FUNCTIONAL CHARACTERIZATION OF MUSCLE FIBRES FROM PATIENTS WITH CHRONIC FATIGUE SYNDROME: CASE-CONTROL STUDY

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Received February 9, 2009 – Accepted March 25, 2009

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Chronic fatigue syndrome (CFS) is a disabling condition characterized by unexplained chronic fatigue that impairs normal activities. Although immunological and psychological aspects are present, symptoms related to skeletal muscles, such as muscle soreness, fatigability and increased lactate accumulation, are prominent in CFS patients. In this case-control study, the phenotype of the same biopsy samples was analyzed by determining i) fibre-type proportion using myosin isoforms as fibre type molecular marker and gel electrophoresis as a tool to separate and quantify myosin isoforms, and ii) contractile properties of manually dissected, chemically made permeable and calcium-activated single muscle fibres. The results showed that fibre-type proportion was significantly altered in CSF samples, which showed a shift from the slow- to the fast-twitch phenotype. Cross sectional area, force, maximum shortening velocity and calcium sensitivity were not significantly changed in single muscle fibres from CSF samples. Thus, the contractile properties of muscle fibres were preserved but their proportion was changed, with an increase in the more fatigue-prone, energetically expensive fast fibre type. Taken together, these results support the view that muscle tissue is directly involved in the pathogenesis of CSF and it might contribute to the early onset of fatigue typical of the skeletal muscles of CFS patients.

Chronic fatigue syndrome (CFS) is a debilitating illness affecting thousands of individuals, with an estimated worldwide prevalence of 0.2% to above 2% of the population (1-2). Clinically, CFS is characterized by extreme fatigue (experienced as exhaustion and extremely poor endurance) of at least six months duration, usually with the onset accompanied by an infection-like illness. Despite considerable research interest, the physiopathology of this condition remains unclear. Although some researchers consider CFS a psychological rather than a physical illness, several observations support an organic origin of this disease. Many patients with CFS are unable to work and drastically reduce their social activities. In CFS patients, the most striking symptoms include debilitating fatigue, muscle pain, and muscle weakness, indicative of neuromuscular dysfunction (3). Previous studies revealed that

Key words: CSF, single muscle fibres, muscle mechanics, myosin isoforms, force, maximum shortening velocity, myofibrillar calcium sensitivity

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0394-6320 (2009)

some CFS patients during incremental exercise showed a decreased anaerobic threshold (3). Serum acylcarnitine deficiency in CFS patients may lead to a decrease in oxidative metabolism and higher levels of plasma lactate (4). Such metabolic defects may contribute to the reduced physical endurance of CFS patients. During dynamic exercise, CFS patients reach exhaustion much more rapidly than control subjects despite CFS patients to have normal muscle strength and either normal or slightly reduced muscle endurance (5).

However, there is no evidence that exercise therapy may worsen outcomes of these syndrome (6). Mammalian skeletal muscles are heterogeneous, composed of fibres with different molecular architecture and specific functional properties, such as contraction speed, maximum shortening velocity and resistance to fatigue. The physiological properties of muscles, and among them resistance to fatigue, are determined by the proportions of phenotypically distinct fibre types. Among the proteins that are differentially expressed in distinct fibre types, myosin heavy chains (MyHC) are often used as molecular markers because they are i) the most abundant protein, representing about 50% of the whole protein complement; ii) determinants of several functional properties, such as maximum shortening velocity and ATPase activity; and iii) responsible for the differential histochemical ATPase staining after acid or alkali pre-incubation, a procedure used for many years to classify fibre types (7). In adult human skeletal muscles, three MyHC isoforms encoded by separate genes are expressed: two fast isoforms, 2A and 2X, and one slow isoform, designated type 1 or β-cardiac isoform, (8). Accordingly, three main fibre types are present in human skeletal muscles: slow fibres expressing type 1 MyHC and characterized by an oxidative metabolism, fast 2X fibres expressing 2X MyHC and characterized by glycolytic metabolism and fast 2A fibres expressing 2A MyHC and characterized by intermediate metabolic properties (9). Which gene is expressed in a given muscle fibre depends on intrinsic programs related to the myoblast lineage from which the fibre develops, and extrinsic influences, such as neural, hormonal and mechanical factors, including muscle activity (7).

