

## Association of Fibromyalgia With Altered Skeletal Muscle Characteristics Which May Contribute to Postexertional Fatigue in Postmenopausal Women

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**Objective.** To identify muscle physiologic properties that may contribute to postexertional fatigue and malaise in women with fibromyalgia (FM).

**Methods.** Healthy postmenopausal women with (n = 11) and without (n = 11) FM, ages 51–70 years, participated in this study. Physical characteristics and responses to self-reported questionnaires were evaluated. Strength loss and tissue oxygenation in response to a fatiguing exercise protocol were used to quantify fatigability and the local muscle hemodynamic profile. Muscle biopsies were performed to assess between-group differences in baseline muscle properties using histochemical, immunohistochemical, and electron microscopic analyses.

**Results.** There was no significant difference between healthy controls and FM patients in muscle fatigue in response to exercise. However, self-reported fatigue and pain were correlated with prolonged loss of strength following 12 minutes of recovery in patients with FM. Although there was no difference in percent succinate dehydrogenase (SDH)-positive (type I) and

SDH-negative (type II) fibers or in mean fiber cross-sectional area between groups, FM patients exhibited greater variability in fiber size and altered fiber size distribution. In healthy controls only, fatigue resistance was strongly correlated with the size of SDH-positive fibers and hemoglobin oxygenation. In contrast, FM patients with the highest percentage of SDH-positive fibers recovered strength most effectively, and this was correlated with capillary density. However, overall, capillary density was lower in the FM group.

**Conclusion.** Peripheral mechanisms, i.e., altered muscle fiber size distribution and decreased capillary density, may contribute to postexertional fatigue in FM. Understanding of these defects in fibromyalgic muscle may provide valuable insight with regard to treatment.

Fatigue is a hallmark of functional somatic syndromes including fibromyalgia (FM) (1–3). FM is a common disorder: epidemiologic studies show that it is found in ~2–5% of the US population, with a 9-fold higher prevalence rate in women than in men (4,5). The condition is characterized by chronic widespread musculoskeletal pain, tenderness, fatigue, nonrestorative sleep, dyscognition, and multiple other somatic symptoms (6–8). Although there has been considerable research in recent years, there is no consensus on a single etiopathogenesis of FM, and current theories include both central and peripheral mechanisms (9).

Although this remains somewhat controversial, peripheral defects that may contribute to both pain and fatigue, including muscle fiber dysfunction due to oxidative damage, chronic inflammation, and vasomotor dysregulation (10–12), have been reported in FM. Mitochondrial abnormalities in FM have been described, including abnormalities in shape, volume, orientation, and distribution that occur very rarely in healthy subjects (13–15). Mitochondrial function may also be reduced, as

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lower levels of ATP and phosphocreatine have been found in the muscle of patients with FM (16). Defects in capillary microcirculation (17), lower capillary density per muscle fiber, reduced capillary permeability, and changes in capillary endothelium thickness have been described as well (18,19). The current study was designed to test the hypothesis that reduced muscle oxidative capacity associated with decreased capillary density contributes to exercise-induced fatigue and malaise in FM.

## PATIENTS AND METHODS

**Subjects.** Twenty-three healthy women and 14 women who had been diagnosed by a rheumatologist as having FM according to the American College of Rheumatology 1990 criteria (8) participated in a parent study (20) and were initially enrolled for the present study. The women ranged in age from 51 to 70 years, and all were postmenopausal. The racial and ethnic composition of the study group was representative of the Kentucky population. Written informed consent was obtained from each subject prior to participation, and all experimental procedures were performed in accordance with the University of Kentucky Institutional Review Board. The final study population reported herein was limited to 22 subjects (11 FM patients and 11 healthy controls), due to inadequate quality of biopsied muscle from some subjects and in order to match groups for age, height, weight, and body mass index (BMI).

FM was characterized by number of tender points, subjective fatigue and pain determined using the Multi-dimensional Assessment of Fatigue (MAF) (21), Borg rating of perceived exertion (RPE) (22), visual analog scale (VAS) for fatigue (23) and pain (24), global rating of change scale (GRCS) (25), and Fibromyalgia Impact Questionnaire (FIQ) (26). Dual x-ray absorptiometry (DXA) scanning was performed for assessment of body composition including total percent body fat and thigh mineral-free lean mass (27). Further, during the consent process the frequency, intensity, type, and duration of weekly exercise were assessed, using the International Physical Activity Questionnaire (IPAQ) (28) to closely match for activity levels between the 2 groups.

Participants with FM who were taking nonsteroidal antiinflammatory drugs (2 of 11) were instructed to discontinue these medications at least 3 days prior to the muscle biopsy. Several subjects were taking fish oil supplements (3 of 11) and/or low-dose aspirin (2 of 11), which were also discontinued at least 3 days prior to the procedure. Compliance with these instructions was confirmed prior to the biopsy. Other medications for treatment of FM were continued; these included antidepressants (9 of 11), medications for sleep (5 of 11), opioid-containing analgesics (4 of 11), antiepileptic/alpha-2-delta ligands (3 of 11), and stimulants (1 of 11).

**Maximal isometric strength testing.** Prior to strength testing, subjects participated in a session to become familiar with the performance of maximal voluntary isometric contractions (MVICs). Following familiarization, each subject's central activation was quantified as previously described (29) (central activation ratio = MVIC/total force, where total force = MVIC plus superimposed involuntary twitch). The

central activation ratio did not differ between the healthy control group and the FM group (mean  $\pm$  SD  $0.99 \pm 0.01$  and  $0.99 \pm 0.02$ , respectively), suggesting that subjects in both groups were able to maximally and voluntarily activate their quadriceps muscle.

