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Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise

Y. JAMMES, J. G. STEINBERG, O. MAMBRINI, F. BRÉGEON & S. DELLIAUX
From the Laboratoire de Physiopathologie Respiratoire (UPRES EA 2201), Faculté de Médecine, Institut Fédératif de Recherche Jean Roche, and Service des Explorations Fonctionnelles Respiratoires, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France

Abstract. Jammes Y, Steinberg JG, Mambrini O, Brégeon F, Delliaux S (Institut Fédératif de Recherche Jean Roche and Assistance Publique-Hôpitaux de Marseille, Marseille, France). Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. *J Intern Med* 2005; **257**: 299–310.

Objectives. Because the muscle response to incremental exercise is not well documented in patients suffering from chronic fatigue syndrome (CFS), we combined electrophysiological (compound-evoked muscle action potential, M wave), and biochemical (lactic acid production, oxidative stress) measurements to assess any muscle dysfunction in response to a routine cycling exercise.

Design. This case–control study compared 15 CFS patients to a gender-, age- and weight-matched control group ($n = 11$) of healthy subjects.

Interventions. All subjects performed an incremental cycling exercise continued until exhaustion.

Main outcome measures. We measured the oxygen uptake ($V\dot{O}_2$), heart rate (HR), systemic blood pressure, percutaneous O_2 saturation (SpO_2), M-wave recording from vastus lateralis, and venous blood sampling allowing measurements of pH (pHv), PO_2 (PvO_2), lactic acid (LA), and three markers of the oxidative stress (thiobarbituric acid-reactive substances, TBARS, reduced glutathione, GSH, and ascorbic acid, RAA).

Results. Compared with control, in CFS patients (i) the slope of $V\dot{O}_2$ versus work load relationship did not differ from control subjects and there was a tendency for an accentuated PvO_2 fall at the same exercise intensity, indicating an increased oxygen uptake by the exercising muscles; (ii) the HR and blood pressure responses to exercise did not vary; (iii) the anaerobic pathways were not accentuated; (iv) the exercise-induced oxidative stress was enhanced with early changes in TBARS and RAA and enhanced maximal RAA consumption; and (v) the M-wave duration markedly increased during the recovery period.

Conclusions. The response of CFS patients to incremental exercise associates a lengthened and accentuated oxidative stress together with marked alterations of the muscle membrane excitability. These two objective signs of muscle dysfunction are sufficient to explain muscle pain and postexertional malaise reported by our patients.

Keywords: chronic fatigue syndrome, incremental exercise, M-wave, metabolic response, oxidative stress.

Introduction

Chronic fatigue syndrome (CFS) is an illness characterized by a persistent fatigue at rest which is made worse by exercise [1]. As recommended by the US Center for Disease Control and Prevention [2], at least four symptoms of inability must be concurrently present before evoking the CFS diagnosis.

The aetiology of CSF remains unclear. Because there has often been an absence of changes in light and electron microscopy of muscle biopsies and in

physiological or biochemical data, many clinicians conclude that CFS is not a myopathy but that psychological/psychiatric factors appear to be of greater importance [1]. Supporting the hypothesis of a 'central fatigue' in CFS patients, some physiological studies testing peripheral (stimulated muscle contraction, M-wave recording) and central (twitch interpolation technique, maximal voluntary contraction) components of muscle fatigue have suggested the absence of any failure of the peripheral force production [3–5]. By contrast, other studies focusing on the changes in postexercise physiological events in CFS patients have shown a significant reduction of the muscle excitability in response to motor stimulation [6] with an impaired recovery of the maximal isometric torque [7]. A recent study by Fulle *et al.* [8] also showed in CFS patients a deregulation of the Na^+/K^+ and Ca^{2+}-ATPase pumps and alterations in ryanodine channels in the sarcoplasmic reticulum membranes.

Muscle metabolism at rest, during, and after muscle contraction was explored in CFS patients using measurements of oxygen uptake ($\dot{V}O_2$), ^{31}P nuclear magnetic resonance spectroscopy, and/or blood dosages of lactate or markers of the oxidative stress. Data are often contradictory. Some studies, based on measurements of a reduced maximal oxygen uptake (VO_{2max}) [9, 10], on an accelerated glycolysis during contraction [11], and on an impaired postexercise phosphocreatine resynthesis [12], have claimed that the aerobic capacity was reduced in CFS patients. By contrast, numerous other studies lead to the conclusion that CFS patients have either higher submaximal oxygen uptake [13] or no deficit in the oxidative metabolism [14–19]. These discrepancies could result either from the limited maximal performances of CFS patients, resulting in a marked reduction of exercise bout duration and in consequence of their energetic demand, or from the existence of subgroup of CFS patients having an enhanced postexercise lactate production and impaired mitochondrial oxidative phosphorylation [20, 21].

An enhanced oxidative stress has been implicated in the pathogenesis of several muscle diseases including CFS [22]. In CFS patients at rest, several studies suggest the existence of increased oxidative stress and decreased antioxidant defences related to the extent of symptomatology [23–26]. We did not find any data on an enhanced postexercise oxidative stress in these patients. However, there are some data in CFS patients on the exercise-induced cytokine release which often results from an accentuated production of reactive oxygen species (ROS). Peterson *et al.* [27] reported significant postexercise increases in TGF-β in CFS patients after walking at 1 mph for 30 min. By contrast, Cannon *et al.* [28] did not measure any significant increase in IL-6 and IL-1β plasma concentrations in their patients after stepping up and down on a platform. Once more, the exercise protocols were very specific and the magnitude of changes in muscle energetics ($\dot{V}O_2$) was not determined.

