EXTENDED REPORT

Increased DNA fragmentation and ultrastructural changes in fibromyalgic muscle fibres

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Objective: To determine whether there is evidence of increased DNA fragmentation and ultrastructural changes in muscle tissue of patients with fibromyalgia (FM) compared with healthy controls.
Methods: Muscle tissues from 10 community residents with FM and 10 age and sex matched healthy controls were examined ''blindly'' for the presence of DNA fragmentation by two different methods: terminal deoxynucleotidyl transferase (TdT) staining (TUNEL) and the FragEL-Klenow DNA fragmentation detection kit. Ultrastructural analysis of tissue was performed by electron microscopy.
Results: DNA fragmentation was detected by both methods in 55.4 (SEM 2.5)% of the nuclei in muscle tissue of patients with FM compared with 16.1 (4.1)% ($p<0.001$) of the nuclei in healthy controls. Contrary to expectation, no typical features of apoptosis could be detected by electron microscopy. The myofibres and actin filaments were disorganised and lipofuscin bodies were seen; glycogen and lipid accumulation were also found. The number of mitochondria was significantly lower in patients with FM than in controls and seemed to be morphologically altered.
Conclusion: The ultrastructural changes described suggest that patients with FM are characterised by abnormalities in muscle tissue that include increased DNA fragmentation and changes in the number and size of mitochondria. These cellular changes are not signs of apoptosis. Persistent focal contractions in muscle may contribute to ultrastructural tissue abnormalities as well as to the induction and/or chronicity of nociceptive transmission from muscle to the central nervous system.

Fibromyalgia (FM) is a common, chronic musculoskeletal disorder that is characterised by widespread pain and enhanced pain sensitivity.[1] The aetiopathogenesis of FM is not understood, but abnormalities have been found in muscle tissue as well as in the neuroendocrine and central nervous system (CNS).[2–4] We have developed a model of the pathogenesis of abnormal pain sensitivity in FM that incorporates current findings for dysregulation of CNS functions involved in pain transmission and inhibition.[5]

A large number of investigations have produced important findings on the dysregulation of CNS functions that may contribute to abnormal pain sensitivity in people with FM.[6–8] However, efforts to identify abnormalities in the structure or metabolic activity of muscle tissue that may also contribute to the pain sensitivity in people with FM have generally produced conflicting results. For example, early studies using electron microscopy found that the muscle tissue of patients with FM is characterised by high frequencies of ''rubber band'' morphology, mild myofibrillar separation, papillary projections, and subsarcolemmal accumulation of glycogen.[9–11] In contrast, electromyographic (EMG) mapping studies determined that although patients with FM have a reduced voluntary capacity for work, their biochemical response to work and recovery seemed to be normal.[12] On the other hand an increased EMG activity in resting patients with FM was demonstrated by Anders *et al* and discussed as possible stressor of FM muscle.[13]

Studies with ^{31}P magnetic resonance imaging have reliably identified several abnormalities in the muscle tissue of patients with FM. For example, Park and colleagues found metabolic abnormalities in the quadriceps muscle tissue of patients with FM, such as relatively low levels of phosphocreatine and ATP at rest and low phosphorylation potential and total oxidative capacity during rest and exercise.[14] We recently found significantly higher levels of phosphodiesters and inorganic phosphate in the spectra of patients with FM compared with controls.[15] Jubrias and colleagues reported similar findings.[16]

It is possible, for example, that structural abnormalities in the muscle tissue of patients with FM may be produced by the production of several intracellular second messengers, such as nitric oxide (NO).[17] Indeed, our laboratory and other investigators have produced evidence that patients with FM, compared with controls, are characterised by increased synthesis of NO.[18] Prolonged release of high levels of NO in people with FM[19] may induce accelerated apoptosis and abnormal cell death in muscle tissue, which might contribute to the structural and metabolic defects that have been identified in the muscle of patients with FM.[20]

It should be noted that apoptosis is associated with a cascade of molecular and biochemical events, including the activation of endogenous endonucleases that cleave DNA into oligonucleosomes detectable as a ladder of DNA fragments in agarose gels.[21 22] Endonucleases generate free 3′-OH groups at the end of these DNA fragments that can be end labelled with molecular and histochemical techniques.[23–25] Therefore, this study aimed at using these methods and electron microscopy to study if people with FM, compared with healthy controls, have evidence of increased DNA fragmentation characteristic for apoptosis and other ultrastructural changes in muscle tissue.

