

Chronic fatigue syndrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses

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Abstract. Jammes Y, Steinberg JG, Delliaux S, Brégeon F (Université de la Méditerranée and Pulmonary Function Laboratory, North Hospital, Assistance Publique – Hôpitaux de Marseille, France). Chronic fatigue syndrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses. *J Intern Med* 2009; doi: 10.1111/j.1365-2796.2009.02079.x

Objectives. As heat shock proteins (Hsp) protect the cells against the deleterious effects of oxidative stress, we hypothesized that Hsp expression might be reduced in patients suffering from chronic fatigue syndrome (CFS) who present an accentuated exercise-induced oxidative stress.

Design. This case–control study compared nine CFS patients to a gender-, age- and weight-matched control group of nine healthy sedentary subjects.

Interventions. All subjects performed an incremental cycling exercise continued until exhaustion. We measured ventilation and respiratory gas exchange and evoked compound muscle potential (M-wave) recorded from *vastus lateralis*. Repetitive venous blood sampling allowed measurements of two markers

of oxidative stress [thiobarbituric acid reactive substances (TBARS) and reduced ascorbic acid (RAA)], two cytokines (IL-6 and TNF- α) and two Hsp (Hsp27 and Hsp70) at rest, during maximal exercise and the 60-min recovery period.

Results. Compared with controls, resting CFS patients had low baseline levels of RAA and Hsp70. Their response to maximal exercise associated (i) M-wave alterations indicating reduced muscle membrane excitability, (ii) early and accentuated TBARS increase accompanying reduced changes in RAA level, (iii) absence of significant increase in IL-6 and TNF- α , and (iv) delayed and marked reduction of Hsp27 and Hsp70 variations. The post-exercise increase in TBARS was accentuated in individuals having the lowest variations of Hsp27 and Hsp70.

Conclusions. The response of CFS patients to incremental exercise associates a lengthened and accentuated oxidative stress, which might result from delayed and insufficient Hsp production.

Keywords: chronic fatigue syndrome, cytokines, heat shock proteins, incremental exercise, oxidative stress.

Introduction

Chronic fatigue syndrome (CFS) is characterised by persistent and unexplained fatigue, often associated with pain in muscles and several joints, resulting in severe impairment in daily activity for which there exists no clear aetiology [1, 2].

In a previous study [3], we reported marked alterations in biological response to maximal exercise in CFS patients compared to a matched group of healthy sedentary subjects. The changes combined post-exercise alterations of muscle membrane excitability (M-wave) together with an early and lengthened exercise-induced oxidative stress, measured by

an increased plasma concentration of thiobarbituric acid reactive substances (TBARS) and lowered consumption of antioxidant (reduced ascorbic acid, RAA). Other authors also reported a correlation between musculoskeletal symptoms and TBARS [4] or isoprostanes levels [5] and an accentuated lipid peroxidation in resting CFS patients [6]. Recent general reviews on CFS genesis suggest a possible role of excessive exercise-induced oxidative stress [7, 8] and perhaps also of innate immune imbalance with excessive production of inflammatory mediators and cytokines, which might have the most influence on chronic inflammation in resting CFS patients [8]. However, data on altered innate cytokine immune system in CFS patients only concern the baseline plasma cytokine levels and are often contradictory [9–13]. We regret the absence of data on post-exercise changes in plasma cytokine levels in CFS patients because in healthy subjects, maximal exercise represents a situation, which promotes, in various proportion, the plasma release of both inflammatory and anti-inflammatory cytokines, unbalancing the innate immunity [14, 15]. Depending on the strength of the effort, submaximal exercises inconstantly elicit an immune response [14].

The cellular redox status primarily is represented by the balance between cellular oxidant and reductant levels. Numerous recent data suggest that heat shock proteins (Hsp) would complement the existing endogenous antioxidants during and following the cellular oxidative stress, thereby, protecting cells against the deleterious effects of reactive oxygen species (ROS). In healthy sedentary subjects, a close interrelationship exists between cellular expression of heat shock proteins (Hsps) and redox status. Indeed, the Hsp expression reduces the ROS generation through the activation of antioxidants and, in turn, the oxidant and antioxidant levels increase plasma Hsp level [16]. Hsp20, Hsp27 and Hsp 70 formation occurs in contracting muscles [17]. Close interactions also exist between the activation of Hsp gene expression and IL-6 production. Indeed, Hsp induces inflammatory processes including the IL-6 production [18] and, reversely, IL-6 activates Hsp gene expression [19]. As a redox imbalance exists in patients with CFS, and

given the above-mentioned link between Hsp and redox status, an altered Hsp response in CFS patients seems highly probable. We found no information on the baseline plasma levels of Hsp 27 and 70 nor their changes after a maximal exercise in CFS patients.

