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Overweight and erythrocyte polyunsaturated fatty acid changes in menopause

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Abstract

Lipid disorders have been implicated in overweight and menopause. However, evidence on lipidomic analysis of fatty acids in erythrocytes of menopausal women is scarce. The aim of this study was to investigate the relationship between the body mass index within or beyond 5 years of menopause and erythrocyte fatty acid profile. This case-control study was conducted on out of 37 menopausal women total patients, 22 with body mass index ≥25 and 12 matched controls (body mass index <25). Experimental procedures were performed on the blood through robotic equipment for isolation of erythrocyte and cell membrane fatty acids were analyzed by using gas-liquid chromatography. Results showed that erythrocyte membranes did not change significantly in lipid composition between case and control group. However, the percentage of women who had a physiological content of saturated fatty acids was lower in case than in control group, and the percentage of women who had a physiological content of monounsaturated fatty acids and polyunsaturated fatty acids was lower in control than in case group. Woman with BMI>25 and non-physiological content of fatty acids, were richer in percentage of saturated fatty acids and poorer of monounsaturated fatty acids and polyunsaturated fatty acids than women with BMI<25. The percentage of physiological n-6/n-3 polyunsaturated ratio was lower in women with BMI>25 than in women with BMI<25. Interestingly, the percentage of patients that had physiological values of lipids beyond 5 years of menopause increased in comparison patients within 5 years of menopause. Notably, n-6/n-3 polyunsaturated fatty acids physiological ratio beyond 5 years of menopause increased in both case and control patients, indicating normalization over time. In conclusion erythrocytes fatty acids composition may be related to the body mass index and to the time from menopause.

Keywords: Menopause; BMI; lipidomic; fatty acids; omega 6; omega 3

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Introduction

Overweight/obesity is characterized by excessive body weight due to the accumulation of fatty tissue with negative effects on the health. It represents the sixth most important risk factor contributing to the overall burden of disease worldwide (1). For the World Health Organization (WHO), obesity is a complex and multi-factorial pathology, which involves environmental, social, cultural, genetic, physiological, metabolic, psychological and comportamental factors (2). In fact, the obese suffer from social bias, prejudice and discrimination, and different comorbidities including dyslipidaemia, coronary heart disease, hypertension and stroke, cancer, diabetes mellitus, osteoarthritis, and pulmonary diseases (2). In Italy, in 2015, more than a third of the adult population (35,3%) is overweight, while one in ten people is overweight (9,8%); overall, 45,1% of individuals aged 18 or over is overweight even if the analysis of the trend shows a decline of childhood overweight and obesity between 2008-2016 (3).The most used indicator in the clinical evaluation and in the classification of overweight and obesity is the BMI (Body Mass Index) and it is the numerical value obtained from the ratio between the weight in Kg and the height expressed in meters squared (normal weight, BMI 18.5-24.99; overweight, 25-29,99; obe-

sity class I, 30-34,9; obesity class II, 35-39,9; obesity class III, ≥40). Although BMI is a more accurate measurement method of total fats than simple body weight, it has some limits. In fact, with BMI measurement total body fat is overestimated in subjects with highly represented muscle mass and is underestimated in subjects affected by muscle loss (4). Moreover, BMI underestimates health risk for obese patients (5). The limit of BMI to indicate obesity in postmenopausal women does not seem to be appropriate. In fact, a study carried out on 1329 postmenopausal women and in which fat mass was measured by Dual-Energy X-Ray absorptiometry (DEXA) has indicated that for this category of subjects the limit of BMI should be 24,9 to define the obesity state (6). On the other hand, with advancing age women tend to lose bone mass and muscle mass and to increase visceral fat and this, together with a decrease in energy expenditure, can explain the higher risk of hypertension, lipid changes, diabetes and cardiovascular diseases that occur in postmenopausal women than in premenopausal women (7).

Cell membrane represents the fundamental structure of the cells in living organisms. They are constituted by proteins, glicoproteins, and lipids of which the main ones are phospholipids. The last are formed by a glycerol molecule with two fatty acid molecules and a phosphate group linked to different bases as choline, ethanolamine, serine, and inositol. Due to the structure, phospholipids have an amphipathic behaviour with the polar heads facing extracellular and intracellular aqueous environment, and the hydrophobic tails facing each other to form the thickness of the membrane.