Abbreviations: ACR, American College of Rheumatology; CNS, central nervous system; DAB, 3,3′-diaminobenzidine; EMG, electromyographic; FM, fibromyalgia; NO, nitric oxide; TBS, Tris buffered saline; TdT, terminal deoxynucleotidyl transferase

SUBJECTS AND METHODS

This study was approved by the Institutional Review Board of the University of Alabama at Birmingham. Volunteers were paid ($100).

Subjects

Muscle tissue was obtained from two groups of volunteers (table 1). The first group consisted of 10 white (nine women, one man) Alabama community residents who had recently served as ''non-patients'' in other investigations in our laboratory. That is, these subjects contacted our laboratory in response to newspaper advertisements for volunteers with muscular aches and pains who had not sought treatment for their symptoms during the past 10 years. As described in previous reports,[7 26] all potential subjects were extensively interviewed about their symptoms and use of the healthcare system. Whenever possible, we reviewed the medical records of potential subjects who received their primary medical care at our institution to verify that they had had no previous treatments for FM symptoms. All potential subjects who met our interview criteria for FM and who had not sought treatment were invited to our general clinical research centre to undergo a clinical evaluation and tender point examination by one of the project rheumatologists (GSA). Individuals who met the American College of Rheumatology (ACR) criteria for FM,[27] did not meet criteria for any other rheumatological diagnosis, and had not sought medical or chiropractic treatment for their symptoms during the past 10 years were asked to participate in the study. It should be noted that none of the subjects were aware that they met criteria for FM until they had completed the screening procedures for our study.

We chose to study these FM ''non-patients'', rather than rheumatology clinic patients with FM, for two reasons. Our primary reason for studying ''non-patients'' with FM was that all of these subjects were characterised by an insidious onset of painful symptoms. Thus, it was highly unlikely that any structural abnormalities in the muscle tissue in these subjects would have been produced by traumatic physical injury. A second reason was an earlier finding in our laboratory that ''non-patients'' with FM report significantly lower levels of fatigue and functional disability than patients with FM recruited from the rheumatology clinic.[26] This reduced the likelihood that any structural abnormalities found in the muscle tissue of the FM ''non-patients'' were produced by disuse. Table 1 shows that the mean (SEM) age of our ''non-patients'' with FM was 45.0 (4.4) years (range 24–62), and the mean time since the onset of their symptoms was 15.1 (4.6) years (range 1–50).

The second group of volunteers comprised 10 sex and ethnically matched healthy controls with a mean (SEM) age of 49.7 (4.3) years (range 30–68). There was no difference in age between the controls and the ''non-patients'' with FM ($p = 0.53$). These subjects had no symptoms of muscular disease and considered themselves to be healthy (table 2).

Table 2 Individual data of the healthy controls

Control	Age	TP	Fatigue[7]
01	37	4.5	1.78
02	61	5.1	2.89
03	41	3.5	4.22
04	57	5.9	2.56
05	47	4.3	2
06*	68	2.4	1.89
07†	30	–	–
08	35	–	–
09	66	3.2	2.22
010	55	–	–

TP, pain thresholds of pressure at tender points in kg/1.54 cm^2.
*The electron micrograph of subject 06 is shown in fig 3A; †male subject.

Clinical assessment

After obtaining informed consent, we assessed each subject's pain threshold levels for dolorimeter stimulation at 10 of the 18 ACR tender points using a procedure that is standard in our laboratory.[7 26] We then administered the Fatigue Severity Scale[28] to all subjects. We also administered the McGill Pain Questionnaire[29] to our subjects with FM in order to assess the intensity of their clinical pain during the preceding week.

