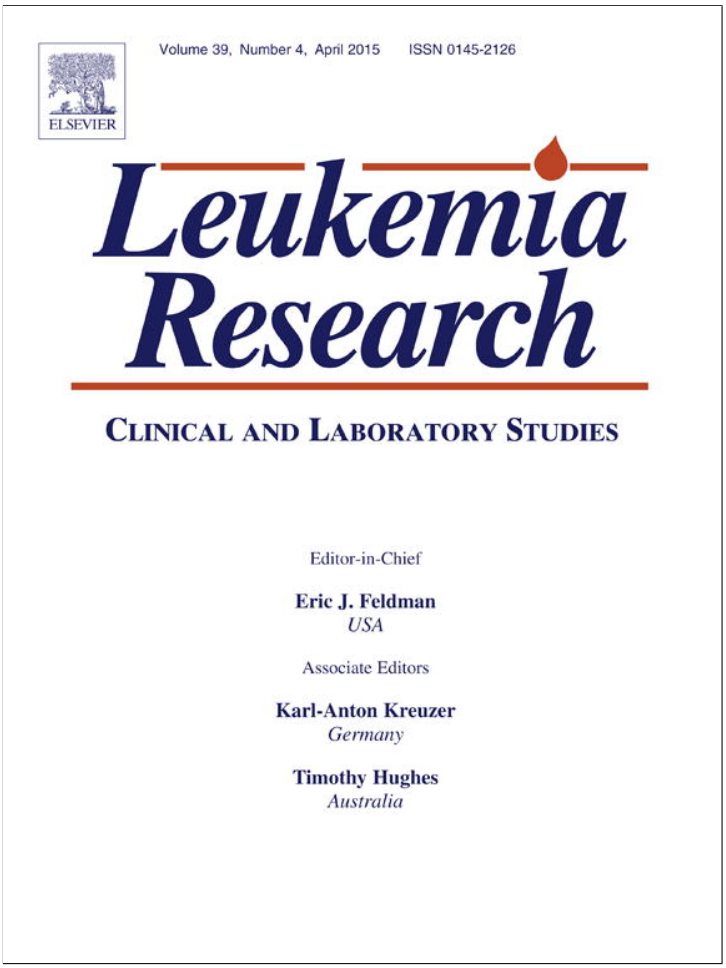

Leukemia Research 39 (2015) 400–405

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Plasma fatty acid profile in multiple myeloma patients

Artur Jurczyszyn[a,*,1], Jacek Czepiel[b,1], Joanna Gdula-Argasińska[c,1], Paweł Paśko[d], Anna Czapkiewicz[e], Tadeusz Librowski[c], William Perucki[f], Aleksandra Butrym[g,h], Jorge J. Castillo[i], Aleksander B. Skotnicki[a]

[a] Department of Hematology, University Hospital, Krakow, Poland
[b] Department of Infectious Diseases, Jagiellonian University Medical College, Krakow, Poland
[c] Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
[d] Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
[e] Faculty of Management, AGH University of Science and Technology, Krakow, Poland
[f] Department of Medicine, John Dempsey Hospital, University of Connecticut, Farmington, CT, USA
[g] Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Medical University, Wroclaw, Poland
[h] Department of Physiology, Medical University, Wroclaw, Poland
[i] Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA, USA

ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 1 December 2014
Accepted 16 December 2014
Available online 24 December 2014

Keywords:
Multiple myeloma
Fatty acids
Plasma
Gas chromatography
Desaturase activity

ABSTRACT

New membrane formation in the proliferating tumor cells consequently results in hypermetabolism of fatty acids (FA), as seen in many cancer patients, including multiple myeloma (MM). The FA composition of plasma reflects both endogenous synthesis as well as the dietary supply of these compounds. Additionally, obesity is a risk factor for the development of MM. The aim of this study was to compare the FA composition of plasma in 60 MM patients and 60 healthy controls. We noted significant differences in the FA profile of plasma from patients with MM when compared to the control group. Increased levels of saturated and n-6 polyunsaturated fatty acids in MM patients suggest that there may be increased endogenous synthesis of these fatty acids, likely due to increased expression of desaturase and elongase. Furthermore, cluster analysis showed differences in the distribution of FA in plasma from MM patients compared to controls. Dietary fat and a deranged endogenous FA metabolism may contribute to cancer-associated inflammation through an abnormal arachidonic acid metabolism, caused by pro-inflammatory derivatives. Our study supports further research on the biochemistry of lipids in patients with MM.

1. Introduction

Malignant transformation requires changes to glucose and lipid metabolic pathways, among others. Lipogenesis is a result of the activity of fatty acid synthase (FAS), and increased FAS expression is observed in the cells of a variety of malignancies [1]. It appears that *de novo* fatty acid (FA) synthesis plays an important role in this process. Preventing FAS activity by inhibiting its formation is a potential mechanism against cancer, as previously described [2–5]. It is evident that during the course of several neoplastic processes there are disturbances of lipid metabolism. Lysophosphatidic Acid (LPA) and Phosphatidic Acid (PA) are phospholipids involved in signal transduction and lipid biosynthesis. Isoform-specific functional LPA inhibitors induce apoptosis in MM cells by damaging poly-ADP-ribose (PARP), even in the presence of IL-6, IGF-1 and stromal cells [6].

