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INVITED REVIEW

Adverse plasma fatty acid composition in patients with femoral neck fracture[1]

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Abstract: Our study aimed to examine the status of plasma fatty acids (FAs), inflammatory markers, and lipid peroxidation in patients with femoral neck fractures. The study included 20 patients (64–86 years) with femoral neck fractures indicated for surgery and a control group of 17 elderly subjects without fractures or serious chronic diseases. Plasma was obtained during the first 12 h postfracture and presurgery and 7 days postop. Compared to the control, patients had significantly higher saturated FA (SFA) and monounsaturated FA as well as increased TNF-α and IL-6. Opposite to that, levels of individual and total *n*-6 polyunsaturated FA (PUFA), individual and total *n*-3 PUFA, *n*-6/*n*-3 ratio, and levels of thiobarbituric acid reactive substances (TBARS) were markedly lower in the patient than in the controls. On the seventh day after the surgery, we showed a further rise in the SFA, oleic acid, and TNF-α and reductions of *n*-6 PUFA and IL-6. Taken together, our results suggest that altered FA status, especially reduced PUFA, may influence hip fracture repair and even contribute to femoral fracture susceptibility in the elderly. A potential benefit from nutritional intervention with PUFA in prevention and (or) fracture healing should be considered.

Key words: femoral neck fracture, presurgery, postsurgery, fatty acid, arachidonic acid, TNF-α, IL-6, thiobarbituric acid reactive substances.

Résumé : Nos travaux visaient à étudier l'état des acides gras, marqueurs de l'inflammation et de la peroxydation lipidique, chez des patients présentant une fracture du col du fémur. L'étude portait sur 20 patients (de 64 à 86 ans) présentant une fracture du col du fémur avec indication d'intervention chirurgicale et un groupe témoin de 17 sujets âgés sans fracture ni maladie chronique grave. Nous avons obtenu du plasma dans les 12 premières heures suivant la fracture et avant l'intervention chirurgicale, ainsi que 7 jours après celle-ci. Les patients présentaient des taux nettement plus élevés d'acides gras saturés et monoinsaturés, ainsi que de facteur de nécrose tumorale α (TNF-α) et d'interleukine 6 (IL-6) que les témoins. À l'opposé, les taux d'acides gras *n*-6 individuels et polyinsaturés totaux, d'acides gras *n*-3 individuels et polyinsaturés totaux, le ratio *n*-6/*n*-3 et les taux de substances réagissant à l'acide thiobarbiturique (TBARS pour « thiobarbituric acid reactive substances ») étaient nettement moins élevés chez les patients que chez les témoins. Au 7e jour après l'intervention chirurgicale, nous avons montré une augmentation supplémentaire des taux d'acides gras saturés, d'acide oléique et de TNF-α, avec des diminutions en matière d'acides gras polyinsaturés *n*-6 et d'IL-6. Dans l'ensemble, nos résultats laissent entendre que les modifications sur le plan des acides gras, en particulier l'abaissement des acides gras polyinsaturés, pourraient influencer la réparation de la fracture de la hanche et même contribuer à la susceptibilité aux fractures fémorales chez les personnes âgées. On devrait prendre en compte les bienfaits éventuels d'interventions de nature alimentaire sur les acides gras polyinsaturés dans la prévention ou la guérison des fractures. [Traduit par la Rédaction]

Mots-clés : fracture du col du fémur, avant l'intervention chirurgicale, après l'intervention chirurgicale, acide gras, acide arachidonique, TNF-α, IL-6, substances réagissant à l'acide thiobarbiturique.

Introduction

Femoral neck fractures as a common source of morbidity and mortality, especially in the elderly, represent a global health challenge. Treatment of injuries is complex and usually requires surgery (Ossendorf et al. 2010). Femoral fractures can be caused by an injury and (or) low bone density, with women with osteoporosis being in the highest risk (Kopperdahl et al. 2014). Other risk factors are smoking, high low-density lipoprotein levels, increased homocysteine, and fat mass (Martínez-Ramírez et al. 2007).