Muscle biopsy

After the clinical assessment, each subject underwent a procedure in which the project neurologist/myopathologist (SJO) or one of his fellows in training surgically removed muscle tissue from the left deltoid area. After local anaesthesia, an incision of 3–4 cm in length was made and a segment (0.5×1.5 cm) of the deltoid muscle was removed. Stitches were used to close the incision and an antibiotic and an analgesic were given to the subject to minimise the risk of infection and reduce discomfort. Immediately after excision of the muscle, we stretched the removed tissue with pins at each end on plastic strips for 5 minutes before we performed the procedures below.

After stretching the muscle tissue, a section of tissue was snap frozen in OCT TissueTek embedding medium (Miles, Elkhart, IN) and stored at −70℃. Another section of tissue was fixed in 10% buffered formalin (Fisher Scientific, Norcross, GA) for 4 hours at room temperature and then processed in several steps of increasing butanol concentrations (Fisher Scientific, Norcross, GA) for 17 hours at 40℃ under vacuum and, finally, in soft paraffin (Sargent-Welch Scientific Comp, Skokie, IL) for 5 hours at 60℃ under vacuum in the Hypercenter XP 739 (Shandon Inc,

Table 1 Demographic and clinical data of the ''non-patients'' with FM

Subject	Age (years)	Onset of symptoms (year(s))	TP	McGill[26]	Fatigue[7]
12*	48	20	3.3	23.52	7.0
13	43	10	2.8	9.01	5.89
14	62	28	1.4	26.66	3.11
15	55	50	1.4	40.58	4.67
16†	61	8	2.0	5.51	6.0
17	24	14	3.7	7.66	4.89
18‡	32	4	2.5	44.2	4.33
19	51	4	2.4	41.83	5.89
110	50	12	2.8	26.08	5.0
111§	24	1	2.4	25.18	4.11

TP, pain thresholds of pressure at tender points in kg/1.54 cm^2.
*The electron micrograph of subject 12 is shown in fig 3D; †the electron micrograph of subject 16 is shown in fig 3C; ‡male subject; §the electron micrograph of subject 111 is shown in fig 3B.

Pittsburgh, PA). The project neurologist/myopathologist examined each muscle tissue in a ''blinded'' fashion using light microscopy and haematoxylin and eosin, adenosine triphosphatase, succinate dehydrogenase, NADH-tetrazolium reductase, periodic acid-Schiff, and glucose phosphorylase stains to rule out any other morphological diseases of the muscle.[30]

Increased DNA fragmentation

The hallmarks of apoptosis are chromatin condensation, nuclear fragmentation, plasma membrane blebbing, and/or cell shrinkage. DNA fragmentation which does not result in apoptosis will not be visible by electron microscopy. The FragEL-Klenow DNA fragmentation detection kit and the TUNEL kit are both enzymatic detection methods and were applied to the tissues to show nuclei of muscle cells with DNA fragmentation. The Klenow kit has the advantage that it counterstains ''healthy'' nuclei within the same procedure. It is then possible to calculate relations between DNA fragmented and ''healthy'' nuclei as we performed in our study.

Staining with FragEL-Klenow DNA fragmentation detection kit (Klenow)

