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CHARACTERIZATION OF FATTY ACID COMPOSITION IN BONE MARROW FLUID FROM POSTMENOPAUSAL WOMEN: MODIFICATION AFTER HIP FRACTURE

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Abstract

Bone marrow adipose tissue (BMAT) is associated with low bone mass, although the functional consequences for skeletal maintenance of increased BMAT are currently unclear. BMAT might have a role in systemic energy metabolism, and could be an energy source as well as an endocrine organ for neighboring bone cells, releasing cytokines, adipokines and free fatty acids into the bone marrow microenvironment. The aim of the present report was to compare the fatty acid composition in the bone marrow supernatant fluid (BMSF) and blood plasma of postmenopausal women women (65 to 80 years old). BMSF was obtained after spinning the aspirated bone marrow samples; donors were classified as control, osteopenic or osteoporotic after dual-energy X-ray absorptiometry. Total lipids from human bone marrow fluid and plasma were extracted, converted to the corresponding methyl esters, and finally analyzed by a gas chromatographer coupled with a mass spectrometer. Results showed that fatty acid composition in BMSF was dynamic and distinct from blood plasma, implying significance in the locally produced lipids. The fatty acid composition in the BMSF was enriched in saturated fatty acid and decreased in unsaturated fatty acids as compared to blood plasma, but this relationship switched in women who suffered a hip fracture. On the other hand, there was no relationship between BMSF and bone mineral density. In conclusion, lipid composition of BMSF is distinct from the circulatory compartment, most likely reflecting the energy needs of the marrow compartment.

Keywords

Fatty acids composition; Bone marrow fluid; Bone marrow microenvironment; Osteoporosis; Hip fracture

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Introduction

The functional consequences for skeletal maintenance of increased bone marrow adipose tissue (BMAT) are currently unclear although recently the focus of several studies in humans and rodents. In many circumstances, BMAT is associated with low bone mass, as in aging, osteoporosis and anorexia nervosa. However, high BMAT is also present in the long bones of healthy individuals, although in complex age-, sex-, and site-specific associations (Moore et al, 1990; Yeung et al, 2005; Bredella et al, 2009). It is suspected that BMAT has a role in systemic energy metabolism (Krings et al, 2011; Lecka-Czernik, 2012) (Wren et al, 2011; Di Iorgi et al, 2010), and could be an energy source, as well as an endocrine organ, for neighboring bone cells, releasing cytokines, adipokines and free fatty acids (FA) into the bone marrow microenvironment (Ahima and Flier, 2000; Frühbeck et al. 2001; Rosen and Bouxsein, 2006; Lecka-Czernik, 2012 (Rosen et al, 2009; Kawai et al, 2012; Lecka-Czernik, 2012; Gillet et al, 2015). Furthermore it has been demonstrated that fatty acid uptake in the bone marrow is substantial following oral feeding (Niemeier et al, 2008).

Since, osteoblasts and adipocytes in the bone marrow arise from a common precursor cell, the mesenchymal stem cells (MSCs), a reciprocal relationship between the two differentiation pathways has been postulated; as such any perturbations in lineage allocation would facilitate adipose accretion in the bone marrow at the expense of osteoblast formation, with the consequent decrease in bone mass (Rosen and Bouxsein, 2006; Rodriguez et al, 2008; Rosen et al, 2009). The bone marrow of osteoporosis patients often exhibits this imbalance (Nuttall and Gimble, 2004; Moerman et al, 2004; Rosen and Bouxsein, 2006). Histomorphometric studies on bone biopsy samples (Meunier et al, 1971; Verma et al, 2002) and more recently, proton magnetic resonance spectroscopy of vertebral marrow fat (Yeung et al, 2005) has shown that BMAT is increased in osteoporosis. Moreover, alterations in the osteo-/adipogenic processes are also observed in other bone loss conditions, such as aging, immobilization, microgravity, ovariectomy, diabetes, and glucocorticoid or tiazolidindione treatments, highlighting the harmful consequence of marrow adipogenesis in osteogenic disorders (Wronski et al, 1986; Forsen et al, 1999; Moerman et al, 2004; Zayzafon et al, 2004). However, it is unknown whether high BMAT represents a storage depot or a reserve for increased energy demands.