Many human, animal, and in vitro studies have led to better understanding of biochemical processes during the fracture healing (Einhorn 2005). Generally, bone healing goes through four stages: inflammation, soft callus formation, hard callus formation, and remodeling (Marsell and Einhorn 2011). Recently, the potential roles of polyunsaturated fatty acids (PUFA) in inflammatory regulation of bone remodeling, including gene transcription, have been highlighted. Arachidonic acid (AA) (C20:4 *n*-6) increases expression of peroxisome proliferator activator receptor gamma

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(PPARγ), a transcription factor that inhibits the osteoblast proliferation (Maurin et al. 2005). Due to high binding affinity, docosahexaenoic acid (DHA) (22:6 *n*-3) reduces PPAR expression and favors osteoblastogenesis (Mangano et al. 2013). Furthermore, AA increases gene expression of several proinflammatory cytokines, including interleukines (IL) IL-1α and IL-1β, transforming nuclear factor α (TNF-α), and macrophage colony stimulating factor in human osteoblast-like cells in vitro (Priante et al. 2002). During the early inflammatory phase of fracture healing, a short, highly regulated secretion of TNFα, IL-1, IL-6, IL-11, and IL-18 has been detected (Marsell and Einhorn 2011). This early inflammatory response reaches its maximum after 24 h and ends after 7 days. IL-1 is believed to promote the primary cartilage growth, angiogenesis, and production of IL-6 in osteoblasts (Marsell and Einhorn 2011). IL-6 stimulates angiogenesis, vascular endothelial growth factor production, and the differentiation of osteoblasts and osteoclasts (Yang et al. 2007).

Furthermore, AA can be metabolized by cyclooxygenase to prostaglandin E2, an important factor in bone resorption, genesis of osteoblasts, and new bone production (Keila et al. 2001). AA is synthesized from linoleic acid (LA) (18:2 *n*–6) via elongase and desaturase activity (Ristic-Medic et al. 2013). Since LA and α-linolenic acid (ALA) (18:3 *n*–3) are essential FA that cannot be synthesized by human tissues (Salari et al. 2008), studies suggest that FA intake and status may be related to bone health (Farina et al. 2012). It has been shown that saturated FA (SFA) intake may significantly increase hip fracture risk, whereas monounsaturated (MUFA) and PUFA, particularly *n*-3 PUFA, intake may decrease total fracture risk (Orchard et al. 2010; Chen et al. 2017). In addition, serum levels of *n*-3 PUFA were positively correlated with bone mineralization in mice and humans, while the deficiency of essential FA may be associated with pathological fractures in rats (Kruger et al. 2010). Considering the potential effects of PUFA on bone metabolism and fracture healing, we hypothesized that low PUFA status, particularly of *n*-3 PUFA, in patients is related to appearance of femoral neck fracture. Secondary hypotheses were that PUFA status during early fracture healing would improve as compared to the preoperative status and that better PUFA status would be associated with lower markers of inflammation. The aim of this study was to determine the level of plasma FA, TNF-α, IL-6, and lipid peroxidation in elderly patients with femoral neck fractures during the early stages of healing, i.e., within 12 h after the fracture and on the seventh postoperative day.

Materials and methods

This study included 20 patients aged 64–86 years with femoral neck fractures and indicated for surgery. Patients were recruited from Orthopedic Clinic, Clinical Centre Kragujevac, during 2015. Exclusion criteria were age below 64 years, serious liver, kidney, or heart diseases, malignant diseases, use of statins, use of fish oil or other supplements that influence FA metabolism, and unsuitability for prosthesis. None of the patients had damage to the spinal cord or peripheral nerve injury (quadriplegia, paraplegia, hemiplegia). The study protocols were approved by the Ethical Committee of the Clinical Centre of Kragujevac in accordance with the Declaration of Helsinki and principles of Good Clinical Practice. All subjects gave written participation consent. Given that all patients were older than 64 years (average 73.4), trophic changes in bone density could be classified as primary osteoporosis due to age. The control group were 17 subjects without fractures or serious chronic diseases and comparable in age and sex.

Measurements and laboratory data

Blood samples were taken within 12 h after the fracture and on the seventh day after the operative reposition of the fracture. The FA profile in plasma total lipids was isolated by the method of Glaser et al. 2010 with some modifications. Individual FA were determined by gas chromatography comparing their retention times with those of commercial standards PUFA-2 (Supelco, Inc., Bellefonte, Pennsylvania, USA). The results are presented as percentage of total FA (Ristic-Medic et al. 2009). The activities of enzymes involved in long-chain FA syntheses were estimated as we previously described (Tepsic et al. 2013).

Table 1. Demographic and clinical characteristics of the study population.