In the present study, we aimed to document the oxidative stress, cytokine and Hsp levels before and after maximal cycling exercise in CFS patients. Based on previous data showing an increased oxidative stress response to exercise in CFS [3], we hypothesized that this dysregulation might be associated with an altered cytokine response and decreased Hsp production compared to healthy controls. We compared nine CFS patients to an age, sex-matched control group of healthy sedentary subjects at rest, during, and after a maximal cycling exercise allowing to measure the maximal oxygen uptake (VO_{2max}). Plasma dosages of markers of oxidative stress, cytokines (IL-6, TNF α) and heat shock proteins (Hsp 27 and Hsp 70) were performed in all subjects. Neuromuscular function was explored in *vastus lateralis* to confirm the altered muscle excitability already reported in CFS patients [3].

Methods

A total of eighteen subjects were explored. Nine Caucasian subjects (four female subjects/fourteen male subjects; mean age: 38 ± 5 y; mean weight: 74 ± 4 kg) complained for more than 1 year of fatigue – muscle pain – post-exertion malaise – unrefreshing sleep, the four symptoms addressing for the diagnosis of CFS according to the US Centers for Disease Control and Prevention Criteria for CFS [20]. They were addressed by clinicians of different specialities and most often by general practitioners. Six of them had practised sport at high level (>6 h per week) for more than 4 years before the symptoms had occurred. Data were compared to those obtained in a gender-, age-, and weight-matched control group of nine Caucasian healthy volunteers (four female subjects/five male subjects; mean age: 39 ± 5 y; mean weight: 74 ± 3 kg) who consulted for a medical check up and came from the same type of socioeconomic circumstances. They were considered as sedentary

subjects because they did not participate in regular formal exercise, some of them maximally practising tennis for only 2 h each week. This control group was helpful to determine the Hsp response to an incremental maximal cycling exercise in comparison with the simultaneous determination of the changes in oxidant-antioxidant balance and the cytokine response. Indeed, these control data are absent in the literature. Procedures were carried out with the adequate understanding and written consent of the subjects. The protocol was approved by the Ethics Committee of our institution and was performed in accordance with the Declaration of Helsinki.

The functional examinations at rest consisted of lung function testing with measurements of arterial blood gases, electrocardiogram (ECG) recording, arterial blood pressure measurements using a sphygmomanometer, and venous blood measurements of TBARS, reduced ascorbic acid (RAA), IL-6, TNF- α , Hsp 27 and Hsp 70 concentrations. The protocol of incremental cycling exercise was the same as in our previous CFS study [3], including measurements of cardiorespiratory variables, surface electromyogram (EMG) from *vastus lateralis* and venous blood levels.

Physiological measurements

Twelve ECG leads were recorded continuously (Cardiognost Hellige, Stuttgart, Germany) and the arm arterial blood pressure was measured using a sphygmomanometer. During the exercise trial, the heart rate was computed from standard ECG leads by the software system and data were obtained for each respiratory cycle. Blood pressure was measured each two workloads.

At rest, arterial blood gases, including arterial partial pressures of oxygen and carbon dioxide, (P_{aO_2} , P_{aCO_2}) as well as arterial pH (pHa), were analyzed in 100 μ L arterialized blood sampled from the ear lobes (Corning-Chiron model 860; Bayer Corporation, East Walpole, MA USA). Percutaneous oxygen saturation (SpO_2) was continuously measured throughout the exercise challenge and the recovery period using an infrared analyser (Nellcor model N3000; Boulder, CO, USA).

A face mask (dead space: 30 mL) was designed to form an air-tight seal over the patient's nose and mouth, with all the inspired and expired gas going into a turbine flowmeter (Triple V digital volume transducer; Sebac MSR, Gennevilliers, France) giving measurements of minute ventilation (V_E). A side pore of the face mask was connected to fast-response differential paramagnetic O_2 and infrared CO_2 analyzers (90% response time in 100 ms), which measured the end-tidal partial pressures of O_2 and CO_2 respectively. Calibration procedure for flowmeter and gas analyzer systems was carried out before each test. Exercise trials were performed in the morning on an electrically braked cycle ergometer (Ergometrics ER 800; Jaeger, Bunnik, the Netherlands) connected to a microcomputer software (OXYCON BETA; Jaeger, Bunnik, the Netherlands). The exercise bout was preceded by a 2-min 0-Watt pedalling period. The load was increased as a ramp (20 W min^{-1}) until the subject decided to interrupt the exercise bout, then he continued to pedal for the first 5 min of the 30-min recovery period. Throughout the incremental exercise trial, the software (OXYCON BETA, Jaeger) computed breath-by-breath data of V_E , O_2 and CO_2 consumption ($\dot{V}O_2$ and $\dot{V}CO_2$), and the ventilatory equivalents for O_2 ($V_E/\dot{V}O_2$) and CO_2 ($V_E/\dot{V}CO_2$) and heart rate (HR) was averaged for 10-s consecutive periods. The ventilatory threshold (V_{Th}) corresponded to the $\dot{V}O_2$ value at which $V_E/\dot{V}O_2$ exhibited a systematic increase without a concomitant increase in $V_E/\dot{V}CO_2$ [21]. The criteria to establish $\dot{V}O_{2max}$ was to obtain a plateau of $\dot{V}O_2$, to reach the predicted maximal values of $\dot{V}O_2$ and HR and to measure respiratory quotient value higher than 1.1 [20]. As shown in Table 1, the maximal exercise power and $\dot{V}O_{2max}$ did not differ between the two groups.

