

Determination of Fatty Acid Levels in Erythrocyte Membranes of Patients with Chronic Fatigue Syndrome

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(Received 22 December 2002; Revised 24 March 2003; In final form 19 October 2003)

Chronic fatigue syndrome (CFS) is an illness characterized by persistent and relapsing fatigue, often accompanied by numerous symptoms involving various systems of whole body. The etiology of CFS remains unclear. Literature reported whether the concentrations of the essential fatty acids in red cell membranes of CFS patients were decreased is controversial. In our study, Forty-two patients who fulfilled the diagnostic criteria defined by Centers for Disease Control and Prevention (CDC). Thirty-seven age- and sex-matched controls were selected from healthy medical staffs and volunteers. After lipid analysis, we found that the levels of the arachidonic acid (ARA) and docosahexanoic acid (DHA) were decreased in patients suffered from CFS. However, the levels of the palmitic acid and oleic acid were increased. We speculated that there are two possible mechanisms—one of which is that oxidative stress has led to an excessive oxidation and resulting in the above fatty acids. Alternatively, insufficiency of ingestion of fatty acids might not be the major cause.

Keywords: Chronic fatigue syndrome; Erythrocyte membrane; Fatty acid; Gas chromatography

INTRODUCTION

Chronic fatigue syndrome (CFS) is a multisystem disorder with unexplained prolonged fatigue, myalgia, sleep disturbance, anxiety, depression and impaired concentration. A viral infection often acts as a precipitating factor and the term “post-viral fatigue syndrome (PVFS) has been considered synonymously with CFS at one time, which suggested certain enteroviruses and/or the EB virus might be implicated in a proportion of patients (Behan and Bakheit, 1991). There is no effective treatment for

most of CFS patients at present. One of the recent studies has shown that patients with PVFS appeared to show some decreased levels in essential fatty acids (EFAs) and elevated levels of saturated fatty acids (SFA) in erythrocyte membranes as compared to controls (Behan *et al.*, 1990). However, from another repeated study by using the Oxford Criteria to define CFS, it was found that levels of erythrocyte fatty acids between patients and the matched controls did not reveal any significant differences. Also, there were no significant differences between the erythrocytic fatty acid profiles of patients and placebo groups at the beginning and the end of studies (Warren *et al.*, 1999). The differences between the two results might be due to the differences existed in the diagnostic criteria or the source of included patients. Here, we applied a more well-known criteria from the Centers for Disease Control and Prevention (Strauss, 1998), to screen patients suffered from CFS, and determined the fatty acid levels in erythrocyte membranes of them to study whether any alterations existed on the levels of essential fatty acids in erythrocyte membranes.

SUBJECTS AND METHODS

Forty-two patients diagnosed with CFS were included in this study. There were 20 men and 22 women, aged from 19 to 51 years (mean age 35.2 ± 7.8). All works were performed by a medical team including experienced, certificated neurologists and psychologists, and each patient came from a specific out-patient clinic setting. A detailed

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physical and neurological assessment was carried out. The routine laboratory investigations were carried out which included urinalysis, complete blood count, blood sedimentation rate, blood electrolytes, creatine, liver function tests, muscle enzymes, thyroid function tests, chest X-ray, electrocardiography and serological tests for common viruses, including Coxsackie B group, Herpes simplex I and II, cytomegalovirus and the Epstein-Barr virus. Immunological studies included immunoglobulin levels, antinuclear antibodies and rheumatoid factors. Some necessary CT scan and MRI of the skull were done to exclude multiple sclerosis or potential abscess. After the screening tests by SCL-90, CCDIR-2 depression criteria and Hamilton Depression Scale, those patients who had primary depression or somatic symptoms scores, but their scores were more than 2.5 in SCL-90, were excluded. Thirty seven age- and sex-matched controls were selected as volunteers, 18 men and 19 women, aged from 19 to 50 years (mean 32.0 ± 10.6). All controls were healthy subjects who have received no treatment for any current illness.

Preparation of the Erythrocyte Membranes

Five milliliter aliquots of whole blood were taken into heparin-containing tubes and the erythrocytes were separated and washed in saline. The pure erythrocytes were dissolved and washed in the Tris-HCl solution. Centrifuge for 20 min at 9000 r/min. Under 4°C to enable the erythrocyte membranes precipitate. Wash repeatedly for 3 to 5 times, and get white samples of erythrocyte membrane.

After being lyophilized, the red cell membranes were stored at -30°C, until all procedures of the study were completed (Zhang, 1997).