On the test day, subjects were asked to warm up for 5 minutes on a semirecumbent elliptical apparatus (Biostep 2; Biodex) at a comfortable rate and workload. After the warm-up period, MVIC in the right leg was determined using a dynamometer (Biodex System 4 Quick-Set), with the subject in a seated position (seat angle  $85^\circ$ ) with the lateral femoral epicondyle aligned to the center of the dynamometer shaft. To minimize the use of muscles other than the knee extensors, subjects were stabilized with 2 shoulder straps and a waist strap. Three maximal practice trials were performed, followed by 3 maximal test trials. MVIC (knee angle  $90^\circ$ ) force was recorded as the highest force generated over 3 trials held for 4 seconds, with a 3-minute rest period between attempts. MVIC force was recorded at baseline, every 2 minutes during the fatigue protocol (see below), immediately after exercise, and at time points 3, 6, 9, and 12 minutes during recovery.

**Fatiguing exercise protocol.** After maximal isometric strength testing, subjects immediately started a fatiguing exercise protocol. Participants were asked to perform 6 sets of 12 isometric contractions with a 40% duty cycle (4-second contraction and 6-second relaxation), with each set followed by MVIC as described above. The initial intensity was set at 20% MVIC and increased by 10% every set, eventually reaching an intensity of 70% MVIC. Thus, there were 78 total contractions over the period of exercise. Percent loss of MVIC and repetition to exhaustion during the fatigue protocol were the primary and secondary measures of fatigability, respectively.

**Tissue blood oxygenation.** The optical sensor placed on the quadriceps was connected to a commercial near-infrared tissue oximeter (Imagent; ISS) for tissue blood oxygenation measurements. Absolute deoxy-hemoglobin (Hgb) and oxy-hemoglobin (HgbO<sub>2</sub>) concentrations were determined from the measured tissue blood absorption coefficients at the 2 wavelengths (830 and 690 nm) and 4 source-detections (2.0, 2.5, 3.0, and 3.5 cm) (30). The absolute values of Hgb and HgbO<sub>2</sub> during the first 6 seconds after exercise were normalized to baseline (before exercise) to calculate their relative change.

**Muscle biopsy.** Within 60 minutes of recovery from the fatiguing exercise protocol a biopsy specimen from the vastus lateralis muscle of the nondominant, nonexercised leg was obtained,  $\sim$ 15 cm above the proximal patella edge, using a 5-mm Bergstrom biopsy needle with applied suction. Standard local anesthesia and aseptic procedures were used. Approximately 50 mg of vastus lateralis muscle tissue was mounted and oriented in tragacanth gum, snap-frozen in isopentane precooled with liquid nitrogen, and stored at  $-80^\circ\text{C}$  for histochemical analysis. Approximately 10 mg of muscle was fixed and embedded for electron microscopy analysis. Baseline physiologic properties of muscle that may contribute to fatigue and were not expected to be altered by the single bout of acute exercise were recorded.

**Histochemical and immunohistochemical analyses.** Frozen muscle samples were sectioned with a cryostat (Microm HM 525) at  $7\ \mu\text{m}$  thickness and used for succinate dehydrogenase (SDH), lectin, nitrotyrosine, and dystrophin detection and fiber typing. All steps were performed at room tempera-

ture unless noted. SDH activity in the muscle sections was visualized by incubation for 1 hour at 37°C in a mixed solution containing nitroblue tetrazolium (Sigma), succinate acid disodium (Sigma), and 0.2M of phosphate-buffered saline (PBS) in a dark chamber. Sections were rinsed with acetone (30%/60%/30% for 1 minute each) and distilled water and mounted with Vectashield mounting medium (Vector).

For fiber typing, sections were rehydrated in PBS and incubated with 3 different isoform-specific myosin heavy chain antibodies. Because the primary antibodies were of different isotypes, they were applied in combination for 90 minutes. BA.D5 IgG2b (1:75; Developmental Studies Hybridoma Bank), SC.71 IgG1 hybridoma supernatant (provided by Stefano Schiaffino, University of Padua, Padua, Italy), and 6H1 IgM hybridoma supernatant (provided by J. Y. Hoh, Sydney University, Sydney, New South Wales, Australia) were used for myosin heavy chain types I, IIA, and IIX expression, respectively. Alexa Fluor 647-conjugated goat anti-mouse IgG2b (1:250), Alexa Fluor 488-conjugated goat anti-mouse IgG1 (1:500), and biotin-conjugated goat anti-mouse IgM (1:150) secondary antibodies (all from Invitrogen) were applied together and incubated for 1 hour. Subsequently, streptavidin-Texas Red (1:150; Vector) was applied for 15 minutes. Sections were postfixed in methanol for 5 minutes and mounted with Vectashield.

For identifying capillaries, sections were rehydrated in PBS, blocked for 30 minutes in 2.5% normal horse serum (Vector), and incubated with tetramethylrhodamine

isothiocyanate-conjugated lectin (1:50; Sigma) for 1 hour. Sections were washed 3 times with Tris buffered saline and mounted with Vectashield.