Electromyographic (EMG) recording and analysis

As in our previous studies [3, 22, 23], bipolar (30 mm centre-to-centre) Ag-AgCl surface electrodes (Dantec, 13 L 20) were used to measure EMG voltage from the *vastus lateralis* muscle on the dominant side of the body (all subjects were right-handed). This muscle group plays a key role in leg extension during cycling. The electrodes were placed between the motor point and the proximal tendon. Inter-electrode

Table 1 Characteristics of the nine CFS and nine control subjects and their cardiorespiratory response to incremental exercise

	Age (years)	Weight (kg)	$\dot{V}O_{2\max}$		Maximal power (Watt)
			absolute (%) predicted)		
Controls	38	76	30	101	167
	6	4	3	5	12
CFS	39	74	33	104	190
	5	3	4	6	15

CFS, chronic fatigue syndrome; $\dot{V}O_{2\max}$, maximal measured oxygen uptake (absolute value and percentage of predicted one); maximal power. $\dot{V}O_2$ values are expressed in mlSTPD $\text{min}^{-1} \text{kg}^{-1}$.

impedance was kept below 2 kohm by careful skin shaving and abrasion with an ether pad. The EMG signal was amplified (Nihon Kohden, Tokyo, Japan; common mode rejection ratio, 90 dB; input impedance, 100 mohm; gain, 1000 to 5000) with a frequency band ranging from 10 to 2000 Hz. Compound muscle mass action potentials (M-waves) were evoked by direct muscle stimulation, using a monopolar technique. The monopolar technique was preferred to the bipolar one because it allowed to obtain the most reproducible M-wave amplitude [3, 23]. A constant-current neurostimulator (S88 model; Grass, Quincy, MA, USA) delivered supramaximal shocks with 0.1-ms rectangular pulses through an isolation unit (SIU 5 model; Grass, Quincy, MA, USA). One small (1×1 cm) negative silver electrode was applied on the main motor point of the *vastus lateralis* muscle and a large (3×3 cm) positive silver electrode was placed on the opposite side of the thigh. The main motor point of this muscle was identified as the location of the cathode yielding the strongest contraction with the lowest pulse amplitude. To prevent any change in the electrode impedance throughout the whole challenge, the pulse intensity was set about 15% above the level yielding an M-wave of maximal amplitude during the 0-Watt pedalling period preceding the exercise bout. The EMG signal was fed to a digital oscilloscope (model DSO 400; Gould, Ballainvilliers, France), sampling the EMG signal every one ms. This oscilloscope permitted to average the M-waves from eight successive potentials and to calculate the peak M-wave amplitude and duration,

and the conduction time, that is the time between the stimulus artefact and peak. As already described [3], to average M-waves elicited at constant muscle length, a trigger signal from a magnetic sensor coupled to the crank gear mechanism of the cycloergometer indicated the onset of leg extension and thus of *vastus lateralis* contraction. It was used to trigger within a delay of 100 ms the neurostimulator. As the pedalling rate was set at 60 min^{-1} , each leg extension was performed every second and lasted for 500 ms. Thus, the stimulation frequency was set at 1 Hz and eight successive evoked muscle potentials was averaged, i.e. eight successive *vastus lateralis* contractions. For each epoch of the protocol (the 0-Watt 2-min period preceding the incremental exercise, the exercise itself and its recovery period), M-wave recordings were always performed during cycling at the same pedalling rate. Thus, at different epochs of the post-exercise recovery period, the subject was asked to pedal again for 30-s periods.