Previous reports have suggested that the composition of BMAT could have clinical relevance in addition to the size and volume of marrow fat. Such conclusion is derived from *in situ* noninvasive magnetic resonance imaging (MRI) - based analysis of bone marrow, in patients with chronic diseases such as osteoporosis (Yeung et al, 2005), type 2 diabetes mellitus (Baum et al, 2012), or diabetic and nondiabetic women with prevalent fragility fractures (Patsch et al, 2013). These studies demonstrated significantly lower unsaturation of bone marrow lipids in patients than corresponding controls. Moreover, Patsch and colleagues showed that low unsaturation and high saturation levels of lipids was accentuated in diabetic patients with prevalent fractures (Patsch et al, 2013).

In contrast, the lipid composition in the interstitial compartment surrounding cells in the bone marrow is unknown, as well as its physiological significance, or its variation under a bone stress situation, or its relationship with systemic lipids. Measurement of physiological

levels of molecules in the human bone marrow milieu has been particularly difficult not only because of tissue seclusion but also because of the complicated anatomy and blood perfusion of bone. Thus, knowledge on the availability to bone marrow cells of metabolites or regulatory compounds is scarce, limited to some pathologic condition or estimated from measurements in plasma (Wiig et al, 2004; Iversen and Wiig, 2005; Lee et al, 2002; Khosla et al, 1994), in part, because such measurements require invasive bone marrow sampling. Formerly, we evaluated in the bone marrow supernatant fluid (BMSF) the concentration of some significant signaling molecules revealing distinctive differences between non-osteoporotic and osteoporotic elderly women (Pino et al, 2010; Xian et al, 2012; Li et al, 2012), while substantiating that measurement in the BMSF reliably reflects the physiologically significant concentrations in the bone cell microenvironment, albeit dissimilar from circulating plasma or whole blood.

The aim of the present report was to test the hypothesis that the bone marrow milieu differs in its fatty acid composition from the circulation and that osteoporotic individuals have a characteristic lipidomic signature. To this end we compared the fatty acid composition in the BMSF and blood plasma of postmenopausal women, conjecturing that the fatty acids composition in the first fraction may more reliably reflect the physiologically relevant activity of interstitial adipose cells. Accordingly, the fatty acid composition in BMSF was dynamic and distinct from blood plasma, implying there may be significance in the locally produced lipids. The BMSF fraction showed enrichments in saturated fatty acids and decreases in unsaturated fatty acids as compared to blood plasma, although this relationship was statistically different in women with a hip fracture. On the other hand, there was no relationship between BMSF and bone mineral density.

Materials and methods

Subjects

Bone marrow samples and peripheral blood were obtained from 37 postmenopausal women. Bone marrow samples (20 ml) were obtained by iliac crest aspiration while undergoing orthopedic surgery as part of their therapeutic treatment at the Trauma Section of Hospital Sótero del Río, Santiago, Chile. Samples from osteoporotic patients with fracture were obtained after 2 to 7 days after hip fracture. The day before surgery a fasting blood sample (10 ml) was extracted.

Informed signed consent to participate in this study was obtained from each patient, prior to surgery. The study protocol and patient informed consent forms were approved by the Ethics Committee from the Instituto de Nutrición y Tecnología de Alimentos (INTA) and the Hospital Sótero del Río. Subject demographics data including age, body mass index (BMI), bone mass density (BMD) and T-score are shown in Table 1.

Measurement of the bone mass density.

Dual energy X-ray absorpiometry (DXA) of the lumbar spine (L2–L4) was performed on all subjects using a DXA system (LUNAR, Prodigy, General Electric Medical Systems, Madison, WI, USA). DXA was performed 2-3 days after surgery. Subjects were grouped

into three categories: control, osteopenia or osteoporosis according their bone mass density values at L2-L4. Control donors had a T-score greater than −1.0, osteopenia had a T-score between −1.0 and −2.5, and osteoporosis had a T-score less than −2.5 (Raisz, 1997).

Bone marrow supernatant fluid (BMSF)

Bone marrow supernatant fluid was obtained after spinning the bone marrow–aspirated sample (approximately 2 mL) for 5 minutes at 600 × g (Pino et al, 2010). Approximately 500 to 800μL of bone marrow supernatant fluid was collected and kept at −20°C until fatty acids measurement.