Paraffin embedded sections of muscle tissue (6 µm) were deparaffinised in several steps using xylene and ethanol. The slides then were subjected to proteinase K digestion (20 µg/ml) at room temperature for 20 minutes. After rinsing with Tris buffered saline (1×TBS, 20 mM Tris pH 7.6, 140 mM NaCl), the specimens were covered with 100 µl of 3% H_2O at room temperature for 5 minutes. The specimens were rinsed again with 1×TBS and covered with 100 µl of 1× Klenow equilibration buffer at room temperature for 10 minutes. Next, 60 µl of Klenow labelling reaction mixture (58.4 µl Klenow labelling reaction mix and 1.6 µl Klenow enzyme) were applied to each specimen and incubated at 37℃ for 1.5 hours (Oncogen Research Products, Cambridge, MA). The reaction was stopped with 100 µl of stop solution at room temperature for 5 minutes. The specimens then were rinsed with 1×TBS and covered with 100 µl of blocking buffer at room temperature for 10 minutes. After removing the blocking buffer from the specimen, 100 µl of diluted 1×conjugate (2 µl 50×conjugate and 98 µl blocking buffer) was applied to each specimen (room temperature, 30 minutes). Then 3,3′-diaminobenzidine (DAB) solution was added (one tablet of DAB and one tablet of H_2O_2/urea in 1 ml of H_2O) and incubated at room temperature for 10 minutes. The slides were rinsed with double distilled H_2O and counterstained with methyl green at room temperature for 3 minutes. After washing in ethanol and xylene, each specimen was coverslipped with Paramount (mounting medium, Dako) (fig 1). Measurement by light microscopy was performed in a ''blinded'' fashion. Each tissue section was analysed for DNA fragmentation in nuclei of muscle cells in at least 20 areas by two ''blinded'' investigators (HS, SS), and the mean number of DAB stained nuclei was calculated. The original magnification for nuclei counting was ×200.

DNA fragmentation staining with terminal deoxynucleotidyl transferase (TUNEL)

The method described by Gavrieli *et al* was used to perform this procedure.[31] Snap frozen muscle tissue sections (6 µm) were fixed in 10% buffered formalin (Fisher Scientific, Norcross, GA) for 30 minutes. The slides were washed six times with double distilled water (Fisher Scientific, Norcross, GA), then subjected to proteinase K digestion (10 µg/ml; Boehringer, Germany) at room temperature for 5 minutes. The slides were washed again with double distilled water and incubated with freshly prepared deoxynucleotidyl transferase (TdT) reaction mix (0.3 µl TdT (20 U/µl), 4.0 µl 5×TdT buffer (both from Promega, Madison, WI), and digoxigenin-11-dUTP (Boehringer, Germany)) at 37℃ for 1 hour. After washing with Dulbecco's phosphate buffered salt solution (1×phosphate buffered saline), non-specific binding was blocked with 2% horse serum at room temperature for 30 minutes. Next, the incorporated digoxigenin-dUTP was detected by incubation with anti-digoxigenin-AP, Fab fragments (Boehringer, Germany) 1:100 in phosphate buffered saline at room temperature for 45 minutes. Visualisation of the reaction product was performed with Sigma *FAST* BCIP/NBT buffered substrate tablet (Sigma BioSciences, St Louis, MO). Each tissue section was analysed for DNA fragmentation in the nuclei of muscle cells in at least 20 areas by two ''blinded'' investigators (HS, SS). The original magnification for nuclei counting was ×200. The mean number of counted cells with stained DNA fragments was then calculated. This procedure was performed to confirm the data from the Klenow kit.

Electron microscopy

Immediately after surgical removal of the muscle tissue specimens, they were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.35, at 4℃ for 1 hour. The tissue was washed in the same buffer at 4℃, cut into slices 0.5 mm thick, post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer at room temperature for 1 hour, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr resin.[32] Ten non-numbered embedding procedures were performed with each tissue specimen. The slides were cured overnight in a 60℃ oven and afterwards the capsules were snapped off. The examiner randomly chose three of 10 possible sections for cutting. Semithin sections were stained with 1% toluidine blue, longitudinal sections were identified, and the blocks were randomly trimmed for ultrathin sectioning. Ultrathin sections were made with a diamond knife on an LKB ultratome and then stained with uranyl acetate and lead citrate. Ten sections of each tissue were examined at 75 kV in a Hitachi H7000 electron microscope.

A final magnification of 2.2×14 000 was used for determining the number of mitochondria and structural features in all samples. Two investigators independently counted the mitochondria without knowing the origin of the samples, and the average of the two readings was used to calculate group means.