Certain lipid compounds are not endogenously produced and must be ingested. Hence, it is important to understand the potential influence that an individual's diet has on the development of cancer. Current evidence supports an association between of obesity in MM [7]. In a large case–control study, Fritschi et al. [8] found protective effects of fresh fish consumption on the development of myeloma. Eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) are provided primarily by a diet rich in fish, and it is believed to effectively inhibit arachidonic acid (AA) transformation. Eicosanoids, formed from AA, take part in many carcinogenetic processes and

Abbreviations: FA, fatty acids; MM, multiple myeloma; SFA (SAT), saturated fatty acids; UNSAT, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic fatty acid; DHA, docosahexaenoic fatty acid; AA, arachidonic acid; LA, linoleic acid; ALA, α-linolenic acid; D5D, Δ5-desaturase; D6D, Δ6-desaturase.

* Corresponding author at: Department of Hematology, University Hospital, ul Kopernika 17, 31-501 Kraków, Poland. Tel.: +48 124247600.
E-mail address: mmjurczy@cyf-kr.edu.pl (A. Jurczyszyn).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.leukres.2014.12.010
0145-2126/

are important in both the development and progression of cancer. It is postulated that an appropriate relationship between n-3 and n-6 FA might be a protective factor against neoplastic development [9].

The implications of circulating essential FA on the inflammatory risk profile and clinical outcome of MM are not well understood. FA, particularly n-3 and n-6 polyunsaturated FAs (PUFAs), mediate a number of biologic processes including eicosanoid production, cell membrane physiology, cellular signaling, inflammation, gene regulation, and gene expression [9–12]. Therefore, accurate measurement of plasma FA might have important physiological and clinical implications. A better understanding of the distribution of FA in the plasma of patients could provide important insights into lipid metabolism as well as guide future biomarker selection [13–16]. The aim of our study was to compare the FA composition in the plasma of MM patients to those of healthy controls.

2. Materials and methods

2.1. Reagents

Butylated hydroxytoluene (BHT), 14% BF_3 and FAME standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical grade chloroform, methanol and n-hexane were obtained from Merck (Darmstadt, Germany). Water (18.2 MΩ, TOC < 5 ppm) was ultrapurified and filtered through a Milli-Q Plus system filter (Millipore, Bedford, MA, USA). Gases for chromatography of a 5.5 purity level were purchased from AirLiquide (Poland).

2.2. Patients

The patients were cared for at the Department of Hematology University Hospital in Krakow. Diagnosis of MM was based on current WHO criteria; patients with MM had greater than 10% plasma cells in the bone marrow (BM) aspirate and the significance presence of monoclonal protein in the serum or urine [17]. Patients were treated according to the guidelines of the Polish Myeloma Group [18]. Exclusion criteria included the presence of acute or chronic inflammatory.

2.3. Sample collection

Venous blood samples were collected in K_2-EDTA-containing tubes. Erythrocytes were separated from plasma by centrifugation (1500 × *g*, for 10 min). 10 μl of 0.05% BHT was added to each plasma sample. The study was conducted in accordance with the Declaration of Helsinki (1975) and approved by the Local Ethics Committee.

2.4. Lipid analysis

Total lipid from plasma was analyzed as described earlier [19]. Results were expressed in relative percentage of total FA. We calculated total saturated FA (SFA), monounsaturated FA (MUFA), n-6 and n-3 polyunsaturated FA (PUFA n-6, PUFA n-3) as well as *trans*-FA. Additionally, we calculated the n-3/n-6 ratio as well as the content of desaturases. D5 Desaturase was calculated as the ratio of 20:4n-6/20:3n-6, D6 Desaturase was calculated as the ratio of 20:3n-6/18:2n-6, D9 SCD1 Desaturase was calculated as the ratio of 16:1/16:0, and D9 SCD2 Desaturase was calculated as the ratio of 18:1n-9c/18:0.

2.5. Statistics

All data are presented as means ± standard deviation (SD). Differences between study groups were determined using the *F*-test, or the Mann–Whitney *U*-test if normality was not observed. Analysis of similarities between content of the FA in plasma of healthy people and MM patients was performed using clustering methods. Ward's method with Euclidean distance matrix was used to group a set of objects in such a way that objects in the same group are more similar to each other than to those in other groups. Calculations were performed using Statistica 10 (StatSoft®, Poland) software, and statistical significance was defined as $p \leq 0.01$.

3. Results

Our study included 120 patients. The control group was composed of 34 men and 26 women, and the MM group of 31 men and 29 women. The average time from MM diagnosis was 2.5 years. IgG kappa was identified in 52% of patients, IgG lambda in 15%, IgA kappa and IgA lambda in 7% each, 17% were light chain only, and 3% non-secretory. At the time of this report, four patients (7%) have died. Clinical characteristics of the MM patients are presented in Table 1. Healthy controls had no metabolic diseases, and had mean age of 57 years. Both group had normal weight (body mass index [BMI] 18.5–25 kg/m^2), and typical Western diet. Plasma FA composition in patients with MM and controls are shown in Table 2. The plasma SFA index (35.3% and 32.5%), and MUFA index (27.6% and 24.1%, $p = 0.001$) were both respectively higher in MM patients compared to the control group. Total plasma n-6 acids were significantly higher in the MM group compared to the control group (28.0% and 25.1%, respectively, $p = 0.004$).