Gas chromatography analysis of fatty acids

Total lipids derived from human bone marrow fluid and plasma were first extracted in chloroform–methanol (2:1 v/v) containing nonadecanoic acid (C19:0) as an internal standard for fatty acid quantification. The fatty acids present in the extracted lipids fraction were then converted to the corresponding methyl esters using anhydrous methanol with hydrochloric acid 1.2 N under N_2 at 80 °C for 2 hours. The fatty acid methyl esters were analyzed in gas chromatographer Agilent 7890, coupled with a Mass Spectrometer Agilent 5975 MSD. The capillary column HP-Innowax (length 25 meters, 0.2 mm internal diameter, 0.2mm film) was used to preferentially separate the long fatty acid chain. The injector temperature was maintained at 220°C and oven ramp was linear from 150°C × 1 min to 200°C at a 15°C/min rate maintained for 5 min and then increasing from 200°C to 260°C at a 12°C/min rate maintained for 5 min. A mass detector was used at electronic impact ionization mode with ionization potential of 70eV and the injection was at split mode (1:20). Data register was in SCAN mode and chromatography peak integration was done by using the MS ChemStation software. Fatty acid methyl esters were identified by comparing retention times to known standards and by matching them up with what is provided in the mass spectra profile held in the fatty acid library NIST (NIST DataBase Library). The identification of each fatty acid peak was then quantified by normalizing its integration area against the chromatography peak integration of the internal standard.

Fatty acids composition was expressed as the percentage of molar individual fatty acids respect to the total fatty acids.

Statistical analysis

Data were expressed as mean ± standard deviation unless otherwise stated. Paired T-test was used to test for differences in fatty acid composition between blood plasma and bone marrow supernatant fluid. Statistical analysis was performed using Mann-Whitney test, assuming non parametric distribution. A p-value of < 0.05 was considered statistically significant, using GraphPad Prism 5.01 software.

Results

Demographics and clinical characteristics

Patient demographics and clinical characteristics are presented in Table 1; ten osteoporotic patients had hip fractures. Data are listed by category, control, osteopenic, osteoporotic

without fracture, osteoporotic with fractures; i.e.the osteoporotic subjects grouped according to whether they had or not suffered a hip fracture. Not surprisingly, osteoporotic patients with fracture were significantly older ($p<0.01$) and had lower T-score ($p< 0.05$) and BMD (< 0.01) than osteoporotic patients without fracture. Mean BMI and percentage of fat did not differ among the four groups.

Comparison of the fatty acid composition in the bone marrow supernatant fluid (BMSF) and in blood plasma for the entire cohort.

The composition of fatty acids in BMSF was compared to that found in the blood plasma of all women studied. As shown in Table 2 fatty acid content in BMSF was distinct from that found in blood plasma: total saturated fatty acids were 15 % higher ($p<0.05$) while polyunsaturated fatty acids were 23% ($p<0.01$) lower than the corresponding values in blood plasma. Consequently, in the BMSF and blood plasma the total unsaturated to saturated fatty acids ratio was 1.44 ± 0.55 and 1.71 ± 0.41, respectively ($p<0.04$). The difference relates to the content of some individual fatty acids; thus, in the BMSF the content of palmitic, stearic and oleic acids was higher by 15, 12 and 9 %, while linoleic was 24 %, lower than in blood plasma (Table 2). The palmitic (C16:0) to linoleic acid (C18:2) ratio in BMSF and blood plasma was 1.39 ± 0.89 and 1.05 ± 0.40, respectively ($p<0.05$). Other PUFAs detected showed peak values below the range of quantification.

Composition of fatty acid in BMSF and blood plasma according to bone mineral density

Table 3 provides the breakdown of BMSF and blood plasma in three categories. Fatty acid composition was compared between BMSF and blood plasma samples according to their classification as control, osteopenia, or osteoporosis, based on lumbar spine BMD T- scores. Results in Table 3 show no significant variation in the content of fatty acids according to patient's t score, in both BMSF and blood plasma. Although the content of total saturated fatty acids tended to be higher in BMSF than in plasma samples, it did not reach statistical significance. The content of polyunsaturated fatty acid tended to be lower in BMSF than in blood plasma, but such difference was statistically significant only in the osteoporosis group (Table 3). With respect to the content of individual fatty acids, in osteoporotic individuals, oleic acid was 16 % higher in BMSF than in plasma ($p<0.05$), while in osteopenia and osteoporosis linoleic acid was 29 % and 21 % lower ($p<0.05$), respectively in BMSF vs blood plasma (Table 3).