Biochemical analyses

A catheter (Neofly 21G, Viggo-Spectramed, Miami, FL, USA) was inserted in an antecubital vein. Six millilitre of heparinized blood were sampled at different sequences of the protocol to measure biochemical variables. Plasma TBARS and RAA were analyzed according to procedures already published elsewhere [3, 15, 24, 25] and based on the original methods by Uchiyama and Mihara [26] for TBARS and Maickel [27] for RAA. Plasma IL-6 and TNF- α were measured with high-sensitive enzyme-linked immunosorbent assay kit (Human Quantikine IL-6 Immunoassay D6050 and Human Quantikine TNF- α DTA00C Immunoassay; R & D Systems Europe, Lille, France). The limits of detection of IL-6 and TNF- α assays were $<0.70 \text{ pg mL}^{-1}$ and 1.6 pg mL^{-1} respectively. Plasma Hsp27 and Hsp70 levels were measured with high-sensitive enzyme-linked immunosorbent assay kits (Human Hsp27 Total: BioSource International, Inc., Camarillo, CA, USA, supplied by Invitrogen, Eragny sur Oise, France; Hsp70: High Sensitivity EIA Kit from Assay Designs, supplied by Tebu-Bio SAS, Le Perray en Yvelines, France). The limits of detection of Hsp27 and Hsp70 assays were $<0.3 \text{ ng mL}^{-1}$

and $<0.09 \text{ ng mL}^{-1}$ respectively. All measurements were made in duplicate and the coefficient of variation was inferior to 5%.

Exercise protocol

The protocol consisted of (i) a 30-min rest period, during which all variables were measured and venous and arterial blood samples collected, (ii) a 2-min 0-Watt work load period used to reach a 60 revs min^{-1} cycling frequency, (iii) a work period, and (iv) a 30-min recovery period. The work period started at a work load of 20 Watt and the load was increased by 20 Watt every 1 min until the subject could not maintain the required pedal rate. At determination of V_{Th} and at the maximal work rate reached, venous blood was sampled and M-wave recorded. The ergometer was then unloaded and the subject continued to cycle for a 5-min recovery period to facilitate the venous blood return from the legs. During the post-exercise recovery-period, venous blood samplings and M-wave recordings were performed at 5, 10, 30 and 60 min.

Statistical analyses

Data are presented as means \pm standard error of means (SEM). An independent Student's *t*-test was performed to compare baseline levels of biochemicals between CFS patients and control subjects. For temporally repeated data during exercise bout and the recovery period, the changes over time in each group were determined using repeated measure-ANOVA when variables were normally distributed or a Friedman's test for repeated measures when they were not. When data were normally distributed, a two-way analysis of variance for repeated measures (RM-ANOVA 2) (i.e., group and time) was used to determine in CFSs and controls the significance of changes throughout the cycling trial and the post-exercise recovery period. *Post-hoc* tests with Bonferroni correction were used to compare time-point specific differences between groups. The relations between variables were evaluated with simple linear regression with 95% confidence intervals. In all cases, significance was set at the 0.05 level.

As intergroup differences in maximal exercise power and exercise duration between control subjects and CFS patients could simply explain intergroup differences, the maximal individual variations of biochemicals in percentage of the corresponding $\dot{V}O_{2max}$ was also expressed.

Results

Resting levels of variables

No significant differences in M-wave amplitude and duration were measured between CFS patients and control subjects (Fig. 1). As shown in Table 2, baseline

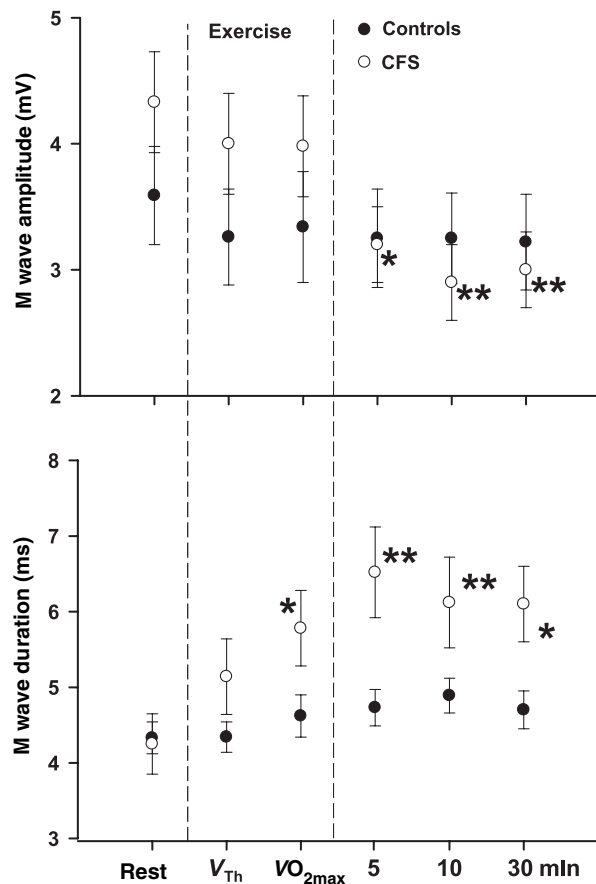


Fig. 1 The changes in M-wave amplitude and duration during and after a maximal incremental cycling exercise in nine chronic fatigue syndrome (CFS) patients and nine healthy sedentary subjects. Asterisks denote significant changes from rest (* $P < 0.05$; ** $P < 0.01$).

