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Reduced oxidative phosphorylation and proton efflux suggest reduced capillary blood supply in skeletal muscle of patients with dermatomyositis and polymyositis: a quantitative ^{31}P-magnetic resonance spectroscopy and MRI study

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Summary

Quantitative MRI and phosphorus magnetic resonance spectroscopy (^{31}P-MRS) were used to investigate skeletal muscle metabolism *in vivo* in patients with dermatomyositis (DM) and polymyositis (PM) in order to evaluate the role of mitochondrial abnormalities in the pathogenesis and clinical expression of these conditions. Nine patients with DM (mean age ± SD, 57 ± 14 years) and five with PM (42 ± 12 years) and with age at disease onset 53 ± 16 and 38 ± 12 years, respectively, were included in the study together with 18 age-matched controls. Post-exercise ^{31}P-MRS indices of muscle oxidative metabolism were all impaired in DM and PM. In both groups of patients, the phosphocreatine and adenosine diphosphate recovery half-times were almost twice as long as in controls ($P < 0.05$ for each variable) and the maximum rate of mitochondrial ATP production was half that found in normal subjects ($P < 0.001$). The rate of proton efflux from muscle fibres was significantly reduced in DM ($P < 0.001$) and PM ($P = 0.02$). The impairment of ^{31}P-MRS recovery indices in DM and PM patients was similar to that found in a group of 10 patients with a primary mitochondrial disorder that showed a normal proton efflux rate. There was no correlation between the MRS-detectable abnormalities and the degree of inflammation or fatty infiltration of the muscle, as measured by MRI. The *in vivo* findings in DM and PM patients indicate impaired muscle aerobic function, which, considering the reduced proton efflux, is likely to be secondary to an impaired blood supply. Our results suggest that the abnormal mitochondria seen in some muscle biopsies are unlikely to be the primary cause of the oxidative insufficiency in these patients.

Keywords: dermatomyositis; polymyositis; ^{31}P-MRS; quantitative MRI; oxidative phosphorylation

Abbreviations: CK = creatine kinase; COX = cytochrome *c* oxidase; Cr = creatine; CSA = cross-sectional area; DM = dermatomyositis; H^+ = proton; IIM = idiopathic inflammatory myopathies; LBM = lean body mass; MM = mitochondrial myopathy; MRS = magnetic resonance spectroscopy; PCr = phosphocreatine; ^{31}P-MRS = phosphorus magnetic resonance spectroscopy; Pi = inorganic phosphate; PM = polymyositis; s-IBM = sporadic inclusion body myositis; $t_{1/2}$ = half-time; TCr = total creatine

Introduction

Idiopathic inflammatory myopathies (IIM) form a heterogeneous group of acquired muscle disorders characterized by muscle weakness and chronic inflammatory infiltrates within the skeletal muscle and are thought to have an autoimmune origin. They include three conditions that can be distinguished by clinical and histological features: (i) dermatomyositis (DM), involving a humoral immune process against muscle capillaries and secondary muscle fibre necrosis; (ii) polymyositis (PM), believed to involve cell-mediated cytotoxicity; and (iii) sporadic inclusion body myositis (s-IBM), in which antigen-directed cytotoxicity mediated by T cells is the proposed mechanism of damage (Dalakas, 1991).

Although mitochondrial abnormalities similar to those found in patients with primary mitochondrial disorders (DiMauro and Moraes, 1993) are commonly found in skeletal muscle of IIM patients, the contribution of mitochondrial dysfunction to the pathogenesis and clinical symptoms of IIM is still undefined and controversial. Histological studies of skeletal muscle in these disorders have revealed the presence of ragged-red fibres, cytochrome *c* oxidase (COX)-negative fibres and increased succinic dehydrogenase staining (Rifai *et al*., 1995; Chariot *et al*., 1996). These changes are more pronounced and consistently reported in s-IBM (Oldfors *et al*., 1995; Rifai *et al*., 1995; Chariot *et al*., 1996; Santorelli *et al*., 1996; Lodi *et al*., 1998) than in DM and PM. In DM patients, abnormal numbers of ragged red fibres and COX-negative fibres were shown to be more frequent than in PM patients (Chariot *et al*., 1996), where they have been found only in rare (Rifai *et al*., 1995) or atypical (Blume *et al*., 1997) cases. Reduced respiratory chain enzyme complex activities (Campos *et al*., 1995; Chariot *et al*., 1996; Santorelli *et al*., 1996) have been reported in the skeletal muscle of PM and s-IBM patients but not in DM (Miro *et al*., 1998). Abnormal accumulation of mitochondrial DNA deletions has been reported in s-IBM and PM patients (Oldfors *et al*., 1995; Santorelli *et al*., 1996; Blume *et al*., 1997).

Phosphorus magnetic resonance spectrometry (^{31}P-MRS) is an ideal method to study skeletal muscle bioenergetics *in vivo*, especially mitochondrial function. Previous studies in thigh muscles of patients with DM (Park *et al*., 1990, 1995; Newman and Kurland, 1992) and juvenile DM (Park *et al*., 2000), showing altered concentrations of phosphorylated compounds at rest and abnormal work–cost relationships during exercise, have suggested defective oxidative phosphorylation that was hypothesized to be secondary to capillary abnormalities. However, similar changes in resting and exercising muscle have been described in s-IBM (Argov *et al*., 1998; Lodi *et al*., 1998) and Becker muscular dystrophy (Lodi *et al*., 1999), conditions associated with normal mitochondrial respiration, as shown by normal phosphocreatine (PCr) and ADP recovery rates after exercise.