The aim of the present study was to propose a poorly invasive protocol to explore both the muscle metabolism and the response to electrical stimulation of CFS patients at exertion. Because invasive methods (needle EMG, muscle biopsies) had previously failed to demonstrate any muscle abnormalities, our patients expressly asked for biological support of their symptoms which were too often attributed to psychological or psychiatric disorders. During and after an incremental cycling exercise bout maintained until exhaustion, routinely used in our laboratory [29–31], we searched for altered responses of the muscle metabolism ($\dot{V}O_2$, arteriovenous oxygen difference, venous pH, and blood lactate), exercise-induced oxidative stress, and/or neuromuscular excitability (evoked muscle action potential, i.e. M-wave and potassium efflux). The data obtained in CFS patients were compared with those measured in a gender-, age-, and weight-matched group of healthy sedentary volunteers.

Methods

A total of 26 subjects were explored at work. Fifteen Caucasian subjects (six females; mean age: 48 ± 3 years; mean weight: 67 ± 4 kg) complained for more than 6 months of muscle pain, multi joint pain, postexertional malaise and unrefreshing sleep – the four symptoms addressing for the diagnosis of CFS. They were addressed by clinicians of different specialties and most often by general practitioners. Medical, including psychiatric, evaluation has shown no other explanation of their illness. Namely, needle electroneurography and electromyography (15/15), muscle biopsies (13/15), and ^{31}P NMR spectroscopy of forearm muscles (6/15) failed to demonstrate any neuromuscular disease. None of

the CFS patients had a genetic background of the same pathology. Data were compared with those obtained in a gender-, age- and weight-matched group of 11 Caucasian healthy sedentary volunteers (three females; mean age: 43 ± 3 years; mean weight: 71 ± 3 kg) who consulted for a medical checkup and came from the same type of socioeconomic circumstances.

Procedures were carried out with the adequate understanding and written consent of the subjects. The protocol was approved by the Ethics Committee CCPPRB Marseille 1.

The functional examinations at rest consisted in lung function testing with measurements of arterial blood gases, ECG recording, arterial blood pressure measurements using a sphygmomanometer, and venous blood measurements of oxygen partial pressure (PvO_2), pH (pHv), lactate, potassium (K^+), TBARS, GSH and RAA concentrations. All the resting data of the subjects, even of those suffering from fatigue, were in a normal range. The protocol of the examination during an incremental cycling exercise, which included measurements of cardiorespiratory variables, surface EMG from vastus lateralis, and venous blood dosages, is routinely used in our laboratory for the diagnostic of dyspnoea at exertion [29–31].

Physiological measurements

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

At rest, arterial blood gases (PaO_2, $PaCO_2$ and pHa) were analysed in 100 μL arterialized blood sampled from the ear lobes, and venous PO_2 (PvO_2) and pH (pHv) were measured in a venous blood sample (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; Nellcor Puritan Bennett, Hertogenbosch, the Netherlands).

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 for continuous measurements of inspired and expired volume. It was calibrated with a 1-L syringe, and gave 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 analysers (90% response time in 100 ms) which measured the endtidal partial pressures of O_2 and CO_2 respectively. Throughout the incremental exercise trial, the software (Oxycon beta; Jaeger, Bunnik, The Netherlands) computed breath-by-breath data of V_E, $V\dot{O}_2$, $V\dot{C}O_2$, and the ventilatory equivalents for O_2 ($V_E/V\dot{O}_2$) and CO_2 ($V_E/V\dot{C}O_2$), and HR was averaged for 10-s consecutive periods. It also allowed to obtain relationships between each variable and time or work load in each individual. The ventilatory threshold (V_{Th}) corresponded to the $V\dot{O}_2$ value at which $V_E/V\dot{O}_2$ exhibited a systematic increase without a concomitant increase in $V_E/V\dot{C}O_2$ [32]. V_{Th} was expressed in absolute value of oxygen uptake related to body weight.

EMG recording and analysis

Bipolar (30 mm centre-to-centre) Ag-AgCl surface electrodes (Medtronic, 13 L 20 Skovlunde, Denmark) were used to measure EMG voltage from the vastus lateralis muscle on the dominant side of the body. The electrodes were placed between the motor point and the proximal tendon. Inter-electrode impedance was kept below 2000 Ω 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 mΩ; gain, 1000–5000) 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 [29, 30]. A constant-current neurostimulator (Grass, Quincy, MA, USA) delivered supramaximal shocks with 0.1-ms rectangular pulses through an isolation unit. 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. The supramaximal stimulation was defined as the pulse intensity level about 15% above the level yielding an M-wave of

maximal amplitude. The signal was fed to an oscilloscope (model DSO 400; Gould, Ballainvilliers, France), permitting 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.

