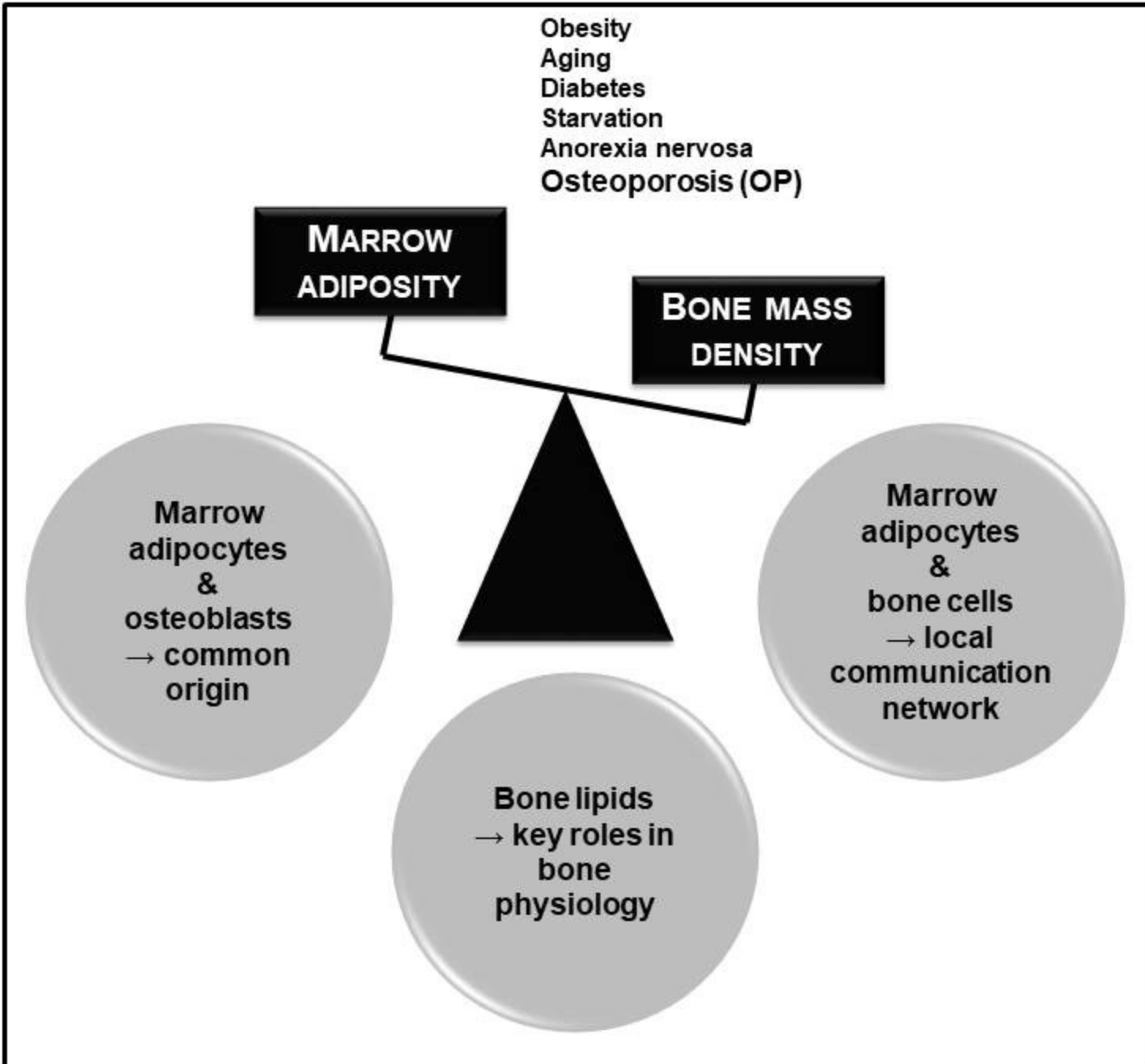
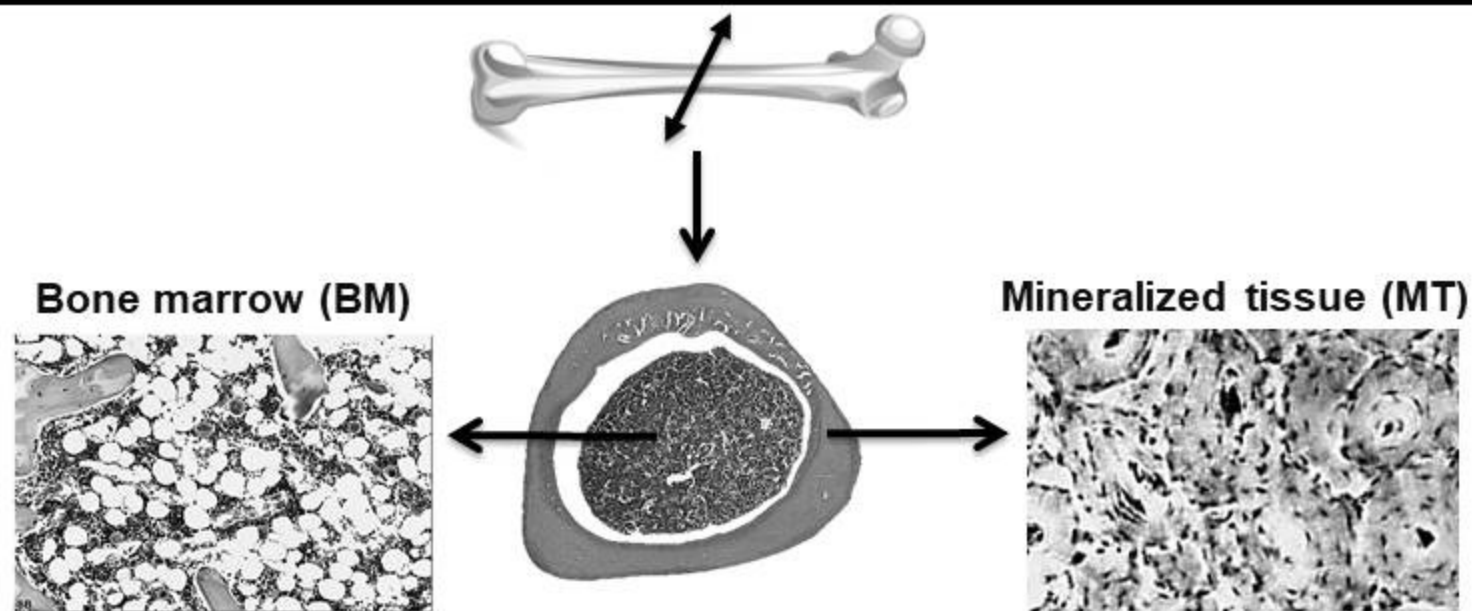