In the present study, we used quantitative ^{31}P-MRS and MRI to establish whether an *in vivo* deficit of skeletal muscle mitochondrial function or other bioenergetic abnormalities are present in DM and PM, and their role in the pathogenesis and clinical expression of these disorders. In order to distinguish between primary and secondary (reduced blood supply) mitochondrial dysfunction, the rate of proton extrusion from muscle fibres was also assessed. ^{31}P-MRS findings in patients with DM and PM were also compared with those in a group of patients with a primary mitochondrial disorder.

Patients and methods

We studied nine patients with DM (seven female) and five with PM (three female), aged 57 ± 14 (mean ± SD) and 42 ± 12 years, respectively. Diagnosis was made on the basis of clinical and histological criteria and the response to treatment. Relevant clinical features are summarized in Table 1. All patients had clear muscle weakness and, although some of them complained of generalized weakness, only Patients 7, 8 and 9 had mild calf weakness on examination. In the DM group, four patients were acute without treatment, one was a relapsing case without treatment, and four were chronic patients on treatment. In the PM group, four were chronic patients on treatment and one was an acute case without treatment. All chronic DM patients and all PM patients led normal everyday lives, with the exception that some had difficulty in activities using the proximal lower limb muscles, such as climbing stairs or rising from a sitting position. All patients on treatment had been unable to stop immunosuppressive treatment without relapsing. At the time of the MRI examination, all the patients had biopsy-proven disease except Patient 7, who had a normal deltoid muscle biopsy, although she had a typical DM clinical picture and a plasma creatine kinase (CK) concentration of 19 000 IU/l (normal range 70–120 IU/l). All patients had normal sensory and motor nerve conduction but EMG showed a myopathic pattern and typical irritative signs of myositis, such as fibrillation potentials and positive sharp waves. COX-negative fibres were found in Patients 1 and 3 but constituted no more than 0.4% of the total, which is considered normal for their age.

Normal control subjects were 18 healthy, age-matched volunteers (two male and 16 female, aged 51 ± 11 years). None of the controls was undergoing any type of physical training.

^{31}P-MRS data from the patients and normal controls were compared with results from a group of 10 patients with primary mitochondrial myopathy (MM), comprising three patients with Leber's hereditary optic neuropathy, four patients carrying the A3243G mitochondrial DNA mutation, two brothers with a clinical and biopsy-proven mitochondrial myopathy and one patient with progressive external ophthalmoplegia with a single 5.17 kb deletion. MRS studies of MM patients were carried out under conditions identical to those described for IIM and normal controls.

Table 1 *Clinical data of patients with DM and PM at the time of study*

Patient	Sex	Age at onset (years)	Age at study (years)	Skin rash	Muscle weakness*					COX fibres	CK	Medications (mg)†	
					General	Neck	Shoulders	Hips	Quadriceps		(IU/l)	Prednisolone	Others
DM													
1	F	55	57	+	–	4+	5	4+	4+	+	66	60 a.d.	Azathioprine 150
2	F	20	31	+	–	4+	4+	4+	5	n.a.	256	45 a.d.	Azathioprine 120
3	F	58	67	+	–	5	4+	4	5	+	200	–	Methotrexate 12.5 w
4	F	39	40	–	+	4	4	4	5	n.a.	1400	80	Azathioprine 125
5	F	57	64	–	+	5	5	4	4	–	318	–	
6	M	69	69	+	+	4	3	3	3+	–	4534	–	
7	F	63	63	+	+	4	4	4	4	–	19 000	–	
8	F	46	46			4	4	4	4+	–	1670	–	
9	M	72	72	+	+	4	4	4–	4+	–	750	–	
PM												–	
10	M	40	46	–	–	5	4	4	4+	–	248	20 a.d.	
11	F	36	45	–	–	5	5	4	5	–	260	10	Cyclophosphamide 25
12	F	43	50	–	–	4	4	4	4	–	158	35 a.d.	Azathioprine 200
13	F	19	21	–	–	5	5–	5–	5	–	150	5	Azathioprine 125
14	M	50	50	–	–	5–	5–	4	4+	–	6218	–	

*Muscle weakness is expressed on the modified MRC scale; †dosing frequency was daily unless indicated as a.d. (alternate days) or w (weekly). F = female; M = male; n.a. = not available.

Informed consent was obtained from each patient and normal volunteer, and studies were carried out with the approval of the Central Oxford Research Committee.

Magnetic resonance

A 2.0 T superconducting magnet (Oxford Magnet Technology, Eynsham, Oxford, UK) interfaced to a Bruker spectrometer (Bruker, Coventry, UK) was used for ^{1}H-MRI and ^{31}P-MRS.

^{1}H-MRI

An axial MRI of the right leg was acquired using an 18 cm diameter quadrature birdcage coil. Three sagittal gradient-echo images were obtained in order to identify the position of the calf at maximal cross-sectional area (CSA). MRI parameters were: repetition time (TR) 302 ms; echo time (TE) 12 ms; matrix size 256 × 128; and field of view (FoV) 50 × 25 cm.

A three-point Dixon encoding method (Xiang and An, 1997) (echo offsets 0, 0.442, 0.883 ms) combined with a multi-echo spin-echo sequence that employed non-slice-selective refocusing pulses (Hardy and Yue, 1997) was then used to obtain images at maximal CSA in a transaxial orientation. This combined method has been developed by us to produce separate images from fat and water (each of which can contribute to signal intensity in any individual pixel) whilst simultaneously providing a determination of transverse relaxation times (T_2) of each component. MRI parameters were: TR 2.0 s, TE 20, 40, 60, 80, 100, 120 ms; matrix size 256 × 128; FoV 25.6 × 12.8 cm; slice thickness 7 mm. Total acquisition time was 12 min 48 s.