	Patients (*n* = 20)		Controls (*n* = 17)	
Age (years)	73.4±8.2	64–86	71.5±6.7	64–79
Male (%)	20	4 (20)	23.5	4 (17)
Female (%)	80	16 (20)	76.5	13 (17)
History of osteoporosis (%)	35	7 (20)	35.3	6 (17)

Index of lipid peroxidation

The degree of lipid peroxidation in plasma was estimated by measuring thiobarbituric acid reactive substances (TBARS) using the method of Zeb and Ullah (2016).

Measurement of the plasma levels of cytokines TNF-α and IL-6

The cytokine concentrations in the plasma were determined using commercial ELISA assays (Human IL-6 DUOSET ELISA development kit and Human TNF-α/TNFSF1A DUOSET ELISA development kit; R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's protocol.

Statistical analysis

The statistical analysis was performed using the statistical package SPSS 20.0 for Windows. The results are presented as means ± SD. The normality was tested by the Shapiro–Wilk test. The differences between orthopedic patients and the control group were assessed with the Student *t* test and Mann–Whitney *U* test. Comparisons between preoperative and postoperative values in orthopedic patients were performed by paired *t* test and Wilcoxon test for nonnormally distributed variables. Pearson's correlation analysis was used to study correlations between cytokines and FA. The alpha level for significance was set to $p < 0.05$.

Results

Demographic characteristics of the study population are presented in Table 1. There were no differences between groups of patients and the control group in terms of ages, gender, and osteoporosis.

Plasma lipids FA profile

The plasma lipid FA profiles in patients with femoral neck fracture before surgery (preop) and 7 days after the surgery (postop) and the control group are presented in Table 2. As can be seen, the plasma FA profiles in patients, determined within 12 h after the fracture, markedly differed from those in the control group. Levels of palmitic acid (16:0) and total SFA as well as level of oleic (18:1 *n*-9), vaccenic acids (18:1 *n*-7), and total MUFA were significantly higher in patients than in the control subjects. On the other hand, levels of γ-linolenic (18:3 *n*-6), AA, and docosatetraenoic acid (22:4 *n*-6) as well as total PUFA and *n*-6 PUFA were significantly lower in patients with femoral fractures than in the control group. Further, all identified *n*-3 FA, including ALA, eicosapentaenoic acid (20:5 *n*-3), docosapentaenoic acid (22:5 *n*-3), DHA, and total *n*-3 PUFA were markedly lower in the patient group when compared with the controls (Table 2.). The *n*-6/*n*-3 PUFA ratio was very high in all patients, unlike in the control subjects.

On the seventh day after the surgery, plasma FA profiles in patients noticeably changed in comparison with the profiles before surgery. Levels of palmitic acid and total SFA significantly increased, while the level of stearic acid (18:0) decreased during this period. In addition, the level of oleic acid, which is the most

Table 2. Fatty acid profile in total plasma lipids in patients with fracture and the control group.

Fatty acid	Preop (%) (n = 20) (mean ± SD)	Postop (%) (n = 20) (mean ± SD)	Control group (%) (n = 17) (mean ± SD)
16:0	30.65±2.28$^{\#\#\#}$	33.33±1.44***	27.31±1.23
16:1	1.96±0.84	1.90±0.72	1.52±0.56
18:0	13.53±1.17	12.66±1.91*	13.40±1.28
18:1 n-9	15.91±1.81$^{\#\#}$	16.84±1.98*	13.83±1.35
18:1 n-7	2.24±0.55$^{\#\#\#}$	2.23±0.55	1.60±0.24
18:2 n-6	24.53±3.33	22.65±2.81***	23.41±2.53
18:3 n-6	0.44±0.29$^{\#\#\#}$	0.42±0.25	0.92±0.24
20:3 n-6	1.93±0.88	1.91±0.67	2.29±0.50
20:4 n-6	6.47±1.43$^{\#\#\#}$	5.91±1.56	11.77±1.20
22:4 n-6	0.31±0.14$^{\#\#\#}$	0.32±0.27	0.57±0.15
18:3 n-3	0.22±0.07$^{\#\#}$	0.17±0.08*	0.45±0.20
20:5 n-3	0.26±0.18$^{\#}$	0.21±0.11	0.44±0.19
22:5 n-3	0.33±0.09$^{\#\#}$	0.32±0.14	0.60±0.25
22:6 n-3	1.22±0.52$^{\#\#}$	1.13±0.34	1.91±0.52
SFA	44.18±2.17$^{\#\#\#}$	45.99±2.35**	40.70±1.67
MUFA	20.12±2.63$^{\#\#\#}$	20.98±2.16	16.95±1.52
PUFA	35.17±3.71$^{\#\#\#}$	33.03±2.61***	42.35±2.22
n-6	33.67±3.69$^{\#\#\#}$	31.20±2.51***	38.96±2.68
n-3	1.95±0.79$^{\#\#\#}$	1.83±0.41	3.39±0.87
n-6/n-3	18.04±5.02$^{\#\#\#}$	17.92±4.39	11.65±3.93

