

# Chronic fatigue syndrome with history of severe infection combined altered blood oxidant status, and reduced potassium efflux and muscle excitability at exercise

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## ABSTRACT

**It is documented that chronic fatigue syndrome (CFS) combines enhanced oxidative stress with altered muscle excitability. We hypothesized that these disorders may be accentuated when severe infection preceded the CFS symptoms. This case-control study compared 55 CFS patients to a matched control group of 40 healthy subjects. In twenty-five CFS patients, severe infection was reported within the three to seven month period preceding the CFS symptoms. The others had practiced sport at high level. Plasma concentrations of potassium, a marker of lipid peroxidation (thio-barbituric acid reactive substances, TBARS), and an endogenous antioxidant (reduced ascorbic acid, RAA) were measured. Action potential (M-wave) was evoked in the *vastus lateralis* muscle to explore the muscle membrane excitability. All subjects performed a maximal incremental cycling exercise. Compared to control subjects, all CFS patients presented an elevated resting TBARS level and, during and after exercise, an altered M-wave configuration. History of infection was associated with marked significant increase in resting TBARS level, enhanced M-wave alterations, and also reduced exercise-induced potassium efflux. The magnitude of exercise-induced M-wave alterations was proportional to the baseline TBARS level. Severe infection preceding CFS seems to constitute a stressor inducing altered blood oxidant status and a reduced muscle excitability at work.**

**Keywords:** Chronic Fatigue Syndrome; Infectious Diseases; Muscle Excitability; Oxidative Stress

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## 1. INTRODUCTION

The Chronic Fatigue Syndrome (CFS) results in a severe impairment in sports and daily activities [1]. Exercise-induced CFS pathology is suspected [2,3]. However, CFS often follows severe bacterial or viral infection [4-6] and various suspected pathogens, like Epstein-Barr virus, cytomegalovirus, human herpes virus, enterovirus, parvovirus and mycoplasma, have been considered. Previous studies have reported marked alterations in biological and physiological responses to maximal exercise in CFS patients compared to healthy sedentary subjects [7-12]. These differences included marked post-exercise alterations of the muscle membrane excitability [7,8], an early and lengthened exercise-induced oxidative stress [7-13], and an attenuated heat shock proteins response (HSP) [8,13].

An excessive production of reactive oxygen species (ROS), responsible for membrane lipid peroxidation [14], exerts well known effects on isolated human and animal tissues, including the loss of membrane excitability due to an altered activation of potassium ( $K^+$ ) channels in cardiac myocytes [15] and skeletal muscle fibres [16]. In healthy humans, muscle biopsies have demonstrated that the strength of membrane excitation is proportional to the  $K^+$  efflux [17], detectable in plasma [18]. An increased ROS production exerts an inhibitory action on the  $Na^+K^+$  pump activity [14], reducing the potassium outflow and the muscle membrane excitability. The CFS study by Fulle *et al.* [19] has confirmed a deregulation of the  $Na^+K^+$  and  $Ca^{2+}$ -ATPase pumps and the alterations of ryanodine channels in the sarcoplasmic reticulum membranes. These authors [19] have hypothesized that the deregulation of pump activities could result from an increased fluidity of the sarcoplasmic membrane due to the ROS-induced formation of lipid hydroperoxides. It is

well documented that severe bacterial [20] and viral [21-25] infections, including the H1N1 influenza [24,25], induce an oxidative stress. Thus, infection could be responsible for an altered membrane excitability due to reduced  $K^+$  exchanges through the muscle membrane.

Recording the compound evoked muscle action potentials (M-wave) with surface electrodes is a non-invasive tool, commonly used to explore the muscle membrane excitability in resting and exercising humans [7,26,27]. An impaired excitation of the muscle fibres is assessed when the M-wave declines and becomes broader. In healthy sedentary subjects, modest changes in M-wave duration and amplitude occur during and after a maximal incremental cycling exercise [7,26,27]. On the opposite, CFS patients present a significant lengthening of the neuromuscular conduction time (CT), a reduction of the M-wave amplitude, and lengthened M-wave duration, the altered muscle excitability often persisting at the end of a 30-min recovery period [7].

We hypothesized that the disorders of blood oxidant status and altered muscle membrane excitability could be accentuated in CFS patients with history of severe infection.

