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Free Radical Biology & Medicine 39 (2005) 584 – 589

www.elsevier.com/locate/freeradbiomed

Original Contribution

Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms

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Received 1 September 2004; revised 21 February 2005; accepted 15 April 2005
Available online 6 May 2005

Abstract

The aetiology of chronic fatigue syndrome (CFS) is unknown; however, recent evidence suggests excessive free radical (FR) generation may be involved. This study investigated for the first time levels of 8-iso-prostaglandin-$F_{2\alpha}$-isoprostanes alongside other plasma markers of oxidative stress in CFS patients and control subjects. Forty-seven patients (18 males, 29 females, mean age 48 [19–63] years) who fulfilled the Centres for Disease Control classification for CFS and 34 healthy volunteers (13 males, 21 females, 46 [19–63] years) were enrolled in the study. The CFS patients were divided into two groups; one group had previously defined cardiovascular (CV) risk factors of obesity and hypertension (group 1) and the second were normotensive and nonobese (group 2). Patients had significantly increased levels of isoprostanes (group 1, $P = 0.007$; group 2, $P = 0.03$, unpaired t test compared to controls) and oxidised low-density lipoproteins (group 2, $P = 0.02$) indicative of a FR attack on lipids. CFS patients also had significantly lower high-density lipoproteins (group 1, $P = 0.011$; group 2, $P = 0.005$). CFS symptoms correlated with isoprostane levels, but only in group 2 low CV risk CFS patients (isoprostanes correlated with; total symptom score $P = 0.005$; joint pain $P = 0.002$; postexertional malaise $P = 0.027$, Pearson). This is the first time that raised levels of the gold standard measure of in vivo oxidative stress (isoprostanes) and their association with CFS symptoms have been reported.

Keywords: Oxidative stress; Chronic fatigue syndrome; Free radicals; Cardiovascular risk factors

Introduction

Chronic fatigue syndrome (CFS) is a condition characterised by debilitating fatigue and other nonspecific symptoms resulting in significant disability, and its pathophysiology continues to remain elusive. A number of biological systems have been implicated and there is mounting evidence that oxidative stress [1–5] and, more specifically, lipid peroxidation contribute to the disease process [6] and to some of the symptoms in the illness [1]. Oxidative stress has been defined as a disturbance to the equilibrium status of prooxidant and antioxidant systems in favour of prooxidation. The term oxidative stress is used to describe a number of chemical reactions involved in the production of free radicals and other reactive molecules that are potentially able to induce cellular injury.

While free radicals may generate tissue oxidative injury it is also evident that other oxidative by-products, especially peroxidised lipids such 8-iso-prostaglandin $F_{2\alpha}$, may be even more pivotal in the pathological process. 8-Iso-prostaglandin $F_{2\alpha}$ is a member of the F_2-isoprostane family and can exert potent biological activity, such as platelet activation, and act as a powerful vasoconstrictor of the peripheral vasculature [7,8]. Such biological effects may be instrumental in the development of some of the vascular features that characterise patients with CFS [9,10].

Abbreviations: CFS, chronic fatigue syndrome; CV, cardiovascular; oxLDL, oxidised low-density lipoproteins; HDL, high-density lipoproteins; CDC, Centres for Disease Control; CVD, cardiovascular disease; BMI, body mass index; MAP, mean arterial pressure; GSH, glutathione; LDL, low-density lipoprotein; NF-κB, nuclear factor-kappa B; HSV, 1-herpes simplex virus type 1.

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doi:10.1016/j.freeradbiomed.2005.04.020

A further indication of the in vivo consequences of increased lipid peroxidation would be higher levels of oxidised low-density lipoproteins (oxLDL) accompanied by low levels of high-density lipoproteins (HDL), which are associated with the development of atherosclerosis [11]. This study set out to investigate, for the first time, levels of 8-iso-prostaglandin $F_{2\alpha}$ alongside other markers of oxidative stress and antioxidant status in well-defined CFS patients and comparable control subjects, and to relate these levels to reported clinical symptoms of CFS.