Note: Preop, postfracture and preoperative; Postop, postoperative; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with presurgery; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the control group.

Table 3. The estimated plasma desaturase and elongase activities in patients with fractures and the control group.

Desaturase and elongase	Preop (n = 20) (mean ± SD)	Postop (n = 20) (mean ± SD)	Control group (n = 17) (mean ± SD)
18:0/16:0 (elongase)	0.44±0.06	0.38±0.06***	0.48±0.06
18:1/18:0 (Δ9 desaturase)	1.19±0.20$^{\#}$	1.38±0.40*	1.06±0.13
18:3 n-6/18:2 n-6 (Δ6 desaturase)	0.02±0.01$^{\#\#\#}$	0.02±0.01	0.04±0.01
20:4 n-6/20:3 n-6 (Δ5 desaturase)	3.79±1.34$^{\#\#}$	3.40±1.31	5.46±1.45

Note: Preop, postfracture and preoperative; Postop, postoperative; $^{*}p < 0.05$, $^{***}p < 0.001$ compared with presurgery; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with control group.

common MUFA, increased and levels of LA, total PUFA, and n-6 PUFA were significantly reduced at the end of the study. Among n-3 PUFA, only ALA significantly decreased 7 days after surgery, while other PUFA remained unchanged.

Estimated activity of plasma desaturase

As shown in Table 3, the estimated activity of plasma elongase decreased, while the estimated activity of plasma Δ9 desaturase significantly increased in patients with fracture during 7 days after intervention. When compared with the control group, the estimated activity of plasma Δ9 desaturase was higher, while activities of Δ6 and Δ5 desaturases were significantly lower in the patients.

Level of TNF-α, IL-6, and TBARS

Figures 1, 2, and 3 display the levels of TNF-α, IL-6, and TBARS before and 7 days after the surgery. We found a significantly ($p < 0.05$) increased level of TNF-α and a significantly decreased ($p < 0.001$) level of IL-6 7 days after surgery.

Table 4 shows the correlation of FA with inflammatory markers and lipid peroxidation in this study. Our results have shown a moderate inverse correlation of IL-6 with MUFA ($r = -0.509$) and a positive correlation with DHA and n-3 PUFA ($r = 0.605$ and 0.490, respectively). TNF-α was inversely associated with MUFA as well ($r = -0.469$) and in positive correlation with PUFA ($r = 0.435$) and

Fig. 1. The plasma level of TNF-α in the control and patient groups. [Colour online.]

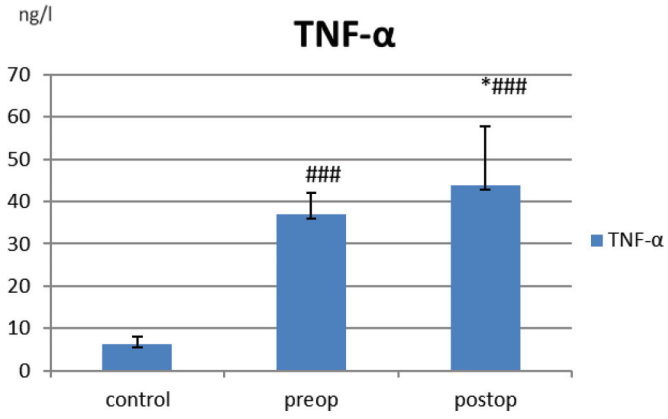


Fig. 2. The plasma level of IL-6 in the control and patient groups. [Colour online.]

