

Fatty Acid Profile in Plasma and Red Blood Cells in Chronic Fatigue Syndrome Patients

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Abstract: Chronic Fatigue Syndrome is an illness of unknown aetiology that has been associated with abnormalities in essential fatty acid metabolism, however this still remains unclear. We have compared the fatty acid profile in plasma and red blood cells and their dietary intake between a group of 20 patients diagnosed with CFS (18 women and 2 men) and 20 aged- sex- diet- low activity- matched controls. Compared to the controls, CFS patients show a decrease in the proportion of linoleic acid (t: 2.137, p: 0.039) and an increase in that of stearic acid (t: 2.602, p: 0.013) in plasma, however such differences have not been observed in the fatty acid profile of red blood cells. In these cases, no direct relationship has been observed between the dietary intake and fatty acid profile. Therefore, these results do not give support that long-chain polyunsaturated fatty acids may contribute in the better course of the pathology.

Keywords: Chronic fatigue syndrome, Essential fatty acids, Docosahexaenoic acid, Eicosapentaenoic acid, ω -3 Fatty acids.

INTRODUCTION

Patients affected by Chronic Fatigue Syndrome (CFS) are characterized by persistent or relapsing fatigue lasting six or more consecutive months, accompanied by musculoskeletal and multijoint pain, sore throat, headaches, sleep disturbance, postexertional malaise lasting more than 24 h and impairment in short-term memory or concentration severe enough to cause substantial reduction in previous levels of occupational, social or personal activities [1].

Omega-3 fatty acids are essential for normal growth and development; they may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis and other inflammatory and autoimmune disorders [2]. Ingestion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish or fish oil leads to decreased production of prostaglandin E₂ metabolites, thromboxane A₂ and leukotriene B₄ that favour platelet aggregation and vasoconstriction and induce inflammation [3]. In contrast, EPA and DHA ingestion increase the concentrations of thromboxane A₃, prostaglandin I₃, leukotriene B₅, that are weaker platelet aggregators, vasoconstrictors and inducers of

inflammation; at the same time decrease the production of interleukin-1 (IL-1) and tumor necrosis factor (TNF α) which are proinflammatory factors [4]. Also, polyunsaturated fatty acids are important in cell signalling molecules, they can act as second messengers or substitute for the classic second messengers of the inositide phospholipid and cyclic AMP signal transduction pathways. They can also act as modulator molecules mediating responses of the cell to extracellular signals [5-7].

Some studies have suggested that a dietary enriched regimen with ω -3 fatty acids could be of benefit for these patients [8], and another ones have shown that EPA-rich fatty acid supplementation in CFS patients has beneficial effects because of a possible abnormal membrane phospholipid metabolism in the brain [9, 10]. Moreover, some recent studies have shown that patients with Post Viral Fatigue Syndrome (PVFS) [11] and CFS [12, 13] appeared to have lower levels of essential fatty acids (EFAs) and elevated levels of saturated fatty acids in erythrocyte membranes as compared to controls. In fact, such infections are likely to impair the ability of the body to biosynthesise ω -3 and ω -6 long-chain polyunsaturated fatty acids, by inhibiting the δ -6 desaturation of the precursor essential fatty acids, α -linolenic acid and linoleic acid. This would therefore impair the membrane structure and functioning, and in turn diminish the production of eicosanoids [14]. However, another study found that levels of erythrocyte fatty acids

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between patients and controls did not reveal any significant differences [15], and another one showed that the arachidonic acid level was significantly lower in the CFS group than in the controls ($P > 0.05$) and that there were no differences in linoleic acid [16].

The aim of this study was to compare the fatty acid profile, both in plasma and red blood cells (RBC), between the CFS patients and normal individuals, and their relation to the habitual diet of the participants.