Total n-3 FA were statistically significantly decreased in the plasma of patients with MM as compared to the control group (7.7% and 14.5%, respectively, $p = 0.001$). In the MM group, we observed decreased linolenic acid (1.7%), EPA (2.1%), and docosapentaenoic acid (0.3%) levels when compared to the control group (C18:3n-3 6.0%, EPA 3.8%, C22:5n-3 1.4%, $p = 0.001$).

We identified three *trans*-FA in the plasma of patients with MM and in the control group (3.8% and 1.4% respectively). The plasma n3/n6 ratio was statistically significantly decreased (two-fold) in patients with MM (ratio 0.3) compared to the control group (ratio 0.6). In addition, plasma activity of D6 desaturase and D9 SCD1 was decreased in the plasma of MM patients. However, the activity of plasma D5 and D9SCD2 desaturase was similar in both groups (Table 2).

To determine the covariant behavior of the measured variables, we analyzed variables to identify which best discriminate the FA profile in plasma from healthy people and patients with MM (Fig. 1). Variables were separated into four unique clusters. In the plasma of the cluster 1 control group, 22:5n-3, 20:3n-3, DHA as well as 14:0, 14:1, 15:0, 15:1, 17:0, 17:1 and 20:1 FA were present. In cluster 2 we observed grouping of n-6 FA, 22:0, 22:1, 22:2 and *trans* FA variants. In cluster 3, the follow FA variants grouped together: 16:1, n-3 FA 18:3 and EPA as well as 18:2n-6 and 20:2n-6. Cluster 4 included FA

Table 1
Clinical data of MM patients. Means ± SD are presented. Females: $n = 29$, males: $n = 31$.

Sex	BMI [kg/m^2 ± SD]	Hb [g/dl ± SD]	Calcium [mmol/l ± SD]	MP [g/l ± SD]	Plasmocytes [% ± SD]	ISS stage	Immunoglobulin
F	22 ± 3	11 ± 1	2 ± 0	33 ± 3	33 ± 10	I – 10 II – 10 III – 9	IgG kappa – 16 IgG lambda – 3 IgA lambda – 1 LCD – 5 Non-secretory – 2
M	24 ± 2	11 ± 1	2 ± 0	34 ± 9	42 ± 10	I – 7 II – 7 III – 17	IgG kappa – 15 IgG lambda – 6 IgA kappa – 4 IgA lambda – 1 LCD – 5

F, females; M, males; BMI, body mass index; MP, monoclonal protein; SD, standard deviations.

Table 2
Plasma fatty acid profile as a % of peak area in control ($n = 60$) and patients with MM ($n = 60$). Means ± SD.