Composition of fatty acids in BMSF and blood plasma in osteoporotic women with or without hip fractures

Considering the difference in the demographic and clinical data between osteoporotic patients with and without hip fracture, the content of fatty acids was further analyzed in osteoporotics according to the presence or absence of hip fracture. Results in Table 4 show that in the blood plasma of patients with or without fracture, no significant difference in the content of fatty acids was observed, except for stearic acid that was decreased in the fracture group ($p<0.05$). Conversely, in BMSF several differences in the fatty acids content was appreciated among women with or without hip fracture (Table 4), thus, total saturated fatty acids decreased by 19 %, while total unsaturated fatty acids increased by 16 % in women with fracture. Therefore, total unsaturated to saturated fatty acids ratio showed significantly

higher in BMSF of women with hip fracture (1.65 ± 0.47 vs 1.14 ± 0.31, $p<0.05$). With respect to the content of specific fatty acids, the content of oleic acid increased significantly (21.3%, $p<0.05$), while the content of stearic, eicosatrienoic and arachidonic acids decreased significantly by 37%, 88%, and 67%, respectively ($p<0.001$) in BMSF of women with fracture (Table 4).

Discussion

Tracer studies in mice have demonstrated bone as the second most important organ after liver for the clearance of radiolabeled postprandial lipoproteins (Neimeier et al, 2008). As such, it is clear that skeletal and marrow cells utilize fatty acids as substrates for their energy needs. On the other hand, the function of marrow adipocytes is not known, nor has it been determined whether fatty acids stored in the marrow adipocytes are necessary for bone remodeling and hematopoiesis. In this report, we tested the hypothesis that bone marrow serum reflects the uniqueness of the marrow compartment in respect to the sequestration and utilization of fatty acids. In fact, we found a specific pattern of fatty acid composition in the bone marrow milieu of elderly women that was characterized by higher saturated and decreased unsaturated fatty acids compared to that of the circulation. Furthermore the fat composition displayed unique characteristics in pathologic states such as osteoporosis and hip fracture. Specifically we found that the proportion of fatty acids in the BMSF, varied in the range of lipids in plasma, but its relative levels of unsaturated and saturated fatty acids compares well to those observed in the human marrow fat tissue (Yeung et al, 2008; Patsch et al, 2013), implying a distinct lipid content in BMSF which replicate relevant fatty acids derived from the activity of adipocytes and other marrow cells, rather than those provided by blood plasma. With respect to the fatty acid content in blood plasma, our data are in the range reported for adult women (Hodson et al, 2008). Bearing in mind that fatty acids in plasma are considered markers of recent dietary fatty acid intakes, particularly saturated fatty acids, n-6 and n-3 polyunsaturated fatty acid (Hodson et al., 2008), it can be concluded that fat intake amongst women in this study was comparable and appropriate.

For the entire cohort of women studied there appears to be an active exchange of lipids between marrow cells (Table 2), thus the surplus of saturated fatty acids in BMSF could result from palmitic and stearic acids supplied mainly by adipocytes, while marrow cells apparently preserve their pool of unsaturated fatty acids, except for oleic acid which is higher in BMSF than in plasma.

In contrast, when the content of fatty acids in BMSF and blood plasma was evaluated according to women's BMD, no consistent differences in fatty acid composition were apparent in BMSF, nor in plasma, which is analogous to former observations on the composition of bone fat tissue (Yeung et al, 2005; Baum et al, 2012; Griffith et al, 2009). In this analysis, the osteoporosis group included all women with t-score lower than −2.5, with and without hip fracture; but results were not significantly different if data from women with fracture were not included. Notwithstanding, there was a trend towards higher saturation and lower unsaturation in BMSF fatty acids as compared to plasma. In addition, in BMSF of the osteoporosis group a larger proportion of fatty acids were oleic and linolenic acids implying an active distribution of unsaturated fatty acids.