Fatty acids (FAs) are divided into saturated and unsaturated fatty acids (SFA, USFA). SFA have the typical linear and rigid structure and USFA have a folded and slightly fluid structure. The most represented FA in mammalian membranes is palmitic acid (C16:0) whose chain can be stretched by specific elongases. The family of elongases includes elongases 1,3 e 6 that preferentially elongate saturated and monounsaturated fatty acids, whereas elongases 2,4 and 5 that elongate polyunsaturated fatty acids. SAT and monounsaturated fatty acids (MUFA) are also substrates for desaturates that are responsible for the polyunsaturated fatty acids (PUFA) synthesis. Moreover, FAs are divided into four classes based on the length of the monocarboxylic chain: short-chain FAs with 4 carbon atoms, medium-chain FAs with 6-12 carbon atoms, long-chain FA with 14-18 carbon atoms, and very long-chain FAs with 20-36 carbon atoms (8). As the affinity of SFA for desaturase enzymes is very high, long chain SFA cannot be formed in high amounts, and their increase can become an indicator of dysmetabolic lipid pathways (9).

Taken together, the studies above reported indicate the change of lipid tissue in menopause and the characteristic of FAs in cell membrane, but their relationship is not completely clarified. Therefore, we aimed to investigate : 1) the relation between FA composition of cell membrane and menopause in relation to the BMI; 2) the change of cell membrane FAs in the first 5 years and over 5 years after menopause.

Materials and Methods

Patients

37 menopausal women without cardiovascular, ipertensive, thyroid, diabete, and cancer disease were included in the present study authorized from the Local Health Unit Company Umbria (Cod. PG263274). All patients signed the informed consent. A specific questionnaire was carried out with the patients to find out: BMI, pregnancies, menarche age, pregnancies, physical activity, smoker, age of onset of menopause, years since onset of menopause. BMI was assessed according to the World Health Organization criteria and the study above reported (6).

Blood samples were collected between October 1, 2018, and February 28, 2019 by using standardized procedures.

Erythrocyte fatty acid profile analysis

The blood sample was taken by puncturing and dripping blood from the finger until collecting 0,5 ml in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) in the fasting state. Samples were stored at 4 °C until analysis. For mature erythrocyte selection, blood samples were centrifuged and entered a specific automatized procedure in which cell fraction was isolated on the basis of high density of the aged cells (9) The robotics performs all the subsequent steps for the cell lysis, isolation of the membrane pellets, phospholipid extraction from pellets and treatments (9). Erythrocyte cell membrane lipid analysis was performed by Fatpharmacy test that permits a lipidomic analysis with molecular check up of membrane lipids. The analysis includes capillary column gas chromatography (GC). Gas chromatography is the gold standard method for the determination of fatty acids, and it is performed under optimal separation condition, to identify a group of 10 cis fatty acids and 2 trans fatty acids (called lipidomic cluster). This cluster is always present in biological membranes because the fatty acids that make it up are part of the fundamental structure of the hydrophobic layer of the membrane. The lipidomic cluster of healthy subjects and the optimal intervals, to be used as a reference for the object of study samples, were obtained from an examination of the literature together with the study of the values obtained by Italian subjects present in the Lipinutragen database (10). The amount of each FA was calculated as a percentage of the total FA content (relative %), being > 97% of the GC peaks recognized with appropriate standards (9).

Statistical analysis

We consider two samples $\mathcal{S}_1$ (control sample with BMI<25) and $\mathcal{S}_2$ (case samples with BMI>25) of n_1 (15) and n_2 (22) patients, respectively, chosen among menopause women. One asks, whether the differences between the obtained experimental data are accidental, or the second sample is significant with respect to the first.

For $i = 1,2, \dots, r$ and $j = 1, 2$, let $y_{i,j}$ be the mean of the data concerning the concentration of the acid X_i under the treatment $\mathcal{T}_j$, and set

$$\overline{\mathbf{y}}_j = \begin{pmatrix} y_{1,j} \\ y_{2,j} \\ \cdots \\ y_{r,j} \end{pmatrix}.$$

Moreover, let $\mu_{i,j}$ be the mean corresponding to the world menopause women populations associated with the concentration of the acid X_i under the treatment $\mathcal{T}_j$, and put

$$\boldsymbol{\mu}_j = \begin{pmatrix} \mu_{1,j} \\ \mu_{2,j} \\ \cdots \\ \mu_{r,j} \end{pmatrix}.$$