For detection of nitrotyrosine and dystrophin, sections were rehydrated in PBS and permeabilized for 5 minutes with 0.3% Triton X-100 (Sigma), then blocked in 10% normal goat serum (The Jackson Laboratory). Primary mouse antidystrophin (1:100; Vector) and rabbit antinitrotyrosine (1:100; Millipore) were applied for 2 hours. Texas Red-conjugated goat anti-mouse secondary antibody (1:200; Rockland Immunochemicals) and Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (1:500; Invitrogen) were applied for 1 hour. Sections were postfixed for 10 minutes with 4% paraformaldehyde and counterstained with Vectashield DAPI. As a positive control for the nitrotyrosine staining, oxidative stress was induced in mouse muscle by mechanical overloading of the plantaris muscle. Briefly, mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and synergistic ablation was performed as previously described (31); 24 hours after overloading, muscle was dissected and snap-frozen. All histochemical and immunohistochemical images were captured using a Zeiss upright microscope (Axio Imager.M1) and analyzed with AxioVision Rel, version 4.8.

**Electron microscopy.** The muscle biopsy samples were fixed for 2 hours at 4°C in buffer containing 0.1M cacodylate (pH 7.4) with 3.5% glutaraldehyde and 4% paraformaldehyde. Samples were postfixed for 2 hours at 4°C with 1% OsO<sub>4</sub> and

**Table 1.** Characteristics of the healthy controls and the patients with FM\*

	Healthy controls (n = 11)	FM patients (n = 11)	P
Age, years	59.2 ± 5.2	60.9 ± 5.9	0.489
Height, cm	164.5 ± 5.5	163.2 ± 6.4	0.615
Weight, kg	69.9 ± 8.3	69.4 ± 17.9	0.942
No. of tender points	2.0 ± 3.4	15.8 ± 1.7	<0.001
Body composition			
BMI, kg/m <sup>2</sup>	25.9 ± 3.1	25.9 ± 5.4	0.988
% body fat	37.1 ± 6.6	38.9 ± 9.1	0.604
Thigh mineral-free lean mass, kg	3.9 ± 0.5	3.6 ± 0.7	0.244
Baseline questionnaires			
IPAQ, median (IQR) MET/minute/week <sup>-1</sup>	4,758.0 (3,424.8–6,102.8)	5,055.0 (1,671.0–12,219.4)	1.000
FIQ	12.2 ± 12.2	55.5 ± 15.6	<0.001
VAS pain score pre-exercise	7.3 ± 12.6	49.1 ± 26.4	<0.001
Self-reported exertion, fatigue, and pain levels with exercise			
RPE	13.4 ± 1.0	16.2 ± 2.8	0.005
MAF	11.5 ± 9.2	36.8 ± 6.4	0.001
VAS fatigue score	24.5 ± 20.1	63.6 ± 28.7	0.001
VAS pain score postexercise	15.8 ± 18.6	57.0 ± 28.3	<0.001
VAS pain score after 12 minutes of recovery	6.5 ± 13.3	52.5 ± 27.2	<0.001
GRCS pain postexercise	-0.5 ± 0.8	-2.3 ± 2.5	0.046
Strength assessment			
MVIC pre-exercise, Nm	131.5 ± 30.5	114.5 ± 20.1	0.137
MVIC postexercise, Nm	96.2 ± 29.2	79.5 ± 13.7	0.149
MVIC after 12 minutes of recovery, Nm	109.4 ± 29.1	92.0 ± 15.0	0.131

\* Except where indicated otherwise, values are the mean ± SD. FM = fibromyalgia; BMI = body mass index; IPAQ = International Physical Activity Questionnaire (data available on 9 controls and 9 patients; higher scores represent higher physical activity levels); IQR = interquartile range; MET = metabolic equivalents; FIQ = Fibromyalgia Impact Questionnaire (0 [low impact or fewer/smaller problems related to FM] to 100 [high degree of impact or greater problems related to FM]); VAS = visual analog scale (0 [least pain or fatigue] to 100 [greatest pain or fatigue]); RPE = Borg rating of perceived exertion (6 [easy or little-to-no effort] to 20 [maximum exertion]); MAF = Multidimensional Assessment of Fatigue (0 [least fatigue] to 50 [greatest fatigue]); GRCS = global rating of change scale (-7 [feeling a great deal worse after exercise] to +7 [feeling a great deal better after exercise] [0 = no change]); MVIC = maximal voluntary isometric contraction.

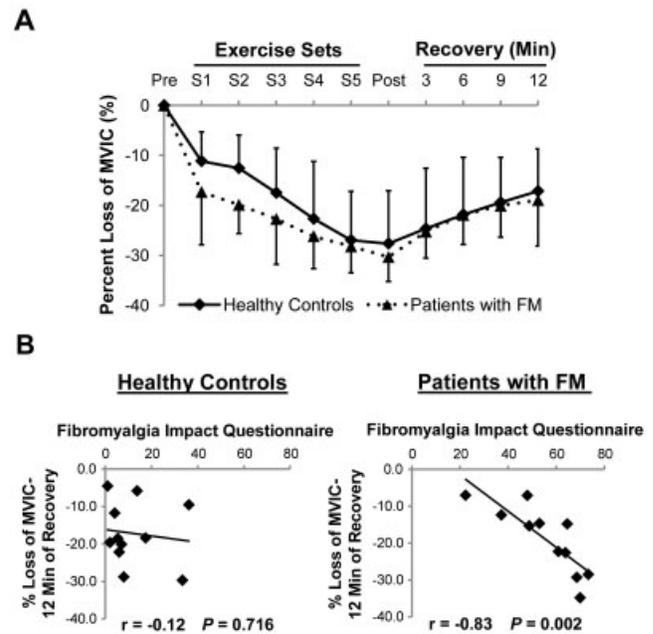
dehydrated with a series of graded ethanol (10 minutes at each dilution) and then propylene oxide (twice for 20 minutes each time). Finally, the samples were incubated overnight with a 1:1 dilution of resin with accelerator and propylene oxide, placed into pure resin with accelerator twice for 1 hour each time, and then embedded in resin with accelerator and placed in a 60°C oven for 48 hours. Thin cross-sections (60–90 nm) were cut, and 20–30 muscle fibers per subject were analyzed to determine the density of interfibrillar mitochondria, by point counting according to standard stereologic methods (32) with  $10 \times 10$  grids (132 points/image) using MetaMorph Advanced Image Analysis software, version 7.7.3.0 (Molecular Devices). The large number of fibers counted per subject randomizes the effect of fiber type. Images were captured at  $\times 13,000$  magnification and data represented as the percent fiber volume occupied by mitochondria on each image. In each subject, the percent of fibers that exhibited mitochondrial clumping was also determined.