Table 2 Baseline and maximal post-exercise variations of the blood markers of oxidative stress, interleukin 6 (IL-6), and heat shock proteins (Hsp 27 and 70) in control and chronic fatigue syndrome (CFS) subjects

	CFS		Controls	
TBARS (nmol mL⁻¹)				
Rest	1.23		1.37	
	0.09		0.15	
Peak increase	3.50***	\$\$	2.14*	
	0.33		0.26	
Δ TBARS/ VO_{2max}	0.07***	\$	0.03*	
	0.01		0.01	
RAA (nmol mL⁻¹)				
Rest	65.3	\$	122.0	
	14.0		19.0	
Lowest value	42.2		75.0**	
	11.9		16.0	
Δ RAA/ VO_{2max}	-0.67	\$	-1.52**	
	0.11		0.34	
IL-6 (pg mL⁻¹)				
Rest	8.08		4.36	
	2.23		0.96	
Peak increase	11.56		7.54**	
	2.48		1.45	
Δ IL-6/ VO_{2max}	0.11	\$\$	0.11**	
	0.06		0.04	
TNF-α (pg mL⁻¹)				
Rest	17.0		14.2	
	0.9		1.8	
Peak increase	15.0	\$\$	22.0	
	0.5		2.0	
Δ TNF- α / VO_{2max}	0.45	\$\$	0.73	
	0.07		0.05	
Hsp 27 (ng mL⁻¹)				
Rest	5.34		6.47	
	0.94		1.13	
Peak increase	10.97*	\$	18.01***	
	1.40		2.47	
Δ Hsp 27/ VO_{2max}	0.17*	\$\$	0.38**	
	0.09		0.09	
Hsp 70 (ng mL⁻¹)				
Rest	0.95	\$	1.56	
	0.09		0.18	

Table 2 *Continued*

	CFS		Controls	
Peak increase	1.27*	\$\$\$	2.09*	
	0.16		0.23	
Δ Hsp 70/ VO_{2max}	0.010*	\$	0.018*	
	0.03		0.004	

STPD, standard temperature dry air condition; TBARS, thiobarbituric acid reactive substances. Maximal post-exercise variations are also related to the corresponding maximal oxygen uptake (VO_{2max}) (expressed in mL O_2 STPD mL⁻¹ kg⁻¹). Symbol \$ indicate significant intergroup differences and asterisks are used to depict significant post-exercise peak variations.

levels of TBARS, IL-6, TNF- α and Hsp 27 did not differ between CFS patients and controls. There was only a tendency for an elevated IL-6 level in resting CFS patients, but statistical analysis failed to show any significance. However, baseline RAA and Hsp70 levels were significantly lowered in CFS patients.

Response to exercise

During the exercise bout and the post-exercise recovery period, the M-wave characteristics were not significantly affected in healthy subjects. By contrast, in CFS patients, the M-wave amplitude declined and the M-wave duration was lengthened, the changes persisting for the first 20 min of the recovery period (Fig. 1).

The exercise-induced oxidative stress was markedly accentuated compared to controls (Table 2 and Fig. 2) in CFS patients. Then, the TBARS increase occurred earlier, i.e. 1 to 2 min before the exercise had ended (corresponding to V_{Th} measurement), and the accentuated peak TBARS value (absolute value and related to VO_{2max}) was significant. No significant post-exercise decrease in RAA level was measured in CFS patients whilst the antioxidant response was always present in controls.