Biochemical analyses

A catheter (Neofly 21G, Viggo-Spectramed) was inserted in an antecubital vein. Three millilitres of heparinized blood was sampled at different sequences of the protocol in order to measure all the biochemical variables: blood lactate ([LA]) and potassium concentration ($[K^+]$) (Corning-Chiron model 860; Bayer Corporation), and three blood markers of the oxidative stress (thiobarbituric acid-reactive substances, TBARS, and two endogenous antioxidants, plasma-reduced ascorbic acid, RAA, and erythrocyte reduced glutathione, GSH). The plasma TBARS concentration was assessed according to the method by Uchiyama and Mihara [33]. After centrifugation of heparinized blood samples (1500 ***g*** at 4 °C for 10 min), 300 μL of treated plasma was deproteinized in the same volume of 10% trichloroacetic acid (TCA) containing 2.90 mmol of ethanolic butylated hydroxytoluene (Sigma-Aldrich Co., Saint Quentin Fallavier, France) to avoid further peroxidation. After vortexing and centrifugation (2500 ***g*** at 4 °C for 15 min), the supernatants were stored at −80 °C for further analysis. In test tubes containing 200 μL aliquots, we added successively 200 μL of 8.1% sodium dodecylsulfate, 1.5 mL of 20% acetate buffer (pH = 3.5), 1.5 mL of freshly prepared 0.8% thiobarbituric acid (Sigma-Aldrich Co.) and 600 μL of water. The test tubes containing glass beads were heated at 100 °C for 60 min, then cooled in tap water at room temperature. Then, we added in each tube 4 mL of n-butanol and 1 mL of water. After vortexing for 5 min, the mixture was centrifugated (2000 ***g*** for 3 min) to obtain a rapid separation between organic and aqueous phases. The upper organic phase was pipetted and the pink pigment was measured using a spectrofluorimeter at an excitation wavelength of 515 nm and an emission wavelength of 553 nm (Shimadzu model RF-5000; Shimadzu, Kyoto, Japan). A standard curve of TBARS was obtained after an overnight hydrolysis at room temperature of a solution containing 1 mmol of tetraethoxypropane (Sigma-Aldrich Co.) in 0.1 N HCl. Erythrocyte GSH was assayed spectrophotometrically using a commercially available kit (GSH-400; Oxis International Inc., Biomedical Diagnostics, Marne-la-Vallée, France). We used the method by Yoshida [34]. Briefly, 200 μL of erythrocyte pellet was pipetted and extracted in 800 μL of 6.25% metaphosphoric acid. The extract was vortexed and centrifuged (3000 ***g*** at 4 °C for 10 min.). The supernatants remained stable at −80 °C in metaphosphoric acid for a maximum of 3 weeks before analysis. To the test tube containing 100 μL of supernatant, we added 800 μL of potassium phosphate buffer (pH 7.8) containing 0.2 mmol L^{-1} diethylenetriamine-penta-acetic acid (DTPA) and 0.025% lubrol, 50 μL of 1.2×10^{-2} mol L^{-1} chromogen (4 chloro-1-methyl-7-trifluoromethyl-1-quinolinium sulphate) in 0.2 N HCl, and 50 μL of 30% NaOH. After vortexing, the solution was incubated for 10 min at 25 °C in darkness. The optical density was estimated at a wavelength of 400 nm. A standard curve was obtained with reduced glutathione in 5% metaphosphoric acid. Plasma RAA concentration was estimated spectrophotometrically using the method by Maickel [35] based on the reduction of iron by ascorbic acid in the presence of orthophosphoric acid and α-α′-dipyridyl, leading to the formation of a red-orange chromophore. One millilitre of plasma was deproteinized in an equal volume of 10% TCA and vortexed. After centrifugation (2500 ***g*** at 4 °C for 15 min), the supernatants were then stored for a maximum of 2 months at −80 °C until biochemical analyses were performed. We added 60 μL of 0.5% orthophosphoric acid, 1 mL of 0.5% α-α′-dipyridyl and 200 μL of 1% ferric chloride into a test tube containing 200 μL of ascorbic acid extract in 5% TCA. After vortexing, the mixture was left for 10 min at room temperature in the dark. The optical density was measured at 525 nm using a spectrophotometer (Spectronic Genesys 2; Milton Roy Company, Rochester, NY, USA). A freshly prepared standard curve was obtained with ascorbic acid in 5% TCA.

Exercise protocol

The protocol consisted of (i) a 2-min rest period, during which all variables were measured and

venous and arterial blood samples collected, (ii) a 2-min 0-W work load period used to reach the 1 Hz cycling frequency, (iii) a work period, and (iv) a 30-min recovery period. The work period started at a work load of 20 W and the load was increased by 20 W every 1 min until the subject stopped pedalling. 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 recovery, venous blood samples were drawn and M-wave recorded at 5, 10, 20 and 30 min.

Statistical analyses

ANOVA for repeated measures was used to determine the significance of changes in TBARS, GSH and RAA values throughout the cycling trial and the postexercise recovery period. Significance was accepted when $P < 0.05$.

In each group, linear regression analyses with determination of 95% confidence intervals for ordinates and slopes were first established throughout the entire exercise bout between breath-by-breath measurements of $V\dot{o}_2$ (related to body weight) or HR averaged for consecutive 10-s epochs and the corresponding work loads. Relationships were also established between systolic and diastolic blood pressure or biochemical data collected at rest, V_{Th}, $V\dot{o}_{2max}$, and the corresponding oxygen uptake. ANOVA allowed to test for the significance of intergroup differences in the time course of changes in physiological and biochemical variables during the exercise bout. Regression analyses were also performed during the recovery after the exercise but the significance of intergroup differences between ordinates did not reveal any intergroup difference in slopes.