^{31}P-MRS

After imaging, ^{31}P spectra were collected during a protocol of rest, incremental exercise and recovery, using a 2 s interpulse delay. Subjects lay supine with a 6 cm diameter surface coil under the maximal CSA identified by previous MRI. Exercise consisted of plantar flexion performed at 0.5 Hz, lifting 10% of lean body mass (LBM; calculated from body weight and skin-fold thickness) (Durnin and Womersley, 1974) through a distance of 7 cm. After 4 min the workload was incremented by 2% LBM for each further minute until the PCr reached ~50% of the pre-exercise level, when the areas of the inorganic phosphate (Pi) and PCr peaks are similar by visual estimation. At this point mitochondrial function has been adequately stimulated for recovery analysis (see below). Spectra were acquired at rest (64 scans), during exercise (16 scans) and in recovery. Immediately after the last 16-scan exercise spectrum, an eight-scan spectrum was recorded to be used as the zero time-point for the recovery phase. Exercise was then stopped and data were collected for 10 min (four 8-scan spectra followed by four of 16, three of 32 and two of 64 scans).

Data analysis

^{1}H-MRI

The gastrocnemius–soleus complex region was outlined manually in the transaxial image at maximal CSA using the Xtip software package (Bruker, Ettlingen, Germany), excluding subcutaneous fat and major blood vessels. Fat and water contributions to the total signal were determined as described above for the soleus and medial and lateral gastrocnemius muscles and for the gastrocnemius–soleus complex as a whole. The gastrocnemius–soleus complex is the group of

muscles involved in the plantar-flexion exercise protocol, and spectroscopy data were acquired in this region with the surface coil. Therefore, the CSA of the gastrocnemius–soleus complex was correlated with the LBM with the purpose of determining whether the work done by patients and controls was comparable. The T_2 relaxation time was also measured in this area and was compared with the ^{31}P-MRS findings.

^{31}P-MRS

Metabolite concentrations and pH. Relative concentrations of Pi, PCr and ATP were obtained by a time-domain fitting routine (van der Veen *et al*., 1988) using the AMARES algorithm and MRUI software (van den Boogaart, 1997*a*, *b*) and corrected for magnetic saturation. Absolute concentrations were calculated assuming that the concentration of ATP in normal muscle is 8.2 mM (Arnold *et al*., 1985). Intracellular pH was determined from the chemical shift of the Pi peak relative to PCr (Gadian *et al*., 1981). Free [ADP] and the free energy of ATP hydrolysis (ΔG_{ATP}) were calculated, as described previously, by adjusting the CK equilibrium constant to the ionic conditions of the cell in order to avoid the errors that can arise at low pH (Golding *et al*., 1995). The equilibrium constant for the CK reaction was taken as 1.75×10^9 M^{-1} at rest and the equilibrium constant for the ATP hydrolysis reaction as 0.129 (Harkema and Meyer, 1997). The concentration of free creatine ([Cr]) used in determining [ADP] was calculated assuming (i) a [PCr]/([PCr] + [Cr]) ratio of 0.85 in resting muscle (Jeneson *et al*., 1996) and (ii) that total creatine ([TCr] = [PCr] + [Cr]) remained constant throughout the test.

Muscle bioenergetics in exercise. Rates of change for [PCr] and pH were calculated for each subject with respect to the duration of exercise and work performed for three time-points: initial (midpoint, 0.25 min); the last time point at which all subjects but one were still exercising (midpoint, 6.1 min); and the end of exercise.

Rates of ATP production from PCr hydrolysis (R_{PCr}) and glycogenolysis (R_{Gly}) were calculated (Kemp *et al*., 1994) and expressed with respect to work output. The sum of R_{PCr} + R_{Gly} is the contribution of anaerobic metabolism (R_{An}) to cellular energy production. R_{PCr} was calculated from the time-dependent changes in PCr. R_{Gly} was derived from the pH change over the relevant time interval, assuming that each mole of ATP synthesized glycolytically, when coupled to ATP hydrolysis, is accompanied by the production of 1.5 mol protons (H^+) from lactic acid (Kemp *et al*., 1993*b*).

At the onset of exercise, the contribution of oxidative metabolism to energy production is minor (Kemp *et al*., 1994). R_{An} at the start of exercise was therefore used as a measure of the total rate of ATP production necessary for a given unit of power, the energy cost. Assuming that the energy cost remains directly proportional to power output throughout exercise, any change in R_{An} must be compensated for by an equivalent change in oxidative metabolism in the opposite direction.

Corrections were made for H^+ consumption from the net change in [PCr], H^+ efflux from the cell and intracellular buffering (β) (Kemp *et al*., 1993*b*). The value of β, expressed in slykes (mmol H^+/unit change in pH), was taken as $20 + \beta_{Pi}$, where β_{Pi} is the contribution of inorganic phosphate in mM to the total intracellular buffering capacity determined according to the standard formula (Conley *et al*., 1998):

$$\beta_{Pi} = \frac{2.303 \cdot [H^+] \cdot K \cdot [Pi]}{(K + H^+)^2}$$

where K is the dissociation constant of the buffer and is equal to 1.77×10^{-7}.

Muscle pH is modified by H^+ efflux by various membrane transport systems, but it is not possible to measure this parameter during exercise because the intracellular pH represents a balance between lactic acid production, H^+ efflux and the H^+ consumed by PCr breakdown. However, H^+ efflux can be determined from the recovery data (see Results, Recovery).