The former conclusion is supported by further analysis of data on the composition of fatty acids in both BMSF and plasma in the osteoporosis group, in relation to the presence or not of hip fracture. A switch towards decreased content of total saturated versus unsaturated fatty acids was observed in BMSF of women with fracture, emphasizing a dynamic relationship between the composition of fatty acid in the bone microenvironment and the metabolic requirements of cells. We found that in the BMSF of women with fractures, the content of stearic acid decreased significantly while oleic acid content increased concomitantly, implying a substrate to product relationship to fulfill specific requirements for unsaturated fatty acids. Moreover, the polyunsaturated eicosatrienoic and arachidonic fatty acids were deleted, suggesting increased activity of cyclooxygenase (COX), COX-2.

It is difficult to define the origin (adipocyte or other marrow cells production) and type of lipids that change the fatty acid content of the BMSF fraction after bone fracture, in part because the measurement was limited to the total fatty acid pool in the soluble fraction of the bone marrow. With a hip fracture bone cell metabolism is involved in fighting against inflammation and repairing the damaged to bone tissue as well as extracting a huge demand for energy. Interestingly, the lipid fraction in BMSF of fractured women appears adjust to such conditions, thus diminished level of saturated fatty acid could result from increased supply of energetic substrate to marrow cells (bone, fat and hematopoietic), while an increased exchange of oleic and polyunsaturated fatty acids could be related to their regulatory functions.

Fatty acids influence bone formation and resorption thus, physiological concentrations of palmitate and oleate differently modulate cell death and function in bone (Gillet et al, 2015; Drosatos-Tampakaki et al, 2014; Yeh et al, 2014). Also, palmitate negatively affects differentiation, bone nodule formation and mineralization, reducing transcriptional activities of beta-catenin and Runx2 (Gunaratnam et al, 2014). Lipid mediators have a critical role in the mechanical-signaling pathway. As an early response to strain there is upregulation of phospholipase-mediated membrane release of fatty acids, markedly arachidonic acid (AA, 20:4n-6), the substrate for prostaglandin E2 (PGE2) synthesis, and expression of the inducible form of COX-2, which oxidizes arachidonic acid to PGE2 (Smith and Clark, 2005). This compound is liberated within seconds of mechanical loading of bone by osteocytes and mature osteoblasts (Bonewald, 2006). The importance of arachidonic acid and PGE2 in regulating bone remodeling is well established, but the participation of long chain PUFAs in the control of bone metabolism may be much broader than is currently recognized (reviewed in Poulsen et al, 2007).

Our observations add to those of Patsch et al. (2013), who observed by magnetic resonance imaging in the spine, that fracture and type 2 diabetes were associated with low bone marrow fat unsaturation and high bone marrow fat saturation levels independent of age, race, and local BMD. To make clear the apparent divergence with the former study, two experimental issues must be considered: measurement in BMSF portrays the composition of fatty acid in the soluble milieu surrounding bone cells, collecting fatty acids actively provided by both bone cells and blood plasma, dissimilar to the stationary *in situ* determination of bone marrow fat content by MRI. Furthermore, in the present study samples were obtained within hours after hip fracture, while Patsch et al. (2013) results refer

to varied fracture history. These independent observations in women with bone fracture coincide with the risk of limited availability of unsaturated fatty acids, mainly long-chain PUFAs, in the present report. This conclusion appears particularly appealing in the light of epidemiologic data, which indicate an inverse association between dietary intake of polyunsaturated lipids and hip fracture risk in older adults (Farina et al, 2011).

In conclusion, in the current study we characterized in BMSF the local relationship of fat composition within the bone marrow milieu. The fatty acid composition in BMSF was dynamic and distinct from that of blood plasma, implying the significance of locally produced lipids. The fatty acid composition in the BMSF was enriched in saturated fatty acid and decreased in unsaturated fatty acids as compared to blood plasma, but this relationship switched in women who suffered a hip fracture. Whether changes in saturated and unsaturated fatty acids in the local bone marrow milieu influences skeletal fragility in elderly women will require further studies. However, it is clear that the lipid composition of BMSF is distinct from the circulatory compartment, almost certainly reflecting the energy needs of the marrow compartment.

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Table 1

Demographic and densitometric characteristics of study participants (n = 41).