Compared to controls, where we measured a significant increase in post-exercise levels of both IL-6 and

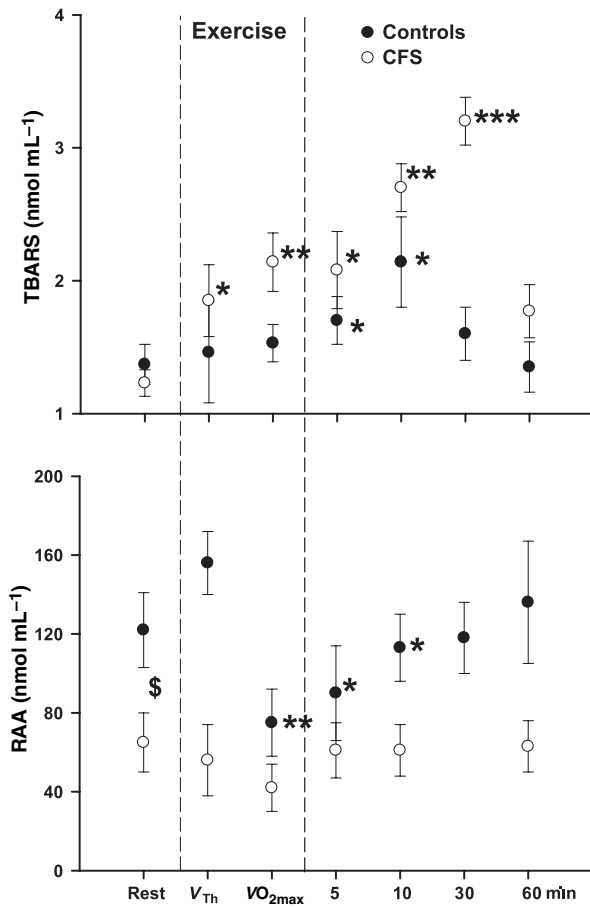


Fig. 2 Time course of changes in plasma levels of a marker of lipid peroxidation [thiobarbituric acid reactive substances (TBARS)] and endogenous antioxidant [reduced ascorbic acid (RAA)] in chronic fatigue syndrome (CFS) patients and control subjects completing an incremental cycling exercise allowing to determine the ventilatory threshold (V_{Th}) and maximal oxygen uptake (VO_{2max}). Symbol \$ indicates significant intergroup difference at rest. Asterisks are used to depict significant variation from baseline level throughout the exercise bout (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

TNF- α , no significant post-exercise variations of both cytokines could be detected in CFSs (Fig. 3).

In controls, the Hsp27 increase appeared earlier (at V_{Th} measurement) during the exercise bout, preceding both the TBARS increase and RAA consumption (Figs 2 and 4), and persisted until the end of the 60-min recovery period. A significant post-exercise increase in Hsp70 level was measured, but the response was delayed compared to the Hsp27

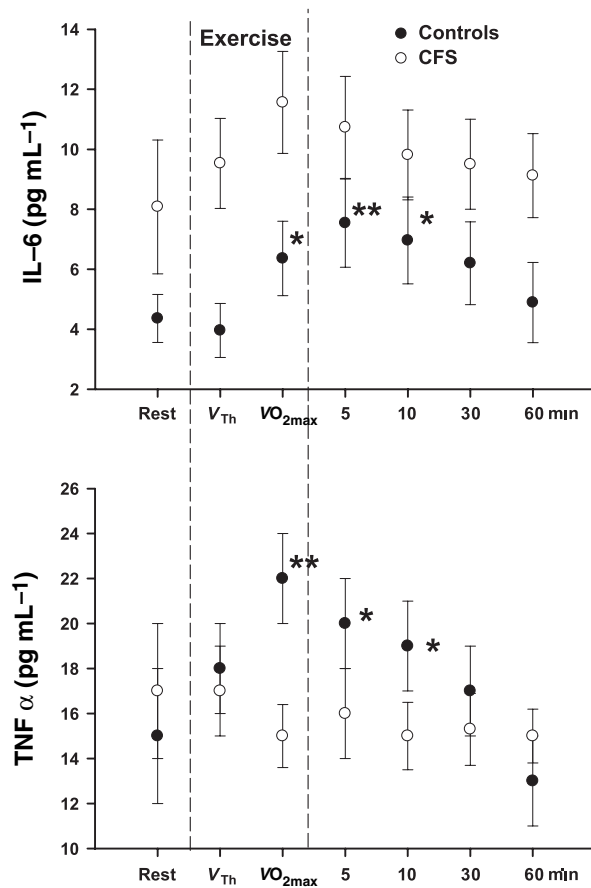


Fig. 3 Time course of changes in plasma levels of cytokines (IL-6, TNF- α) in chronic fatigue syndrome (CFS) patients and control subjects completing an incremental cycling exercise allowing to determine the ventilatory threshold (V_{Th}) and maximal oxygen uptake (VO_{2max}). Asterisks are used to depict significant variation from baseline level throughout the exercise bout (* $P < 0.05$; ** $P < 0.01$).

changes (Fig. 4). In CFS patients, the Hsp 27 response to exercise began only at the 5th min of the post-exercise recovery period and only persisted for a further 5-min period. Compared to controls, the maximal post-exercise variations of both the Hsp27 and Hsp70 were significantly less in CFS patients (Table 2).