Statistical analyses

The counts for mitochondria of the subjects with FM and the healthy controls then were compared using the non-parametric Mann-Whitney test for independent samples (SPSS for Windows version 7.5.2G, SPSS Inc, Chicago, IL). All other statistical comparisons were performed using Student's *t* test. An α level of 0.05 was used for all comparisons.

RESULTS

Clinical assessment

The subjects with FM exhibited significantly lower mean (SEM) pressure pain thresholds (2.47 (0.23) kg/1.54 cm^2) at the ACR tender points than the controls (4.13 (0.45) kg/1.54 cm^2) ($p = 0.009$). In addition, the subjects with FM produced a mean (SEM) total score on the McGill Pain Questionnaire (25.02 (4.52)) and displayed a significantly higher mean score on the Fatigue Severity Scale (5.09 ± 0.35) than the controls (2.51 ± 0.32; $p = 0.001$).

Muscle tissue studies

Light microscopy showed no histological features indicative of myopathy, polymyositis, or denervation processes in any of the subjects. Succinate dehydrogenase and NADH stainings

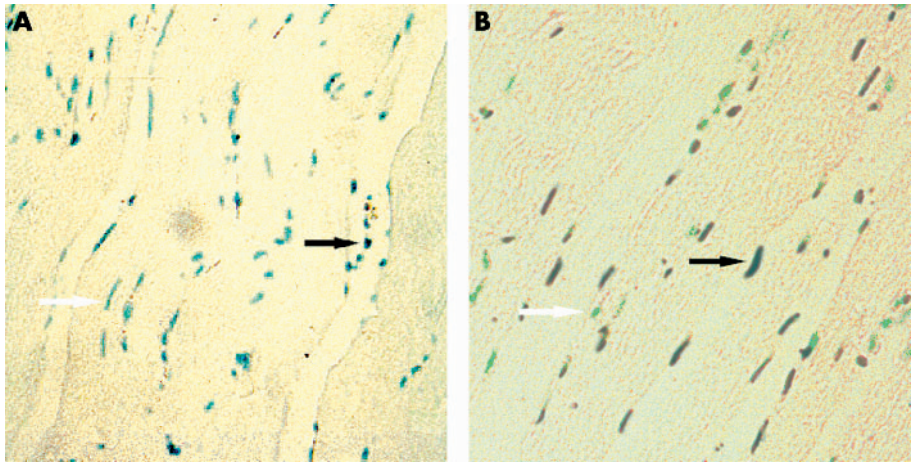

Figure 1 DNA fragmentation staining (dark dots, black arrow) with the Klenow method in muscle tissue of subjects with FM (B) compared with healthy controls (A). Blue staining (white arrow) shows nuclei without DNA fragmentation (original magnification ×200).

showed no abnormal findings except type II fibre atrophy in one of the subjects of each group. However, our primary finding was produced by the enzymatic detection method, which showed increased DNA fragmentation in the muscle tissue of the subjects with FM compared with the muscle tissue of healthy subjects (fig 1). Among the subjects with FM, 55% of the muscle cell nuclei were positive for DNA fragmentation as compared with 16% in the controls (table 3).

Contrary to our expectation, electron microscopy did not reveal the typical features of apoptosis. There was no evidence of chromatin condensation, nuclear fragmentation, plasma membrane blebbing, or cell shrinkage in any of the muscle specimens. However, because the enzymatic DNA fragmentation assays both showed positive results it must be concluded that the positive DNA fragmentation in nuclei of FM muscle cells does not reflect apoptosis. Nevertheless, electron microscopy did find several abnormalities in the muscle tissue of the subjects with FM. The myofibres and actin filaments were disorganised in all samples from these 10 subjects (fig 2). Interfibrillar lipid and lipofuscin deposits were also seen in the tissue of all subjects with FM. Interfibrillar glycogen was found in the tissue of five of the subjects with FM, and moth-eaten destruction of muscle fibres appeared in the tissue of three of these subjects. In contrast, no myofibre disorganisation was seen in the tissue of any of the healthy controls. Only 2 of the 10 controls showed glycogen in their tissue and only one showed an accumulation of lipid or other tissue abnormalities (fig 3).