Let $\mathbf{S}_j$ be the covariance matrix associated with $\mathcal{S}_j$, $j = 1,2$, and define a pooled-sample covariance matrix by $\mathbf{S}$

$$\mathbf{S} = \frac{n_1\mathbf{S}_1 + n_2\mathbf{S}_2}{n_1 + n_2 - 2}. \qquad (1)$$

We compute $\mathbf{S}$ by means of the Excel functions COVAR or COVARIANZA. Since these functions deal with population covariance rather than sample covariance, in (1) we take the product $n_1\mathbf{S}_1 + n_2\mathbf{S}_2$ rather than the product $(n_1 - 1)\mathbf{S}_1 + (n_2 - 1)\mathbf{S}_2$ in ([1, §6.4 (3)]). We test the null hypothesis $\boldsymbol{\mu}_1 = \boldsymbol{\mu}_2$ (H_0), without specifying the common value, versus the alternative hypothesis $\boldsymbol{\mu}_1 \neq \boldsymbol{\mu}_2$ (H_1). Note that, in general, it would not be advisable to test H_0 by taking each acid separately (see also [1, Exercise 6.4.7]). In our samples n_1=22, n_2=15, r=12, and hence $n_1+n_2 > r+1$. This condition guarantees that the matrix $\mathbf{S}$ is non-singular, and so the matrix $\mathbf{S}^{-1}$ is well-defined (see also [1]).

We consider the statistic ϕ defined by

$$\phi = \frac{n_1 + n_2 - r - 1}{(n_1 + n_2 - 2)r} \frac{n_1 n_2}{n_1 + n_2} (\overline{\mathbf{y}}_1 - \overline{\mathbf{y}}_2)^T \mathbf{S}^{-1} (\overline{\mathbf{y}}_1 - \overline{\mathbf{y}}_2). \qquad (2)$$

Without loss of generality, we can assume that the total world population of menopause women has a normal distribution, and that the samples $\mathcal{S}_1$ and $\mathcal{S}_2$ are independent. The studied corresponding test statistic is

$$F = \frac{n_1 + n_2 - r - 1}{(n_1 + n_2 - 2)r} \frac{n_1 n_2}{n_1 + n_2} (\overline{\mathbf{y}}_1 - \overline{\mathbf{y}}_2 - (\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2))^T \mathbf{S}^{-1} (\overline{\mathbf{y}}_1 - \overline{\mathbf{y}}_2 - (\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)),$$

which has a Fisher-type distribution with r and n_1+n_2-r-1 degrees of freedom (see also Theorem 6.4.1) (11). We compute the value of the statistics ϕ in (2). The Excel functions FDIST and DISTRIB.F compute the *p-value* related to F and ϕ, that is the quantity

$$Pr(\{F \geq \phi | H_0\}), \qquad (3)$$

where the symbol in (3) denotes the probability that the test statistic F is greater than or equal to ϕ, when the null hypothesis H_0 is satisfied. The p-value is the smallest significance level at which the obtained data lead to reject the null hypothesis. As we consider the significance levels $\alpha_1 = 0.05$ or α_2=0.01, the null hypothesis is rejected if and only if the p-value is less than or equal to α_1 (or α_2). Thus, a p-value $p \leq \alpha_1$ (or $p \leq \alpha_2$) indicates that the sample $\mathcal{S}_2$ is significant (resp. highly significant) with respect to $\mathcal{S}_1$, or equivalently that $\mathcal{S}_1$ is significant (resp. highly significant) with respect to $\mathcal{S}_2$.

Results

Population characteristics

The analysis of BMI revealed that of 37 menopausal women object of study, 15 had a BMI value <25 and 22 >25 (Tab.1). According to the World Health Organization criteria and the study showing the value of reference of BMI in menopause (6), the control samples were considered women with BMI <25 and case samples women with BMI >25. The media ±SD of BMI showed significant differences between the two groups (*p<0.001). There were no significant differences in age. Also percentage values of smokers, physical activity, and pregnancies were similar.