ANOVA for repeated measure was used to test the significance of the changes in M-wave and the markers of ROS production during the exercise bout and the recovery period with respect to the corresponding resting values.

Because large intergroup differences in the maximal exercise power and the exercise duration between control subjects and CFS patients could simply explain the intergroup differences, we also expressed the maximal individual variations of each biochemical variable in percentage of the corresponding $V\dot{o}_{2max}$.

Student's *t*-test was used to assess intergroup differences (CFS versus control subjects) between resting values and exercise-induced variations of physiological and biochemical variables.

Results

The majority (11/15) of our CFS patients were able to withstand the exercise test without any major exacerbation of fatigue and of the symptoms of their illness.

Cardiorespiratory responses to exercise

As shown in Table 1, $V\dot{o}_{2max}$ of CFS patients was significantly ($P < 0.01$) lower than that of control subjects. In control subjects, $V\dot{o}_{2max}$ was close to the predicted values (108 ± 5%) whereas it was only 84 ± 7% of predicted $V\dot{o}_{2max}$ in CFS patients. Because the maximal work rate was lower in CFS patients, the total duration of the incremental exercise was reduced in these patients (7 min in

Table 1 Characteristics of chronic fatigue syndrome (CFS) and control subjects and their cardiorespiratory response to incremental exercise

Age (years)	Weight (kg)	V_{Th} absolute	$V\dot{o}_{2max}$ absolute (%)	Maximal power (W)	$V\dot{o}_2$ versus work load	HR versus work load
Control ($n = 11$)						
43	71	25	37 (108)	209	0.152	0.341
3	3	2	3 (5)	17	0.006	0.038
CFS ($n = 15$)						
48	67	16	24 (84)	133	0.139	0.362
3	4	1**	**1 (7)*	9**	0.008	0.040

V_{Th}, value of oxygen uptake measured at determination of the ventilatory threshold; $V\dot{o}_{2max}$, maximal measured oxygen uptake (absolute value and percentage of predicted one); maximal power; mean slopes of $V\dot{o}_2$ or HR versus work load relationships. $V\dot{o}_2$ values are expressed in mL STPD min^{-1} kg^{-1}. *$P \leq 0.05$; **$P \leq 0.01$.

CFS vs. 10 min in control). The ventilatory threshold (V_{Th}) was measured at a lower Vo_2 level in CFS patients but, when expressed in percentage of measured Vo_{2max}, it did not differ between groups (CFS: 67% Vo_{2max}; control: 68% Vo_{2max}). Comparing CFS patients to control subjects, we found that the slope of their Vo_2 versus work load relationship did not significantly differ from control.

There were no intergroup differences between the slopes of the HR versus work load relationship (Table 1) and no intergroup differences in the relationships between systolic blood pressure and oxygen uptake (Fig. 1). In CFS patients, as in control subjects, diastolic blood pressure did not vary in proportion to oxygen uptake (Fig. 1).

As already stated, we cannot compare the post-exercise recovery of Vo_2, HR, and systolic blood pressure values because the ordinates of regression lines differed too much between groups.

There were no significant changes in SpO_2 value throughout the exercise bout in the two groups. Figure 2 shows the time courses of PvO_2 and pHv fall, and LA increase during the exercise. The slopes of PvO_2 versus Vo_2 relationship differed significantly ($P < 0.01$) (CFS patients: -0.72 ± 0.14 mmHg mL^{-1} $STPDo_2$ min^{-1} kg^{-1}; control: -0.26 ± 0.12 mmHg mL^{-1} $STPDo_2$ min^{-1} kg^{-1}). However, these slopes cannot be compared because the ordinates of regression lines also differed (CFS: rest $PvO_2 = 42 \pm 3$ mmHg; control: 36 ± 4, $P < 0.05$). Nevertheless, Fig. 2 clearly