## 2. METHODS

### 2.1. Subjects

Fifty five CFS patients were explored. The criteria to include them were 1) clinical [1]: persistent or relapsing fatigue for at least 6 consecutive months or longer and four or more of the following symptoms: post-exertional malaise, impaired memory or concentration, unrefreshing sleep, muscle pain, multi-joint pain, tender cervical or axillary lymph nodes, sore throat, headache; and 2) bio-

logical: the combination of exercise-induced alteration of the muscle membrane excitability [7,8] and an early and lengthened exercise-induced oxidative stress [7-13]. Depression patients and chronic disease patients were excluded in this study. Retrospectively, two groups of CFS patients were identified considering their history of sport practice and severe acute infection. Thirty of them had practiced sport at high level (>6 h per week) for more than six years before the symptoms had occurred and no history of severe infection was reported by their practitioners. In twenty five patients, who did not regularly practice any sport, severe infection (H1N1 influenza: 5; pneumonia: 7; encephalomyelitis: 6; sepsis: 4; unidentified: 3) was reported within the three to seven month period preceding the onset of CFS symptoms. The two groups of CFS patients were called NI (not infected) and I (infected), respectively. The data were compared to those obtained in a gender, age-, and weight-matched control group of forty Caucasian healthy volunteers who consulted for a medical check up and were coming from the same type of socioeconomic class. Additional biochemical analyses in venous blood samples were approved by the Ethics Committee of our institution (CCPPRB Marseille 1) and the protocol was performed in accordance with the Declaration of Helsinki. The procedures were carried out with the adequate understanding and written consent of the subjects. The morphological characteristics of NI and I CFS patients and healthy subjects are collected in **Table 1**.

### 2.2. Physiological Measurements

The functional examinations at rest consisted in ECG recording, measurements of the arterial blood pressure

**Table 1.** Comparison of controls and CFS patients. Morphological characteristics, maximal performances at work, baseline and maximal variations of venous plasma concentrations of lactic acid (LA), potassium ( $K^+$ ), and markers of oxidant/antioxidant status (TBARS, RAA) in healthy (control) subjects and CFS patients having history or not of recent severe infection (Infected or I CFS, non infected or NI CFS).

|   | Controls    | NI CFS group                 | I CFS group              |
|---|-------------|------------------------------|--------------------------|
| N   | 40          | 30                           | 25                       |
| F/M ratio   | 14/26       | 10/20                        | 12/13                    |
| Age, y  | 44 ± 5      | 39 ± 3                       | 40 ± 3                   |
| Weight, kg  | 71 ± 4      | 71 ± 2                       | 66 ± 2                   |
| VO <sub>2</sub> max, mlSTPD·min <sup>-1</sup> ·kg <sup>-1</sup> | 31 ± 3      | 29 ± 2                       | 27 ± 1                   |
| LA rest, mmol·l <sup>-1</sup>                                   | 1.30 ± 0.16 | 1.43 ± 0.11                  | 1.40 ± 0.10              |
| Δ LAmax, % rest   | +382 ± 21   | +381 ± 51                    | +328 ± 43                |
| K <sup>+</sup> rest, mmol·l <sup>-1</sup>                       | 4.02 ± 0.04 | 4.00 ± 0.05                  | 3.93 ± 0.06              |
| Δ K <sup>+</sup> max, % rest                                    | +29 ± 3     | +25 ± 2*                     | +17 ± 4 <sup>#</sup>     |
| TBARS rest, nmol·ml <sup>-1</sup> ##                            | 1.36 ± 0.07 | 1.87 ± 0.21 <sup>#,***</sup> | 3.48 ± 0.40 <sup>#</sup> |
| Δ TBARSmax, % rest  | +57 ± 11    | +149 ± 25 <sup>#,***</sup>   | +66 ± 18                 |
| RAA, rest nmol·ml <sup>-1</sup>                                 | 148 ± 10    | 134 ± 15                     | 157 ± 19                 |
| Δ RAAMax, % rest  | -51 ± 8     | -30 ± 5                      | -33 ± 7                  |

Values are mean ± SE. Symbol <sup>#</sup> indicates significant difference between controls and CFS patients (<sup>#</sup>: p < 0.05) and asterisk depicts intergroup CFS differences (\*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001).