Table 1. Differences in population characteristic between women with BMI<25 (control samples) and BMI >25 (case samples). *p<0.001 against control group (BMI<25)

	BMI<25	BMI>25
Patients n° (%)	15 (41)	22 (59)
BMI (media±SD)	22±1.9	29±3.4*
Age years (media±SD)	57±10.8	59±7.88
Smoker (%)	13	13.6
Physical activity (%)	33	31.8
Pregnancies (%)	80	90

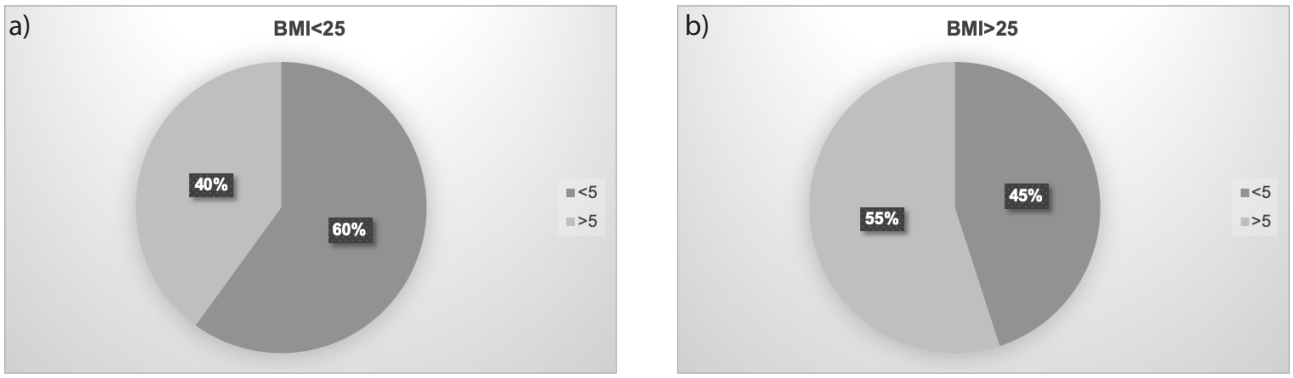

Figure 1. Distribution of population in relation to the time away from menopause. a) BMI<25; b) BMI>25.

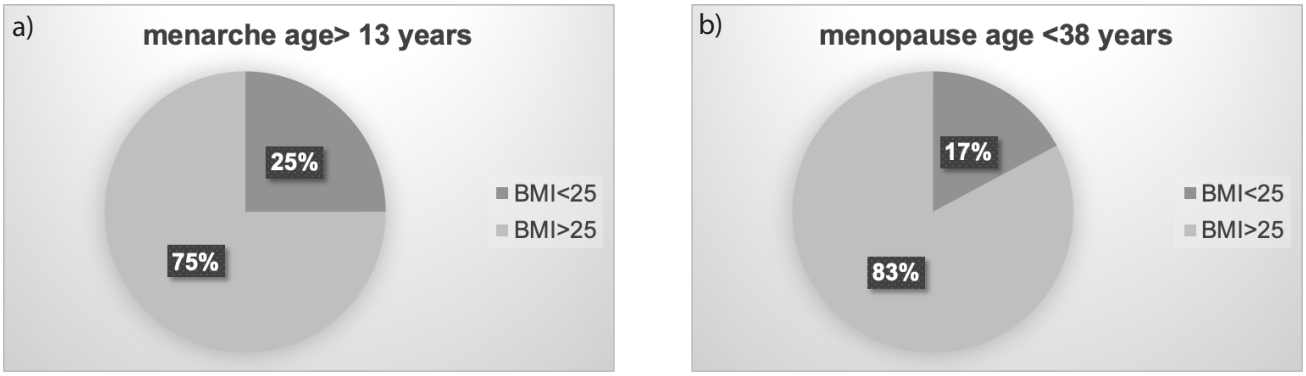

Figure 2. Distribution of population in relation to the time of onset; a) menarche age >13; menopause age <38.

We then analysed the time from menopause and divided samples between those who were within and those who were over 5 years after menopause (**Fig.1**). 60% (n°9) of control samples were within 5 years and 40% (n°6) over 5 years (**Fig.1a**); 45% (n°10) of control samples were within 5 years and 55% (n°12) over 5 years (**Fig.1b**)

We then analyzed the percentage of the population with an age of appearance of the menarche greater than 13 years and the onset of menopause at the age less than 38 years (**Fig.2**). Interestingly, the results showed that among women with menarche age >13 years, 75% were case samples (**Fig.2a**) and among women with the age of onset the menopause <38, 83% were case samples (**Fig.2b**).