Fatty acids	Control	MM	p
C14:0 myristic	2.0 ± 1.0 (0.4–5.1)	1.9 ± 1.1 (0.8–2.8)	
C15:0 pentadecanoic	2.0 ± 0.8 (0.1–2.3)	0.5 ± 0.5 (0.3–0.6)	0.001
C16:0 palmitic	15.6 ± 3.4 (11.9–18.8)	22.6 ± 3.1 (19.0–27.0)	0.001
C17:0 heptadecanoic	1.9 ± 1.3 (0.2–6.1)	1.1 ± 0.5 (0.5–2.2)	
C18:0 stearic	9.9 ± 2.5 (3.8–9.5)	9.2 ± 2.0 (6.2–13.6)	
C20:0 behenic	1.0 ± 0.4 (0.0–1.0)	0.1 ± 0.1 (0.0–0.3)	0.001
Total SFA	32.5 ± 4.4 (23.7–34.9)	35.3 ± 4.0 (29.5–43.5)	
C14:1n-5 myristoleic	2.5 ± 1.0 (0.4–9.0)	3.8 ± 1.3 (1.7–6.0)	
C15:1 cis-10-pentadecanoic	1.7 ± 0.9 (0.0–4.7)	5.6 ± 3.8 (1.6–15.1)	0.001
C16:1c palmitoleic	4.0 ± 1.6 (0.8–14.4)	2.1 ± 1.0 (0.9–3.6)	0.001
C17:1 cis-10 heptadecanoic	1.7 ± 1.5 (0.2–5.3)	0.7 ± 0.4 (0.2–1.2)	0.001
C18:1n-9c oleic	10.6 ± 2.4 (4.8–12.6)	10.1 ± 1.7 (7.4–12.6)	
C18:1n-7	1.0 ± 0.8 (0.0–3.1)	0.7 ± 0.2 (0.4–1.1)	
C20:1n-9 cis11eicosaenoic	1.7 ± 1.3 (0.0–5.1)	3.1 ± 1.3 (1.0–4.7)	0.001
C22:1n-9 erucic	0.9 ± 0.2 (0.0–3.6)	1.4 ± 0.9 (0.0–3.4)	0.001
C24:1 nervonic	0.1 ± 0.1 (0.0–0.2)	1.0 ± 0.5 (0.0–1.6)	0.001
Total MUFA	24.1 ± 5.4 (12.4–37.3)	27.6 ± 3.5 (21.5–35.1)	0.001
C18:2n-6 linoleic	5.2 ± 2.0 (1.1–10.0)	8.1 ± 2.8 (6.3–11.8)	0.004
C18:3n-6 γ-linolenic	0.9 ± 0.8 (0.0–3.4)	5.4 ± 1.1 (1.7–10.2)	0.001
C20:2n-6 cis 11,14eicosadienoic	2.3 ± 2.2 (1.0–6.4)	2.4 ± 1.5 (0.5–5.8)	
C20:3n-6 dihomo-γ-linolenic	2.3 ± 1.7 (1.7–6.0)	1.2 ± 0.8 (0.1–2.6)	0.001
C20:4n-6 arachidonic	12.2 ± 2.1 (9.9–21.6)	9.9 ± 2.5 (9.0–11.8)	
C22:2n-6 cis-13,16-docosadienoic	1.2 ± 0.7 (0.2–1.9)	0.1 ± 0.1 (0.0–0.2)	0.001
C22:4n-6 adrenic	1.0 ± 0.4 (0.6–2.5)	0.9 ± 0.5 (0.6–1.4)	
Total PUFA n-6	25.1 ± 8.0 (13.6–45.8)	28.0 ± 6.0 (16.0–33.3)	0.004
C18:3n-3 linolenic	6.0 ± 1.8 (4.0–19.5)	1.7 ± 0.7 (0.0–2.8)	0.001
C20:3n-3 cis11,14,17 eicosatrienoic	1.4 ± 1.3 (0.2–2.7)	1.8 ± 0.6 (0.0–3.1)	
C20:5n-3 EPA	3.8 ± 1.7 (2.5–9.9)	2.1 ± 0.8 (0.8–3.4)	0.001
C22:5n-3 docosapentaenoic	1.4 ± 1.0 (0.7–3.8)	0.3 ± 0.2 (0.0–0.6)	0.001
C22:6n-3 DHA	1.9 ± 1.2 (0.2–7.8)	1.8 ± 1.2 (1.2–5.1)	
Total PUFA n-3	14.5 ± 5.7 (4.7–33.2)	7.7 ± 2.9 (5.0–12.5)	0.001
C16:1n-9t palmitelaidic	0.5 ± 0.6 (0.0–1.2)	0.4 ± 0.4 (0.5–2.4)	
C18:1n-9t elaidic	1.7 ± 1.4 (0.0–2.1)	0.3 ± 0.4 (0.0–1.3)	0.001
C18:2n-6t linolelaidic	1.5 ± 1.5 (0.0–1.7)	0.7 ± 0.2 (0.4–1.1)	0.01
Total *trans*	3.8 ± 4.1 (0.0–5.1)	1.4 ± 1.0 (0.5–2.8)	0.001
n-3/n-6	0.6 ± 0.3 (0.4–1.5)	0.3 ± 0.1 (0.2–0.7)	0.001
D5 Desaturase	5.9 ± 1.9 (1.7–9.9)	7.7 ± 1.8 (2.5–13.2)	
D6 Desaturase	0.6 ± 0.2 (0.1–2.0)	0.1 ± 0.1 (0.1–0.7)	0.001
D9 SCD1 Desaturase	0.3 ± 0.2 (0.2–1.0)	0.1 ± 0.0 (0.1–0.2)	0.001
D9 SCD2 Desaturase	1.1 ± 0.3 (0.4–1.6)	1.1 ± 0.3 (0.8–1.7)	

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.% peak area calculated from fatty acid methyl esters. Different letters denotes statistical significant differences of fatty acid between groups. D5Desaturase was calculated as 20:4n-6/20:3n-6 ratio, D6 Desaturase was calculated as 20:3n-6/18:2n-6 ratio, D9 SCD1 Desaturase was calculated as 16:1/16:0 ratio, and D9 SCD2 Desaturase was calculated as 18:1n-9c/18:0 ratio.

variants 18:1, 18:0, 20:4n-6, and 16:0 (Fig. 1, top). The distribution of variants was different in the plasma of MM patients when compared with the control group. In cluster 1, 20:3n-3 and EPA, 14:0, 16:1 cis and trans dominated. The cluster 2 grouping included DHA, 18:3n-3, 14:1, 20:1 and 20:2n-6. Cluster 3 grouped primarily n-6 FA (18:2n-6, 20:3n-6 and 22:4n-6) as well as15:0, 17:0, 17:1 and 24:0. n-6 FAs were present in the cluster 4 (18:3n-6, 20:4n-6, and 18:2n-6), as well as 15:1, 16:0, 18:1, and 18:0 (Fig. 1, bottom).

4. Discussion

In an earlier study, we showed differences in FA content of the erythrocyte membranes in MM patients compared to healthy subjects [19]. In the present study, we showed that plasma FA profile also differs significantly between MM patients and controls. The SFA index, the plasma concentration of trans-FA, D6 desaturase activity were higher, while the n-3/n-6 ratio and D5 desaturase activity were lower in MM patients compared to controls. Additionally, there is distinct clustering of the variants of individual FA in plasma between MM patients compared to controls.

Both dietary intake and metabolic pathways influence the FA composition of the plasma [20–24]. Arachidonic acid originates from both diet and the elongation-desaturation process of its precursor, linoleic acid (LA 18:2n-6). The D5 and D6 desaturases are key enzymes of this pathway and are also involved in the n-3 fatty acid pathway, which favors the conversion of α-linolenic acid (ALA) into eicosapentaenoic acid (EPA). D5D and D6D are encoded by *FADS1* and *FADS2* genes, respectively. The use of a product to precursor ratio (AA/LA or EPA/ALA) as a surrogate measure to estimate desaturase activity is well established. Thus, indirect information can be gathered from the analysis of plasma lipid composition that provides a simple, suitable model for studying FA metabolism [25].