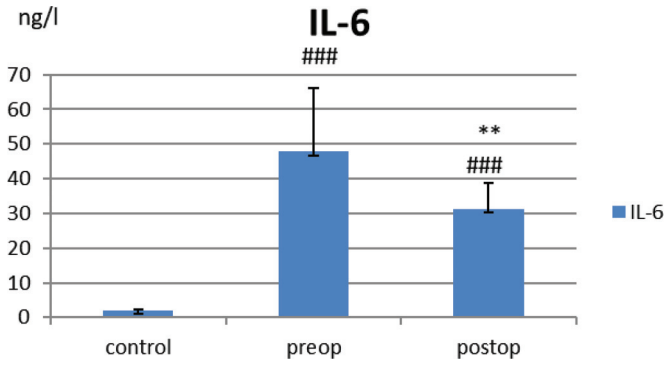


Fig. 3. The plasma level of TBARS in the control and patient groups. [Colour online.]

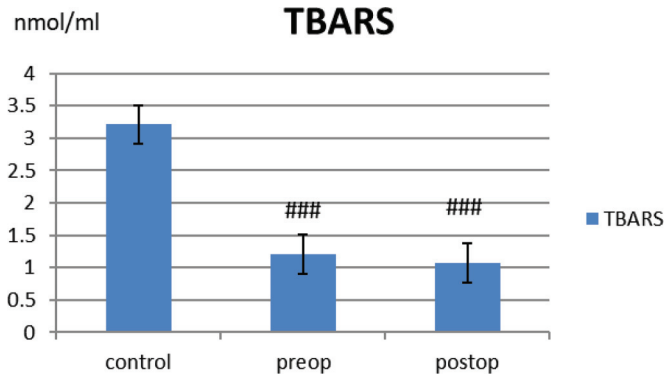


Table 4. Correlation of fatty acids with inflammatory markers and lipid peroxidation.

Fatty acid	TNF-α, r (p)	IL-6, r (p)	TBARS, r (p)
Preop (n = 20)			
SFA			−0.446 (0.043)
MUFA	−0.469 (0.032)	−0.509 (0.019)	
PUFA	+0.435 (0.043)		
Postop (n = 20)			
16:1 n-7	+0.462 (0.035)	+0.605 (0.004)	+0.524 (0.015)
18:3 n-3		+0.490 (0.024)	
22:6 n-3			
Total n-3			

palmitoleic acid (r = 0.462). TBARS was inversely correlated with SFA(r = −0.446) and positively with ALA (r = 0.524).

Discussion

This study demonstrated marked differences in FA profiles between elderly patients with femoral neck fractures and age- and sex-matched control subjects. Further alterations were found during the followup after the surgery.

The group of patients had significantly higher levels of palmitic acid and total SFA, which were further increased after the surgery. Since these FA are highly atherogenic and associated with an increased risk of cardiovascular mortality and morbidity (Stanley et al. 2007), these changes are unfavorable. According to a clinical case control study in a Chinese population, a diet rich in total fats and SFA might increase the risk of hip fractures in the elderly (Zeng et al. 2015). Although the FA status in patients before fracture was unknown, further elevation of SFA may have a negative effect on bone healing.

Further, we detected higher levels of oleic and vaccenic acid and total MUFA in patients with femoral neck fractures than in the control group. The literature data on the effect of dietary MUFA or their status in plasma on bone health in animals and humans are sparse. However, some authors suggest a positive correlation between MUFA in the diet and bone density (Orchard et al. 2010). Martínez-Ramírez et al. (2007) reported an association between higher dietary intake of MUFA and reduced risk of fracture in the elderly. Thus, the herein observed increase in Δ9 desaturase activity that converts SFA into MUFA may have a protective role and aid in recovery after the surgery.

PUFA may influence bone health preferably via gene expression but also via reduction of proinflammatory cytokines (Maurin et al. 2005; Priante et al. 2002), stimulation of osteoblastic differentiation by increasing insulin-like growth factor-1 and parathyroid hormone (Shen et al. 2006), and by increased generation of nitric oxide (Rahman et al. 2009). A few hours after fractures, patients in our study had lower levels of n-6 gamma-linolenic acid (GLA), AA, and docosatetraenoic acid (DTA) as well as total PUFA and n-6 PUFA compared to the control group. Seven days after the surgery, total PUFA and n-6 PUFA further decreased and LA also decreased in comparison to the level after the fracture. Furthermore, when we compared FA profiles of patients with or without a history of osteoporosis, there were no significant differences. However, although approximately a third of the patients were diagnosed with osteoporosis before the fracture, all patients were older than 64 years and thus we can assume that all of them had primary osteoporosis.