Erythrocyte membrane fatty acid profile

SFA, MUFA, PUFA, and n-6 PUFA/n-3 PUFA levels in erytrocyte membrane were considered (**Table 2**). The results showed that the percentage of women who had physiological levels of SFA was lower in case samples than control samples, and percentage of physiological levels MUFA and PUFA was higher. All samples that did not have physiological levels, they always had higher values and never lower. Therefore, erythrocyte membrane of woman with BMI>25 were enriched in SFA and poorer in MUFA and PUFA than woman with BMI<25. Physiological level of n-6/n-3 PUFA ratio was lower in woman with BMI>25 than in woman with BMI<25, by indicating a high level of n-6 PUFA (**Table 2**).

Table 2. Differences in percent of patients that had physiological level of SFA, MUFA, PUFA, n-6/n-3 PUFA between control group (BMI<25) and case group (BMI>25).

		BMI<25	BMI>25
	range	% patients	% patients
SFA	30-45	80	68
MUFA	13-23	33	45
PUFA	28-39	27	59
n-6PUFA/n3 PUFA	3,5-5,5	47	27

Table 3. Differences in percent of patients that had physiological level of SFA, MUFA, PUFA, n-6/n-3 PUFA between case group and control group.

		BMI<25		BMI>25	
	range	%<5 years	%>5years	%<5 years	%>5years
SFA	30-45	89	67	60	75
MUFA	13-23	33	33	40	50
PUFA	28-39	22	33	50	67
n-6PUFA/n3 PUFA	3,5-5,5	33	67	0	50

Then, we analyzed the percentages of patients who had physiological values by dividing them between women who were within 5 years of menopause and those who were over 5 years of menopause (**Table 3**). As you can see, the percentages of physiological values increased over time, with the exception of the SFA of women belonging to the control group, indicating an improvement of the parameters over time regardless of the BMI.

However, if you consider the absolute values of SFA, MUFA, PUFA there are no significant variations between the case and the group control both in reference to the total of women and between women who are within 5 years of menopause and those who are beyond 5 years after menopause

Notably, the value of n-6/n-3PUFA ratio of control group, that was within the physiological range (**Table 3**), was similar if you consider the time within 5 years and beyond 5 years after menopause (Fig.4a). Differently, medium values (5.5-7 range) was reached by woman who were in menopause within 5 years and high value (>7) by woman who were in menopause beyond 5 years (Fig.4a). As above reported, no woman with BMI >25 who were in menopause within 5 years had physiological values of n-6/n-3PUFA ratio but this was achieved after 5 years, indicating a possible normalization over time (**Table 3**, **Fig. 4b**). Medium and high values were similar within and beyond 5 years after menopause (**Fig.4b**)

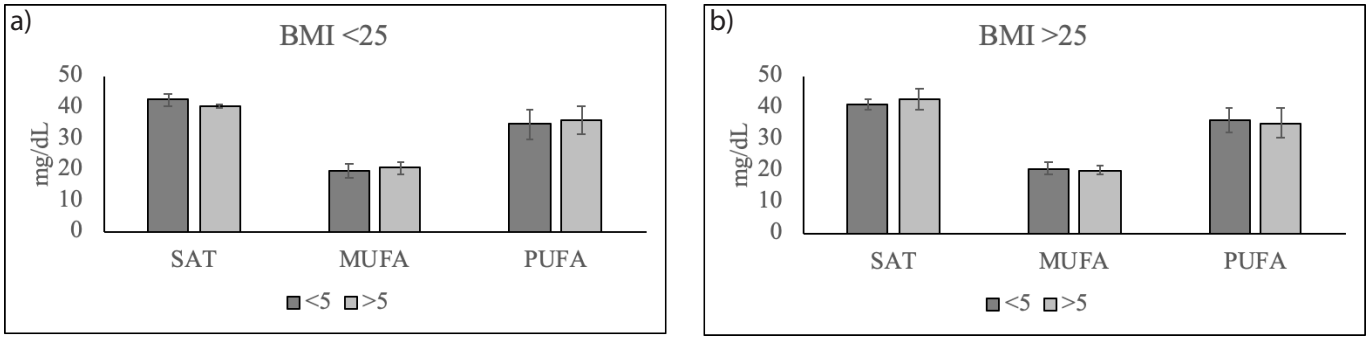

Figure 3. SFA, MUFA, and PUFA levels in control group (a) and case group (b).