Inflammation in the tumor microenvironment has been recognized as one of the hallmarks of cancer. Endogenously produced lipid autacoids, locally acting small molecule lipid mediators, play a central role in inflammation and tissue homeostasis, and have recently been implicated in cancer. These lipid mediators are collectively referred to as eicosanoids and are generated by distinct enzymatic systems initiated by cyclooxygenases, lipoxygenases and cytochrome P450 [12,26–29].

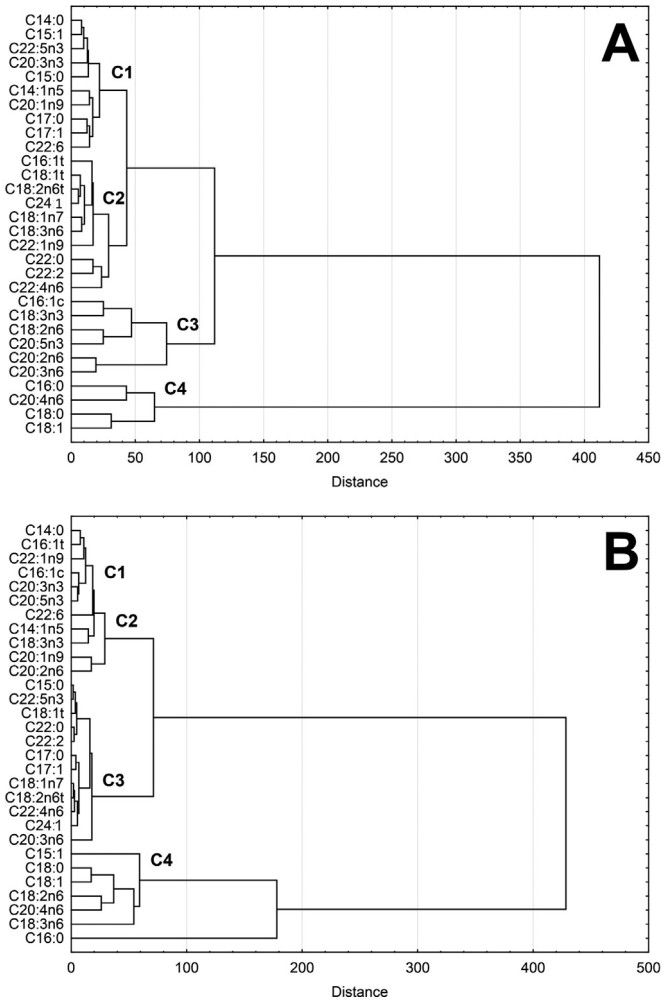

Fig. 1. Cluster analysis of the FA profile in plasma from healthy people (top) and from multiple myeloma patients (bottom). Data were segregated into 4 unique clusters of variables by hierarchical cluster analysis.

Several reports have indicated that MM pathophysiology is supported by a strong interaction between the clonal plasma cells and the surrounding bone marrow microenvironment. There is an increasing interest in the function of marrow fat including its capabilities to modulate marrow microenvironment to support bone remodeling and contribute to insulin-dependent FA metabolism [30]. Marrow fat may participate in lipid metabolism by clearing and storing circulating triglycerides, thereby providing a localized energy reservoir for osteogenesis [31]. It has been argued that the abnormal direction of differentiation of bone marrow cells such as osteoblast and adipocyte will have distinct impact on the process of bone formation. As a whole, adipocytic transdifferentiation is driven by an intricate and well-orchestrated signaling cascade. It involves regulated changes in the expression and/or activity of several key transcription factors, most notably PPARγ and several members of the CCAAT/enhancer-binding family of proteins (C/EBPs) [32]. Fish oil is a natural agonist for PPARγ and thus may exert its actions through this pathway. Long-term fish oil diet rich in omega-3 FAs could have a favorable impact on fat mass, skeletal integrity and bone loss with age, *i.e.* bone mass and trabecular bone microarchitecture in vertebrae [33]. Understanding what mechanisms and how pathways mediating the transdifferentiation between osteoblasts and adipocytes *in vivo* are modulated should be of relevance to the development of therapeutic control of bone loss in osteoporosis or high bone resorption seen in MM patients.

The n-3/n-6 ratio in plasma was lower in patients with MM compared to the control group, but showed higher level of D5 desaturase. However, activity of D6 desaturase and D9 SCD1 was significantly lower in patients with MM. The activity of the desaturases is regulated by PUFAs and can be affected by diabetes as well as cancer. Interestingly, a potential association between diabetes and hematologic malignancies has been proposed [34]. n-3 PUFAs exert anti-inflammatory actions by inhibiting pro-inflammatory signal transduction pathways, whereas AA (n-6 PUFA) facilitate inflammation. AA mediates inflammatory signals and serves as a precursor of pro-inflammatory eicosanoids. Moreover, in our study we observed significantly lower EPA/AA and n-3/n-6 ratio in the plasma of MM patients than in controls. These lower ratios in MM patients are probably associated with AA-related inflammatory effects [19,28,35,36].

Perhaps an imbalance of pro- and anti-inflammatory cytokines, caused by changes in the relative n-3/n-6 PUFA ratio, is one of the factors that might promote survival and growth of cancer cells. In particular, the n-3/n-6 PUFA ratio may play a role in influencing lymphoma risk, as these essential FA compete in the release of anti- and pro-inflammatory eicosanoids, respectively, and n-3 PUFAs suppress pro-inflammatory cytokine production. Previous studies have also shown a positive association of dietary saturated fatty acid (SFA) intake with higher lymphoma risk. For other cancers and diseases, n-3 PUFAs, found largely in fish oils, have been shown to be protective, while n-6 PUFAs, abundant in a typical Western diet, appear to be pro-inflammatory [11,36,37].