An early study on the contractile properties of muscles of CFS patients revealed no consistent correlations between symptoms and changes in fibre-type proportion, fibre size, degenerative or regenerative features. Moreover, that study found that the contractile properties of quadriceps (maximal isometric force, force generation pathway, and relaxation rate), examined using symptom-limited incremental exercise tests, were not significantly altered relative to normal controls (10). However, a more recent report on a larger CFS patient population in which biopsy samples from quadriceps muscle were evaluated was aimed to determine the muscle fibre characteristic and lactate responses to exercise in CFS patients. The authors reached a conclusion that patients with abnormal lactate responses to exercise had significantly lower proportion of mitochondria rich type 1 muscle fibres (3). In the present study we analysed the force, cross sectional area (CSA), maximum shortening velocity and calcium sensitivity of single CFS fibres to understand if the contractile properties of CFS muscle fibres and their proportion were changed.

MATERIALS AND METHODS

Subjects

CFS patients were recruited at the National Reference Centre for CFS Study of Chieti-Pescara University (diagnosis with CDCP criteria).

Ten patients were enrolled in this study: five women (mean age 44.8±3.4) and five men (mean age 37.0±3.2), mean±standard error (M±SE).

Inclusion criteria for all subjects were: (a) age 25–55 years; (b) no current pathology other than CFS, as assessed by clinical history, physical examination and routine laboratory tests; (c) no pregnancy (fertile women); (d) Type I dietary intake (American Heart Association Guidelines, 2002); (e) no medication for at least one month prior to examination; and (f) informed, written consent for participation.

Control subjects were sedentary healthy volunteers of the medical and nursing staff at the same centre. An important criterion for controls was the lack of any current or past history of diffuse musculoskeletal pain/fatigue lasting more than ten consecutive days. Four healthy women (mean age 35.0±5.0) and five healthy men (mean age 43.0±8.0) were recruited as controls. The patients and controls were selected very carefully; for all patients, the diagnosis was confirmed and there was a record of several years with clear symptoms. The female subjects were

affected by the syndrome by 5.4 ± 0.7 years. The male subjects were affected by the syndrome by 7.8±1.9 years. The controls were chosen to match age, sex and life-style of the patients.

Experimental plan

The entire experimental plan has been approved by the local ethics committee. It consisted in the study of skeletal muscle derived from CFS patients. The experimental plan has been designed as translational study involving molecular and cellular aspects of the same muscle. The plan is based on two different sets of experiments on each biopsy sample: (i) transcription profile analysis of entire muscle fragment, (ii) measurement of tension development and fibre-type characterization.

Biopsy specimens of *vastus lateralis* (VL) muscle were taken at the level corresponding to one-third of the distance from the upper margin of the patella to the anterior superior iliac spine under local anaesthesia (1% lidocaine) using a 5-mm muscle biopsy needle with applied suction. Each biopsy was divided into two portions. The first portion was immediately immersed in ice cold skinning solution (see below), partially dissected into small fibre bundles and stored at -20°C in skinning solution containing 50% glycerol. The study on this biopsy fragment is reported in the present paper. A second portion of each sample was stored in 2X Lysis Buffer (Applied Biosystems, od. no. 4305895) and frozen at -80°C for RNA extraction for transcriptional profile (Pietrangelo et al, 2009 IJIPP in press).

Electrophoretic and Western blot analyses

MyHC isoform composition was determined in biopsy sample fragments or single muscle fibres. Denatured proteins stored in sodium dodecylsulfate (SDS)-containing sample Buffer were separated by SDS-polyacrylamide gel electrophoreses (SDS-PAGE) on 8% gels (18 cm x 16 cm x 1 mm) using a procedure derived from Talmadge & Roy (26). Gels electrophoresis at 70 V for 1.5 h and at 230 V for the remaining time and then silver stained. Three separate bands were detected in the 200 kD region, corresponding to MyHC-1, MyHC-2A and MyHC-2X, in order of migration (from fastest to slowest).

For purposes of quantifying myosin isoform proportion, densitometry analyses of silver-stained bands were performed on at least two independent electrophoretic runs of each biopsy sample fragment. The mean value was used as an individual measurement. Gels were digitized with an EPSON 1650 scanner (1200 dpi resolution). Each band was characterized by a value of Brightness-Area Product (BAP) using a constant threshold after black/white inversion using Adobe Photoshop Software. In each gel, the BAP values for bands identified as myosin heavy chain (MyHC) were summed and the BAP value for each isoform was expressed as a percent of the total. The reproducibility of the procedure was confirmed by calculating isoform ratios of selected samples from gels loaded with different amounts of sample.