**Statistical analysis.** Data are presented as the mean  $\pm$  SD. Differences between groups were analyzed by repeated-measures analysis of variance with rank test, independent *t*-test, and Mann-Whitney rank sum test as appropriate. Distribution analyses were performed with the Kolmogorov-Smirnov test, and correlation analyses with Pearson's correlation. Statistical analyses were performed using SigmaPlot software, version 12.0, build 12.0.0.182 and SAS software, version 9.3. *P* values less than 0.05 were considered significant.

## RESULTS

**Subject characteristics.** Age, height, weight, BMI, percent body fat, thigh mineral-free lean mass, and IPAQ total physical activity score were not significantly different between the control and FM groups. Patients with FM had significantly more tender points, a higher FIQ, higher subjective scores for fatigue (MAF and VAS), greater perceived exertion during the fatiguing exercise task (RPE), a greater sensation of pain change or malaise postexercise (GRCS), and more pain pre- and postexercise (VAS) compared to healthy control subjects (Table 1).

**Strength loss with fatiguing exercise.** To investigate muscle fatigue in response to exercise, subjects performed a fatiguing exercise protocol with strength loss quantified over time. Percent loss of strength, evaluated by MVIC before, during, and after exercise, demonstrated the effectiveness of the protocol in inducing fatigue both in healthy controls and in patients with FM ( $-27.7 \pm 10.5\%$  versus  $-30.3 \pm 4.9\%$ ;  $P = 0.453$ ) (Figure 1A). There was a trend toward a more rapid onset of fatigue in the patients with FM (indicated by the decrease in MVIC during the early stages of fatiguing exercise [sets 1 and 2]), suggesting possible differences in physiologic muscle properties in FM patients. However, there was no significant difference in the percent loss of strength following the fatiguing exercise protocol or



**Figure 1.** Correlation between the percent loss in maximal voluntary isometric contraction (MVIC) after the fatiguing exercise protocol and the score on the Fibromyalgia Impact Questionnaire (FIQ) in patients with fibromyalgia (FM). **A**, MVICs in healthy controls and FM patients, recorded at each time period (pre-exercise, after each exercise set [S1–5], and during recovery). Values are the mean  $\pm$  SD. **B**, Analyses of the correlation between the FIQ score and the percent loss of MVIC in controls and FM patients following 12 minutes of recovery from fatiguing exercise.

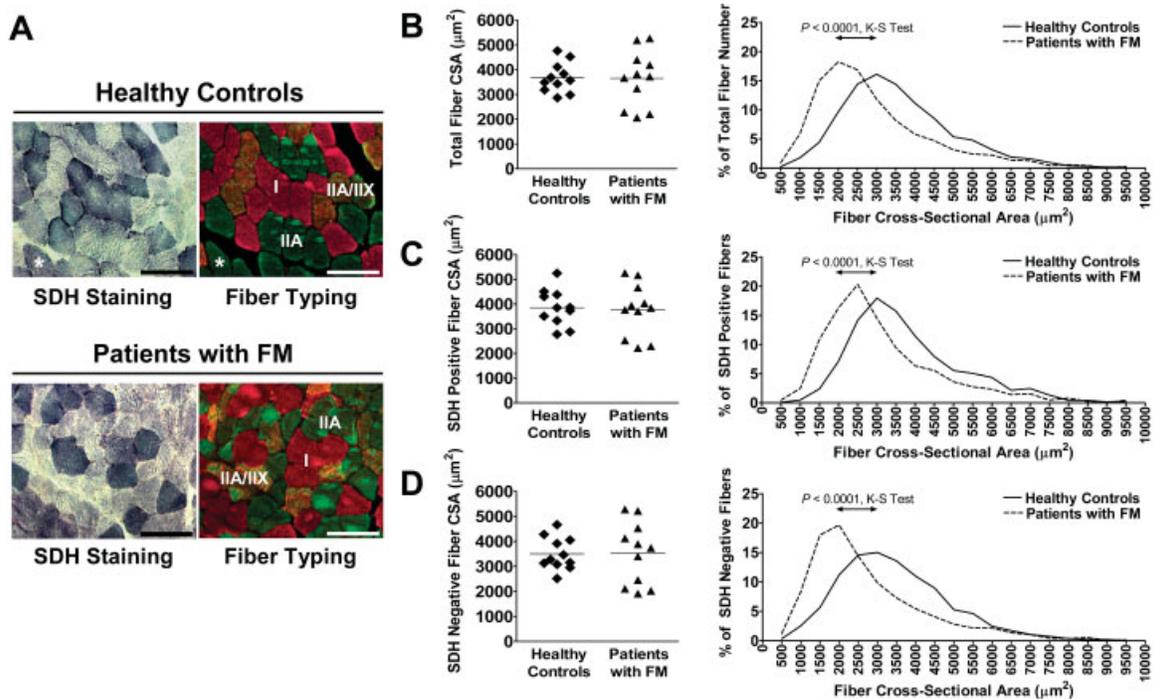
recovery period, or in time to failure to achieve MVIC (data not shown), between the patients and controls. In addition, the absolute MVICs (pre-exercise, immediately postexercise, and following 12 minutes of recovery) appeared lower in the FM patients, but the difference did not reach significance (Table 1). The trend toward lower absolute strength may be due to a trend toward reduced muscle mass of the thigh in patients with FM as measured by DXA, although other intrinsic muscle properties may also have contributed.