In our study, we observed distinct clustering of the individual FA variants in the plasma from MM patients compared to controls, particularly pronounced for long-chain FAs. This may indicate changes in gene expression of the elongases and desaturases during the development of the MM disease. These results are consistent with our previous work [19] and suggested significant roles of desaturases and elongases in the pathogenesis of cancer. The SFA index was significantly higher in the plasma of patients with MM than in controls. Stearic acid is an SFA that is converted into oleic acid by the liver microsomal desaturase system. Increased D9 desaturation and low saturation index have been observed in colorectal carcinoma, bronchogenic carcinoma, lymphoma, leukemia and malignant liver neoplasms. A prognostic significance in colorectal carcinoma has been suggested [38,39].

Alterations in the fatty acid profiles of plasma lipids are quite common in cancer and have been shown in a variety of neoplastic processes. All saturated, monounsaturated, and essential FA as well as their polyunsaturated derivatives are decreased, apparently as a consequence of the disease itself [26,40]. The major metabolic differences detected in MM patients at the time of diagnosis include increased levels of aminoacids such as isoleucine, phenylalanine, arginine, and tyrosine, as well as acetate. Decreased levels of 3-hydroxybutyrate, cholesterol, lysine, lipids, choline, and glutamine were also observed [40].

An unbalanced diet, particularly one high in fat, could contribute to homeostatic dysregulation of metabolic pathways, leading to diet-related health problems as obesity, diabetes, dyslipidemia, cardiovascular disease, inflammation, and also can be one of the factor important in cancer developing. Previous observations have shown that animal fat intake, not protein intake, as an influential factor for the development of lymphoma and other cancers [41–44]. On the other hand, Fernandez et al. [45] suggested that consumption of fresh fish significantly reduced the risk of MM, possibly because long chain polyunsaturated n-3 fatty acids inhibit the use of arachidonic acid, an n-6 fatty acid, for the production of prostaglandins and leukotrienes. A ratio of n-3/n-6 fatty acid intake $\geq$0.5 may be important in reducing cancer risk. Our study can provide insights on the dietary habits of the investigated groups. As an example, there was a significant difference in the

plasma n-3/n-6 PUFA ratio between the control group and the MM group. Subjects in the control group had a higher ratio than MM patients, indicating that the latter group consumed less n-3 unsaturated FAs in their diet.

FAs play an important biological and immunological role besides their energetic and storage functions. PUFAs are an important part of one's diet, as they are not synthesized by the organism. Thus, diet in combination with other elements such as an immunological disorder, may play a role in human illness. The potential influence of lipids on neoplastic development may be due to one of three mechanisms. These mechanisms include a potential influence on the metabolism of neoplastic cells, the function of lipids as intercellular messengers, or as mediators that take part in the inflammatory reaction. Studies evaluating the role of diet on cancer risk have been conducted, but with inconclusive results. Some reports have shown a beneficial role of fish-rich diet on neoplastic development [45]. Other have shown lack of effect [46], and others even an increase in the risk of developing cancer [47]. EPA and DHA are provided primarily by a diet rich in fish, and it is believed that they effectively inhibit AA transformation. Eicosanoids, formed from AA, take part in many carcinogenetic processes and are important in both the development and progression of cancer. It is postulated that an appropriate relationship between n-3 and n-6 FA may be a protective factor against neoplastic development [41].

The cytokine network is important in the development and survival of tumor cells as well as the progression of MM [19,48]. It is still unclear whether the neoplastic process is initiated by disruption of the network, or if it is the result of ongoing carcinogenesis. Cancer cells arise in every human being throughout life; a well-functioning immune system prevents their survival. The human diet is wholly dependent on each individual. Perhaps an imbalance of pro- and anti-inflammatory cytokines, caused by changes in the relative n-3/n-6 ratio, is one of the factors that may initiate the survival and further growth of cancer cells in the human body.

Among the potential risk factors that have been investigated, dietary FAs are thought to alter inflammatory responses and contribute to lymphomagenesis. Animal fats have been implicated as one dietary factor related to MM. A similar observation in men, regarding high consumption of milk, was made in patients with non-Hodgkin lymphoma [36,41]. The authors postulated that animal fat intake, not protein intake, is the influential factor in this case [10]. Correlation between animal fat and malignant disorders may be influenced by hormonal changes caused by the previously mentioned food products. However, Fernandez et al. [41] suggested that consumption of more than 2 servings of fish significantly reduced the risk of MM. According to recent studies, fresh fish and shellfish should be an important part of an MM patient's diet, as sources of n-3 unsaturated fatty acids and vitamin D. *Brassicaceae* vegetables, such as broccoli, cauliflower, cabbage and Brussels sprouts, are a source of antioxidants, sulfur compounds and vitamin C, and have protective effects against MM. A population-based case–control study pointed out that tomatoes and lettuce may also reduce the risk of MM [42,43,46]. However, nutrition recommendations based on retrospective diet recollection should be interpreted with caution. Quantitative prospective analysis of daily food portions would be a more accurate research method. Further study in this area is required, but we believe the data presented above should be carefully discussed with patients.