Single fibre mechanics

On the day of the experiment, muscle fibre bundles were removed from the freezer (-20°C) and washed repeatedly with skinning solution. Single fibres were manually dissected from fibre bundles under a stereomicroscope (10-60 x magnification). Dissected fibres were bathed for 1 h in a skinning solution containing 1% Triton X-100 (Sigma) to ensure complete membrane solubilisation. Segments of 1-2 mm length were then cut from the fibres and light aluminum clips were applied at both ends.

Skinning, relaxing, pre-activating and activating solutions used in single fibre mechanical experiments were prepared as previously described (11). Skinning solution had the following composition: 150 mM potassium propionate, 5 mM magnesium acetate, 5 mM sodium ATP, 5 mM EGTA and 5 mM KH_2PO_4. Relaxing solution was composed of 100 mM KCl, 20 mM imidazole, 5 mM $MgCl_2$, 5 mM Na ATP and 5 mM EGTA. Pre-activating solution was the same as the relaxing solution except that the EGTA concentration was reduced to 0.5 mM and 25 mM creatine phosphate and 300 U/ml creatine phosphokinase were added. A series of activating solutions with distinct pCa values were prepared by adding variable amounts of $CaCl_2$ to the relaxing solution. The pH of all solution was adjusted to 7.0 at the temperature at which solutions were used (12°C). Protease inhibitors (E64 10 μM and leupeptin 40 μM) were added to all solutions.

Once the clips were applied, the fibre segment was transferred to the experimental setup (see below) and mounted in a drop of relaxing solution between the force transducer (AME-801 SensorOne, Sausalito, California) and the electromagnetic puller (SI, Heidelberg, Germany) equipped with a displacement transducer. The signals from the force and displacement transducers, after A/D conversion (interface CED 1401 plus, Cambridge, UK), were fed into a personal computer and stored on the hard disk. The software package, Spike 2 (CED, Cambridge, UK), was used for data storage, recall and analysis.

The experimental setup was composed by a movable aluminum plate with seven pedestals (height 0.5 mm) placed on the stage of an inverted microscope (Axiovert 25, Zeiss, Germany). Glass coverslips, glued to the upper surface of each pedestal, were positioned beneath a second cover slip connected to a movable arm. The distance between the upper surface of the pedestal-mounted coverslip and the movable arm-mounted coverslip was 2

mm. A drop of solution (70 µl) of varying composition was kept between the two coverslips: the drop on the first pedestal was composed of relaxing solution, the second was composed of pre-activating solution, and subsequent pedestals were composed of activating solutions containing decreasing pCa (increasing calcium concentrations) down to pCa 4.6 on the last pedestal. The upper surface of each pedestal had a central opening through which fibre segments in solution could be viewed via a 40x objective in conjunction with a 10x eyepiece. The fibre segment could be also viewed (10-60x) using a stereomicroscope (Konus, Verona, Italy) placed above the inverted microscope; this second microscope was used for mounting and removing the fibre segment.

The fibre segment was mounted in relaxing solution and length, diameters and sarcomere length were measured. The segment was then stretched by approximately 20% and video-images were collected. The fibre segment was subsequently transferred to the pre-activating solution for at least 2 min and then activated by transferring to solutions containing progressively higher free calcium concentrations (decreasing pCa); maximal activation was recorded at pCa 4.6. During maximal activation, isometric tension (Po) was measured and unloaded shortening velocity (Vo) was determined according to the slack-test procedure (12).

The pCa-tension curve was obtained by measuring tension development at each calcium concentration. Tension was normalized to tension developed at the highest calcium concentration (pCa = 4.6) and plotted versus pCa values. The resulting sigmoidal curve was fitted to the Hill's equation:

$$relative\ tension = 100 / \left[1 + 10^{[(pCa - pK) \times n_H]}\right]$$

The parameters pK, corresponding to the pCa value at which relative tension is 50% of the maximum, and n_H, corresponding to the Hill slope of the curve, were determined using a least-squares fitting procedure.