measurements using a sphygmomanometer, the percutaneous measurement of oxygen saturation (SpO<sub>2</sub>) using an infrared analyser (Nellcor model N3000, Tx, USA), and ventilation and respiratory gas exchange. A face mask 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, Jaeger, Germany) giving measurements of minute ventilation. Fast-response paramagnetic O<sub>2</sub> and infrared CO<sub>2</sub> analyzers measured the end-tidal partial pressures of O<sub>2</sub> and CO<sub>2</sub>, respectively. Exercise trials were performed 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-W pedalling period then the load was increased as a ramp (20 W/min) until the peak O<sub>2</sub> uptake (VO<sub>2</sub>max) was measured. The criteria to establish VO<sub>2</sub>max were to obtain a plateau of VO<sub>2</sub>, to reach predicted maximal values of VO<sub>2</sub> and HR, and to measure a respiratory quotient value higher than 1.1 [28].

### 2.3. Electromyographic (EMG) Recording and Analyses

As in our previous studies [7,26,27], bipolar (30 mm center-to-center) 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. The electrodes were placed between the motor point and the proximal tendon. Inter-electrode impedance was kept below 2000 ohm 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 action potentials (M-waves) were evoked by direct muscle stimulation, using a monopolar technique. A constant-current neurostimulator (S88 model Grass, Quincy, MA, USA) delivered supramaximal shocks with 0.1-ms rectangular pulses through a Grass 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. 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-W pedalling period preceding the exercise bout. The EMG signal was fed to a numerical oscilloscope (model DSO 400, Gould, Ballainvilliers, France), permitting to aver-

age the M-waves from 8 successive potentials and to calculate 1) the peak M-wave amplitude; 2) its duration; and 3) the conduction time, that is the time between the stimulus artefact and the peak EMG response. 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 the leg extension and thus of the *vastus lateralis* contraction. It was used to trigger within a delay of 100 ms the neurostimulator delivering 0.1-ms-long rectangular pulses. Because the pedalling rate was set at 60 min<sup>-1</sup>, one leg extension was performed every sec and the stimulation frequency was set at 1 Hz. For each epoch of the protocol (the 0-W cycling period preceding the incremental exercise, incremental exercise, and recovery period), eight successive evoked muscle potentials were averaged. Because M wave recordings must be performed during cycling at the same pedalling rate, the subject was asked to pedal again for several epochs during the recovery from exercise.

### 2.4. Biochemical Analyses

A catheter (Neofly 21 G, Viggo-Spectramed) was inserted in an antecubital vein. Eight ml of heparinized blood were sampled at different sequences of the protocol in order to measure biochemical variables. Lactic acid (LA) and potassium (K<sup>+</sup>) concentrations were measured using specific electrodes (Corning-Chiron model 860, Bayer Corporation, East Walpole, MA USA). A plasma marker of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and an antioxidant (reduced ascorbic acid, RAA) were analyzed according to the original methods by Uchiyama and Mihara [29] for TBARS and Maickel [30] for RAA. All the measurements were made in duplicate.

### 2.5. Exercise Protocol

The protocol consisted of 1) a 30-min rest period, during which all the variables were measured and venous blood samples collected; 2) a 2-min 0-W work load period; 3) a work period, and 4) a 30-min recovery period. When VO<sub>2</sub>max was reached, venous blood was sampled and M-wave recorded. During the post-exercise recovery-period, venous blood samplings and M-wave recordings were performed at 5 and 30 min.

### 2.6. Statistical Analyses

Data are presented as means + standard error (SE). An independent Student's t-test was performed to compare the baseline levels of biochemicals between the two groups of CFS patients and control subjects. For temporally repeated data during rest, exercise, and recovery periods,

the changes over time were determined using ANOVA when the 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.* groups and time) was used to determine the significance of changes throughout the cycling trial and the post-exercise recovery period. Post-hoc independent t-tests with Bonferroni corrections were used to compare time-point specific differences between groups. In all cases, significance was set at the two sides 0.05 level.

### 3. RESULTS

#### 3.1. Examination at Rest

No intergroup differences were noted between baseline values of LA, K<sup>+</sup>, and RAA concentrations whereas the mean value of resting TBARS level was significantly higher in CFS patients and almost doubled in the I CFS group (**Table 1**). The M-wave characteristics measured in the *vastus lateralis* at rest did not significantly differ between groups (**Table 2**).