Muscle bioenergetics in recovery from exercise. PCr and ADP recovery half-times ($t_{1/2}$) were calculated from the slopes of semi-logarithmic plots. Initial rates of PCr resynthesis after exercise (V, in mM/min) were determined from the exponential rate constant of PCr recovery ($k = \ln(2)/t_{1/2}$) and the net decrease in [PCr] during exercise (Δ[PCr]) as $V = k\Delta[PCr]$. V is a good estimate of the end-exercise rate of oxidative ATP synthesis (Kemp *et al*., 1994; Thompson *et al*., 1995), and this has an approximately hyperbolic (Michaelis–Menten) relationship to its driving force, the cytosolic free [ADP] (Kemp *et al*., 1993*c*). Therefore, end-exercise [ADP] ($[ADP]_{end}$) and V were used to calculate the maximum rate of oxidative ATP synthesis:

$$V_{max} = V(1 + K_m/[ADP]_{end})$$

During recovery, as glycolysis is inactive in the absence of muscle contraction, no lactic acid is produced, so the only significant source of H^+ is from the net resynthesis of PCr (Kemp *et al*., 1993*b*). H^+ efflux was calculated as $V_{pH}\beta + H^+$ consumed by PCr breakdown, where V_{pH} is the rate of pH change at the beginning of recovery. Because H^+ efflux is pH-dependent, it was calculated for any given time interval during exercise on the basis of the ΔpH for that interval (Kemp *et al*., 1994).

Statistical analysis

All results are presented as the mean ± standard error of the mean unless otherwise stated. The non-parametric Mann–Whitney *U*-test was used for comparisons between patients

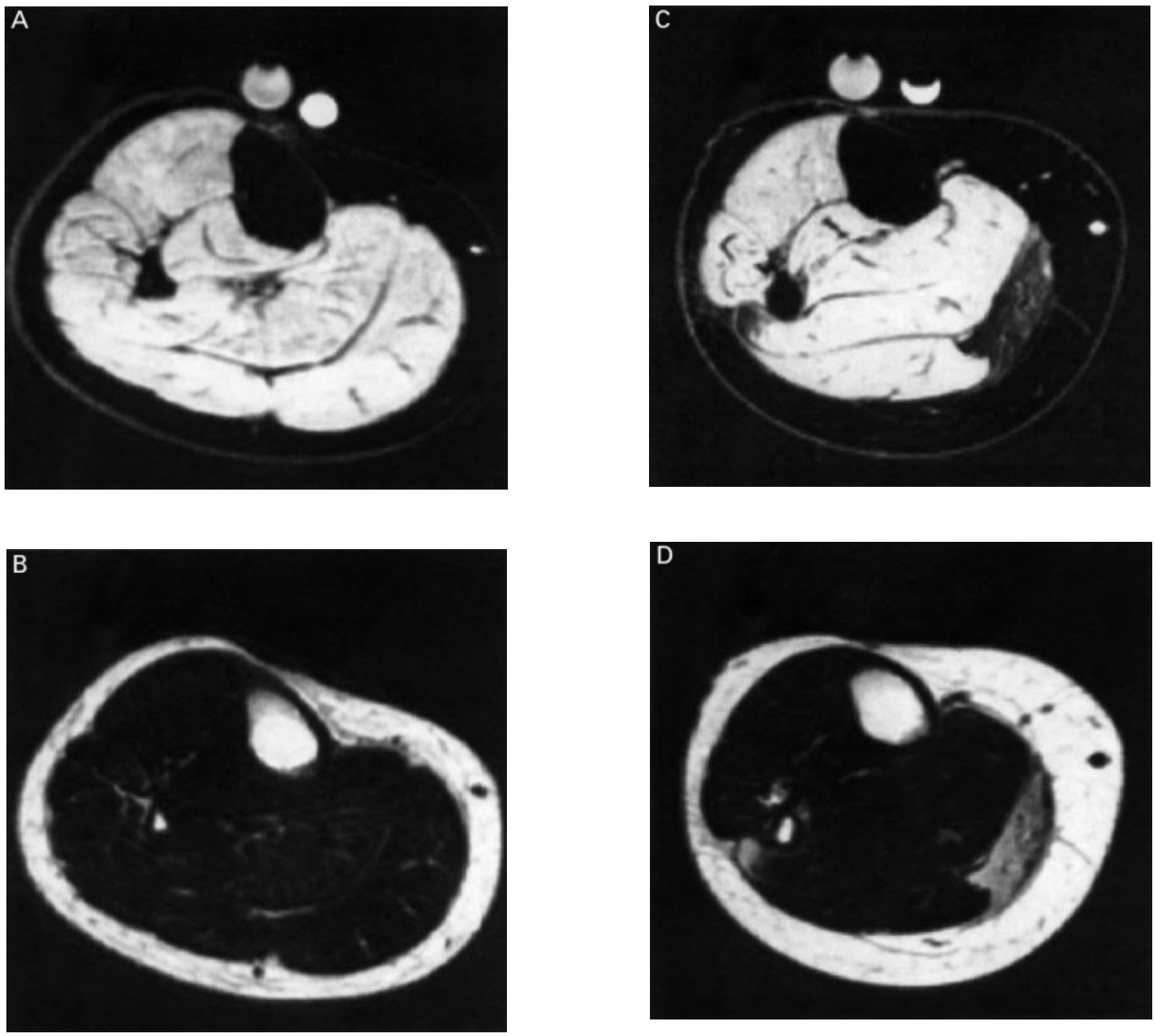

Fig. 1 Muscle water and fat images from the leg of a patient with PM (Patient 11) and a control. (**A**) Images of the muscle water content of the maximal CSA from a normal control and (**B**) fat signal from the same control. (**C** and **D**) Muscle water and fat signal images, respectively, from Patient 11. Two water phantoms can be seen in each water image. Note the fat infiltration of the head of the medial gastrocnemius in the patient. For image parameters see text.

and controls and between groups of patients. Statistical significance was taken as $P < 0.05$.