Electron microscopy also disclosed substantial differences in the number and shape of the mitochondria seen in the muscle tissue specimens between the subjects with FM and the controls. Specifically, the mean (SEM) number of mitochondria found in the muscle tissue of the subjects with FM (7.0 (1.2)) was significantly lower than that found in the muscle tissue of the healthy controls (11.9 (1.0); $p = 0.019$) (figs 2, 3 and table 4). Moreover, the mitochondria found in the tissue of the subjects with FM (for example, fig 3D) were substantially larger than those in the tissue of the controls (for example, fig 3A).

DISCUSSION

As far as we know this is the first study to show evidence of abnormal DNA fragmentation in the muscle tissue of subjects with FM.

Given that FM is associated with enhanced NO levels in circulating blood[19 33] and cerebrospinal fluid,[18] we expected to find evidence of apoptosis in the muscle tissue of our subjects with FM. However, electron microscopy did not identify any of the hallmarks of apoptosis. It must be concluded, then, that increased DNA fragmentation in FM muscle tissues does not reflect apoptosis. Persistent focal contractions in muscle may contribute to ultrastructural tissue abnormalities as well

Table 3 Average of DNA fragmentation stained by the FragEL-Klenow DNA fragmentation detection kit in muscle cells of non-patients with FM (Subjects) and healthy controls (Control)

Subject				Control			
No	Fragmentation	Nuclei	Percentage	No	Fragmentation	Nuclei	Percentage
12	98	161	61	01	0	156	0
13	34	74	46	02	7	27	26
14	130	193	67	03	17	63	27
15	59	129	46	04	46	139	33
16	31	60	52	05	11	93	12
17	59	99	60	06	32	129	25
18	126	216	58	07	5	19	26
19	21	32	66	08	13	105	12
110	72	147	49	09	0	96	0
111	35	72	49	010	0	95	0
Mean (SEM)	66.5 (12.5)*	118.3 (19.2)†	55.4		13.1 (4.8)*	92.2 (14.2)†	16.1

The total number of counted nuclei (Nuclei) and the percentage are given. Statistical differences between subjects with FM and controls are calculated by the Mann-Whitney test. Means (standard errors of the mean) are given.
*$p<0.001$; †$p=0.353$.

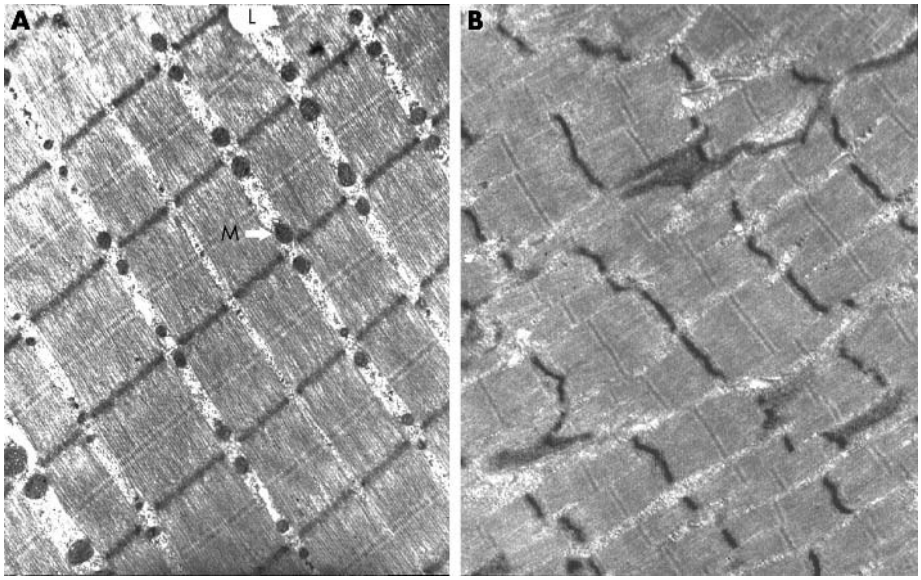

Figure 2 Electron micrograph of muscle tissue from a healthy control (A) and from a subject with FM (B) showing zig-zagging of the Z bands. L shows a lipid droplet. M and the white arrow shows a mitochondrion (A). There are no mitochondria in the selected anatomical region of the subject with FM (B). (Original magnification ×8400.)