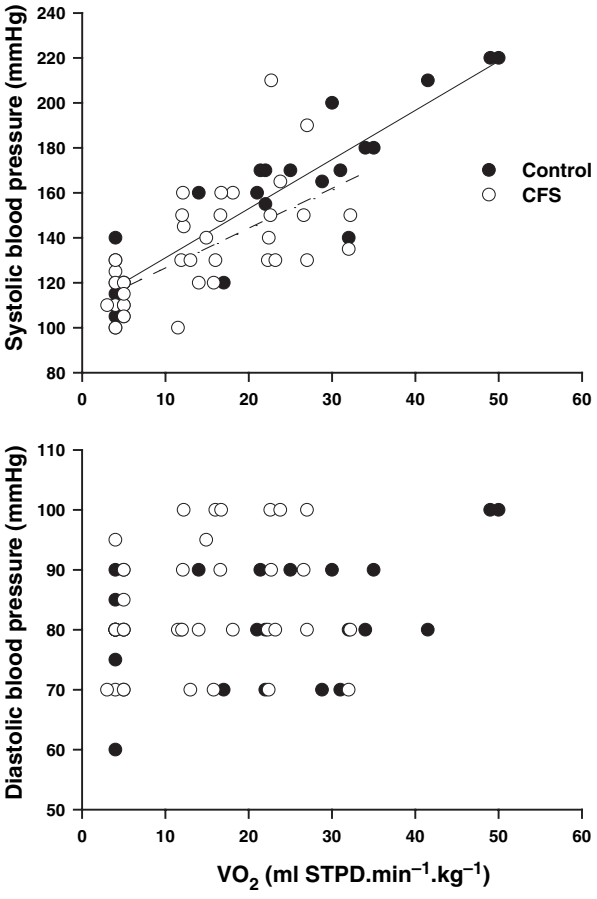

Fig. 1 Relationships between systolic systemic blood pressure and the corresponding oxygen uptake (Vo_2) obtained during the incremental exercise bout in control (normal) and patients suffering from the chronic fatigue syndrome (CFS). Regression lines are drawn (control: continuous line; CFS: dashed line). No correlation exists between diastolic blood pressure and Vo_2.

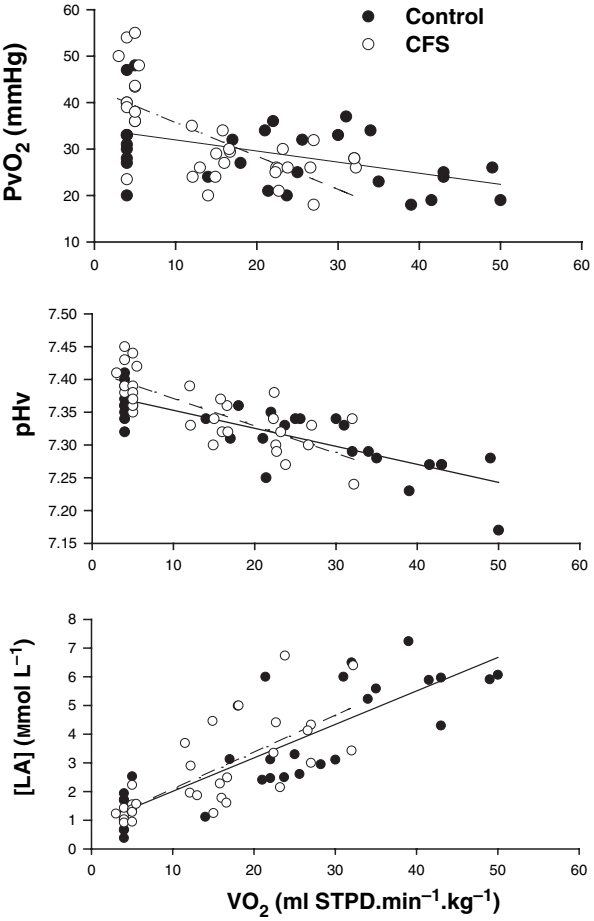

Fig. 2 Relationships between Vo_2 and the partial pressure of oxygen in venous blood (PvO_2), pHv, and lactic acid concentration in venous blood ([LA]) in control and CFS patients performing an incremental exercise. Regression lines are drawn (control: continuous line; CFS: dashed line).

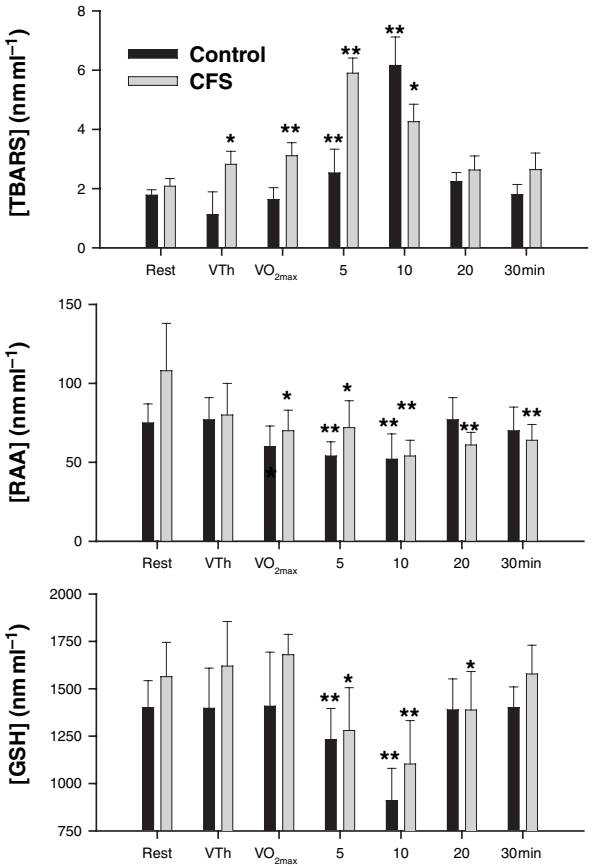

Fig. 3 Mean values (±SEM) of the blood marker of lipid peroxidation (TBARS), plasma-reduced ascorbic acid (RAA), and erythrocyte-reduced glutathione (GSH) measured at rest, during exercise, and the 30-min postexercise recovery period in control and CFS patients. Asterisks indicate values that significantly differ from resting level (*$P < 0.05$; **$P < 0.01$).

indicates that compared with control values, the PvO_2 value was markedly lower at the same work load in CFS patients.