Lipid Analysis

Analytical grade solvents were redistilled in an all-glass system. All glassware was rinsed with chloroform-methanol 2:1 (v/v) and dried under nitrogen. Fatty acid and fatty acid methyl ester standards (Sigma) were certified to be >99% pure (Fig. 1). Unsaturated lipid standards were purchased in packaged ampoules under inert gas to prevent oxidation. 10 mg of erythrocyte membranes was precisely weighed in glass tubes. An internal standard consisting of 80 µg of heptadecanoic acid (C17:0) was added. Analysis was performed by using GC-9A gas chromatography (Shimadzu) equipped with a flame ionization detector. Nitrogen was used as the carrier gas. The split ratio was 6:1. The transesterification method and time program has been described by Lepage and Roy (1986). After the standards of the fatty acids were added to

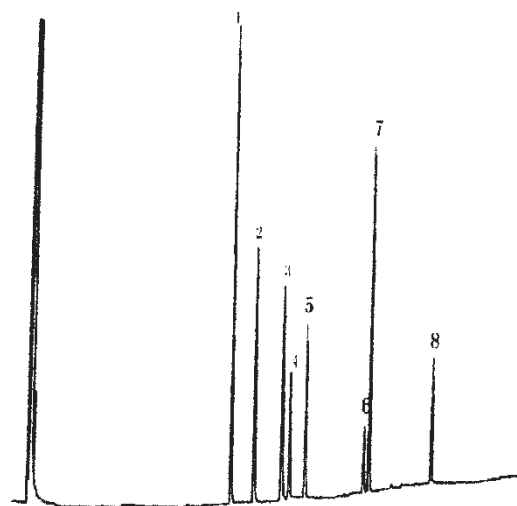


FIGURE 1 Gas chromatogram profiles of the methyl esters of fatty acids of standards.

the samples, the recovery rate of individual fatty acids from biological samples was evaluated, and the results are showed in Table I.

Statistics

Sample size calculations were made prior to the study by using Fisher's Exact test for equal numbers with 0.80 power and 0.05 significance level. Blood fatty acid levels for patients and the controls were compared by independent *t*-test.

RESULTS

By our study we found the levels of essential fatty acids-arachidonic (ARA) and docosahexanoic acids (DHA) decreased significantly in CFS patients (*P* value < 0.01 and 0.05, respectively). On the other hand, the ratios of non-essential unsaturated fatty acid oleic acid and the saturated fatty acid palmitic acid to total determined fatty acids were increased (Figs. 2 and 3). Other fatty acids such as stearic acid, linoleic acid (LA), and dihomo-gamma-linolenic acid (DGLA) acid showed no statistical differences as compared to controls (Table II).

TABLE I Recovery rate (%) of each determined fatty acid

Standards (µg)	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:3}	C _{20:4}	C _{22:6}
20	100.7	95.8	85.5	106.3	94.3	98.6	89.1
60	97.1	98.9	92.4	90.9	92.6	90.0	88.2
100	94.4	98.1	89.6	85.8	98.9	86.3	88.6
160	93.9	92.7	92.5	88.7	98.7	85.3	86.1

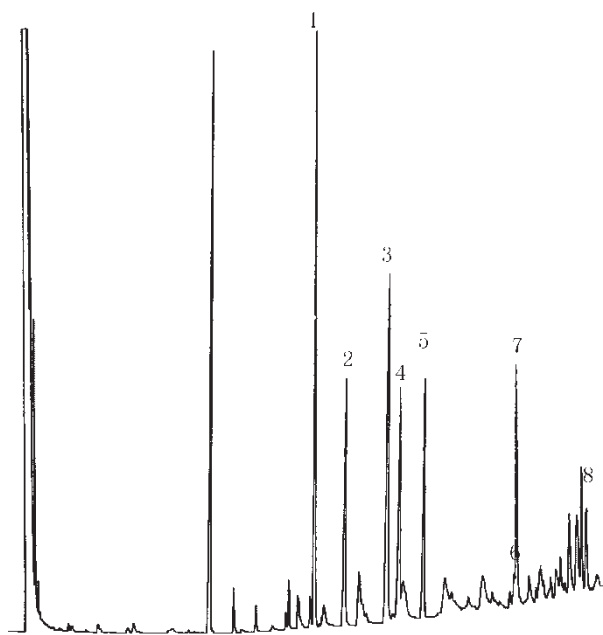


FIGURE 2 Gas chromatogram profiles of the methyl esters of fatty acids in the erythrocyte membranes of patient with CFS.

DISCUSSION

This study appears consistent to the study from Behan *et al.* (1990). As review the recent studies on the pathogenesis about CFS, we considered that the reduction of essential fatty acids ARA and DHA might chiefly based on the following causes.