Subjects and methods

Fifty-four patients were recruited from a register of several hundred local CFS patients. After a medical examination 47 patients (19 males and 28 females, mean age 48 years [19–63 years], 6 current, 3 ex-, and 38 nonsmokers) were found to fulfil the Centres for Disease Control (CDC) classification for CFS [12]. Seven of the patients were excluded: one had diabetes, one had a possible neurological condition underlying the fatigue, one had angina, two patients were unable to undertake the tests, and two did not fulfil the criteria for CFS. The mean length of illness was 9.2 (SD 5.7) years. All patients scored their CFS symptoms (the main 8 Fukuda et al. [12] CFS symptoms) [muscle pain, joint pain, headaches, postexertional malaise, sore throat, tender lymph nodes, headaches, unrefreshing sleep] as either absent (0), mild (1), moderate (2) or severe (3). Hypertension and obesity are both risk factors for cardiovascular disease (CVD), and therefore we divided the CFS patients into two groups for the analyses, low and high risk.

Thirty-four healthy volunteers who were matched (to the patients) for sex, age, and smoking status (13 males and 21 females, 46 [19–63] years, 5 current, 2 ex-, and 27 nonsmokers) were also recruited.

The local medical ethics committee approved the study which was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Each volunteer gave written informed consent to take part. One physician (CU) examined all patients. Height and weight measurements were recorded for all subjects and body mass index (BMI) was calculated. Blood pressure measurements were taken both supine and immediately upon standing and the mean arterial pressure (MAP) was calculated using the formula; MAP = [(2 × diastolic) + systolic] / 3.

A 15-ml blood sample was taken from the antecubital fossa and collected into tubes containing EDTA. All blood samples were taken at the same time of day. The blood was centrifuged for 15 min at 3500 rpm at 4°C, and plasma was then removed, aliquoted, and stored at −70°C until assayed for isoprostanes, oxLDL, cholesterol, and HDL. The buffy coat was removed and the remaining red pellet from the 10-ml EDTA blood tube was washed three times with normal saline (0.9% sodium chloride solution). This resulted in a pellet of packed red blood cells. Levels of the antioxidant glutathione (GSH) were measured on a spectrophotometer from the packed red blood cells by the method of Ellman [13]. Plasma isoprostanes were measured by gas chromatography-mass spectrometry following the method described by Roberts and Morrow [14]. Total cholesterol and HDL levels were measured on a Cobas Bio centrifugal analyser using products from Roche. Plasma oxLDL levels were measured by ELISA (Mercodia, Sweden).

Results

CFS patients in group 1 (CVD risk factor group) [n = 16; 5 males and 11 females, mean age 52.4 years (35–62 years)] were obese (BMI >30) and hypertensive, as defined by the European Society of Hypertension [systolic >140 mm Hg, or diastolic >90 mm Hg] [15]. CFS patients in group 2 [n = 31; 14 males and 17 females, mean age 46 years (19–64 years)] were normotensive and had a BMI of <30. Each patient group was compared with sex- and age-matched controls. Four of the controls had hypertension and they were placed in the control group which matched patient group 1 (CVD risk factor group). At the time of study none of the subjects with hypertension were on any medication to lower BP, although this was subsequently addressed.

The data were normally distributed and an unpaired *t* test was used to compare the mean levels of all the parameters between the groups. Pearson correlation coefficient was used as a measure of linear association.

CFS patient group 1 (CVD risk factor group) had statistically significantly increased BMI ($P < 0.001$), supine blood pressure (systolic $P = 0.049$, and diastolic $P = 0.023$), supine MAP ($P = 0.016$), and 8-iso-prostaglandin $F_{2\alpha}$-isoprostanes ($P = 0.007$), and significantly lower HDL ($P = 0.011$) and GSH ($P = 0.023$) when compared with their control group (Table 1).

CFS patient group 2 (CVD low-risk factor group) had statistically significantly higher levels of oxLDL ($P = 0.02$) and 8-iso-prostaglandin $F_{2\alpha}$-isoprostanes ($P = 0.03$) and significantly lower HDL levels ($P = 0.005$) than their matched control group (Table 2).