The early physiological response to a fracture implies hypoxia and inflammation, which induces synthesis of cytokines and specific growth factors that stimulate cell proliferation and migration into the site of the fracture (Marsell and Einhorn 2011). An important class of mediators in these processes are prostaglandins (PG), produced by osteoblasts, with the amount and the types of synthesized PG depending on different stimuli (Blackwell et al. 2010). The most common precursor for PG synthesis is AA from membrane phospholipids released by activity of phospholipase A2 and then converted into PGH2 and specific prostaglandins such as PGE2, PGD2, PGF2α prostacyclin (PGI2), and thromboxane (Blackwell et al. 2010). PGE2 is the most abundant and most important prostaglandin in bone, which modulates both bone resorption and formation depending on the amount of PGE2 (Li et al. 2007). It has been shown that a low level of PGE2 stimulates bone formation in animals with moderate dietary intake of n-6 PUFA (Kruger et al. 1998). A very low level of AA in patients with a femoral neck fracture could be explained by its intensive conversion into PGE2. In addition, decreased levels of GLA and dihomo-gamma-linolenic acid (DGLA) could be due to their conversion into AA to compensate for higher AA consumption. A reduced level of DTA (22:4 n-6) is also a consequence of low AA level in the patient group. Thus, decreased level of n-6 PUFA, observed immediately after the fracture in our patients compared with the control group, may be a result of intensive synthesis of PG. Nevertheless, we cannot exclude the possibility of altered FA status in these patients before the fracture. On the other hand, the patients had a normal level of LA, which serves as a marker of a good nutritional status at the time of fracture (McCarthy et al. 1991). Seven days after the surgery, we found a decreased level of LA (18:2 n-6), a precursor of all other n-6 PUFA, in plasma lipids. It suggests increased conversion of LA into its products (GLA, DGLA, AA, and DTA), but in spite of this, we found a further decrease of all n-6 PUFA probably due to a high generation of PGE2. In addition to n-6 PUFA, the level of all n-3 PUFA is severely lower in the patients than in the control group after the fracture and even lower 7 days after the surgery. Plasma levels of n-3 PUFA positively correlated with the bone mineralization in both mice and humans and higher n-3 PUFA intake reduces the fracture risk (Harris et al. 2015). It has been found that n-3 PUFA reduce PGE2 synthesis, suppress inflammation, and promote bone repair by enhancing production of insulin-like growth factors, which are potent growth stimulators for bone remodeling (Weitzmann and Pacifici 2006). Additionally, n-3 PUFA have been shown to increase the absorption of dietary calcium needed for fracture healing (Weitzmann and Pacifici 2006). The complex relationships among FA, cytokines/inflammation, and fracture risk and healing are displayed in Fig. 4.