Demographic data	Control (n=10)	Osteopenia (n=10)	Osteoporosis without fracture (n=8)	Osteoporosis with fracture (n=9)
Age (years)	72.7 ± 6.1	72.1 ± 6.8	70.8 ± 3.8	79.3 ± 5.8 **
Height (cm)	155.2 ± 6.8	157.6 ± 4.4	152.4 ± 5.1	149.8 ± 5.7
Weight (kg)	67.1 ± 8.3	65.0 ± 4.8	62.0 ± 15.6	56.8 ± 6.5
BMI	28.0 ± 4.0	26.2 ± 1.8	26.8 ± 7.2	25.6 ± 4.2
T-score (L2-L4)	0.03 ± 0.52	−1.76 ± 0.33	−2.9 ± 0.4	−3.68 ± 0.63 *
BMD (g/cm^2)	1.210 ± 0.06	0.98 ± 0.07	0.882 ± 0.01	0.74 ± 0.08 **
Body Fat (%)	44.1 ± 4.7	41.8 ± 6.6	43.1 ± 2.9	38.0 ± 8.2

BMI: Body mass index; BMD: Bone mass density. Results are expressed as mean ± standard deviation.

* $p<0.05$,

** $p<0.01$, between osteoporosis without and osteoporosis with fracture

Table 2

Fatty acid composition in bone marrow supernatant fluid (BMSF) and blood plasma from postmenopausal women.

Fatty Acids	BMSF	Blood Plasma
	(mol%)	
Saturated	43.2 ± 10.6	37.7 ± 5.6*
Monounsaturated	31.0 ± 5.6	28.8 ± 5.5*
Polyunsaturated	25.8 ± 12.7	33.4± 7.8**
Total Unsaturated	56.8 ± 10.6	62.3 ± 5.6*
Palmitic acid (C16:0)	32.3 ± 8.9	28.1 ± 4.9*
Palmitoleic acid (C16:1 n-7)	2.0 ± 0.9	2.3 ± 1.1
Stearic acid (C18:0)	10.9 ± 3.3	9.7 ± 2.7*
Oleic acid (C18:1 n-9 cis)	27.3 ± 5.3	25.1 ± 5.1*
Elaidic acid (C18:1 n-9 trans)	1.7 ± 1.7	1.5 ± 0.7
Linoleic acid (C18:2 n-6)	22.1 ± 10.3	28.9 ± 6.2**
Eicosatrienoic acid (C20:3 n-6)	0.6 ± 0.7	1.0 ± 0.8
Arachidonic acid (C20:4 n-6)	3.0 ± 3.0	3.5 ± 2.4

The fatty acid analysis was performed in the bone marrow samples from the whole cohort of postmenopausal women donors. Results are expressed as mean ± standard deviation.

* $p<0.05$;

** $p<0.01$.

Table 3

Fatty acid composition in bone marrow supernatant fluid (BMSF) and blood plasma from three groups, control, osteopenia and osteoporosis, based on bone mineral density.

	Control (n=10)		Osteopenia (n=10)		Osteoporosis (n=17)	
Fatty Acids	**BMSF**	**Plasma**	**BMSF**	**Plasma**	**BMSF**	**Plasma**
	(mol%)		**(mol%)**		**(mol%)**	
Saturated	46.2 ± 12.0	38.1 ± 6.5	42.1 ± 12.3	35.4 ± 4.5	41.3 ± 8.5	38.8 ± 5.6
Monounsaturated	29.7 ± 5.1	32.2 ± 6.8	30.1 ± 4.2	26.1 ± 4.6*	32.3 ± 6.5	28.5 ± 4.5
Polyunsaturated	24.0 ± 13.4	29.7 ± 11.5	33.8 ± 9.2	38.5 ± 6.1	25.6 ± 11.6	32.7 ± 4.7*
Total unsaturated	53.8 ± 12.0	61.9 ± 6.5	57.9 ± 12.3	64.6 ± 4.5	57.9 ± 8.8	61.2 ± 5.6
						±
Palmitic acid (C16:0)	34.8 ± 10.8	27.3 ± 5.1	31.3 ± 10.7	26.3 ± 5.0	31.5 ± 6.8	30.4 ± 3.7
Palmitoleic acid (C16:1 n-7)	2.0 ± 0.7	2.6 ± 1.4	1.9 ± 1.1	1.8 ± 0.7	2.1 ± 1.0	2.4 ± 1.0
Stearic acid (C18:0)	11.5 ± 2.0	10.9 ± 2.9	10.8 ± 2.5	9.1 ± 1.9	10.6 ± 4.3	9.3 ± 2.9
Oleic acid (C18:1 n-9 cis)	26.5 ± 4.8	28.1 ± 7.2	26.1 ± 4.3	23.1 ± 4.3	28.5 ± 6.1	24.5 ± 3.6*
Elaidic acid (C18:1 n-9 trans)	1.3 ± 0.8	1.5 ± 0.4	1.2 ± 0.8	1.2 ± 0.5	1.7 ± 0.8	1.6 ± 0.8
Linoleic acid (C18:2 n-6)	22.0 ± 9.2	25.3 ± 8.5	22.6 ± 11.2	31.9 ± 5.7*	23.1 ± 10.0	29.2 ± 4.1*
Eicosatrienoic acid (C20:3 n-	0.8 ± 0.7	0.9 ± 1.0	0.9 ± 0.6	1.3 ± 0.5	0.4 ± 0.6	0.9 ± 0.8
Arachidonic acid (C20:4 n-6)	3.3 ± 2.5	3.5 ± 3.0	4.3 ± 4.3	5.3 ± 1.4	2.0 ± 2.0	2.6 ± 1.9