Figure 3



**Main lipid changes  
in BM of OP bones**

- ↑ TG<sup>a</sup>
- ↑ PC, PS, SM<sup>a</sup>
- ↓ AA (20:4n-6)<sup>a,c</sup>
- ↓ DHA (22:6n-3)<sup>a,c</sup>
- ↑ AA- vs. DHA-  
derived LOX  
products<sup>c</sup>
- ↑ 16:1/16:0 ratio<sup>a</sup>
- ↑ 18:1/18:0 ratio<sup>a</sup>

**Potential effects  
associated**

- Adipocyte hypertrophy?
- PGE<sub>2</sub> overproduction?
- Pro-inflammatory status?
- SCD1 overactivity?
- Aberrant *de novo* lipogenesis?

**Main lipid changes  
in MT of OP bones**

- ↑ TG<sup>a,b</sup>
- ↑ CE<sup>a</sup>
- ↓ SM<sup>a,b</sup>
- ↑ Ceramides<sup>b</sup>
- ↓ AA (20:4n-6)<sup>a</sup>
- ↓ DHA (22:6n-3)<sup>a</sup>
- ↑ PGE<sub>2</sub> metabolite<sup>b</sup>
- ↑ 16:1/16:0 ratio<sup>a</sup>
- ↑ 18:1/18:0 ratio<sup>a</sup>

**Potential effects  
associated**

- Osteocyte malfunction/  
death?
- Improper mineralization?
- Defective mineralization?
- Bone cell apoptosis?
- PGE<sub>2</sub> overproduction?
- LOX-derived products?
- Pro-inflammatory status?
- SCD1 overactivity?
- Aberrant *de novo* lipogenesis?