Our study provides additional insights on the potential role of FA metabolism in the development and growth of MM in humans, and suggests that exogenous and endogenous FA could affect not only the tumor microenvironment but also the immune response to cancer. Certainly, dietary habits not only could help prevent or delay the progression of cancer, and serve as an adjuvant for cancer treatment, but the serum fatty acid profile could also serve as predictive and/or prognostic biomarker. Additional research is needed in this regard.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

Acknowledgments

This project was partially supported by National Science Centre, Poland to the author Joanna Gdula-Argasińska DEC-2011/01/B/NZ7/00038.

Contributors: AJ, JC, JGA conceived and designed the experiments. JGA performed the experiments. JGA, JC, AJ, PP, AC, JJC, TL analyzed the data. AJ, JC, JGA, MD, ABS contributed reagents/materials/analysis tools. AC, JGA involved in statistics. JGA, JC, AJ, WP, JJC wrote the paper.

References

[1] Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pasternack GR, et al. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol 1997;150:201–8.
[2] Gabrielson EW, Pinn ML, Testa JR, Kuhajda FP. Increased fatty acid synthase is a therapeutic target in mesothelioma. Clin Cancer Res 2001;7:153–7.
[3] Pizer ES, Pflug BR, Bova GS, Han WF, Udan MS, Nelson JB. Increased fatty acid synthase as a therapeutic target in androgen independent prostate cancer progression. Prostate 2001;47:102–10.
[4] Menendez JA, Lupu R. Fatty acid synthase-catalyzed de novo fatty acid biosynthesis: from anabolic energy-storage pathway in normal tissues to jack-of all-trades in cancer cells. Arch Immunol Therap Exp 2004;52:414–26.
[5] Tirado-Velez JM, Joumady I, Saez-Benito A, Cozar-Castellano I, Perdomo G. Inhibition of fatty acid metabolism reduces human myeloma cells proliferation. PLoS ONE 2012;7:1–13.
[6] Hideshima T, Chauhan D, Hayashi T, Podar K, Akiyama M, Mitsiades C, et al. Antitumor activity of lysophosphatidic acid acyltransferase-beta inhibitors, a novel class of agents, in multiple myeloma. Cancer Res 2003;63:8428–36.
[7] Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;71:569–78.
[8] Fritschi L, Ambrosini GL, Kliewer EV, Johnson KC, Canadian Cancer Registries Epidemiologic Research Group. Dietary fish intake and risk of leukaemia, multiple myeloma, and non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 2004;13:532–7.
[9] Wendel M, Heller AR. Anticancer actions of omega-3 fatty acids – current state and future perspectives. Anticancer Agents Med Chem 2009;9:457–70.
[10] Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiological evidence. Am J Clin Nutr 2003;77:532–43.
[11] Lands B. Consequences of essential fatty acids. Nutrients 2012;4:1338–57.
[12] Gdula-Argasińska J, Garbacik A, Tyszka-Czochara M, Woźniakiewicz M, Paśko P, Czepiel J. Identification of lipid derivatives in Hep G2 cells. Acta Biochim Pol 2013;60:811–5.
[13] Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw K-T, Wareham NJ, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk cohort. Am J Clin Nutr 2010;92:1214–22.
[14] Zietemann V, Kröger J, Enzenbach C, Jansen E, Fritsche A, Weikert C, et al. Genetic variation of the FADS1 and FADS2 gene cluster and n-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. Br J Nutr 2010;104:1748–59.
[15] Białek A, Tokarz A. Conjugated linoleic acid as a potential protective factor in prevention of breast cancer. Postepy Hig Med Dosw 2013;67:6–14.
[16] Perona JS, Gonzalez-Jimenez E, Aguilar-Cordero MJ, Sureda A, Barcelo F. Structural and compositional changes in erythrocyte membrane of obese compared to normal-weight adolescents. J Membr Biol 2013;246:939–47.
[17] Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia 2009;23:3–9.
[18] Dmoszyńska A, Walter-Croneck A, Usnarska-Zubkiewicz L, Stella-Hołowiecka B, Walewski J, Charliński G, et al. Guidelines of Polish Myeloma Group concerning diagnosis and therapy of multiple myeloma and other plasmacytic dyscrasias for 2013. Acta Haematol Pol 2013;44:3–47.
[19] Jurczyszyn A, Czepiel J, Gdula-Argasińska J, Czapkiewicz A, Biesiada G, Dróżdż M, et al. Erythrocyte membrane fatty acids in multiple myeloma patients. Leuk Res 2014;38:1260–5.
[20] Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta 2002;326:143–9.