Cholesterol levels were not significantly different between either of the patient groups and their controls.

To assess symptom severity we used the CDC 1994 (Fukuda) severity score that we derived for each symptom [16]. We found that for the CFS patients who were normotensive and nonobese, (CFS patient group 2), 8-iso-prostaglandin $F_{2\alpha}$-isoprostane levels significantly positively correlated with joint pain (correlation Pearson coefficient $r = 0.546$, $P = 0.002$), postexertional malaise ($r = 0.411$, $P = 0.027$). Both muscle pain and unrefreshing sleep showed a pattern of association, but this did not reach statistical significance ($r = 0.337$, $P = 0.074$ and $r = 0.338$, $P = 0.073$, respectively).

Table 1
Oxidative stress markers in patients with CFS [CFS patient group 1 (CVD risk factor group)] who are obese (BMI >30) and hypertensive, and healthy age and gender-matched controls

CFS patient group 1 (CVD risk factor group)	CFS patients	Controls	*P* value
Subject numbers	*n* = 16	*n* = 16	
Sex M: F	5:11	5:11	
Age mean (range)	52.4 years (35–62)	52.3 years (35–62)	
Smoking Y:N:Ex	2:14:0	1:14:1	
BMI (kg/m^2)	29.8 (4.5)	23.3 (2.5)	<0.001
Cholesterol (mmol/L)	5.49 (0.82)	5.23 (0.74)	0.356
HDL (mmol/L)	1.35 (0.41)	1.72 (0.38)	0.011
OxLDL (mU/ml)	42.8 (41.0)	33.4 (13.7)	0.065
F2$_\alpha$-isoprostanes (pg/ml)	572 (302)	341 (97)	0.007
GSH (μmol/L)	1210 (186)	1385 (227)	0.023
Supine systolic pressure (mm Hg)	144 (23)	128 (16)	0.049
Supine diastolic pressure (mm Hg)	81 (14)	72 (9)	0.023
Supine mean arterial pressure	103 (15)	90 (11)	0.016
Standing systolic pressure (mm Hg)	133 (21)	126 (16)	0.304
Standing diastolic pressure (mm Hg)	81 (14)	77 (9)	0.346
Standing Mean Arterial Pressure	99 (15)	94 (10)	0.273

Mean [SD] using an unpaired *t* test for between-group differences.

Table 2
Oxidative stress markers in normotensive, nonobese CFS patients and healthy age- and gender-matched controls

CFS patients group 2	CFS patients	Controls	*P* value
Subject numbers	*n* = 31	*n* = 30	
Sex M:F	14:17	13:17	
Age mean (range)	46 years (19–64)	45 years (19–60)	
Smoking Y:N:Ex	4:25:2	5:24:2	
BMI (kg/m^2)	23.9 (3.4)	24.2 (2.8)	0.67
Cholesterol (mmol/L)	5.02 (1.1)	5.10 (0.99)	0.77
HDL (mmol/L)	1.31 (0.30)	1.60 (0.46)	0.005
OxLDL (mU/ml)	38.2 (13.8)	30.8 (10.0)	0.02
F2$_\alpha$-isoprostanes (pg/ml)	406 (192)	318 (113)	0.03
GSH (μmol/L)	1284 (291)	1372 (240)	0.2
Supine systolic pressure (mm Hg)	116 (12)	118 (11)	0.48
Supine diastolic pressure (mm Hg)	70 (10)	71 (10)	0.76
Supine mean arterial pressure	85 (9.5)	87 (9)	0.59
Standing systolic pressure (mm Hg)	118 (14)	121 (15)	0.41
Standing diastolic pressure (mm Hg)	71 (12)	75 (10)	0.17
Standing mean arterial pressure	86 (11)	90 (11)	0.20

Mean [SD] using an unpaired *t* test for between-group differences.