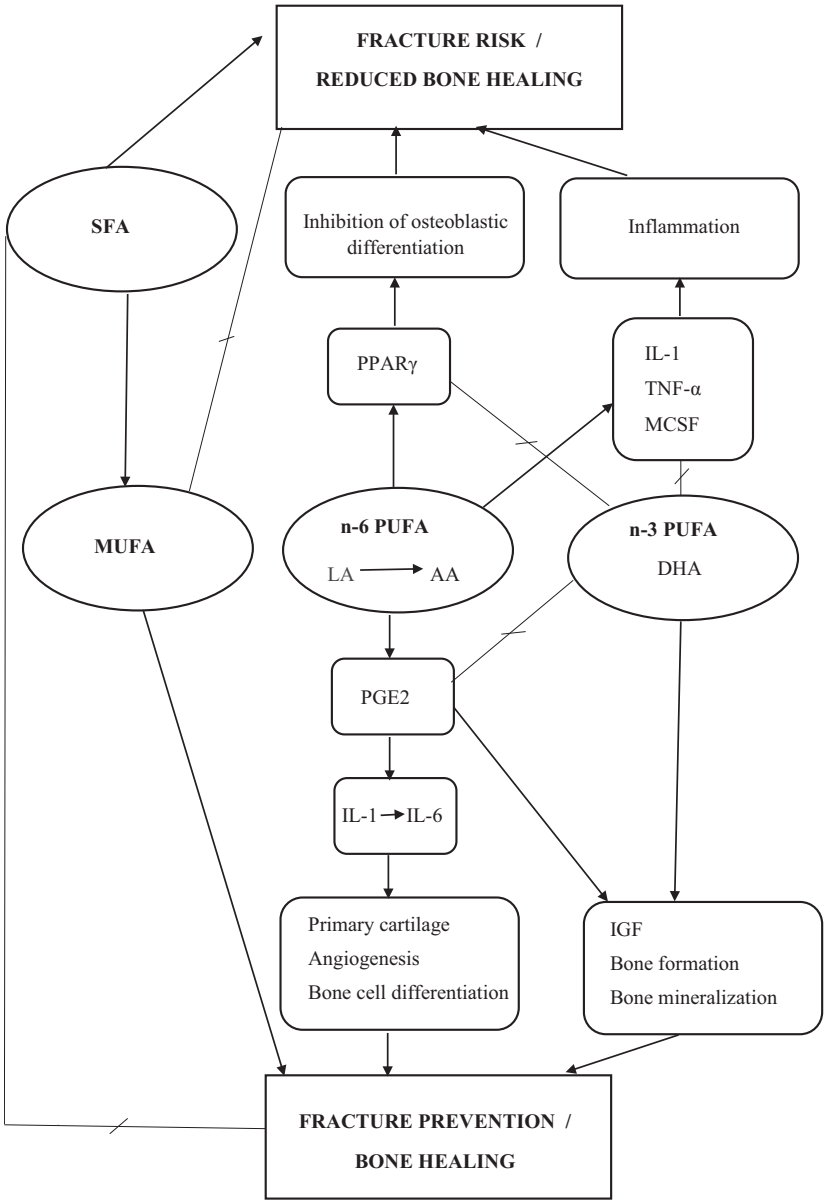
Changes in cytokine levels have shown that the IL-6 level grows quickly after the fracture but significantly declines 7 days after the surgery. On the contrary, TNF-α increased during the postoperative period. Level of TBARS has not changed during the 7 days of postop period and it was lower than in the healthy people.

The inverse correlation between MUFA and markers of inflammation, IL-6 and TNF-α, is in line with previous studies, which concluded that oleic acid did not promote inflammation in healthy adults (Kalogeropoulos et al. 2010). Positive correlation of palmitoleic acid with TNF-α also aligns with previous data, which reported that palmitoleic acid was associated with an adverse adipokine profile and metabolic disorders (Zong et al. 2012). PUFA also positively correlated with TNF-α, and here, it should be emphasized that PUFA is a mix of both pro- and antiinflammatory PUFA. However, positive correlation of DHA and n-3 PUFA with IL-6 is in contrast with the literature data, since the antiinflammatory action of n-3 PUFA is well known (Vucic et al. 2015). Nevertheless, studies are mostly focused on the healthy population, while we conducted this study in patients with a high grade of inflammation. One possible explanation is that in these conditions, high production of IL-6 is conditioned by a high consumption of AA, which altered the FA profile and made the relative concentrations of DHA and n-3 PUFA relatively higher. Further studies in patients with acute inflammation are needed to explore the relationship between FA status and inflammatory markers.

This study provides an insight into the status of FA in plasma lipids of patients with femoral neck fracture. Other studies mostly focused on the relationship between intake and risk of fracture. Although dietary intake influences FA profiles in plasma, the FA metabolism affects the results as well; thus, it is important to check the PUFA status in the blood of elderly people. In line with our primary hypothesis, patients had lower n-3, n-6, and total PUFA status than the control group. However, during the healing, their PUFA status further decreased; that might be a consequence of a high level of PUFA conversion into both pro- and antiinflammatory eicosanoids.

This study has two limitations: a relatively low number of patients and a lack of FA status of the patients before the fracture. Thus, it has given significant inputs for future research: since the time from the fracture to blood drawn was mostly short, it is likely that elderly patients with femoral neck fractures had altered FA status in the plasma before the fracture, in particular low

Fig. 4. Scheme of the relationship between fatty acid profile, inflammatory cytokines, and fracture risk/healing.



n-3 PUFA, which makes them more susceptible for fractures. Identification and PUFA supplementation in these people could help in fracture prevention. Further, nutritional intervention after the fracture may improve the healing process. Future studies should address the potential benefits of high PUFA intake in bone fractures.

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