Results

MRI findings

MRI images of water and fat in the calf of a normal control and a patient are shown in Fig. 1 and illustrate the fatty infiltration of the head of the medial gastrocnemius, which was markedly visible in Patients 5, 11 and 12 and less severe in Patients 3, 9 and 10. This has been described previously for inflammatory myopathy and has been found to be especially prominent in s-IBM (Reimers *et al*., 1994; Lodi *et al*., 1998). Using quantitative chemical-shift MRI, it was found that, although the lateral gastrocnemius and soleus were not different from normal, the medial gastrocnemius in DM and PM contained significantly less water (and therefore less muscle) than normal controls (Table 2), due to replacement of muscle by fat. The CSA of the gastrocnemius–soleus complex was found to be proportional to workload in both patients and normal controls (results not shown), but because of the fatty infiltration it was possible that the actual amount of muscle carrying out the work was less in patients than normal controls. Therefore, the effective CSA determined by adjusting for the fat/water ratio in each image pixel of the gastrocnemius–soleus complex (CSA_{H2O}, representing muscle tissue) was calculated for the three working muscles

Table 2 *MRI and MRS data for resting muscle (mean ± standard error of the mean)*

Variable	DM	PM	Control	*P* versus control*	
				DM	PM
^{1}H-MRI					
Muscle water content (% total signal)					
Lateral gastrocnemius (l)	90 ± 2	91 ± 2	92 ± 1	0.3	0.95
Medial gastrocnemius (m)	81 ± 9	60 ± 17	93 ±1	0.04	<0.1
Soleus (s)	88 ± 2	90 ± 2	93 ± 1	0.4	0.4
All (l + m + s)	88 ± 3	83 ± 4	93 ± 1	0.2	0.1
T_2 (ms)					
Lateral gastrocnemius (l)	38.7 ± 2.3	34.3 ± 1.4	33.3 ± 0.8	0.02	0.4
Medial gastrocnemius (m)	38.5 ± 2.0	41.1 ± 3.1	33.1 ± 0.7	<0.1	0.01
Soleus (s)	39.9 ± 2.3	36.0 ± 2.9	33.4 ± 0.4	<0.1	0.5
All (l + m + s)	39.4 ± 2.1	37.0 ± 2.2	33.2 ± 0.5	<0.1	0.08
^{31}P-MRS					
pH (U)	7.02 ± 0.01	7.01 ± 0.01	7.02 ± 0.01	1.0	0.4
PCr/ATP	3.28 ± 0.23	3.25 ± 0.20	3.50 ± 0.05	0.4	0.2
Pi/ATP	0.50 ± 0.03	0.40 ± 0.02	0.34 ± 0.02	<0.01	0.09
Pi/PCr	0.16 ± 0.0.03	0.13 ± 0.01	0.10 ± 0.004	<0.01	<0.1
PCr (mM)	32.8 ± 2.3	32.5 ± 2.0	35.0 ± 0.4	0.98	0.2
Pi (mM)	5.2 ± 0.3	4.1 ± 0.3	3.5 ± 0.2	<0.01	0.09
ADP (μM)	10 ± 1	9 ± 0.2	10 ± 1	0.6	0.9
ΔG_{ATP} (kJ/mol)	–63.0 ± 0.3	–63.7 ± 0.2	–64.1 ± 0.1	<0.1	0.3

*Significance is given for DM (n = 9) or PM (n = 5) versus controls (n = 10 for MRI, n = 18 for MRS). There were no significant differences between treated and untreated DM patients. Only one PM patient was untreated; his MRI and MRS values were within the range of values of the four treated PM patients. There were no significant differences between DM and PM patients except for Pi (P = 0.03).

and expressed relative to LBM. CSA_{H2O}/kg LBM was not significantly different between patients and normal controls (0.71 ± 0.04 and 0.76 ± 0.04, respectively, P = 0.4). Results from the exercise period are therefore unlikely to have been affected by a difference in the relative workload. It is important to note that any error in this estimation would have not altered results from the recovery period, as metabolic recovery is independent of muscle mass. The degree of fatty infiltration did not correlate with the MRS findings (data not shown).

T_2 is a measure of the relaxation properties of tissue water, and it increases in inflammation (Reimers and Finkenstaedt, 1997). There was evidence of inflammation in the calf muscles of both groups of patients. It can be seen in Table 2 that T_2 tended to be abnormal in all three muscles in the patients, although it reached significance only in the muscles of DM patients and in the medial gastrocnemius in the PM patients. The lower T_2 in the PM group could be the effect of treatment, as four out of the five patients were on treatment. In DM, the untreated group had a slightly longer T_2 compared with the treated group but the difference was not significant. Also, there was much greater variation in T_2 in the patient group than in the control subjects, presumably reflecting variable inflammatory involvement. However, this variation showed no correlation with the MRS findings, which are described below.

MRS findings

Rest

Results are shown in Table 2. At rest, intracellular pH and PCr/ATP were normal in both groups of patients, but Pi/ATP and Pi/PCr were high. When calculated assuming normal levels of ATP and total creatine, the absolute concentrations of PCr and ADP were normal in the patients but Pi was high; this reached significance only in DM. The MM group showed a [Pi] (5.2 ± 0.3 mM) at rest that was significantly increased compared with controls (P = 0.009) but not statistically different from that found in the IIM groups. There were no differences between treated and untreated DM patients.