The diameter of each fibre was obtained by averaging five measurements taken from video images. Cross-sectional areas were calculated from average diameters by assuming that fibres were perfect cylinders. This assumption overestimates the actual area, but this over-estimation is the same for all fibre types as shown by D'Antona (13) . No correction was applied to account for fibre swelling due to permeabilization procedure, which can be estimated to increase diameter by approximately 20% for all fibre types (14).

Myosin composition of each individual fibre segment was assessed by gel electrophoresis, as described above. For mechanical analysis, a total of 80 fibres from each group (~8 from each biopsy) were collected from CFS and control samples.

Statistical analysis

Results were expressed as M±SE, if not otherwise specified. Comparisons between CFS and control samples were made using Student's t-test. One-way ANOVA was used for comparisons among fibre types. A p value < 0.05 was considered significant. Prism software (GraphPad, San Diego, CA, USA) was employed for linear and non-linear fitting and statistical analysis.

RESULTS

The fibre-type proportion in biopsy samples was determined by analyzing MyHC isoforms, considered to be molecular markers of fibre type, using gel electrophoresis and densitometry, an approach previously described and validated (15).

The electrophoretic analysis of MyHC isoforms was made in both whole muscle tissue (biopsy fragments) and single isolated fibres in order to compared the MyHC isoform of the single fibre to the MyHC isoforms expressed by the whole muscle tissue. Examples of electrophoretic patterns are shown in Fig. 1: three MyHC isoforms can be recognized in each sample corresponding to MyHC 1, the β-slow isoform (lowest band, i.e. fastest

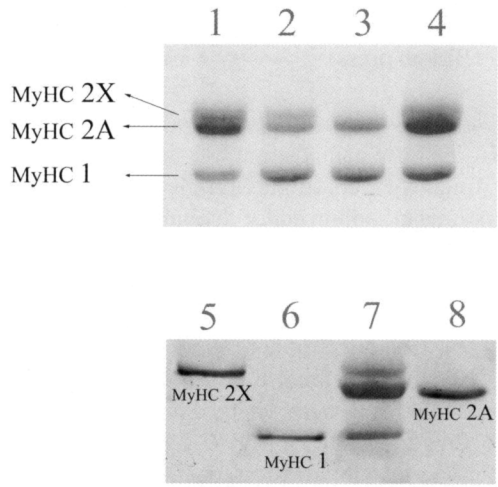

Fig. 1. *Electrophoretic analysis of MyHC isoforms in CFS skeletal muscle. Examples of electrophoretic analysis of MyHC isoforms in single fibres and whole biopsy samples. Upper panel shows four examples (lanes 1, 2, 3, 4) of biopsy samples derived from different CFS patients (staining with Coomassie Blue). In each lane the bands corresponding to different isoforms of MyHC can be recognized. The lower panel shows three single fibres (lanes 5, 6, 8) and a whole sample electrophoresis (lane 7) used as reference (silver staining).*

migrating protein), MyHC 2A (intermediate band), and the fast isoform, MyHC 2X (highest band).

The MyHC isoform proportion has been calculated by densitometric analysis of all patients examined. The results, shown in Fig. 2A, clearly shows that there was considerable variability in isoform proportion between individual patients. For example, the percentage of 2X MyHC varied between 20% and 37%, whereas the percentage of slow fibres (MyHC 1) varied between 17% and 49%. The average values for CFS patients compared with control subjects are shown in Fig. 2B. In both groups, fast 2A fibres were in a similar percentage. The proportion of slow MyHC, however, was significantly lower and that of fast 2X MyHC was significantly higher in the samples from CFS patients compared to the controls.

Interestingly, the fibre-type proportion did not vary in relation to gender. In males, the average values were: MyHC 1: 33.9%, MyHC 2A: 37.2%, MyHC 2X: 28.9%, whereas in females, the average values were: MyHC 1: 32.1%, MyHC 2A: 40.4%, MyHC 2X: 27.5%. The shift in myosin isoform expression is a clear indication of a change in fibre-type proportion, particularly if the CSA of single fibre do not change. We took into account the CSA, measured in μm^2, of CFS and control fibres. In particular, it has been considered not only fibres expressing a unique MyHC isoform (MyHC 1, 2A and 2X) but also hybrid isoforms MyHC 1-2A and MyHC 2A-2X. In any case, we found that there was no significant difference in fibres CSA (Fig. 3).