When data in CFS patients and controls were pooled together, significant negative correlations were obtained between the post-exercise peak increases (maximal plasmatic changes from baseline) in TBARS

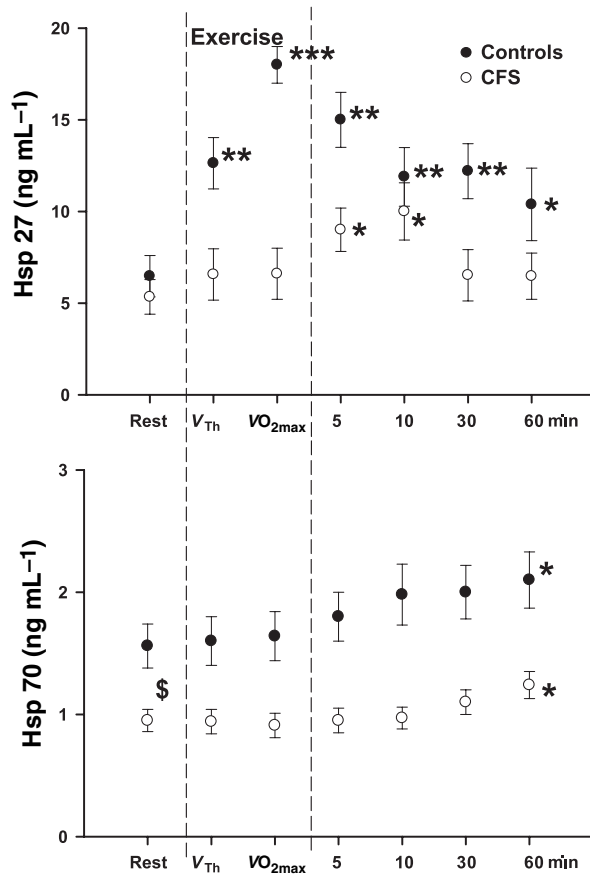


Fig. 4 Time course of changes in plasma levels of heat shock proteins (Hsp27, Hsp70) in chronic fatigue syndrome (CFS) patients and control subjects completing an incremental cycling exercise allowing to determine the ventilatory threshold (V_{Th}) and maximal oxygen uptake (VO_{2max}). Symbol \$ indicates significant intergroup difference at rest. Asterisks are used to depict significant variation from baseline level throughout the exercise bout (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

and Hsp27 or Hsp70 (Fig. 5). However, the relatively low values of correlation coefficients ($r = 0.54$ to 0.59) only suggest a trend for an accentuated post-exercise oxidative stress in individuals having the lowest Hsp response. No correlations were found between peak levels of IL-6, Hsp 27 and Hsp 70.

Discussion

The original findings in this study are that the response to maximal cycling exercise of CFS patients

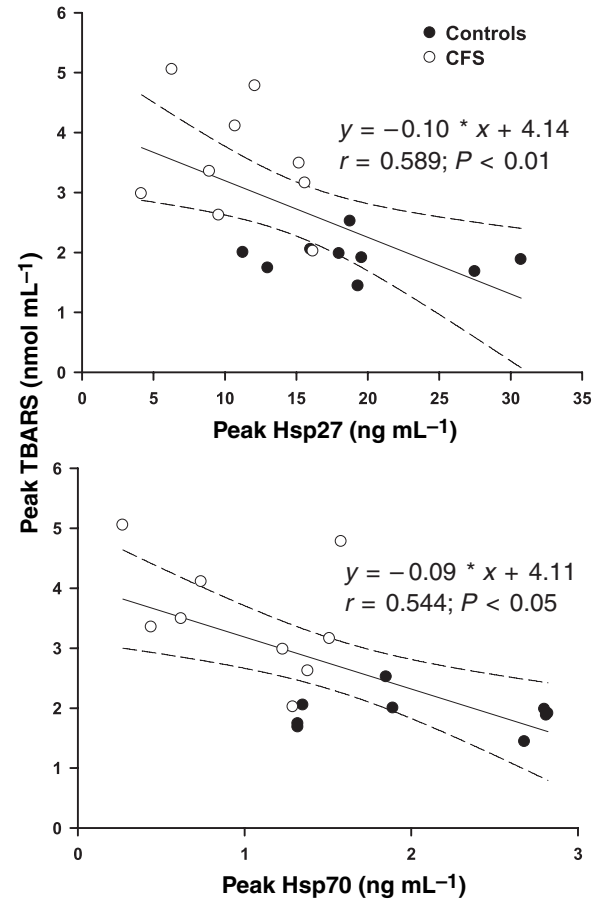


Fig. 5 Correlations between peak post-exercise values of thiobarbituric acid reactive substances (TBARS) and heat shock proteins (Hsp27 or Hsp70) in CFS patients (open symbols) and healthy volunteers (closed symbols). Shown are the regression lines with 95% confidence intervals and their equations with significance against zero.