Exercise

Data from the exercise period are shown in Table 3. At the onset of exercise, the work rate (power), energy cost and β were very similar in patients and normal controls, and so are unlikely to have contributed to any differences between groups. Initial rates of change in PCr were not different between the IIM groups and normal controls, but pH decreased more rapidly in the patients' muscles, consistent with greater glycolytic activity in the patients. The patients exercised for a significantly shorter time than normal controls, so at the end of exercise the total work performed

Table 3 *MRS data from exercise and recovery (mean ± standard error of the mean)*

Variable	DM	PM	Control	*P* versus controls*	
				DM	PM
Initial exercise					
Power (W)	1.5 ± 0.1	1.9 ± 0.2	1.5 ± 0.1	0.7	0.07
ΔPCr, initial rate (mM/min)	–18 ± 3	–20 ± 3	–21 ± 3	0.7	0.6
Energy cost (mM/min/W)	12.4 ± 2.3	10.6 ± 1.8	14.9 ± 2.5	0.4	0.4
ΔpH, initial rate (U/min)	–0.08 ± 0.01	–0.14 ± 0.04	–0.03 ± 0.01	0.02	0.004
Matched time-point (6.1 min)					
Work (kJ)	0.6 ± 0.03	0.8 ± 0.1	0.7 ± 0.0	0.2	0.1
PCr diff. from rest (%)	–47 ± 6	–52 ± 8	–31 ± 2	0.04	0.02
ΔPCr/work (%/kJ)	–79 ± 9	–67 ± 11	–48 ± 4	0.01	0.17
pH diff. from rest (U)	–0.19 ± 0.08	–0.3 ± 0.1	–0.04 ± 0.02	0.2	0.07
ΔpH/work (U/kJ)	–0.29 ± 0.13	–0.3 ± 0.1	–0.05 ± 0.04	0.2	0.05
ΔG_{ATP} (kJ/mol)	–55 ± 1	–55 ± 1	–57 ± 0.3	<0.01	0.06
ADP (μM)	47 ± 4	49 ± 8	35 ± 2	0.02	0.07
End of exercise					
Exercise duration (min)	6.4 ± 1.3	7.2 ± 0.4	10.9 ± 0.4	<0.1	<0.01
Work, total (kJ)	0.9 ± 0.2	1.0 ± 0.1	1.5 ± 0.1	<0.1	0.046
PCr diff. from rest (%)	–59 ± 6[†]	–59 ± 7	–57 ± 3	0.9	0.6
ΔPCr/work (%/kJ)	–87 ± 18	–65 ± 11	–43 ± 4	0.04	0.1
pH diff. from rest (U)	–0.29 ± 0.09[‡]	–0.31 ± 0.10	–0.27 ± 0.04	0.8	0.7
ΔpH/work (U/kJ)	–0.43 ± 0.17	–0.33 ± 0.09	–0.20 ± 0.03	0.5	0.2
ADP (μM)	60 ± 6	55 ± 10	57 ± 4	0.4	0.99
Recovery					
H^+ efflux (mM/min)	5.2 ± 1.0	7.6 ± 1.4	12.5 ± 1.1	<0.01	0.02
PCr $t_{1/2}$ (s)	60 ± 9[§]	54 ± 9	29 ± 3	<0.01	0.02
ADP $t_{1/2}$ (s)	24 ± 4	23 ± 6	14 ± 6	<0.01	<0.1
V (mM/min)	14 ± 2	16 ± 3	35 ± 5	<0.01	0.02
V_{max} (mM/min)	20 ± 2	25 ± 4	47 ± 3	<0.01	<0.01

*P values are given for DM (n = 9) or PM (n = 5) versus controls (n = 18). [†]Difference is significant for treated (n = 4) versus untreated (n = 5) DM (71 ± 4 versus 49 ± 6, P = 0.03); [‡]difference is significant for treated versus untreated DM (–0.51 ± 0.07 versus –0.12 ± 0.08, P = 0.03); [§]difference is significant for treated versus untreated DM (83 ± 10 versus 41 ± 5, P = 0.02). MRS values from the only untreated PM patient were within the range of the four treated PM patients.

was about one-third less. In spite of the reduced exercise period for DM and PM, the three groups reached similar values for [PCr] and intracellular pH. Thus, when scaled to work output, mean PCr consumption and pH decrease were twice as great in DM and 1.5 times as great in PM as in normal controls (although statistical significance was reached only for PCr consumption/work in DM).

Because subjects exercised for different lengths of time, results were also compared at the last time point at which all subjects except one were still exercising (6.1 min; matched time point in Table 3). The three groups had carried out similar amounts of work at this point, but the mean decreases in [PCr] and pH were greater in patients than in controls, both in absolute terms and when scaled to work performed, reaching significance in most cases (see Table 3). There was a mean increase in the relative contribution of anaerobic ATP synthesis to energy production in DM, with R_{An} = 41 ± 12% of the total in DM, 25 ± 6% in PM and 20 ± 4% in controls, but the variation among subjects was large and the differences were not significant.

Significant differences between treated and untreated DM patient groups are indicated in Table 3. In the treated group there was greater PCr depletion and pH decrease at the end exercise and the half-time for PCr recovery was longer. These differences are explained by the longer time of exercise (9.0 min for treated against 5.6 min for untreated patients) and, therefore, the greater amount of work performed by the treated patients (see Patients and methods).