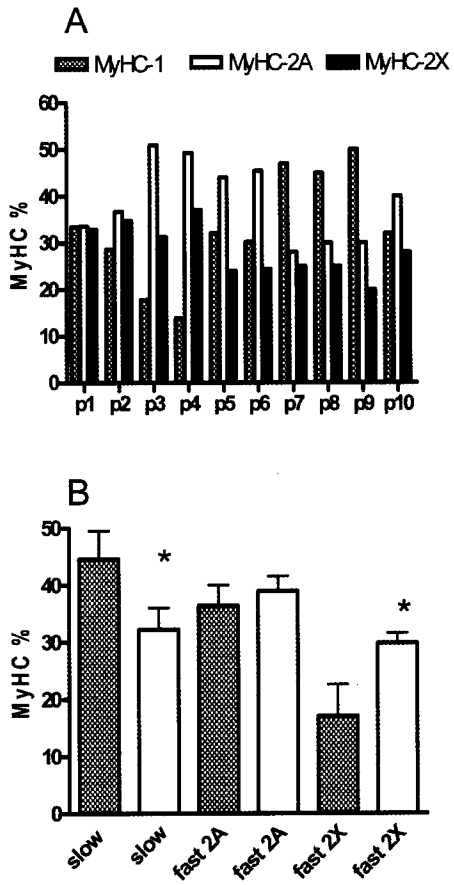

Fig. 2. *MyHC isoform distribution in CFS skeletal muscle. A) MyHC isoform proportion in biopsy samples from ten CFS patients calculated from densitometric analysis. B) Comparison of average distribution of MyHC in biopsy samples from CFS patients (white columns, n = 10) with control subjects (grey columns, n = 9). Data is expressed as M±SE. *denotes statistically significant difference (p < 0.05)*

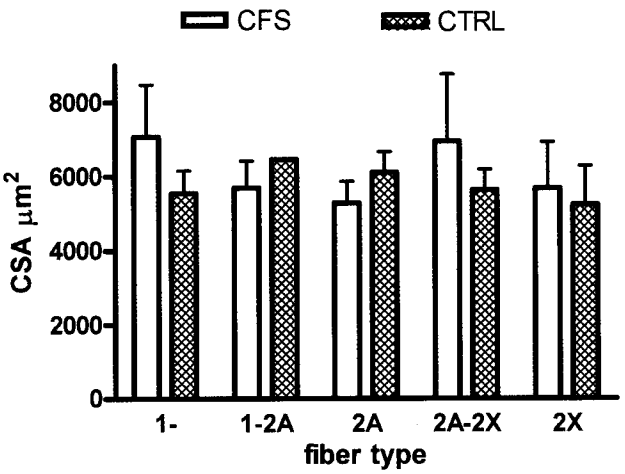

Fig. 3. *Cross-sectional areas of CFS and control fibres studied in mechanical experiments. Cross-sectional areas (CSA) of fibres studied in mechanical experiments, classified on the basis of MyHC composition. CSF (white columns) and control (CTRL, grey columns). Data is expressed as M±SE.*

In the mechanical experiments we evaluated the contractile performance of each fibre. In particular, we measured the maximum shortening velocity, Vo, during maximal activation of unloaded fibres, as described in Methods. The average values of Vo are shown in Fig. 4 A. Because the contractile parameters, and particularly Vo, are strongly dependent on fibre type (8), fibres were grouped on the basis of their MyHC isoform content. White bars

represent CFS fibres while grey bars controls. As can be seen in the graph, the fibre type expressing MyHC 1 and 2A are really homogeneous on both patients and controls, while the hybrid MyHC 1-2A and the MyHC 2X fibres dissected from of CFS muscles present a certain variability in maximum shortening velocity. The hybrid fibres MyHC 2A-2X seem to have a lower Vo with respect to controls but the statistical analysis showed no significant difference. Figure 4 B, shows results obtained measuring the maximal isometric tension, Po, when the skinned fibres were activated with the maximal [Ca^{2+}]. The average values of Po are reported as specific tension mN referred to fibre area in mm^2 (Fig. 4 B). Also in this case, the fibres were grouped on the basis of their MyHC protein and Po was compared in CFS fibres (white bars) and controls (grey bars).

The CFS and control fibres were homogeneous. The statistical analysis showed no significant difference between CFS and control, suggesting that the contractile properties of the muscle fibres were not altered in CFS patients.