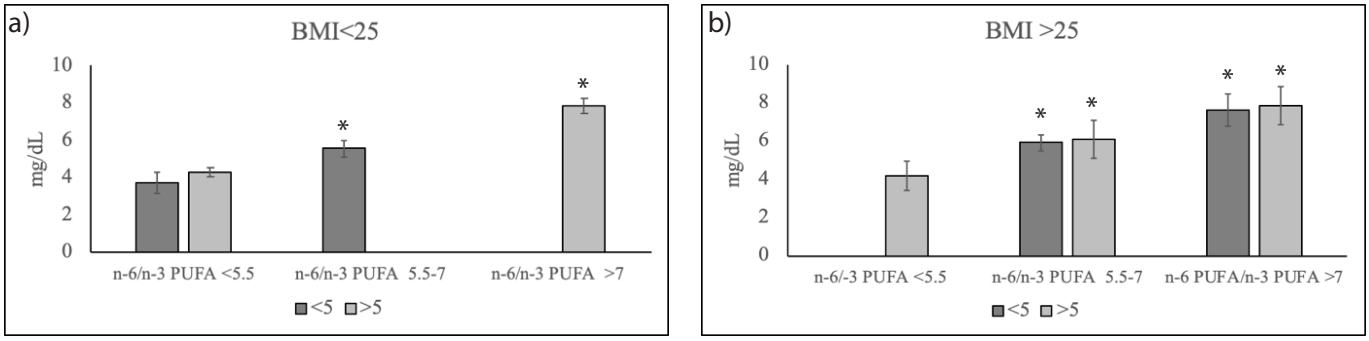

Figure 4. n-6/n-3PUFA ratio value in control group (a) and case group (b). *p<0.001 against n-6/n-3PUFA <5.

Discussion

Metabolically, the composition of isolated membrane from cells is the result of biosynthetic capacities that are put in place thanks to the presence of many cofactors for the enzymatic activities. The mature erythrocyte cannot biosynthesize lipids, so its membrane also depends on the exchanges it makes in vivo with the lipoproteins and with the tissues (12). In addition, the erythrocyte's membrane is composed of all the families of FAs. By considering that the average life of the erythrocyte is 120 days, the analysis in these cells provides a fairly stable picture of what happens in cell membranes. Moreover, the study was performed in erythrocytes because they are ideal cells for functional lipidomic analysis. In this study, we showed that BMI does not influence the composition of total SFA, MUFA and PUFA in cellular membrane but we identified BMI as a critical factor for the normalization of n-6/n-3PUFA ratio beyond 5 years after menopause.

The n-6 PUFA are formed thank to delta-6 desaturase that can be influenced by different cofactors such as Fe, Zn, Mg, and B2, B3, and B6 vitamins (13). Dihomo gamma-linolenic acid (DGLA regulates the n-6 PUFA metabolism, including prostaglandins, thromboxanes, and leukotrienes, molecules essential for anti-inflammatory and coagulative processes (14). Moreover, DGLA is the substrate for the enzyme delta-5 desaturase responsible for arachidonic acid (AA) synthesis (15). AA is the precursor of prostanoids, leukotrienes, and lipoxins, involved in inflammation process (16). The enzyme delta-5 desaturase is regulated by the presence of insulin and cortisol (17).

The omega-3 pathway starts by the action of enzyme delta-6 desaturase (18) with subsequent steps of elongation and desaturation, leading to the synthesis of eicosapentaenoic acid (EPA), and other types of prostanoids and leukotrienes (19). Elongation and desaturation of EPA gives docosahexaenoic acid (DHA) synthesis (19). The role of EPA and DHA is not only to balance the inflammatory effects of AA, but they play several other roles, producing neuroprotectins (from DHA) and resolvins (from EPA), which provide specific protective activity at picomolar concentration in tissues (12). It has been reported that a high n-6/n-3 PUFA ratio is found in today's Western diets and it is responsible for the promotion of the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (17).

Conclusions

In conclusion, our study suggests that the high n-6/n-3 PUFA ratio within 5 years after menopause could be a risk factor for different diseases and this risk decreases in time with the normalization of the ratio value.

Conflict of interest statement

The authors declare no conflict of interest.

Ethical compliance

The study authorized from the Local Health Unit Company Umbria (Cod. PG263274).

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