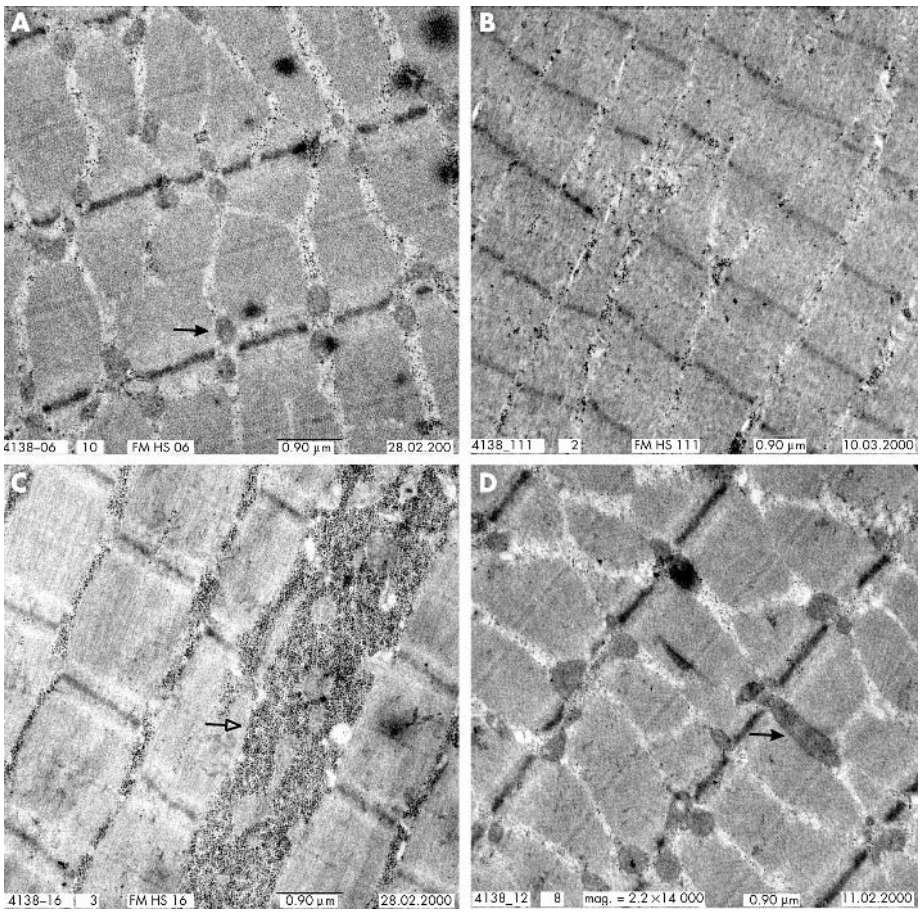

Figure 3 Electron micrograph of muscle tissue from a healthy control (A) for comparison and from subjects with FM (B-D) with different duration of symptoms: (B) 1 year; (C) 8 years; (D) 20 years. Black arrows indicate mitochondria (A, D). The open arrow indicates glycogen accumulation as a sign of disturbed glycogen use in FM (C). Uniformity of size and shape of the mitochondria in the healthy control is shown in (A). In (B) loss of mitochondria is shown in the presented section (see also fig 2B). (C) shows a huge accumulation of glycogen. In (D) enlargement and different shape of mitochondria (arrow) are presented. (Original magnification 2.2×14 000.)