In a selected group of fibres, myofibrillar calcium sensitivity expressed by the tension-pCa curve was also generated. All data points for each MyHC fibre type (I, 2A and 2X) of CFS (dashed line) and control (CTR, continuous line) were interpolated from the sygmoidal curve with variable slope (Fig. 5). The values of the parameters pK and n_H were: type 1 fibres CFS pK = 5.87 ± 0.04, n_H = 2.51 ± 0.61, type 1 CTRL pK = 5.74 ± 0.07, n_H = 2.21 ± 0.54; type 2A fibres CFS pK = 5.97 ± 0.02, n_H = 3.66 ± 0.47, type 2A CTRL pK = 5.98 ± 0.04, n_H = 3.94 ± 0.37; type 2X fibres CFS pK = 6.09 ± 0.04, n_H = 2.41 ± 0.54, type 2X CTRL pK = 6.10 ± 0.03, n_H = 4.27 ± 0.67.

The relative tension generated at submaximal activation by fibres (pCa ranging between 5.5 - 6.5) from CFS muscles was not significantly different from that of control muscles, indicating that the contractile response of the myofibrils

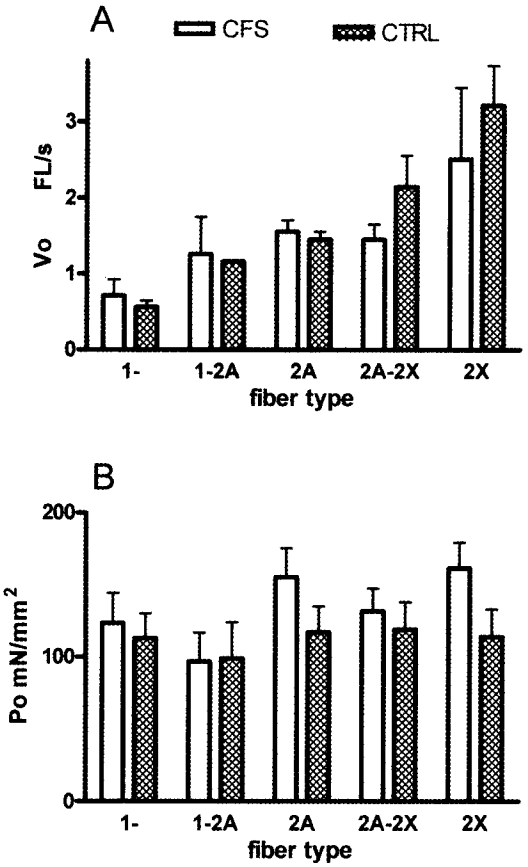

Fig. 4. *Contractile properties of CFS and control fibres. Contractile properties of single muscle fibres analyzed in CFS and control (CTRL) muscle biopsy samples, classified on the basis of MyHC isoform composition. A) Maximum shortening velocity, Vo, and B) isometric tension, Po. Data is expressed as M±SE.*

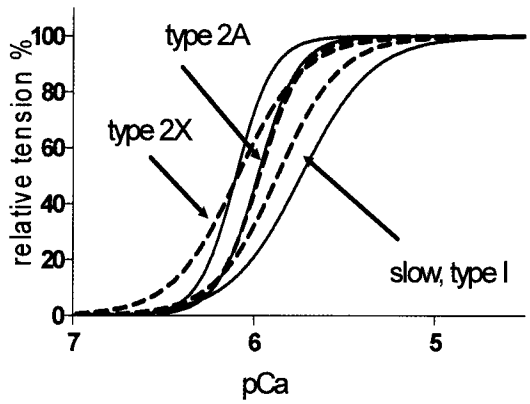

Fig. 5. *Tension-pCa curves of control and CFS fibres. Myofibrillar calcium sensitivity expressed by the tension-pCa curve. All data points for each fibre type were interpolated from the sygmoidal curve (dashed line for CFS and continuous line for CTRL) with variable slope, as described in the Methods section. The numerical values (with SE) of the parameters pK and n_H did not reach statistical significance.*

to calcium stimulation was similar in the two groups.

DISCUSSION

Despite considerable research on the subject, the association between structural alterations or functional changes in skeletal muscles and weakness or reduced resistance to fatigue detectable in CFS patients remains controversial. In this study, our comparative analysis of single fibres from *VL* muscle of CFS patients and age- and sex-matched controls showed a shift in fibre-type proportion in CFS patients towards a greater abundance of fast 2X fibres and reduction in slow fibres compared to controls. There were no differences in muscle fibre CSA, and no differences in the major parameters that characterize muscle contraction - isometric tension, maximum shortening velocity and contractile response to calcium activation.