**Correlation of self-reported assessment of fatigue and pain with strength loss only in patients with FM.** Although there was no significant difference in MVICs following fatiguing exercise and recovery between the groups, correlation analyses revealed that patients with FM who showed the greatest prolonged strength loss after 12 minutes of recovery had the highest scores on the FIQ ( $r = -0.83$ ,  $P = 0.002$ ) (Figure 1B). Fatigue after 12 minutes of recovery was similarly correlated with the MAF ( $r = -0.70$ ,  $P = 0.017$ ) and the VAS pain score ( $r = -0.83$ ,  $P = 0.002$ ) in patients with FM. No such correlations between fatigue

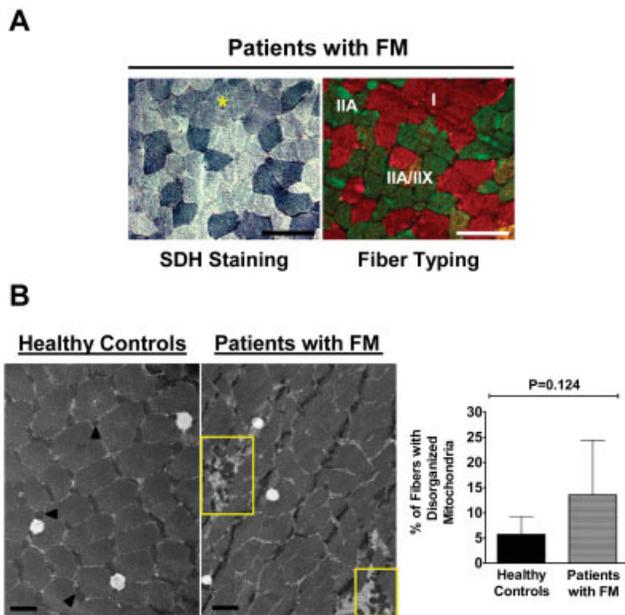
and scores on the FIQ ( $r = -0.12$ ,  $P = 0.716$ ), the MAF ( $r = 0.03$ ,  $P = 0.930$ ), or the pain VAS ( $r = 0.17$ ,  $P = 0.624$ ) were apparent in the control group, suggesting that mechanisms underlying fatigue may differ between healthy subjects and those with FM.

**Differences in fiber size and metabolic activity between healthy controls and patients with FM.** To compare muscle oxidative capacity between the groups, 2 different assays were performed on serial cryosections of muscle biopsy specimens (Figure 2A): SDH staining as an indicator of mitochondrial activity, and fiber typing using fiber type-specific myosin heavy chain antibodies to distinguish slow, type I fibers and fast, types IIA and IIX fibers. These analyses showed that oxidative, SDH-positive fibers were type I fibers both in healthy controls and in FM patients. However, rarely, type IIA fibers demonstrated high SDH activity in healthy controls (Figure 2A). The majority of SDH-negative fibers in both patients and controls coexpressed types IIA and IIX myosin heavy chains.

Quantification of the number of oxidative fibers in the FM patient and control groups showed no difference in the percentage of SDH-positive fibers (mean  $\pm$  SD  $40.4 \pm 11.3\%$  and  $43.3 \pm 9.5\%$ , respectively;  $P = 0.529$ ) and SDH-negative fibers ( $59.6 \pm 11.3\%$  and  $56.7 \pm 9.5\%$ , respectively;  $P = 0.529$ ). These results were supported by the finding that there was no difference in the percentage of type I and types IIA plus IIA/IIX fibers between the groups (data not shown). In addition, there was no significant difference in the mean cross-sectional area (CSA) of total fibers (SDH-positive plus SDH-negative), SDH-positive fibers, or SDH-negative fibers (Figures 2B–D). Although the mean fiber CSA was not different between groups, there was greater variation in fiber CSA among the patients with FM, with very small and very large fibers present. More detailed examination of fiber size distribution showed that FM patients had significantly more small fibers, indicated by the leftward shift in fiber size distribution (Figure 2B). The leftward shift was more pronounced in SDH-



**Figure 2.** Defects in fiber size distribution and loss of correlation with fatigue in patients with fibromyalgia (FM). **A**, Representative histologic results. Succinate dehydrogenase (SDH)-positive fibers (blue; left panels) were strongly correlated with type I fibers (pink; right panels) in both healthy controls and FM patients. Rarely, type IIA fibers (green; upper right panel) were SDH positive in specimens from healthy controls (asterisks). Most type IIX fibers also expressed type IIA myosin heavy chain in specimens from both groups (orange in upper and lower right panels). Bars = 100  $\mu\text{m}$ . **B–D**, Mean fiber cross-sectional area (CSA) and size distribution of total fibers (**B**), SDH-positive fibers (**C**), and SDH-negative fibers (**D**). The distribution of fiber sizes differed significantly as determined by the Kolmogorov-Smirnov (K-S) test (right panels), with more small fibers present in patients with FM. A total of 3,737 fibers from healthy controls (1,425 SDH-positive fibers and 2,312 SDH-negative fibers) and 3,556 fibers from FM patients (1,498 SDH-positive fibers and 2,058 SDH-negative fibers) were measured for CSA quantification. In the left panels, each symbol represents an individual subject; horizontal bars show the mean.