No significant differences were found between the pHv or LA versus Vo_2 relationships in the two groups (Fig. 2). The peak LA increase, always measured at 5 min of the postexercise recovery period in the two groups, was significantly ($P < 0.05$) lower in CFS (+5.24 ± 0.55 mmol L^{-1}) than in control subjects (+7.54 ± 0.71 mmol L^{-1}) but this difference disappeared after the individual peak LA increase was related to the corresponding Vo_{2max} value (CFS: 0.218 ± 0.011; control: 0.205 ± 0.021 mmol L^{-1} mL^{-1} STPDo_2 min^{-1}kg^{-1}).

Exercise-induced oxidative stress

Figure 3 shows the levels of TBARS, RAA and GSH measured at rest, V_{Th}, Vo_{2max}, and postexercise recovery period in the two groups. In control subjects, both the exercise-induced TBARS increase and the consumption of RAA and GSH became significant only at 5 min of recovery of exercise and their variations only persisted until the 10th min of the recovery period. In CFS patients, the TBARS increase and RAA decrease were significant at V_{Th} and Vo_{2max}, respectively, and the consumption of the two antioxidants (RAA and GSH) persisted until the 20th min (GSH) and 30th min (RAA). Table 2 presents the resting levels of ROS blood markers and their maximal postexercise variations, also related to the corresponding Vo_{2max}. This shows that the resting levels of TBARS, RAA and GSH did not differ between CFS and control groups. When related to the corresponding Vo_{2max}, the maximal TBARS increase had a tendency (nonsignificant) to be higher than for control subjects and we measured an accentuated maximal RAA consumption which

Table 2 Baseline and maximal postexercise variations of the blood markers of oxidative stress in control and chronic fatigue syndrome (CFS) subjects

TBARS			RAA			GSH		
Rest nm mL^{-1}	Max nm mL^{-1} (%)	/Vo_{2max}	Rest nm mL^{-1}	Max nm mL^{-1} (%)	/Vo_{2max}	Rest nm mL^{-1}	Max nm mL^{-1} (%)	/Vo_{2max}
Control								
1.78	6.16 (+246)	6.64	75	60 (−19)	−0.52	1401	910 (−38)	−1.03
0.18	0.95 (62)	1.02	12	9 (5)	0.15	142	195 (11)	0.43
CFS								
2.08	5.90 (+184)	7.67	109	54 (−36)	−1.49	1564	1103 (−25)	−1.04
0.24	0.80 (50)	1.05	36	9 (7)*	0.36*	181	174 (6)	0.29

Maximal postexercise variations are also related to the corresponding maximal oxygen uptake (Vo_{2max}) (expressed in %/mL O2STPD mL^{-1} kg^{-1}). *$P \leq 0.05$.

was almost three times higher than in control subjects.

Exercise-induced M-wave variations

As shown in Fig. 4, no significant modification of the neuromuscular conduction time, as well as the M-wave amplitude and duration, were measured in control individuals during both the exercise and the recovery periods. By contrast, in CFS patients a significant lengthening of M wave began at 5 min of the recovery and further M-wave changes developed until the 30th min of the recovery period. These intergroup differences between the changes in M-wave duration were accentuated when expressed in percentage of corresponding resting values (Fig. 5).

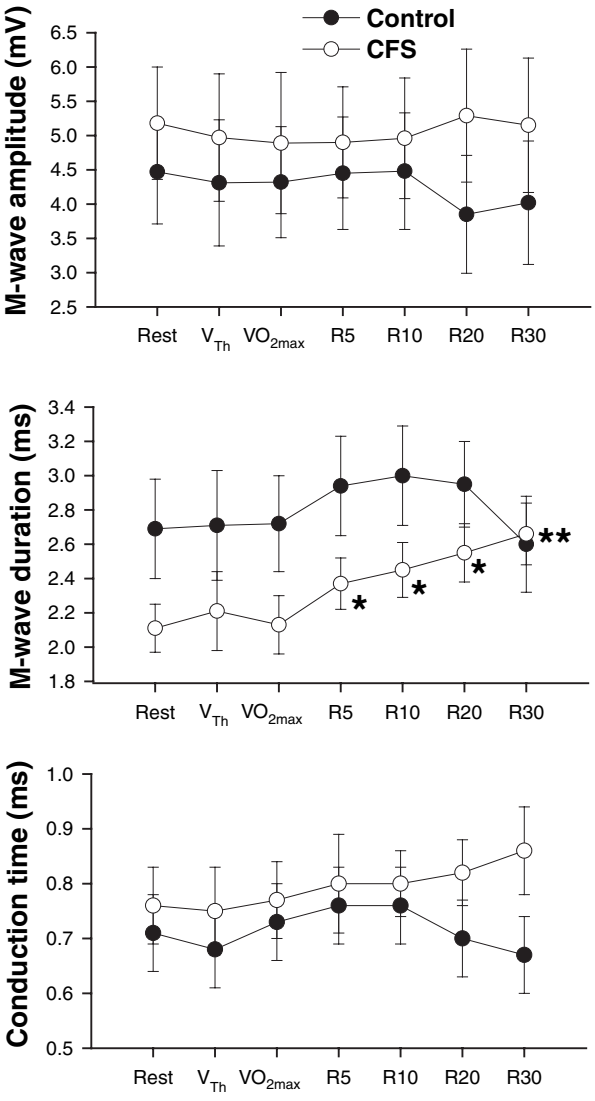

Fig. 4 The changes in characteristics of the compounded-evoked muscle action potential (M-wave) recorded during an incremental exercise bout and the recovery period of exercise in control and CFS patients. Values are mean ± SEM. Asterisks indicate values that significantly differ from resting level (*$P < 0.05$; **$P < 0.01$).