Recovery

Data from the recovery period for PM and DM patients are shown in Table 3. During recovery, ATP synthesis relies exclusively on oxidative metabolism because glycolysis is inactive. Therefore, the kinetics of energy metabolism after exercise characterize mitochondrial function. Patients and controls reached similar end-exercise [PCr], and the mean end-exercise [ADP] of ~60 μM in patients and controls was well above the accepted K_m value of 30 μM for oxidative ATP synthesis (Table 3). For the MM group, [ADP] was 73 ± 9 μM. Under these conditions, the net PCr resynthesis rate during the recovery period is a sensitive index of mitochondrial ATP production. PCr recovery $t_{1/2}$ was increased in both DM and PM compared with normal controls, the initial rate of

PCr re-synthesis after exercise (V) was slow and the maximum rate of mitochondrial ATP synthesis (V_{max}) was low. A prolonged $t_{1/2}$ for post-exercise ADP recovery also indicated a deficit in oxidative metabolism. Similarly, the MM group showed, compared with normal controls, slow PCr recovery $t_{1/2}$ (71 ± 10 s, $P = 0.001$) and reduced V (12.3 ± 1.5 mM/min; $P = 0.0007$) and V_{max} (17.8 ± 2.1 mM/min; $P = 0.0006$) (Fig. 2) and prolonged ADP recovery $t_{1/2}$ (26 ± 2 s; $P = 0.001$).

Glycolysis is inactive in the absence of exercise, so the only significant source of H^+ production during recovery is net PCr resynthesis. Therefore, because PCr recovery was slower in patients, the rate of H^+ production from this source would have been lower and pH recovery would be expected to have been faster. In fact, pH recovery was slower in IIM patients than controls (data not shown) and when the rate of H^+ efflux was calculated for the initial phase of recovery it was significantly reduced in the two patient groups compared with the normal controls (Table 3). H^+ efflux in DM and PM patients was reduced by 53% ($P = 0.01$) and 31% ($P = 0.16$), respectively, compared with the mean value found in MM patients (11 ± 1.5 mM/min) (Fig. 2).

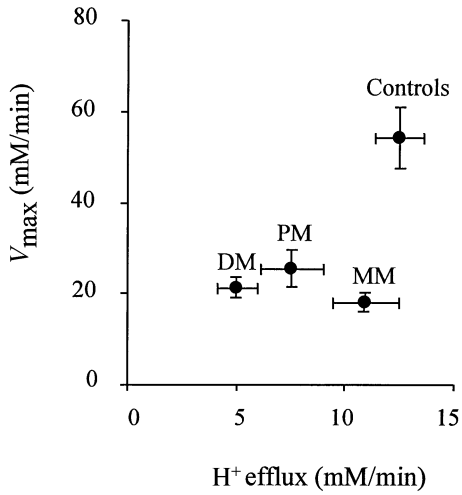

Fig. 2 Plot of the maximum rate of mitochondrial ATP production (V_{max}) and H^+ efflux rate in DM, PM and MM patients and normal controls. Bars show the standard error of the mean.

Discussion

DM and PM share many clinical features, but a different autoimmune pathogenesis has been proposed for each condition: humorally mediated capillary destruction in DM and cell-mediated cytotoxicity in PM. In this study we demonstrated that both disorders have an *in vivo* skeletal muscle defect in aerobic metabolism. However, in contrast to patients with primary mitochondrial disorders, this reduced aerobic capacity is associated with slow H^+ efflux. These findings, present in both treated and untreated patients, all with clinically active disease, indicate that in DM and PM the oxidative metabolism deficit is secondary to impaired blood supply.

Recovery MRS indices of muscle oxidative rate (Arnold *et al*., 1984; Kemp *et al*., 1993*a*) were found to be impaired in PM, DM and the MM groups. In these three groups of patients, the PCr and ADP recovery $t_{1/2}$ values were almost twice as long and V_{max} was half the rate found in normal subjects, indicating a profound impairment of mitochondrial respiration rate. These findings are supported by exercise data showing a faster rate of pH decrease and greater contribution of anaerobic energy production to total ATP synthesis in IIM patients. The results are consistent with a decrease in oxidative ATP production in DM and PM and differentiate these two forms of IIM from s-IBM, in which *in vivo* mitochondrial metabolism is normal (Argov *et al*., 1998; Lodi *et al*., 1998).

Abnormal mitochondrial respiration could be a primary event, possibly associated directly with the mitochondrial abnormalities found in muscle biopsies of DM and PM patients, or secondary to abnormal substrate delivery. In primary mitochondrial disorders arising from mitochondrial DNA defects, a reduced rate of mitochondrial ATP production is typically associated with reduced intracellular acidosis during exercise and an increased or normal rate of H^+ extrusion from the cell during the initial recovery, as we have shown in this study and as reported elsewhere (Argov *et al*., 1987; Bendahan *et al*., 1992; Taylor *et al*., 1994; Chen *et al*., 2001).

In contrast, our PM and DM patients showed an increased rate of acidification during exercise and a reduced rate of H^+ efflux during recovery. An abnormal oxygen supply to the muscle of PM and DM patients is the most likely explanation for the association of oxidative metabolism impairment, the shift towards anaerobic metabolism during exercise and the reduced rate of H^+ efflux. Reduced oxidative phosphorylation rate and slow H^+ efflux is a recognized combination in normal subjects exercising under ischaemic conditions (Kemp *et al*., 1994) and in patients with peripheral vascular disease (Kemp *et al*., 1995). Several abnormalities of capillary vessels have been described in the muscles of patients with DM, including reduced capillary lumen, disrupted capillary membrane and reduced capillary density (De Visser *et al*., 1989; Emslie-Smith and Engel, 1990). These abnormalities, some of which have been described even in the early stages of the disease, could be responsible for the relative hypoperfusion of the muscle of these patients during exercise and recovery, thereby explaining our *in vivo* findings in DM patients. The vascular origin of MRS changes has been suggested previously (Park *et al*., 1995), but as far as we know this is the first *in vivo* evidence indicating a substrate delivery defect. Our findings also suggest an abnormality in perfusion in our PM patients, as they showed MRS changes similar to those found in DM. In PM, results from morphology studies show abnormal regional capillary distribution and, in some patients, thick microvessel walls (De Visser *et al*., 1989; Emslie-Smith and Engel, 1990). Also, it has been found that