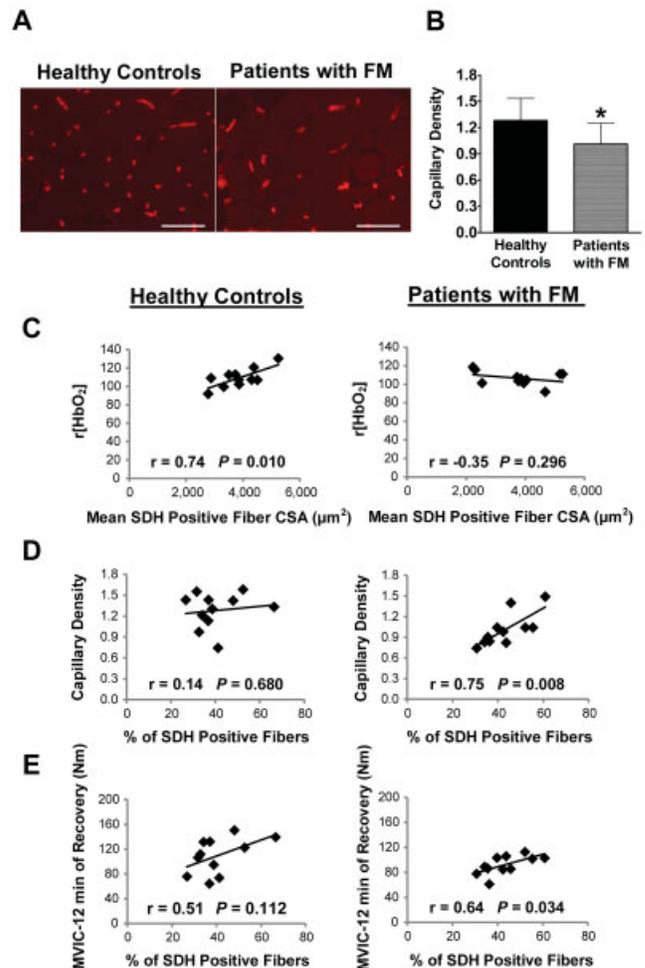


**Figure 3.** SDH staining in type I fibers and mitochondrial distribution in FM muscle. **A**, Representative fiber from a patient with FM, showing weak SDH staining (asterisk; left panel) in a cluster of type I fibers (pink; right panel). Bars = 100  $\mu\text{m}$ ; original magnification  $\times 200$ . **B**, Representative electron micrographs from a healthy control and a patient with FM, showing normal inter-fibrillar mitochondria in the control (arrowheads), compared to disrupted organization in the patient with FM (yellow rectangles). Bars = 1  $\mu\text{m}$ ; original magnification  $\times 13,000$ . Graph shows the mean  $\pm$  SD frequency of fibers with disorganized mitochondria ( $n = 6$  per group). See Figure 2 for definitions.

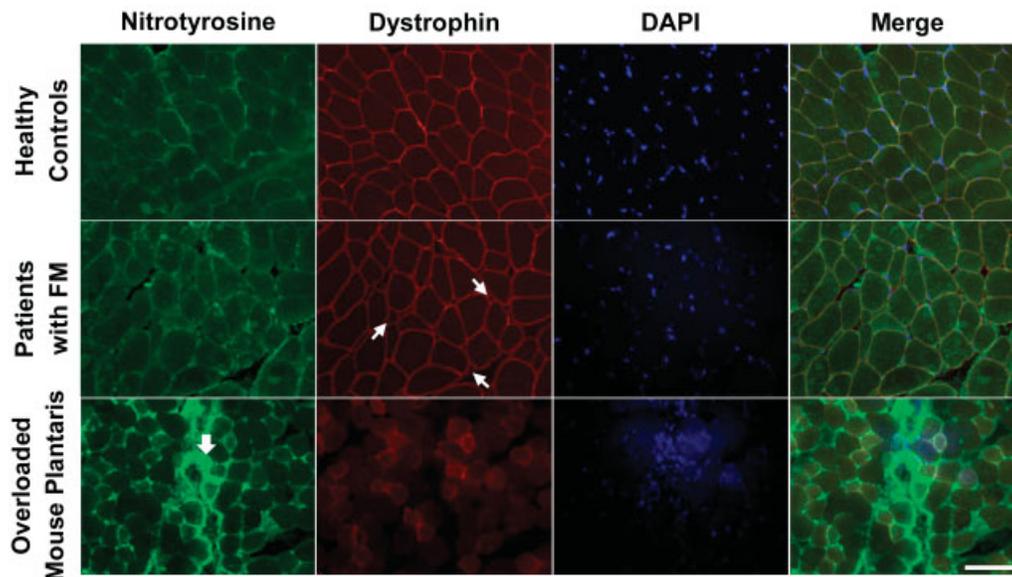
negative fibers (Figure 2D) compared to SDH-positive fibers (Figure 2C). Distribution analyses confirmed that total fiber CSA, SDH-positive fiber CSA, and SDH-negative fiber CSA were significantly different between healthy controls and patients with FM (all  $P < 0.0001$ ) (Figures 2B–D).