SUBJECTS AND METHODS

20 patients diagnosed with CFS were included in the study (CFS group), 18 women and 2 men, aged 18 to 60 years (mean age 38.49 ± 10.72). All fulfilled the CDC criteria for CFS (1) and diagnoses were confirmed in all patients by consensus of two physicians. 20 aged- sex- diet- matched controls were selected as volunteers (control group), 18 women and 2 men, aged from 23 to 55 years (mean age 36.48 ± 9.94). The control group was also physical daily activity- matched using an activity questionnaire to detect those subjects with a low grade of physical activity [17].

All participants were fully informed of the procedures and a written consent was obtained from all of them. The study was approved by the Ethical Committee of the Institut of Research of the Hospital of Bellvitge (IDIBELL- Campus of Bellvitge- Barcelona- Spain).

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Both patients and controls were requested to answer a specific dietetic questionnaire. Blood sample (5 ml) were obtained from all participants from an antecubital vein.

Fatty Acid Profile Determination

The venous blood samples were collected in EDTA-containing tubes. Subjected to centrifugation in order to separate the plasma from the red blood cells, the RBC were washed twice in saline solution and both plasma and RBC were frozen at -20°C until analysed.

Plasma and RBC fatty acids were subjected to transmethylation and the resulting fatty acid methyl esters (FAME) were analysed using a gas

chromatograph (Hewlett Packard, HP6890, USA) equipped with a flame-ionization detector. Peaks were identified based on the retention time in relation to FAME standard (Supelco, Bellefonte, PA), and peak areas were automatically computed. Values were expressed as % of total fatty acids.

Dietary Evaluation

All participants were asked to answer a 3 days 24h recall questionnaire and a specific dietetic questionnaire to evaluate the approximated daily intake of omega 6 and omega 3 EFAs. We have calculated the daily intake of omega 3 fatty acids (Linolenic acid (LIN), Docosapentaenoic acid (DPA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and omega 6 fatty acids (Linoleic acid (LA) and Arachidonic acid (AA); using USDA tables (United States Department of Agriculture Food Search for windows, version 1.0, database version SR15).

The Kolmogorov-Smirnov test was used to establish the normal distribution of the different variables. Differences between the CFS and the Control group in quantitative variables were analysed by Student's t-test for independent group. Data are expressed as mean \pm SD. The level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS v.16 (SPSS Inc., Chicago, USA).

RESULTS

Dietary evaluation: Daily fatty acid intake showed no significant differences between both groups. The mean daily intake of omega 3 and omega 6 fatty acids from CFS and control groups is shown in Table 1.

Table 1. Daily Intake Evaluation

Daily intake	CFS group	Control group
Linolenic acid (LIN)	0.43 ± 0.16	0.48 ± 0.41
Docosapentaenoic acid (DPA)	0.02 ± 0.03	0.04 ± 0.06
Eicosapentaenoic acid (EPA)	0.06 ± 0.05	0.12 ± 0.14
Docosahexaenoic acid (DHA)	0.13 ± 0.11	0.25 ± 0.29
Total omega 3	0.64 ± 0.30	0.89 ± 0.79
Linoleic acid (LA)	42.14 ± 13.1	35.14 ± 10.45
Arachidonic acid (AA)	0.02 ± 0.02	0.03 ± 0.05
Total omega 6	42.15 ± 13.11	35.17 ± 10.44

Dietary intake evaluation

Fatty acid profile: In plasma, we have found a statistical significance decrease in the proportion of