combines (i) an early and accentuated oxidative stress, (ii) the absence of significant increase in both IL-6 and TNF- α , and (iii) delayed and reduced increases of Hsp27 and Hsp70. As the Hsp response to exercise protects the cells against the deleterious effects of oxidative stress, the present data corroborate our primary hypothesis that a reduced Hsp production in CFS patients might explain their accentuated exercise-induced oxidative stress. The severe and prolonged exercise-induced oxidative stress in CFS patients might explain their altered muscle excitability, both events have been already reported in our previous CFS study [3].

The absence of significant difference in $\dot{V}O_{2\max}$ and thus, in total exercise, duration between CFS patients and control subjects enables us to compare the magnitude and kinetics of biochemical response to exercise. The relatively high $\dot{V}O_{2\max}$ in our CFS patients corroborates numerous previous studies. Indeed, $\dot{V}O_2$ measurement in exercising CFS patients indicated normal or increased aerobic function [3, 28–31], especially the relationship between the concomitant increases in $\dot{V}O_2$ and work rate, which were similar to that expected in healthy subjects.

In response to maximal cycling exercise in control subjects, we measured significant increases in plasma levels of IL-6 and TNF- α corroborating our previous observations [15, 24]. However, in CFS patients, we failed to observe any significant post-exercise increase in IL-6 and TNF- α . Cannon *et al.* [10] also noted that the inflammatory response to exercise of CFS patients was not significantly different than controls. We failed to measure any significant difference between the baseline levels of both cytokines in CFS patients compared to control. Indeed, despite we noted a tendency to an elevated baseline IL-6 level in CFSs, the IL-6 values were rather scattered amongst our CFS patients. Some studies, including that by Cannon *et al.*, reported an increase in baseline plasma level of IL-6 in CFS patients [9–11], whilst others found no dysregulation of resting cytokine production [12, 13]. The role played by IL-6 in chronic muscle fatigue and muscle pain is not obvious because one of the main actions of this cytokine is the release of anti-inflammatory cytokines (IL-1Ra, IL-10), whereas TNF- α solely promotes inflammatory reactions [14]. Thus, the depressed cytokine response to exercise in CFS patients might have dual consequences: there may be some benefits of the absence of a post-exercise TNF- α increase, through the reduction of inflammatory reaction, whilst the reduced IL-6 response might attenuate the anti-inflammatory actions. The present observations of a dissociation between an accentuated exercise-induced oxidative stress and absent inflammatory response to exercise in CFS patients are not surprising. Indeed, in healthy sedentary subjects, there is no clear relationship between both the time course and

magnitude of exercise-induced changes in IL-6 and lipid peroxidation [15, 24, 32] because the elevated level of cytokines often preceded the TBARS increase. These data do not favour the hypothesis that the exercise-induced oxidative stress promotes the cytokine release.

The new observations in CFS patients are a delayed, shortened and reduced Hsp27 and Hsp70 responses to exercise and also a lowered baseline Hsp70 level. Indeed, no measurements of Hsp response to exercise in CFS were found in the literature. The present data in healthy sedentary subjects corroborate previous observations, which showed that Hsp27 responds immediately to maximal eccentric exercise with the quadriceps muscle, whilst the post-exercise increase in cytosolic Hsp70 level was only measured 24 h after exercise [33]. The early post-exercise increase in Hsp20 and Hsp27 might result from the observation that low-density heat shock proteins are mostly affected by enhanced phosphorylation process and the consecutive increase in ROS production [34, 35].

It is already documented that an elevated Hsp expression is responsible for a facilitation of antioxidant defences against the oxidative stress in healthy sedentary subjects [16] and also promotes inflammatory processes [18]. Thus, the reduced RAA consumption and the accompanying accentuation of TBARS increase and also the absence of cytokine response in our exercising CFS patients might result from the lowered Hsp27 and Hsp70 production.

The present observations corroborate our hypothesis that a lack of Hsp response to exercise might explain the accentuated oxidative stress in CFS patients. As individuals who exercise frequently are more likely to report a diagnosis of CFS in later life [36], the repetition of exercise bouts at high energetic levels might be the cause of a downregulation of Hsp production and also the reduced cytokines release in some individuals. Further studies are needed in healthy volunteers and perhaps also in animal models to demonstrate that the repetition of exercise bouts might depress the expression of inducible factors of Hsp.

Conflict of interest statement

Nothing to declare.

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