**Dissociation between fatigue and oxidative fiber size in patients with FM.** Fatigue resistance depends largely on the oxidative capacity of muscle fibers. In healthy controls, the CSA of SDH-positive fibers was positively correlated with fatigue resistance (data not shown). Additionally, it was positively correlated with strength recovery 12 minutes after exercise in healthy controls ( $r = 0.75$ ,  $P = 0.008$ ). However, these correlations were not observed in the patients with FM ( $r = -0.08$ ,  $P = 0.808$ ). In addition to the dissociation between oxidative fiber size and fatigue resistance in the patients with FM, many type I fibers in the FM samples showed relatively weak SDH staining (Figure 3A). To determine whether weak SDH activity was related to mitochondrial defects in FM, mitochondrial volume

density was quantified in parallel with mitochondrial distribution, by electron microscopy. There was no apparent difference in mitochondrial density between healthy controls (mean  $\pm$  SD  $2.39 \pm 0.95\%$ ) and FM patients ( $2.59 \pm 0.65\%$ ). Abnormal clumping of mitochondria appeared more frequently in specimens from FM patients; however, the difference between patients



**Figure 4.** Reduced capillary density and altered correlations with tissue oxygenation, oxidative fibers, and strength recovery in FM muscle. **A**, Representative histologic results, with capillaries identified by lectin staining (red). Bars = 100  $\mu\text{m}$ . **B**, Decreased numbers of capillaries per fiber in patients with FM compared to healthy controls. Values are the mean  $\pm$  SD ( $n = 11$  per group). \* =  $P < 0.05$  versus controls. **C**, Correlation between relative change in oxy-hemoglobin level ( $r[\text{HbO}_2]$ ) and mean SDH-positive fiber CSA in healthy controls but not in patients with FM. **D**, Correlation between capillary density and percent SDH-positive fibers in patients with FM but not in healthy controls. **E**, Weak correlation between strength following 12 minutes of recovery, measured as maximum voluntary isometric contractions (MVICs), and percent SDH-positive fibers in patients with FM but not in healthy controls. See Figure 2 for other definitions.



**Figure 5.** Representative histologic findings, showing a lack of apparent muscle morphologic defects in patients with fibromyalgia (FM). Antinitrotyrosine and antidystrophin antibodies (green and red, respectively), together with DAPI staining (blue), were used to visualize muscle fiber structure and nuclei. Overloading-damaged mouse muscle was used as a positive control for nitrotyrosine staining. **Thin arrows** denote small fibers in FM muscle. **Thick arrow** denotes nitrotyrosine accumulation in overloaded mouse plantaris muscle. Bar = 100  $\mu\text{m}$ .

and controls did not reach statistical significance ( $13.54 \pm 10.86\%$  versus  $5.70 \pm 3.57\%$ ) (Figure 3B).

**Association of lower capillary density with altered oxygen delivery in patients with FM.** To investigate oxygen delivery to the muscle in FM, the number of capillaries per fiber (capillary density) was determined. Capillary density was significantly lower among patients with FM compared to healthy controls (Figures 4A and B) (mean  $\pm$  SD  $1.01 \pm 0.24$  capillaries per fiber versus  $1.28 \pm 0.25$  capillaries per fiber;  $P < 0.05$ ). Near-infrared tissue oximetry revealed that, although tissue blood oxygenation was not different between the groups, the relative change in  $\text{HgbO}_2$  concentration following exercise was strongly correlated with the size of oxidative, SDH-positive fibers in healthy controls ( $r = 0.74$ ,  $P = 0.010$ ), but not in patients with FM ( $r = -0.35$ ,  $P = 0.296$ ) (Figure 4C). In contrast, capillary density was positively correlated with the percent of SDH-positive type I fibers in the patients with FM ( $r = 0.75$ ,  $P = 0.008$ ) but not the healthy controls ( $r = 0.14$ ,  $P = 0.680$ ) (Figure 4D). Finally, patients with FM who had a high percentage of SDH-positive type I fibers also exhibited higher MVIC following 12 minutes of recovery ( $r = 0.64$ ,  $P = 0.034$ ) (Figure 4E).

Taken together, these results show that fatigue resistance in patients with FM was associated with the percentage of SDH-positive type I fibers, which was strongly correlated with capillary density. In healthy

controls, overall capillary density was higher, and the size of SDH-positive fibers predicted fatigue resistance.

**Absence of apparent muscle morphologic defects in patients with FM.** Oxidative stress was investigated using nitrotyrosine as a marker of nitrogen species-induced oxidative stress (Figure 5). There was no apparent difference in accumulation of nitrotyrosine between healthy control and FM muscle specimens. An overloaded mouse muscle used as a positive control showed strong nitrotyrosine staining in an area of cell infiltration. No cellular infiltration or signs of inflammation were observed in FM muscle. In addition, an antibody against dystrophin was used to double stain with antinitrotyrosine to delineate muscle fibers. Small fibers were apparent in FM muscle, as noted above, but no difference in muscle membrane integrity between groups was observed.