Exercise-induced blood K^+ variations

During the incremental exercise, there was a normal potassium efflux associated with muscle excitation which increased in proportion of the work rate (Fig. 5). The kinetics of potassium rise as well as that

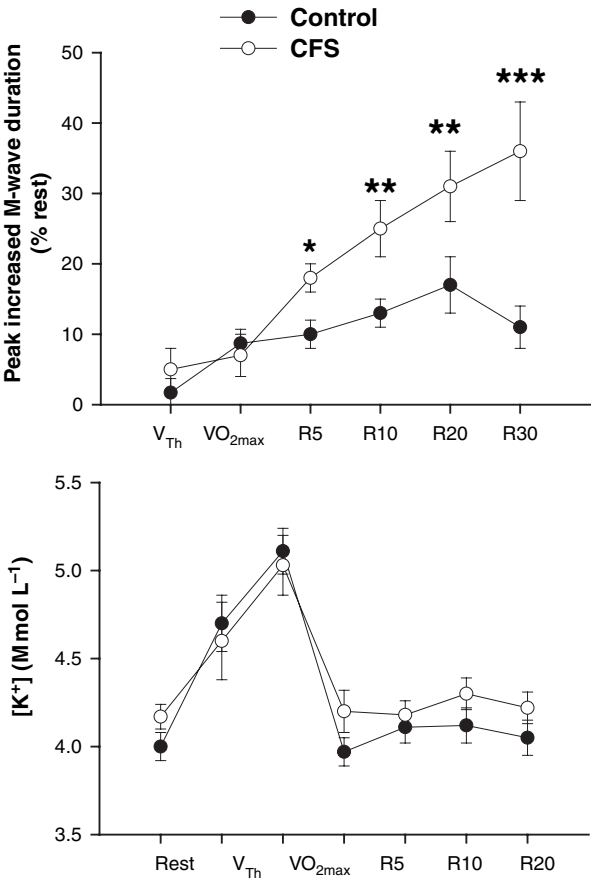

Fig. 5 Upper diagram: the exercise-induced changes in M-wave duration, an index of the muscle membrane excitability, are expressed in percentage of the baseline value measured prior to exercise in control and CFS patients. Lower diagram: absolute values of blood potassium concentration ([K+]) measured in the two groups of subjects. Values are mean ± SEM. Asterisks indicate values that significantly differ from resting level (**$P < 0.01$; ***$P < 0.001$).

of its postexercise decrease were the same in control subjects and CFS patients (Fig. 5). The peak K^+ increase, always measured at the end of the exercise, was significantly ($P < 0.05$) lower in CFS patients ($+0.85 \pm 0.08$ mmol L^{-1}) compared with normal subjects ($+1.27 \pm 0.09$ mmol L^{-1}) but this difference disappeared when the maximal K^+ variations were related to $V\text{o}_{2\text{max}}$ (CFS: 0.035 ± 0.006; control: 0.034 ± 0.008 mmol L^{-1} mL^{-1} STPDo_2 min^{-1} kg^{-1}).

Discussion

The present study in CFS patients shows that their maximal aerobic capacity was lowered because they stopped pedalling earlier than the age-, weight- and gender-matched control individuals. However, the slope of $V\text{o}_2$ versus work load relationship did not significantly differ from control and the simultaneous measurements of SpO_2 and PvO_2 even suggested the occurrence of an accentuated arterio-venous O_2 difference and thus of an elevated oxygen uptake in exercising muscles. By contrast, the resting PvO_2 level, measured in comfortably seated subjects before they were equipped to exercise, was higher in resting CFS patients, suggesting a reduced baseline oxygen uptake by tissues. There was no intergroup difference between resting $V\text{o}_2$ values but they were measured in subjects standing on the bicycle and wearing the complete equipment (face mask, SpO_2 device, ECG leads…), that is in a stressing condition which often increased both ventilation and HR. In contrast, the cardiac and systolic blood pressure response to incremental exercise was the same than in control. We also found that the time course of pHv and LA changes during the exercise did not differ from control and that the peak LA variations were the same in the two groups. Despite no difference in resting levels of TBARS and antioxidants between CFS and control groups, in CFS patients the exercise-induced oxidative stress occurred sooner, that is at the maximal work rate, lasted more, and there was a significant enhanced maximal postexercise decrease in RAA level. This accentuated postexercise oxidative stress was associated with marked alterations in muscle excitability (lengthened M-wave duration), these M-wave changes being totally absent in our control subjects.