The finding that the MyHC isoform proportion was altered is, in our view, a clear indication of an altered fibre-type proportion. MyHC isoforms are considered the molecular markers of fibre type (7-8). In previous studies (15), we have examined the potential correlation of MyHC isoform and fibre-type proportion and reached the conclusion that, in the absence of major changes in fibre CSA, the electrophoretic analysis of MyHC proportion was an indicator of fibre-type proportion. The MyHC isoform proportion found in the *VL* muscle samples from control subjects was comparable to that found by our group in previous studies; slow MyHC represented about 45% of the total, a value similar to that found by Harridge (15) and D'Antona (16). The number of CFS patients studied was limited, but this was in part due to the very precise criteria of inclusion. The shift in fibre type proportion is further confirmed by the observation that similar results were obtained, if the patients are divided in two groups on the basis of their gender.

The decrease in slow MyHC isoform in CFS patients was accompanied by an increase in fast 2X MyHC, with virtually no change in fast 2A MyHC. Further, the proportion of MyHC 2X in samples from CFS patients was clearly greater than that in control subjects of this study and, importantly, of other studies performed independently (13-16). Significantly, the shift in fibre-type proportion from slow to 2X suggested by the shift in myosin isoforms corresponds to a change not only in the proportion of slow and fast fibres, but also in the proportion of oxidative and glycolytic fibres, as, in human muscles, slow fibres are mainly oxidative and 2X fibres are mainly glycolytic (13). The expression profile analysis (Pietrangelo et al, 2009 IJIPP in press) revealed a number of changes in gene expression consistent with a shift from slow to fast fibres. For example, we observed the down-regulation of the genes myogenic factor 6 (MYF6/ MRF4; herculin) and calmodulin 1 (CALM1; phosphorylase kinase delta) and this is consistent with muscle reorganisation directed towards the fast phenotype. In fact, a reduction in the expression of the corresponding proteins suggests that signalling supporting slow fibre phenotype is depressed. In this context, MRF4 has been implicated in regulating the shift of fibre type towards the slow phenotype (17) and CALM1 has been shown capable of deciphering the intracellular calcium oscillations that occur during differentiation toward the slow fibre phenotype (18). Moreover, the expression profile analysis (Pietrangelo et al, 2009 IJIPP in press) show the up-regulation of skeletal muscle myosin light chain kinase 2 (MYLK2), gene encoding a calcium calmodulin-dependent kinase that is highly expressed in regenerating and adult fast fibre (19-20).

Previous studies on CFS patients have provided some indications of a possible change in fibre type proportion. In particular, Lane and co-workers (3, 21) reported that lactate accumulates to a greater extent in the muscles of CFS patients than in controls. According to a widely accepted view, lactate is produced by glycolytic fibres during exercise and taken up by oxidative fibres, and also by cardiomyocytes and hepatocytes (for a recent review see 22). Lane and co-workers have also shown that ATP regeneration by mitochondrial oxidative processes was impaired in a group of CFS patients (3). The expression profile analysis (Pietrangelo et al, 2009 IJIPP in press) revealed a number of changes in gene expression (genes for the pyruvate dehydrogenase kinase PDK4 and enzymes phosphofructo-2-kinase/fructose-2,6-biphosphatase PFKFB3) indicative of an impaired energetic metabolism (23). Although no information

is available on post-transcriptional level, we might hypothesize that in CFS muscle the ATP sources could be the anaerobic glycolysis, which increases the level of lactate that has been shown in CFS muscle. Moreover, the increase of the percentage of glycolytic fibres demonstrated in this work further throw off balance the lactate removal from CFS muscle and favour lactate accumulation (3, 24)