#### 3.2. Response to Incremental Maximal Cycling

VO<sub>2</sub>max and maximal LA concentration did not differ between the control and CFS groups (**Table 1**). **Figure 1** gives the kinetics of plasma TBARS, RAA, and K<sup>+</sup> levels during and after exercise in the three groups. The TBARS level culminated at VO<sub>2</sub>max in all subjects. The maximal RAA decrease was measured at VO<sub>2</sub>max in CFS patients but later in healthy subjects, and the K<sup>+</sup> concentration always culminated at VO<sub>2</sub>max. **Table 1** shows that compared to controls the maximal TBARS increase was almost doubled in NI CFS patients but did not significantly differ in the I CFS group. No intergroup differences were noted in the maximal RAA decrease. The peak K<sup>+</sup> increase was significantly attenuated in the I CFS group.

Marked M-wave changes occurred in CFS patients at VO<sub>2</sub>max and persisted after the exercise had ended. **Figure 2** gives examples of M-wave recordings in a control

subject and a I CFS patient showing a marked depression in the latter. **Figure 3** depicts the kinetics of the M-wave and CT changes in the three groups. The significance of intergroup differences was already present at VO<sub>2</sub>max and persisted at the 5<sup>th</sup> and 30<sup>th</sup> min. **Table 2** shows that the largest exercise-induced M-wave variations occurred in the I CFS group. The M-wave alterations combined a decreased M-wave amplitude and a lengthened duration of both the M-wave and conduction time.

Because our CFS patients were explored 6 to 23 months after the beginning of the muscle fatigue, we searched for relationships between this delay and the magnitude of resting values and exercise-induced variations of TBARS, RAA, K<sup>+</sup> and M-wave characteristics in NI and I CFS groups. We found that the duration of the clinical disorders did not affect the biochemical and electrophysiological variables.

#### 3.3. Relationships between Biochemical and Electrophysiological Variables

As shown in **Figure 4**, the post-exercise changes in M-wave amplitude and CT were proportional to the baseline TBARS level, the highest alterations being measured in the I CFS group. Despite their changes were near concomitant, the maximal M-wave variations were not correlated to the maximal exercise-induced changes in TBARS, RAA, K<sup>+</sup>, and LA.

### 4. DISCUSSION

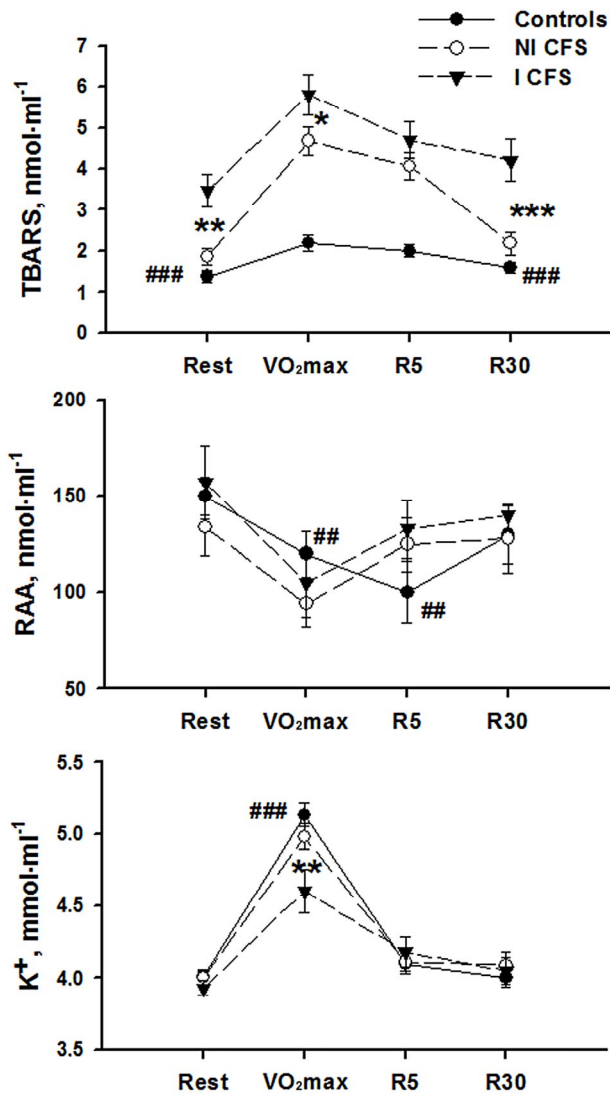
The present study confirms data previously reported in CFS patients, that is an elevation of the blood oxidant status at rest [7-13], an accentuated exercise-induced oxidative stress [7,8] and a reduced muscle excitability [7]. The new finding was the significant accentuation of the blood oxidant status disorders at rest and muscle hypoexcitability at work in CFS patients who reported a recent severe infection (I CFS group). It must be noted that the post-exercise oxidative stress (TBARS increase) was significantly enhanced in NI CFS group but not I CFS one. This might result from the marked elevation of the