Since EFAs could not be synthesized within the human body, minimum consumption should be maintained for proper metabolism. Parent $n - 3$ and

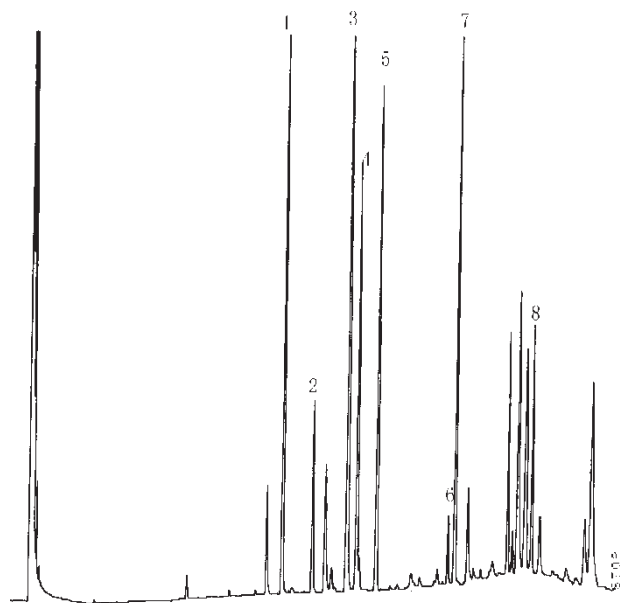


FIGURE 3 Gas chromatogram profiles of the methyl esters of fatty acids in the erythrocyte membranes a control subject: 1, C_{16:0}; 2, C_{17:0}; 3, C_{18:0}; 4, C_{18:1}; 5, C_{18:2}; 6, C_{20:3}; 7, C_{20:4}; 8, C_{22:6}.

TABLE II Erythrocyte membrane fatty acid analysis in patients with chronic fatigue syndrome and age- and sex-matched normal controls

	C16:0	C18:0	C18:1	C18:2	C20:3	C20:4	C22:6
Control	$0.2597 \pm 2.144 \times 10^{-2}$	$0.2136 \pm 1.504 \times 10^{-2}$	$0.1307 \pm 1.031 \times 10^{-2}$	$0.1394 \pm 1.515 \times 10^{-2}$	$2.31 \times 10^{-2} \pm 5.054 \times 10^{-3}$	$0.1718 \pm 2.342 \times 10^{-2}$	$6.12 \times 10^{-2} \pm 2.071 \times 10^{-2}$
Patients	$0.2736 \pm 3.138 \times 10^{-2}$	$0.2195 \pm 1.527 \times 10^{-2}$	$0.1364 \pm 9.613 \times 10^{-3}$	$0.1401 \pm 1.286 \times 10^{-2}$	$2.26 \times 10^{-2} \pm 6.565 \times 10^{-3}$	$0.1552 \pm 3.082 \times 10^{-2}$	$5.26 \times 10^{-2} \pm 1.634 \times 10^{-2}$
P value	0.026	0.083	0.013	0.842	0.659	0.009	0.034

Values are expressed as % of total determined fatty acids (% \pm SD).

$n - 6$ EFAs may undergo a series of desaturations and elongations before various derivative and parent EFAs are incorporated into membrane phospholipids. The etiology of CFS remains unclear. However, a number of studies have shown that oxidative stress may be involved in its pathogenesis, i.e. oxidative stress might play a significant role in the pathophysiology of CFS; and that oxidative stress due to excess free radical formation appears a contributor to the pathology of CFS and was also associated with symptom presentation (Richards *et al.*, 2000; Singh *et al.*, 2002); oxidative stress has led to excessive oxidation and therefore reduction levels of these fatty acids. Moreover, oxidative stress could induce ARA release from human lung cells (Pawliczak *et al.*, 2002). On the other hand, in the patients with CFS, the levels of TNF α were higher than the healthy controls (Moss *et al.*, 1999). Previous studies have shown that ARA could be released from membranes by the action of cytokine TNF α (Zhang and Dziak, 1996). And these patients are always in the state of chronic stress. One study (Maes *et al.*, 2000) found that psychosocial stress might influence the EFA metabolisms in human.

In our study, there were no significant differences between the LA and DGLA (both belong to EFA) of patients and the control group. ARA could be synthesized within the body by desaturations and elongations of LA and DGLA. Therefore, by these evidences it is suggested that the decreased concentrations of ARA and DHA in erythrocyte membranes might not due to the insufficiency of fatty acids ingestion. Besides, we assumed that the increased ratios of palmitic and oleic acid to total fatty acids might be secondary to the decreased levels of essential fatty acids ARA and DHA. Also, the increase level of non-essential acid oleic acid might be related to the maintenance of constant ratio among various unsaturated fatty acids in erythrocyte membranes which is quite essential to keep the normal fluidity and function of cell membranes (Jiang and Huang, 1992).

Previous studies have shown that fatty acids whether released from membrane phospholipids by cellular phospholipases, or formed from the diet, or other aspects of the extracellular environment, are all important cell-signaling molecules. They might act as second messengers of the phosphatidylinositides and cyclic AMP via the signal transduction pathway. They might also act as modulator molecules mediating responses of the cell to extracellular signals (Graber *et al.*, 1994). It has been shown that

fatty acids rapidly and directly alter the transcription of specific genes (Clarke and Jump, 1994), and thus resulting in the disturbance of signal transduction and the response-mediating molecules, which might induce the impairment of immune and circulation functions. Therefore the symptoms of CFS, such as recurrent sore throat, tender lymph nodes, muscle aches, arthralgia, headaches, might share some relationships with above dysfunctions.

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