Our observations of the absence of impaired aerobic metabolism in our CFS patients corroborate several previous observations based on nearly the same exercise protocol [14–19]. These physiological data are supported by the absence of ultrastructural mitochondrial abnormalities in CFS patients [36]. A study by Fulcher and White [13] even reported an accentuated aerobic metabolism in their CFS patients. We cannot come to the same conclusion but the present observation of higher arterio-venous oxygen difference in exercising CFS patients partly supports the conclusions by Fulcher and White [13]. In addition, we did not report any intergroup differences between the progressive blood acidosis (pHv fall, LA increase) during the exercise bout, corroborating previous observations by Barnes *et al.* [14] who did not observe any abnormalities of glycolysis or pH regulation in a large group of CFS patients ($n = 46$) explored using ^{31}P NMR spectroscopy. By contrast, Wong *et al.* [11], who also used ^{31}P NMR spectroscopy in the gastrocnemius muscle, found that the changes in PCr and intramuscular pH occurred more rapidly in CFS patients than in control subjects suggesting an acceleration of glycolysis. However, there may be marked interindividual differences in the metabolic profiles of CFS patients because in their study Barnes *et al.* [14] clearly showed that an increased acidification relative to PCr depletion occurred in six of 46 subjects.

Oxidative stress is highly expressed in skeletal muscles because their antioxidant defences are poor [37]. We already described the changes in the same blood markers (TBARS, RAA and GSH) in response to exactly the same protocol of incremental cycling exercise [31]. In our previous study in a large number of healthy sedentary subjects, we showed that a significant exercise-induced increase in TBARS and consumption of blood antioxidants (RAA and GSH) never occurred before the 5th min of the recovery period and that the three blood markers recovered their resting levels within a maximum of 20 min. Our present data confirm these observations in control subjects. Thus, the early changes in blood redox status here measured in CFS patients during the exercise bout have a real significance. These differences prevail for the changes in plasma RAA concentration. In humans, RAA is the only endogenous antioxidant that completely protects the plasma lipids from any detectable damage induced by the formation of hydroperoxide

radicals [38, 39], trapping all hydroperoxide radicals in the aqueous phase before they can reach the plasma lipids. Data in the literature also indicate an increased blood oxidative stress in resting CFS patients [24, 25]. Another observation [13] is in favour of an increased activity of intramuscular antioxidants (catalase, glutathione peroxidase and transferase) in resting CFS patients. Our study only showed a tendency (nonsignificant) for elevated baseline levels of TBARS, RAA and GSH. The present observations of an accentuated exercise-induced oxidative stress in CFS patients are supported by experimental data in a mouse model of CFS. Indeed, Singh *et al.* [40] reported that antioxidants markedly reduced the increased lipid peroxidation and catalase levels in the whole brain of mice which were forced to swim every day for a 7-day session. Such an accentuated exercise-induced oxidative stress in CFS patients could explain the enhanced oxygen uptake by the exercising muscles suggested by our measurement of an elevated arterio-venous oxygen difference. Indeed, recent data [41, 42] show that superoxide activates the mitochondrial uncoupling proteins and uncoupling processes enhance oxygen uptake through their influence on the mitochondrial respiratory chain.

Recording the compound-evoked muscle action potentials (M-wave) with surface electrodes (SEMG) is a noninvasive means to explore peripheral muscle fatigue in exercising humans. An impaired excitation of the muscle fibres is suspected when the M-wave declines and becomes broader [43]. After an incremental cycling exercise, we observed that the M-wave duration was modestly lengthened in sedentary subjects [29, 30]. The present study in CFS patients reports no change in the neuromuscular transmission (conduction time) but it shows marked alterations of muscle excitability which began early after the exercise had stopped and culminated at the end of the 30-min recovery period. Only Kent-Braun *et al.* [3] recorded the M-wave and analysed its changes in amplitude in CFS patients executing intermittent submaximal contractions of the tibialis anterior muscle. These authors did not measure any significant differences in the M-wave variations between CFS and control subjects but their protocol was limited to a small muscle group and thus cannot be compared with an incremental cycling exercise until $\dot{V}O_{2max}$ which involves the participation of large muscle groups. The postexercise-altered muscle membrane excitability reported here in CFS is not explained by any impairments of the potassium outflow during muscle excitation or of the potassium inflow during the recovery period. As already demonstrated by Marcos and Ribas [44], an extracellular potassium accumulation can act as a negative feedback signal for sarcolemma excitability and this may constitute a possible mechanism for the postexercise M-wave alterations. In CFS patients, the accentuated and prolonged postexercise oxidative stress may be responsible for muscle membrane alterations (for example the formation of lipid hydroperoxides) with the consequence of the impaired membrane excitability described here. To explain their data of altered excitation-contraction coupling in skeletal muscle of CFS patients, Fulle *et al.* [8] suggested that the deregulation of pump activities could result from an increased sarcoplasmic reticulum membrane fluidity and the role played by the formation of lipid hydroperoxides in this process is already well documented [22].

Thus, as in inherited muscular dystrophy in which a variety of cellular abnormalities can be accounted for by free radical-mediated damages including abnormal functions of the sarcolemma and an altered activity of membrane-bound enzymes involved in excitation-contraction coupling, an increased level of free radical damage in CFS may be a contributor to the underlying functional defects and symptom presentation. This should promote further researches towards the goal of an effective treatment of CFS-suffering patients.

Conflict of interest statement

No conflict of interest was declared.

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Correspondence: Pr. Yves Jammes, Laboratoire de Physiopathologie Respiratoire, Faculté de Médecine, Boulevard Pierre Dramard, 13916 Marseille cedex 20, France.
(fax: +33 4 91 69 89 27; e-mail: jammes.y@jean-roche.univ-mrs.fr).