**Table 2.** M-wave characteristics (amplitude, duration) and neuromuscular conduction time (CT) measured during pre-exercise 0-W cycling period (reference, Ref) and their maximal exercise-induced variations in controls and the two groups of CFS patients.

|                                | Controls    | NI CFS group            | I CFS group            |
|--------------------------------|-------------|-------------------------|------------------------|
| M-wave amplitude Ref, mV       | 47 ± 9      | 43 ± 14                 | 34 ± 16                |
| Δ M-wave amplitude max, % rest | -16 ± 6     | 26 ± 6 <sup>**</sup>    | -56 ± 10 <sup>##</sup> |
| M-wave duration Ref, ms        | 4.3 ± 0.7   | 4.2 ± 0.9               | 4.9 ± 1.0              |
| Δ M-wave duration max, % rest  | +8 ± 2      | +25 ± 8 <sup>#,**</sup> | +45 ± 11 <sup>##</sup> |
| CT Ref, ms                     | 0.73 ± 0.07 | 0.61 ± 0.14             | 0.83 ± 0.15            |
| Δ CTmax, % rest                | +5 ± 3      | +15 ± 4 <sup>#,*</sup>  | +30 ± 7 <sup>##</sup>  |

Values are mean ± SE. Symbol <sup>#</sup> indicates significant difference between controls and CFS patients (<sup>#</sup>: p < 0.05; <sup>##</sup>: p < 0.01) and asterisk depicts intergroup CFS differences (<sup>\*</sup>: p < 0.05; <sup>\*\*</sup>: p < 0.01).

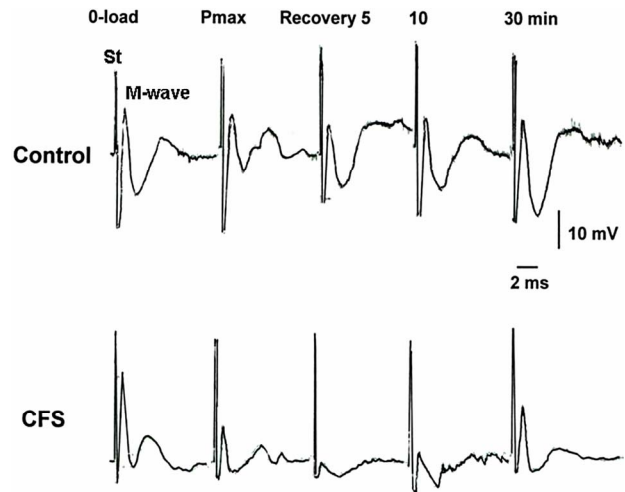




**Figure 1.** Plasma concentration of thiobarbituric acid reactive substances (TBARS) measured in control healthy subjects and both groups of CFS patients (non infected, or NI, and infected, or I) at rest, maximal oxygen uptake (VO<sub>2</sub>max), and during the post-exercise 30-min recovery period. Values are mean ± SEM. Symbol # denotes significant differences between controls and CFSs (##p < 0.01; ###p < 0.001). Asterisk was used to depict significant differences between NI and I CFS groups (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001).

baseline TBARS concentration in I CFS patients which allows to suppose that the disorders of their oxidant status are already near maximal. The chronic elevation of the baseline TBARS level seems to play a key role in muscle excitability at work. Indeed, we measured the highest post-exercise alterations of muscle excitability in the I CFS group. We also noted a significant reduction of the muscle potassium outflow in response to maximal exercise in this group.