the number of capillaries per muscle fibre is less than normal (Carry *et al*., 1986). These abnormalities could explain our MRS findings in PM, but we cannot rule out other immunological or inflammatory factors. If such factors are important, it must be by inducing capillary dysfunction, as MRS findings in the two conditions are so similar. It is interesting to note that all morphological muscle studies report increased capillary density in s-IBM (Carry *et al*., 1986; De Visser *et al*., 1989; Emslie-Smith and Engel, 1990) and that normal *in vivo* mitochondrial function is found in this condition (Argov *et al*., 1998; Lodi *et al*., 1998). These results reinforce our interpretation that the *in vivo* metabolic findings in our patients are related to capillary abnormalities and suggest that the histological, molecular and biochemical mitochondrial abnormalities described in IIM do not play a relevant pathological role in these conditions.

Consistent with previously reported MRS findings in resting muscle, we found a high Pi/PCr ratio (Park *et al*., 1994) but a normal PCr/ATP ratio in both DM and PM. We used the common method for calculating absolute concentrations of intracellular metabolites from MRS data, which utilizes published values of [ATP] and [TCr] obtained from biochemical assay and derives [PCr] and [Pi] from the PCr/ATP and Pi/ATP MRS ratios (Arnold *et al*., 1985). A recent muscle biopsy study found reductions of 23% in ATP and 29% in PCr in a group of 12 patients with IIM (Tarnopolsky and Parise, 1999), but [TCr] was not statistically different from that in controls. The similar extent of reduction in [PCr] and [ATP] content is in keeping with the normal PCr/ATP ratio found in our DM and PM patients at rest, but suggests that the increase in Pi/ATP ratio found in our patients could be due to low [ATP]. Determining intracellular [PCr], [Pi] and [ADP] accurately would depend on precise knowledge of [TCr] and [ATP] obtained from biochemical assay of tissue taken from within the volume of muscle studied by ^{31}P-MRS. If [ATP] is indeed reduced in our patients' muscles, as suggested by the findings of Tarnopolsky and colleagues, we will have overestimated [PCr] and [Pi] by about a quarter and underestimated [Cr] and [ADP] by about three-fold in resting DM and PM muscle (Tarnopolsky and Parise, 1999). In other words, the muscle is likely to be even more abnormal than our results suggest.

It is important to stress that these possible errors in the calculation of metabolite concentrations, and in particular [ADP] and [PCr], do not affect the direction of the comparison of oxidative metabolism between DM and PM patients and MM and normal controls assessed from data collected during recovery from exercise. In particular, a lower [PCr] and higher end-exercise [ADP] in the patients would result in even lower V and V_{max}, and the post-exercise PCr and ADP recovery $t_{1/2}$ values are a kinetic measurement independent of absolute metabolite concentrations.

Besides inadequate oxygen supply, other factors, such as muscle damage and physical deconditioning, may have contributed to the oxidative metabolism deficit found in our patients. Several reports have shown that damaged muscles do not necessarily display abnormal oxidative metabolism. For example, patients with s-IBM or with Becker or limb girdle muscular dystrophy exhibit more prominent structural changes in the calf muscles than DM/PM patients but have normal mitochondrial function, as assessed *in vivo* by ^{31}P-MRS (Lodi *et al*., 1997, 1998, 1999; Argov *et al*., 1998). Physical deconditioning due to reduced physical activity is common in patients with muscle diseases and, although there is no established way to measure it, it has been postulated to influence muscle metabolism in chronic illnesses and sedentary life (Kent-Braun *et al*., 1994). Most data about deconditioning have been obtained in other types of experimental set-ups, such as comparative analyses between trained and sedentary subjects (Takahashi *et al*., 1995), before and after limb immobilization (Appell 1990; Vandenborne *et al*., 1998), or in conditions such as lung or heart disease, where it is difficult to isolate influences on muscle performance (Tada *et al*., 1992; Chati *et al*., 1996). In our study the control subjects were leading ordinary, sedentary lives that were similar in most respects to those of the majority of the IIM patients. Also, if we compared the untreated DM with the treated DM patients or the PM group, who were clearly more active, there was no significant difference, including during recovery from exercise. On the other hand, the studies on s-IBM and muscular dystrophies discussed above were carried out on patients with similar or greater degrees of physical deconditioning than some of our patients, yet recovery was normal (Lodi *et al*., 1997, 1998, 1999; Argov *et al*., 1998).

A direct contribution of inflammation to the ^{31}P-MRS abnormalities found in our PM and DM patients cannot be ruled out. The nitric oxide present in inflammation has been shown to inhibit respiratory enzyme activity, especially in complexes I and II (Zielasek *et al*., 1995). Most of our patients exhibited prolonged T_2 relaxation values, an indicator of muscle inflammation. However, the severity of these changes in T_2 did not correlate with the MRS results. This suggests that if inflammation plays a direct role in the MRS abnormalities it is unlikely to be the dominant effect.

Overall, our data provide a plausible explanation for the symptoms of muscle weakness and fatigue of patients with DM and PM. It is interesting to note that these abnormalities have been found in distal muscle, demonstrating the widespread nature of these conditions. Although different pathological mechanisms are postulated for DM and PM, our patients showed similar ^{31}P-MRS abnormalities, which can be explained as secondary to capillary dysfunction. DM and PM show many similarities with respect to epidemiology, clinical findings and response to treatment. Our studies show that they also have similar metabolic abnormalities, which can be clearly differentiated from s-IBM.

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