Table 2. Fatty Acid Percentages in Plasma and RBC

Fatty acid	Plasma		RBC	
	CFS group	Control group	CFS group	Control group
C16:0	20,08 ± 3,86	22,22 ± 3,27	20,89 ± 2,78	20,62 ± 1,70
C16:1	2,7 ± 2,12	1,69 ± 1,34	0,12 ± 0,30	0,18 ± 0,49
C18:0	11,78 ± 0,94*	10,09 ± 1,19	15,37 ± 2,76	15,60 ± 3,16
C18:1	19,19 ± 1,63	19,5 ± 2,40	15,73 ± 2,00	14,7 ± 1,38
C18:1t	1,85 ± 1,12	2,21 ± 0,92	0,51 ± 0,81	0,57 ± 0,68
C18:2 (LA)	21,78 ± 4,22*	24,26 ± 3,03	10,86 ± 1,86	10,05 ± 1,37
C18:3 (LIN)	0,46 ± 0,38	0,35 ± 0,44	0,15 ± 0,24	0,09 ± 0,18
C20:4 (AA)	9,43 ± 2,15	8,51 ± 1,72	13,2 ± 1,53	13,3 ± 1,03
C20:5 (EPA)	0,79 ± 0,52	0,56 ± 0,35	0,55 ± 0,55	0,42 ± 0,36
C22:6 (DHA)	2,33 ± 0,65	2,11 ± 0,71	3,42 ± 1,67	3,71 ± 1,02

All fatty acid expressed as % of total fatty acids ± SD.

*differences in percentages between Control and CFS plasma samples $p < 0.05$. C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:4 araquidonic acid, C20:5 eicosapentaenoic acid, C22:6 docosaheaxaenoic acid.

linoleic acid (C18:2) ($t = 2.137$, $p = 0.039$) and an increase in that of stearic acid (C18:0) ($t = -2.602$, $p = 0.013$) in CFS group in relation to the control group. Comparisons of RBC fatty acid profile between patients and their matched control did not reveal any significant differences. Table 2 shows the percentages of the different fatty acid profile in plasma and RBC in CFS and control group.

DISCUSSION

Different authors have proposed as a treatment for CFS the supplementation with omega 3 fatty acids [9-12, 14, 18] who have alteration of immune, neuroendocrine and autonomic functions. These fatty acids, that decrease the production of putative mediators of inflammation, may contribute to the amelioration of the functional states of those patients. A few studies have shown an improvement in their symptomatology with an EPA-rich essential fatty acid supplementation [9, 10] and also point out the essential fatty acids antiviral action [11]. These studies have found differences in the fatty acid profile in the erythrocyte membranes of CFS patients compared with those of Control group [11, 12] with an increase in saturated fatty acids like palmitic acid (C16:0) and stearic acid (C18:0) and a decrease in essential fatty acids like arachidonic acid (C20:4) and docosaheaxaenoic acid (C22:6). This may be attributed to a high oxidative stress or to alterations in the fatty acid metabolism observed in chronic diseases such as

CFS [19]. However, other authors did not observe these differences [15]. The discrepancy between studies could be explained by the different patient selection, or could be related to differences in the diet of the subjects. In our study, a population of similar characteristics was chosen: age-, sex-matched and including very low daily activity and similar omega 3 and omega 6 daily intake.

In conclusion, we did not find differences in the fatty acid profile between CFS group and controls in RBC. Although we found some differences in plasma they neither support the idea of a beneficial effect nor contribute to clarify the possible beneficial role of the long-chain polyunsaturated fatty acids. However, other similar studies should be carried out with more patients to find out about possible changes in fatty acid profile in chronic fatigue syndrome patients compared with normal population, to dilucidate their relevance in this disease.

REFERENCES

- [1] Fukuda K, Straus S, Hickie I. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med*. 1994; 121: 953-9.
- [2] Simopoulos A P. Essential fatty acids in health and chronic disease. *Am J Clin Nutr*. 1999; 70 (suppl): 560s-9s.
- [3] Simopoulos A P. Omega 3 fatty acids in inflammation and autoimmune diseases. *J Am Col Nutr*. 2002; 21 : 495-505.
- [4] Lefkowitz J B, Morrison A, Lee V, Rogers M. Manipulation of acute inflammatory response by dietary polyunsaturated fatty acid modulation. *The Journal of Immunology*. 1990; 145: 1523-9.