It is known that muscle disuse can produce a transition from slow to fast fibre type and from oxidative to glycolytic metabolism (9, 25). Thus, the observation that fibre-type proportion is altered in CFS patients raises an important question concerning the origin of CFS, whether the fibre type shift is the cause or the effect of decreased muscle activity. The expression profile analysis (Pietrangelo et al, 2009 IJIPP in press) paper provides some interesting, and potentially illuminating, indications. One down-regulated gene, the nicotinic cholinergic receptor alpha polypeptide 1 (CHRNA1), contains the acetylcholine binding site of the receptor. Reduced levels of this transcript suggest the possibility of a defective nicotinic receptor and reduced efficiency of neuronal transmission at the neuromuscular junction. This deficiency could impair the ability of the end plate to encode the repetitive low-frequency firing of motoneurons essential for slow-fibre-type specification (9, 25) and as a consequence, could result in a reduction of slow fibres number. In addition, some intracellular signalling pathways that likely play a role in transducing the effects of neuronal stimulation may also be down-regulated. For example, the observed down-regulation of the gene for calmodulin (CALM1) could limit the ability of muscle to generate appropriately modulated intracellular calcium oscillations during the differentiation towards the slow fibre phenotype (27). The absence of a change in CFS fibre size deserves some comment. First, although it is at odds with the hypothesis that disuse might contribute to the slow-to-fast shift discussed above, it is in agreement with previous histological studies on CSF patient muscles that were unable to find any significant evidence for muscle atrophy (3, 10).

Our idea is that the increased presence of fast fibre in CFS muscle do not depend on muscle disuse but may have an unknown molecular origin that need to be deeply investigated.

This reported lack of muscle fibre atrophy finds support in the results of the expression profile studies (Pietrangelo et al, 2009 IJIPP in press). In fact, all CFS muscle samples showed down-regulation of the transcripts for forkhead box O3A (FOXO3A), H1 histone family member X (H1FX), ubiquitination factor E4A (UBE4A), proteasome (prosome, macropain) 26S subunit non-ATPase 3 (PSMD3), and proteasome (prosome, macropain) subunit beta type 2 (PSMB2). Collectively, the down-regulation of these genes suggests that the catabolic and atrophic processes that could reduce muscle mass are less active.

The characterization of the contractile properties of muscle fibres from CFS patients at the single fibre level provides a novel contribution to our understanding of the physiopathology of this disease. We found that isometric tension and maximum shortening velocity were not different between CFS muscle and controls. Thus, mechanical power, which is proportional to the product of isometric tension and shortening velocity at zero load (28) would also be unchanged. The same was true for the contractile response of the myofibrillar apparatus to calcium stimulation, as assessed by the force-pCa curve. It is known that the ATPase activity of myosin motors, which represents about 75% of the total energy expenditure of the muscles during contraction, is proportional to maximum shortening velocity and therefore will be greater for fast 2X myosin than for slow myosin (29). The fibre-type shift observed thus implies an increased ATP consumption during contraction. This creates a need for faster ATP regeneration, which can be accomplished by glycolysis and lactate production, with decrease in extracellular pH. This can contribute to worsening muscle condition during activity via acid sensing ion channels found on primary afferent fibres (30).

The determination of the contraction performance was carried out under conditions in which the membranes (sarcolemma and sarcoplasmic reticulum) were permeabilized and contraction was induced by immersion of muscle fibres in solutions containing specific concentrations of free calcium. Accordingly, calcium release and uptake mechanisms would be unavailable and thus no conclusion can be reached about this aspect of contractile regulation. However, previous work from our group has shown that

oxidants accumulates in CSF muscles (31-32) with related impairment of sarcoplasmic reticulum proteins involved in calcium release and uptake (33) and mitochondrial activity underlies an increase in the production of reactive oxygen species that leads to muscle fatigue, a process similar to normal ageing (34-35). The results reported here cannot exclude the possibility that such post-transcriptional changes could contribute to functional alterations, but they do suggest that myofibrillar protein damage is not involved.

Recently, in some CFS patients has been documented a deregulation in immune cells of the 2',5'-oligoadenylate-dependent ribonuclease L (RNase L). This deregulation has been linked to reduced exercise capacity in patients affected by CFS and is consistent with oxidative stress already documented in CFS muscles (35).

In conclusion, this study provides strong evidence to support the idea that skeletal muscle structure and function are altered in CSF patients. Taken together with the results of the transcription profile analysis (Pietrangelo et al, 2009 IJIPP in press) the increase in fast, fatigue-prone 2X fibres is consistent with higher ATP consumption and regeneration primarily through glycolysis and lactate production. Irrespective of the underlying mechanism, the slow-to-fast shift, suggested by the present data, may well contribute to decreased resistance to fatigue typical of the skeletal muscles of CFS patients.

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