The strength of this study was the relative accessibility of the tools used to diagnose CFS by most of the univer-

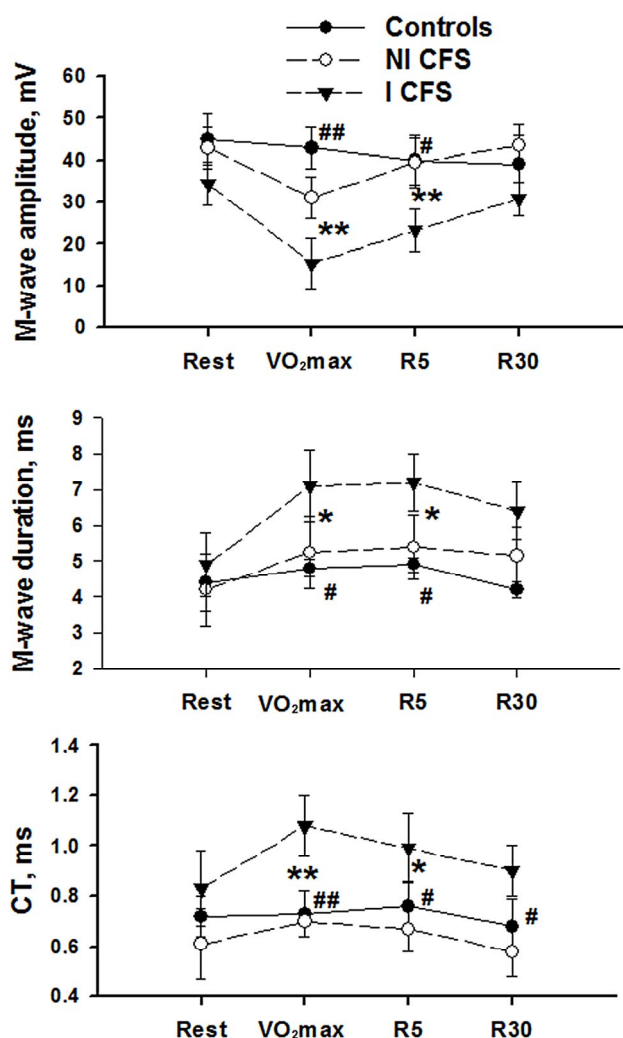


**Figure 2.** Examples of M-wave recordings in *vastus lateralis* muscle in a control (healthy) subject and a CFS patient in the I group at rest, VO<sub>2</sub>max, and during the 30-min post-exercise recovery period. Stimulus (St) and M-wave are indicated.

sity hospitals (M-wave recordings and plasma oxidant-antioxidant status). Its limitation was the absence of data on values of the heat shock proteins (HSP) in our CFS groups. Indeed, our previous studies [8,13] have clearly shown that both the baseline levels and exercise-induced variations of HSPs are significantly lower in these patients. These measurements were not repeated in the present study because HSP measurements with immunoassays kits are relatively expensive and can not be supported by our grants.

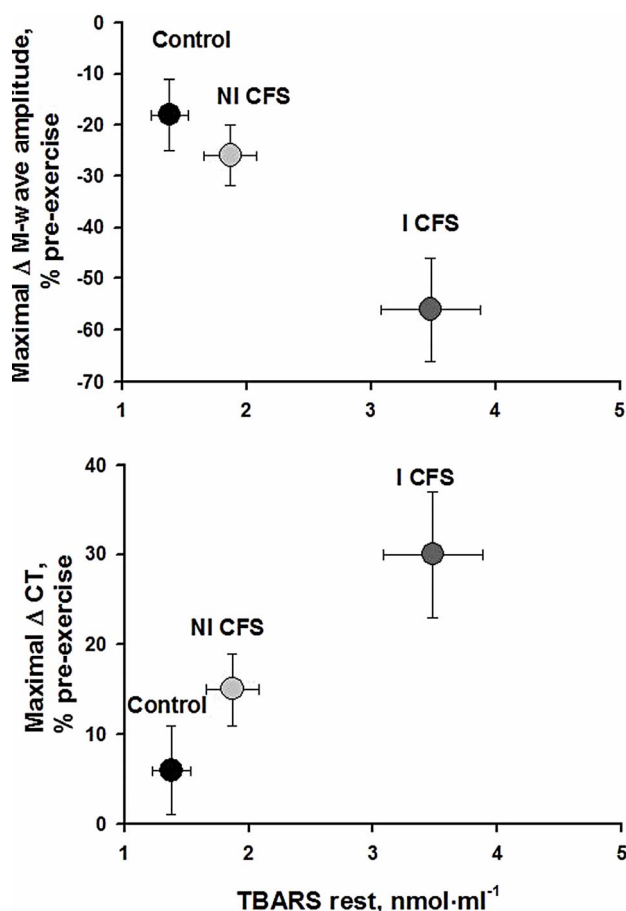
Our comparisons between control subjects and CFS patients at work are valuable because their VO<sub>2</sub>max and maximal exercise power, and thus the total duration of incremental exercise, did not significantly differ. The absence of impaired aerobic metabolism in our CFS patients corroborates several previous observations, based on the same [7] or nearly the same exercise protocol [31, 32]. The accentuated exercise-induced oxidative stress in CFS patients might partly explain the absence of any impairment of their aerobic metabolism at work. Indeed, superoxide activates the mitochondrial uncoupling proteins [33] and the uncoupling processes are well known to enhance the oxygen uptake through their influence on the mitochondrial respiratory chain.