- [5] Mantzioris E, Cleland L G, Gibson R A, Neumann M A, Demasi M, James M J. Biochemical effects of a diet containing foods enriched with n-3 fatty acids. *Am J Clin Nutr*. 2000; 72: 42-8.
- [6] Simmopoulos A P. The importance of the ratio of omega-6/omega-3 essential fatty acids *Biomed Pharmacother*. 2002; 56: 365-79.
[http://dx.doi.org/10.1016/S0753-3322\(02\)00253-6](http://dx.doi.org/10.1016/S0753-3322(02)00253-6)
- [7] Haag M. Essential fatty acids and the brain. *Can J Psychiatry*. 2003; 48: 195-203.
- [8] Tamizi Bfar, Tamizi B. Treatment of chronic fatigue syndrome by dietary supplementation with w-3 fatty acids-a good idea? *Medical Hypotheses*. (2002) 58(3), 249-50.
<http://dx.doi.org/10.1054/mehy.2001.1507>
- [9] Puri B K, Holmes J, Hamilton G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *Int J Clin Pract*. 2004; 58: 297-9.
<http://dx.doi.org/10.1111/j.1368-5031.2004.00073.x>
- [10] Puri BK. The use of eicosapentaenoic acid in the treatment of chronic fatigue syndrome. *Prost Leu Essent Fatty Acids*. 2004; 70: 399-401.
<http://dx.doi.org/10.1016/j.plefa.2003.12.015>
- [11] Behan P O, Behan W M H, Horrobin D F. Effect of high doses of essential fatty acids on the postviral fatigue syndrome. *Acta Neurol Scand*. 1990; 82: 209-16.
<http://dx.doi.org/10.1111/j.1600-0404.1990.tb04490.x>
- [12] Liu Z, Wang D, Xue Q, Chen J, Li Y, Bai X, Chang L. Determination of Fatty Acid Levels in Erythrocyte Membranes of Patients with Chronic Fatigue Syndrome. *Nutr Neuro*. 2003; 6: 389-92.
<http://dx.doi.org/10.1080/10284150310001640356>
- [13] Maes M, Mihaylova I, Leunis JC. In chronic fatigue syndrome, the decreased levels of omega-3 polyunsaturated fatty acids are related to lowered serum zinc and defects in T cell activation. *Neuro Endocrinol Lett*. 2005 Dec;26(6):745-51.
- [14] Puri BK. Long-chain polyunsaturated fatty acids and the pathophysiology of myalgic encephalomyelitis (chronic fatigue syndrome). Review. *J Clin Pathol*. 2007; 60: 122-4.
<http://dx.doi.org/10.1136/jcp.2006.042424>
- [15] Warren G, McKendrick M, Peet M. The role of essential fatty acids in chronic fatigue syndrome: a case-controlled study of red cell membrane essential fatty acids (EFA) and a placebo-controlled treatment study with high dose of EFA. *Acta Neurol Scand*. 1999; 99: 112-6.
<http://dx.doi.org/10.1111/j.1600-0404.1999.tb00667.x>
- [16] Li YJ, Wang DX, Bai XL, Chen J, Liu ZD, Feng ZJ, Zhao YM. Clinical characteristics of patients with chronic fatigue syndrome: analysis of 82 cases. *Zhonghua Yi Xue Za Zhi*. 2005 Mar 16;85(10):701-4.
- [17] FAO/WHO-OMS/UNU Expert consultation report. Energy and Protein Requirements. Technical Report Series 724. Ginebra: WHO/OMS. 1985.
- [18] Tamizi far B, Tamizi B. Treatment of chronic fatigue syndrome by dietary supplementation with w-3 fatty acids – a good idea? *Med Hypotheses*. 2002; 58: 249-50.
- [19] Gray J B, Martinovic A M. Eicosanoids and essential fatty acid modulation in chronic disease and the chronic fatigue syndrome. *Med Hypotheses*. 1994; 43: 31-42.
[http://dx.doi.org/10.1016/0306-9877\(94\)90046-9](http://dx.doi.org/10.1016/0306-9877(94)90046-9)