## DISCUSSION

The goal of this study was to identify muscle properties that may contribute specifically to the post-exertional fatigue and malaise associated with FM. Although the postmenopausal women with FM in this study self-reported experiencing higher levels of fatigue than were reported by healthy controls, loss of strength in response to a fatiguing exercise protocol did not differ significantly between the groups. These results were

similar to those in a previously reported study of premenopausal women with FM, in which no differences in isometric strength and neuromuscular activation during fatiguing resistance exercise were observed (33). However, muscle fatigue, measured as the percent strength loss at the end of the exercise protocol and the magnitude of the deficit 12 minutes after completion of exercise, were highly correlated with self-reported pain and fatigue only in subjects with FM. These results suggest that a central component contributes to fatigue in FM, as was observed in hemodialysis patients in a study using the same type of fatiguing protocol (34).

Examination of muscle phenotype from biopsy specimens showed that, whereas the percentage and mean fiber cross-sectional area of type I and type II fibers were not different in FM compared to control muscle, consistent with an earlier report (19), muscle fibers in women with FM were more variable in size and significantly different in size distribution than fibers in healthy controls. Type I fibers are oxidative, as indicated by high SDH activity, whereas type II fibers are more glycolytic, involved in rapid force generation. The percentage and size of type I fibers are the primary determinants of oxidative capacity and fatigue resistance. Among healthy controls, those individuals with the largest SDH-positive type I fibers were the most fatigue resistant. This correlation was not apparent in patients with FM. The loss of type I fiber size as a contributor to fatigue resistance in FM muscle may have physiologic relevance in terms of exercise prescription (see below).

Oxidative capacity of individual muscle fibers is determined by mitochondrial activity, which may be affected in FM; mitochondrial abnormalities in FM have been described (13–15). Assessment of mitochondrial volume density by electron microscopy in the present study revealed no significant difference between groups; however, a trend toward abnormal mitochondrial distribution was seen in the FM group. No indicators of oxidative stress or inflammation were apparent at the light microscopic level in muscle from patients with FM, consistent with previously reported findings (35).

The most pronounced difference in muscle between patients with FM and healthy controls observed in this study was reduced capillary density in the FM patients. Evidence of reduced capillary permeability and structural changes in the capillary endothelium in FM has also been reported (18,19). Altered microcirculation may result in lower oxygen delivery and waste product clearance, contributing directly to pain and fatigue in FM. This idea is supported by the work of Lund et al, who found a pathologic distribution of tissue oxygen

pressure in patients with FM, using an oxygen multipoint electrode (17). Further, blood flow response during exercise has been reported to be lower in FM patients, as determined by contrast media-enhanced Doppler ultrasonography (36).

Using a near-infrared tissue oximeter, we found that the relative concentration of oxygenated hemoglobin immediately following exercise correlated significantly with SDH-positive fiber size in healthy controls but not in patients with FM, suggesting that impaired oxygen delivery may limit the size of oxidative fibers in FM. Interestingly, we also observed that capillary density was strongly correlated with the percentage of SDH-positive type I fibers only in patients with FM, and this was associated with fatigue resistance. Thus, oxygen availability may dictate the muscle fiber metabolic phenotype in FM, altering mitochondrial oxidative capacity and even limiting the size of oxidative fibers, which may contribute directly to postexertional muscle fatigue. Taken together, these results suggest that approaches to promote angiogenesis and increase muscle vascular density may hold promise in the treatment of FM.

Although exercise has become a common key component in the management of FM, variability in study design and exercise training programs has made appropriate exercise prescription unclear. Our results suggest that combined aerobic and resistance exercise should be prescribed to effectively combat the primary muscle physiologic deficits in FM, i.e., variation in muscle fiber size and reduced capillary density. Exercise in general has been shown to be beneficial in FM (37–39), producing higher effect sizes than pharmacologic treatments (40,41). Aerobic training improves physical function and fitness indices in FM patients (for review, see ref. 37); however, its effects on pain, tender points, and fatigue are equivocal. Our findings suggest that aerobic training programs designed specifically to increase capillary density (42) should be used to maximize beneficial effects in this population.

There has been much less research examining the effects of resistance training on FM symptoms. Some reports suggest that patients with FM have similar trainability to healthy subjects, significantly increasing strength, neuromuscular function, and power (43–45). While some investigators report that patients with FM can tolerate traditional resistance exercise programs designed for muscle hypertrophy that focus on high loads and intensities (46), others report poor adherence and adverse events (for review, see ref. 37). Interestingly, recent research shows that exercise performed at 30% of 1 repetition maximum (30% 1 RM) to momentary fatigue was equally as effective as exercise per-

formed at 90% 1 RM in stimulating myofibrillar protein synthesis (47) and that a slow lifting movement (increased time under tension) stimulated muscle protein synthesis even further (48). In addition, similar hypertrophic responses were observed in young men utilizing 30% compared to 80% 1 RM, as long as the muscle was taxed to momentary failure (49). Thus, we hypothesize that exercise training incorporating multiple resistance exercise sets at a relatively low intensity (30–40% 1 RM) to permit a higher number of slow-lifting repetitions, performed to failure, may be appropriate for individuals with FM. This type of resistance exercise prescription, combined with concurrent aerobic exercise, would be most effective in improving both muscular strength and endurance deficits observed in FM patients, while minimizing adverse events and poor adherence. Such an approach may also avoid the delayed onset of muscle relaxation between contractions reported in patients with FM (50), as well as increased coactivation of the antagonist muscle that may also contribute to the pain and fatigue experienced after exercise (51).

In conclusion, understanding peripheral mechanisms that affect muscle function provides valuable insight for fibromyalgia treatment and symptom management. Further studies on the effectiveness of concurrent aerobic and resistance training are needed.

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#### AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Peterson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Srikuea, Long, Yu, Crofford, Peterson.  
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