Table 4 Mean values of mitochondria in the muscle tissue of subjects with FM and healthy controls

Subject	Mean*	Control	Mean*
12	12.0	01	9.3
13	11.8	02	13.55
14	4.5	03	9.7
15	9.5	04	14.55
16	11.8	05	11.1
17	3.1	06	15.0
18	2.0	07	8.75
19	4.6	08	6.8
110	3.8	09	13.05
111	7.0	010	17.35
Mean (SEM)	7.01 (1.2)		11.9 (1.0)†

*The mean of the results for each patient of the average of the two ''blinded'' investigators who counted the mitochondria independently; †p=0.019.

as to the induction and/or chronicity of nociceptive transmission from muscle to the CNS.

We observed also several ultrastructural abnormalities in the deltoid muscle tissue of our subjects with FM that were consistent with those found by both Danish and American investigators.[9–11] Some of these findings, such as the interfibrillar accumulations of lipids and glycogen, may be related to ATP or other metabolic abnormalities identified in the muscle tissue of subjects with FM.[14 15] Indeed, the relatively low number of mitochondria found in the muscle tissue of our subjects with FM might be related to abnormal metabolism of energy production. The enlargement of the mitochondria found in the muscle tissue of the subjects with FM (fig 3D) may represent a compensatory response to the reduction in the number of these organelles. The cause of these ultrastructural abnormalities is not clear. However, these abnormalities may be produced in part by the high levels of muscle stress we have seen in people with FM by EMG mapping.[13] Further study of an association between muscle stress and ultrastructural abnormalities is warranted.

One might question whether the demographic and clinical variables of our subjects were representative of the general population of subjects with FM. Examination of the demographic variables shows that the ratio of women to men in our sample of FM ''non-patients'' is consistent with that seen in population based studies of the prevalence of FM.[34 35] Age is a particularly important demographic variable, given that it is associated with changes in DNA fragmentation, muscle tissue structure, and mitochondrial oxidative metabolism.[36] Our subjects with FM and our healthy controls had a wide range of ages and there was no significant difference in age between these groups. Thus, it is unlikely that the abnormalities in DNA fragmentation and muscle ultrastructure of our subjects with FM were produced by age related factors.

Examination of the clinical factors of our subjects with FM shows that the reported pain intensity and fatigue levels were substantially lower than those reported by patients with FM in tertiary care centres.[26 37] However, our subjects' pain and fatigue scores are consistent with those of the 52 FM ''non-patients'' we have investigated in previous studies.[7 26 37] Thus, we believe that the pain and fatigue levels of our group of subjects with FM are representative of subjects with FM in the Alabama community who choose not to seek medical care for their FM symptoms.

One might also consider whether physical inactivity might have contributed to some of the ultrastructural changes in the muscle tissue of our subjects with FM. Unfortunately, we cannot rule out the potential influence of this factor on the muscle tissue findings because data on physical activity were not obtained. Nevertheless, we believe that it is unlikely that low physical activity levels alone could have produced the enhanced DNA fragmentation in our subjects as this phenomenon tends to be associated with high levels of activity in healthy people.[38 39] For the two subjects (one in each group) with type II fibre atrophy (a sign of disuse) no significant deviations of DNA fragmentation and mitochondria count from the mean values were seen (tables 3 and 4). At present, then, it appears that the enhanced DNA fragmentation and ultrastructural abnormalities in the muscle tissue of the subjects with FM are not primarily due to aging or low levels of physical activity.

Although our findings are based on small samples of subjects with FM and healthy controls, they do raise the question of whether any treatments might reduce the abnormalities in DNA fragmentation or muscle tissue structure associated with FM. There are currently no treatments targeting such muscular abnormalities (mitochondrial myopathy).[40]

However, we believe that future research should attempt to determine whether pharmacological and behavioural interventions might diminish the DNA fragmentation and other muscle tissue abnormalities identified in our investigation. For example, Hakkinen and colleagues reported recently that muscle strength training significantly improved isometric strength and voluntary neural activation of the trained muscle groups in premenopausal women with FM.[41] Controlled studies of muscle strength and aerobic exercise generally show substantial improvement in functional ability, clinical pain, and pain sensitivity.[42 43]

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