[21] Park Y, Nam S, Yi HJ, Hong HJ, Lee M. Dietary n-3 polyunsaturated fatty acids increase oxidative stress in rats with intracerebral hemorrhagic stroke. Nutr Res 2009;29:812–8.
[22] Matsumori R, Miyazaki T, Shimada K, Kume A, Kitamura Y, Oshida K, et al. High levels of very long-chain saturated fatty acid in erythrocytes correlates with atherogenic lipoprotein profiles in subjects with metabolic syndrome. Diabetes Res Clin Pract 2013;99:12–8.
[23] Takkunen M, Agren J, Kuusisto J, Laakso M, Uusitupa M, Schwab U. Dietary fat in relation to erythrocyte fatty acid composition in men. Lipids 2013;48:1093–102.
[24] Sertoglu E, Kurt I, Tapan S, Uyanik M, Serdar MA, Kayadibi H, et al. Comparison of plasma and erythrocyte membrane fatty acid compositions in patients with end-stage renal disease and type 2 diabetes mellitus. Chem Phys Lipids 2014;178:11–7.
[25] Martinelli N, Girelli D, Malerba G, Guarini P, Illig T, Trabetti E, et al. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. Am J Clin Nutr 2008;88:941–9.
[26] Kurup R, Nair R, Kurup PA. Isoprenoid pathway related cascade in multiple myeloma. Pathol Oncol Res 2003;9:107–14.
[27] Ding J, Tsuboi K, Hoshikawa H, Goto R, Mori N, Katsukawa M, et al. Cyclooxygenase isoenzymes are expressed in human myeloma cells but not involved in anti-proliferative effect of cyclooxygenase inhibitors. Mol Carcinog 2006;45:250–9.
[28] Kelavkar UP, Hutzley J, McHugh K, Allen KGD, Parwani A. Prostate tumor growth can be modulated by dietary targeting the 15-lipoxygenase-1 andcyclooxygenase-2 enzymes. Neoplasia 2009;11:692–9.
[29] Shao J, Li Q, Wang H, Liu F, Jiang J, Zhu X, et al. P-450-dependent epoxygenase pathway of arachidonic acid is involved in myeloma-induced angiogenesis of endothelial cells. J Huazhong Univ Sci Technol Med Sci 2011;31:596–601.
[30] Lecka-Czernik B. Marrow fat metabolism is linked to the systemic energy metabolism. Bone 2012;50:534–9.
[31] Lecka-Czernik B, Stechschulte LA. Bone and fat: a relationship of different shades. Arch Biochem Biophys 2014;561:124–9.
[32] Gao B, Yang L, Luo Z-J. Transdifferentiation between bone and fat on bone metabolism. Int J Clin Exp Pathol 2014;7:1834–41.
[33] Bonnet N, Somm E, Rosen CJ. Diet and gene interactions influence the skeletal response to polyunsaturated fatty acids. Bone 2014;68:100–7.
[34] Castillo JJ, Mull N, Reagan JL, Nemr S, Mitri J. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. Blood 2012;119:4845–50.
[35] Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat 2011;96:27–36.
[36] Morimoto Y, Conroy SM, Ollberding NJ, Henning SM, Franke AA, Wilkens LR, et al. Erythrocyte membrane fatty acid composition, serum lipids, and non-Hodgkin's lymphoma risk in a nested case–control study: the Multiethnic Cohort. Cancer Causes Control 2012;23:1693–703.
[37] Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. J Nutr Metab 2012;5:1–16.
[38] Pandey M, Khatri AK, Dubey SS, Gautam A, Shukla VK. Erythrocyte membrane stearic to oleic acid ratio in carcinoma of the gallbladder: a preliminary study. Eur J Surg Oncol 1998;24:43–6.
[39] Ruiz-Núñez B, Kuipers RS, Luxwolda MF, De Graaf DJ, Breeuwsma BB, Dijck-Brouwer DAJ, et al. Saturated fatty acid (SFA) status and SFA intake exhibit different relations with serum total cholesterol and lipoprotein cholesterol: a mechanistic explanation centered around lifestyle-induced low-grade inflammation. J Nutr Biochem 2014;25:304–12.
[40] Puchades-Carrasco L, Lecumberri R, Martínez-López J, Lahuerta JJ, Mateos MV, Prosper F, et al. Multiple myeloma patients have a specific serum metabolomics profile that changes after achieving complete remission. Clin Cancer Res 2013;19:4770–9.
[41] Ward MH, Zahm SH, Weisenburger MDD, Gridley MG, Cantor KP, Saal MRC, et al. Dietary factors and non-Hodgkin's lymphoma in Nebraska (United States). Cancer Causes Control 1994;5:422–32.
[42] Brown LM, Gridley G, Pottern LM, Baris D, Swanson CA, Silverman DT, et al. Diet and nutrition as risk factors for multiple myeloma among blacks and whites in the United States. Cancer Causes Control 2001;12:117–25.
[43] Vlajinac HD, Pekmezović TD, Adanja BJ, Marinković JM, Kanazir MS, Suvajdzić ND, et al. Case–control study of multiple myeloma with special reference to diet as risk factor. Neoplasma 2002;50:79–83.
[44] Hosgood III HD, Baris D, Zahm SH, Zheng T, Cross AJ. Diet and risk of multiple myeloma in Connecticut women. Cancer Causes Control 2007;18:1065–76.
[45] Fernandez E, Chatenoud L, La Vecchia C, Negri E, Franceschi S. Fish consumption and cancer risk. Am J Clin Nutr 1999;70:85–90.
[46] Tavani A, Pregnolato A, Negri E, Franceschi S, Serraino D, Carbone A, et al. Diet and risk of lymphoid neoplasms and soft tissue sarcomas. Nutr Cancer 1997;27:256–60.
[47] Pobel D, Viel JF. Case–control study of leukaemia among young people near La Hague nuclear reprocessing plant: the environmental hypothesis revisited. BMJ 1997;314:101–6.
[48] Jurczyszyn A, Czepiel J, Biesiada G, Gdula-Argasińska J, Cibor D, Owczarek D, et al. HGF, sIL-6R and TGF-β1 play a significant role in the progression of multiple myeloma. J Cancer 2014;5:518–24.