The present study in CFS patients did not report any difference in resting values of the neuromuscular transmission (conduction time) and muscle membrane excitability compared to healthy subjects. On the other hand, we measured marked alterations of the muscle excitability at work which were already significant at the end of the incremental exercise and persisted during the recovery period. The reduction of the M-wave amplitude and the lengthening of the neuromuscular conduction time (CT) were significantly accentuated in CFS patients with



**Figure 3.** Absolute values of M-wave amplitude and duration, and the neuromuscular conduction time (CT) measured in control healthy subjects and CFS patients (non infected, or NI, and infected, or I) at rest, maximal oxygen uptake (VO<sub>2</sub>max) at the end of cycling exercise, and during the post-exercise 30-min recovery period. Values are mean ± SEM. Symbol # denotes significant differences between controls and CFS groups (#p < 0.05; ##p < 0.01). Asterisk was used to depict significant differences between NI and I CFS groups (\*p < 0.05; \*\*p < 0.01).

history of recent severe infection. The enhanced alterations of post-exercise muscle membrane excitability in our I CFS group were associated with a significant reduction of the maximal potassium outflow measured at the end of exercise. We already reported literature data [15-18] demonstrating close relationships between the oxidative stress, the K<sup>+</sup> exchanges through the sarcolemma, and the muscle excitability. As demonstrated by Marcos and Ribas [18], an extracellular potassium accumulation can act as a negative feedback signal for sarcolemma excitability and might constitute a possible mechanism for the post-exercise M-wave alterations.



**Figure 4.** Mean values (± SEM) of resting TBARS level and the corresponding maximal exercise-induced variations of the M-wave amplitude (upper panel) and neuromuscular conduction time (CT) (lower panel) in controls and CFS patients (non infected, or NI, and infected, or I). **Tables 1** and **2** show the significance of intergroup differences.

The present study indicates that CFS patients with antecedents of severe infection have higher biological and EMG disorders than other patients who solely reported practice of high physical activity before the occurrence of fatigue symptoms. It is well documented that acute infection constitutes a stress responsible for an oxidative stress [21-24]. Our major observation was that infection was responsible for lengthened alterations of the resting oxidant status. Indeed, the episode of infection occurred within the three to seven month period preceding the onset of CFS symptoms and our CFS patients were explored 6 to 23 months after the beginning of fatigue. A persistent enhanced oxidant production is highly suspected to trigger the production of the tumor necrosis factor alpha, the most clearly defined mediator of contractile dysfunction. Some studies failed to demonstrate abnormal plasma cytokine levels in CFS patients at rest compared to healthy subjects [34,35] while others reported an elevation of plasma level of transforming growth fac-

tor-beta level, IL-6, and IL-1 $\alpha$  [36,37].

In conclusion, we verified our hypothesis that severe infection represents a stressor responsible for a lengthened alteration of blood oxidant status which could explain the impaired exercise-induced muscle potassium outflow and the altered membrane excitability in CFS patients. In these patients, free radical damage may be a contributor to the underlying functional defects during exercise and symptom presentation.

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