We found that 8-iso-prostaglandin F$_{2\alpha}$-isoprostane levels are significantly higher ($P = 0.04$) in CFS patients (CFS patient group 2) reporting the most severe postexercise malaise when compared to those reporting mild postexercise malaise (Table 3). We also found that 8-iso-prostaglandin F$_{2\alpha}$ levels are significantly higher in patients reporting more severe joint pain when compared with those who report either no joint pain ($P = 0.002$) or those with mild joint point ($P = 0.012$) (Table 3).

No such associations were found in the CFS patient CVD risk factor group (CFS patient group 1).

Discussion

The novel findings of this study are that patients with CFS have significantly elevated levels of F$_2$-isoprostanes alongside other key markers of oxidative stress, and that these correlate with various CFS symptoms.

This is the first time that elevated levels of isoprostanes have been reported in patients with CFS and the finding is particularly important given their sensitivity, reliability, and association with other measures of lipid peroxidation in vivo [17,18]. F$_2$-isoprostanes are a series of prostaglandin F$_{2\alpha}$ isomers that are described as products of non-cyclooxygenase oxidative modifications of arachidonic acid or circulating low-density lipoprotein (LDL) particles that have resulted from free radical attack of cell membrane phospholipids [8]. In this study we measured plasma levels of 8-iso-prostaglandin F$_{2\alpha}$-isoprostane, because it is the most abundant of the family of isoprostanes, with plasma levels reflecting those in vivo. Isoprostanes not only reflect oxidative stress within integrated systems but they also have potent biological effects associated with the peroxidation of membrane lipid, increased cell permeability and a consequent increase of intracellular calcium [19]. They have also been shown to be powerfully vasoconstricting and are involved in endothelial injury [7,8].

Table 3
Comparisons of isoprostane levels of CFS patients ($n = 31$) (all normotensive and BMI <30) subdivided on basis of self-scoring joint pain and postexertional malaise level

	None	Mild	Moderate	Severe	*P* value
Joint pain (*n*)	8	8	8	7	
Isoprostane levels for this symptom (pg/ml)	318.9* (86.7)	360.6$^{\#}$ (158.5)	339.6 (107.7)	659.2*$^{,\#}$ (219.8)	0.002* 0.012$^{\#}$
Postexertional malaise (*n*)	0	9	12	10	
Isoprostane levels for this symptom (pg/ml)	–	295.2** (82.0)	425.6 (177.2)	492.2** (245.7)	0.04**

Mean (SD) using an unpaired *t* test.
Significance of isoprostane levels between scores for joint pain.
Significance of isoprostane levels between scores for postexertional malaise.
* Severe vs. none $P = 0.002$.
** Severe vs. mild $P = 0.04$.
$^{\#}$ Severe vs. mild $P = 0.012$.

Our data also suggest that there is an association between the raised isoprostane levels and symptoms of CFS patients who are not hypertensive, or obese (patient group 2). The CFS patients in this group also showed a pattern of oxidative stress (increased 8-iso-prostaglandin $F_{2\alpha}$-isoprostanes and oxLDL and decreased HDL) so we suggest that the prooxidant state is a consequence of their illness and not a secondary effect to the presence of any known CVD risk factors. We did not find a similar association between symptoms and markers of oxidative stress in the obese, hypertensive CFS patients (patient group 1); however, obesity and hypertension independently promote $F_{2\alpha}$-isoprostane levels [20] and, as expected, $F_{2\alpha}$-isoprostane levels are greater in our CFS patients with CVD risk factors (patient group 1) compared to those in group 2. Whether obesity is a truly independent variable for oxidative stress has, however, been contested [21]. The close association between obesity and other conditions such as type 2 diabetes, insulin resistance, impaired glucose tolerance, hypertension, and the combination of low HDL cholesterol and high triacylglycerol levels that potentially increase oxidative stress leaves open the possibility of residual confounding; i.e., the association between oxidative stress and obesity may be related to other, unmeasured variables [21]. Antioxidant capacity, represented by glutathione levels, is significantly reduced in the CFS group 1 patients but not in normotensive, nonobese, CFS patients (CFS patient group 2) and this is consistent with other reports of normal glutathione levels in CFS patients [1,22] allowing us to postulate that oxidative stress in CFS is due to excessive free radical formation and not depleted antioxidant reserves.