The fatty acid analysis was performed in BMSF and blood plasma from donors classified according their bone mineral density. The Osteoporosis group includes donors with and without fractures. Results are expressed as mean ± standard deviation.

* $p<0.05$ between BMSF and blood plasma.

Table 4

Composition of fatty acid in bone marrow fluid from osteoporotic donors with and without hip fracture.

	Blood Plasma		Bone Marrow Supernatant Fluid	
Fatty Acids	**Without fracture (n=8)**	**With fracture (n=9)**	**Without fracture (n=8)**	**With fracture (n=9)**
	(mol%)		**(mol%)**	
Saturated	37.6 ± 4.6[a,b]	40.0 ± 6.5	45.6 ± 8.5	37.0 ± 6.3[*]
Monounsaturated	29.5 ± 3.2	27.4 ± 5.5[c]	28.8 ± 5.3	34.1 ± 6.3[*]
Polyunsaturated	32.8 ± 4.3	32.6 ± 5.2	24.1 ± 13.9	26.9 ± 9.8
Total Unsaturated	62.4 ± 4.6	60.0 ± 6.5	54.4 ± 8.5	63.0 ± 6.3
Palmitic acid (C16:0)	29.0 ± 3.7[b]	32.2 ± 3.2	32.4 ± 7.7	30.7 ± 6.2
Palmitoleic acid (C16:1 n-7)	2.7 ± 0.9	2.0 ± 1.0	2.0 ± 1.0	2.3 ± 1.0
Stearic acid (C18:0)	9.4 ± 1.3[a]	9.2 ± 4.0	13.2 ± 4.5	8.3 ± 2.7[*]
Oleic acid (C18:1 n-9 cis)	25.1 ± 3.5	24.0 ± 3.7[c]	24.9 ± 5.0	30.2 ± 5.3[*]
Elaidic acid (C18:1 n-9 trans)	1.8 ± 0.5	1.4 ± 1.0	1.8 ± 0.8	1.6 ± 0.9
Linoleic acid (C18:2 n-6)	28.5 ± 3.5[a]	30.0 ± 4.7	20.4 ± 10.9	25.6 ± 9.0
Eicosatrienoic acid (C20:3 n-6)	1.1 ± 0.8	0.6 ± 0.7[c]	0.8 ± 0.7	0.1 ± 0.1[*]
Arachidonic acid (C20:4 n-6)	3.2 ± 2.3	2.0 ± 1.3	4.6 ± 1.0	1.5 ± 0.8[***]

The Osteoporosis group was divided according the existence or not of hip fracture and each new group was analyzed independently. Results are expressed as mean ± standard deviation.

[a] $p<0.05$; Plasma/without fracture vs BMSF/without fracture;

[b] $p<0.05$; Plasma/without vs Plasma/with fracture;

[c] $p<0.05$; Plasma/with vs BMSF/with fracture;

[*] $p < 0.05$;

[***] $p<0.001$; BMSF/without vs BMSF/with fracture.