In the present study the raised isoprostane levels are accompanied by significantly elevated levels of oxLDL with low levels of the cardio-protective HDL in the presence of normal total cholesterol levels in the CFS patient population. LDL may penetrate the endothelium when it is impaired or injured by smoking, hypertension, or infectious organisms, but it is not toxic to vascular cells even in the high concentrations reached in the hyperlipidemic patient. By contrast oxLDL is toxic to the endothelium, even at low levels. It is involved in the early development of atherosclerosis [23] and it has the ability to stimulate genes associated with antioxidant responses such as the gene transcription factor nuclear factor-kappa B (NF-κB). Free radicals, certain viruses, and inflammatory cytokines can also activate NF-κB and there is recent evidence pointing to an up-regulation of NF-κB in CFS patients [24]. HDL may also have antiviral properties [25] and the significantly reduced levels measured in our CFS patient group may reflect impaired antiviral defence pathways that are characteristic of this patient population [26,27] as well as exposing them to increased cardiovascular risk.

The source of excessive free radical generation in CFS patients which involves oxidation of lipids and proteins [28] may be associated with a variety of altered biological processes. Exercising muscle is a prime contender for excessive free radical generation with recent evidence pointing to good correlations between muscle pain thresholds and fatigue with various blood markers of oxidative injury in CFS patients [5] and further evidence of viral persistence in muscle tissue in at least some patients with the illness [29]. Fulle et al. [30] demonstrated oxidative damage to DNA and lipids within muscle biopsies of CFS patients consistent with metabolic abnormalities to both mitochondria and phospholipids. The data reported here were taken from well-rested subjects and recent research has demonstrated that incremental exercise challenge potentiates a prolonged and accentuated oxidant stress that might well account for postexercise symptoms in CFS patients [31]. CFS is also associated with immune activation [32] and an equally compelling case can be made for excessive free radicals and reactive molecular intermediates being generated by activated white blood cells [33] as a consequence of either persistent infection [34] or environmental stressors [35].

A further consideration is that viral infections are also associated with excessive free radical production [36,37] and, in animal models at least, herpes simplex virus type 1 (HSV-1) infection is associated with significantly elevated levels of F_2-isoprostanes [38]. The problem with CFS is that the diagnostic group contains a highly heterogeneous condition resulting in variable reports of cytokine dysregulation characterised by episodically increased proinflammatory cytokines in plasma and also in the central nervous system [39]. In one study, CFS patients with a moderate magnesium deficiency that was not accounted for by a reduced dietary magnesium intake had a lower antioxidant capacity and this was explained on the basis of inflammation [22]. Elevated levels of plasma and urinary F_2-isoprostanes have been found in a number of inflammatory diseases [40] and we have recently reported raised concentrations of active transforming growth factor β1 and increased neutrophil apoptosis in chronic fatigue syndrome [41]. Neutrophils produce reactive oxygen species via NADPH oxidase and are significant contributors to intravascular oxidant stress and, ultimately, cardiovascular risk. It could be suggested that CFS is an inflammatory condition with many patients in a prooxidant state and this could explain many of the pathological manifestations that underlie the illness.

In this study we have presented evidence of increased in vivo lipid peroxidation and, for the first time, shown raised levels of isoprostanes in CFS patients which are associated with the patient's symptoms. On balance CFS patients have a lipid profile and oxidant biology that is consistent with cardiovascular risk and the presence of high levels of F_2-isoprostanes may explain some of the symptoms of the disease. Obesity and hypertension represent a potential, additional burden to free radical formation and CFS pathology. Supplementation with specific antioxidant medications might help to ameliorate symptoms and any potential cardiovascular complications of the illness.

Acknowledgments

This study was supported by a grant from MERGE (Myalgic Encephalomyelitis Research Group for Education and Support, registered UK charity number 1080201), Perth PH1 5PP, Scotland, UK. J.J.F.B and M.McL receive funding from the Sir John Fisher Foundation.

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