Biochemical and muscle studies in patients with acute onset post-viral fatigue syndrome

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Abstract

Aims—To investigate in detail various biochemical and pathophysiological indices of muscle pathology in acute onset post-viral fatigue syndrome (PVFS).

Methods—Twenty three patients with PVFS (of mean duration 4.6 years) were subjected to needle biopsy for histomorphometry and total RNA contents. Plasma analysis included serology and creatine kinase activities. Indices of whole body mass were also measured—namely, whole body potassium content and plasma carnosinase activities.

Results—About 80% of the patients had serology indicative of persistent enteroviral infection as determined by VP1 antigen assay. Only about 10% of that same group of patients had serological indications of current enterovirus infection by IgM assay; a separate subset of 10% showed antibody changes suggestive of reactivation of Epstein-Barr virus. Quantitative morphometric analysis of skeletal muscle fibres indicated that the quadriceps muscle was normal or displayed only minor abnormalities in 22 patients. The Quetelet's Index (body mass index) and whole-body potassium values (index of lean body mass) were not affected in PVFS. The mean plasma carnosinase and creatinine kinase activities were also generally normal in these patients. The mean muscle RNA composition—mg RNA/mg DNA—was significantly reduced in acute onset PVFS by about 15%. The protein:DNA ratio was not significantly affected.

Conclusions—Patients with acute onset PVFS, therefore, lose muscle protein synthetic potential, but not muscle bulk. Histopathology is consistent with these observations. These perturbations may contribute to the apparent feature of perceived muscle weakness associated with the persistent viral infection in the muscle themselves.

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Patients with acute onset post-viral fatigue syndrome (PVFS), also known as myalgic encephalomyelitis,¹ always complain of muscle fatigue, which is exacerbated by physical or mental activity, or both.¹⁻³ This contrasts with the normal physiological behaviour of

their muscles shown in controlled tests of power and fatigue.^{2,3} Other investigations of muscle, however, have shown subtle abnormalities in patients with myalgic encephalomyelitis,⁴⁻⁷ and these might have profound physiological implications. Widely scattered necrotic muscle fibres without an inflammatory infiltrate have been reported in two patients.⁴ Further abnormalities supporting a diagnosis of peripheral disease include single fibre electromyography, which has been reported to show "jitter" in 75% of cases but without impulse blocking. Standard electromyography has shown no specific abnormality.⁶ Increased intracellular acidosis during exercise has also been documented in one patient with myalgic encephalomyelitis.⁷ There seems, therefore, to be contrasting evidence for peripheral muscle pathology.

Methods

Twenty three women (aged 20-65 years) were selected for study on the basis of a history typical of acute onset post-viral fatigue syndrome, as defined by Smith.¹ Their symptoms had been preceded by a history of a "flu"-like illness and had been either relapsing, remitting, or chronic in nature. Their main symptoms were of muscular fatigue associated with discomfort after exercise, lethargy, lack of concentration and short-term memory dysfunction and were associated with many other lesser symptoms.¹ The duration of symptoms varied from six months to 17 years. These patients had no significant medical or psychiatric history and had been fit and well until the onset of this new disease process. All had had a normal physical examination.

All patients had assays for full blood count, erythrocyte sedimentation rate, white cell differential, and white cell subset assays, a rheumatoid antibody haemagglutination assay, 10 channel SMAC, including estimation of serum glucose and thyroid stimulating hormone. Patients also had viral and anti-nuclear antibody screens. Similar biochemical data were also obtained from control female patients who attended elective surgery or outpatient clinics for non-specific complaints other than PVFS.

STUDIES ON MUSCLES

Muscle biopsy specimens were taken from the vastus lateralis portion of the quadriceps muscle with a UCH biopsy needle under local anaesthesia, as described by Edwards *et al.*⁸

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Table 1 Muscle fibre morphometry in PVFS

Case Number	Type I muscle fibres			Type II muscle fibres		
	Mean	Atrophy	Hypertrophy	Mean	Atrophy	Hypertrophy
	Fibre diameter	Factor (NR < 400)	Factor (NR < 400)	Fibre diameter	Factor (NR < 200)	Factor (NR < 180)
1	58.6	0	214	45.4	52	26
2	62.1	0	333	50.2	37	54
3	54.5	0	343	53.9	0	138
4	51.9	0	37	48.7	15	7
5*	57	0	109	36.9	280†	0
6	54.1	33	100	53.5	0	23
7*	65.9	0	526†	58.2	61	265†
8*	47.9	27	75	28.7	779†	0
9	60.4	0	171	49.5	23	0
10	45.2	11	5	40.3	104	0
11	50.9	11	23	42.2	25	62
12	53.7	0	10	47.3	7	7
13	58.5	0	150	47.4	37	18
14	52.3	0	27	42.3	48	0
15*	71.7	0	1011†	62.9	0	282†
16*	67.7	0	792†	52.1	35	223†
17	48.8	25	23	45.0	50	25
18*	62.7	0	419†	44.5	113	0
19	65.9	0	363	45.1	42	0
20*	54.6	20	290	60.5	0	326†
21*	45.1	0	0	42.9	230†	38
22	54.9	0	25	45.1	61	0
23	60.5	0	337	39.3	0	31
Mean (SEM) (23)	56.6 (1.5)	6 (2)	234 (55)	47.0 (1.6)	87 (35)	66 (22)

*identifies those patients with abnormal muscle fibre morphometry. † = hypertrophy, ‡ = atrophy. Normal ranges (NR) are indicated in parentheses.

Each biopsy specimen was separated into two parts. One part was immediately blotted and stored at -70°C for subsequent RNA and DNA estimation (see below); the other was snap-frozen for histopathological examination. Frozen sections ($6\text{ }\mu\text{m}$) were stained with haematoxylin and eosin, periodic acid Schiff with and without diastase, oil-red O, NADH and myosin Mg^{2+} adenosine triphosphatase (ATPase).⁹ Using the ATPase sections, fibres numbers and fibre diameters for type I and type II fibres were estimated on a Magiscan image analysis system, using an interactive computer program.^{9,10} Atrophy and hypertrophy factors were then calculated, as described before.¹¹ The normal values have been described in previous publications^{9,10} and are also set out in table 1.

Protein RNA and DNA were measured by the spectrometric and fluorometric techniques, described by Preedy and Peters.¹² Processing of muscle was carried out at $0-4^{\circ}\text{C}$, unless otherwise stated. Skeletal muscle biopsy specimens were homogenised in 2 ml distilled water, using an ultrasonic tissue homogeniser with a micro-probe (Northern Media Supply Company, Hull, England). Homogenates were then acid precipitated in 0.2 mol/l perchloric acid and centrifuged ($2000 \times g$ for 20 minutes). The supernatant fluid was discarded and the pellet suspended in 2 ml of 0.2 mol/l perchloric acid, mixed vigorously, and re-centrifuged ($2000 \times g$, for 20 minutes). The supernatant fluid was again discarded and the pellet suspended in 2 ml 0.3 mol/l NaOH for 1 hour. This was acid-precipitated with 0.5 ml, 2 mol/l perchloric acid and centrifuged ($2000 \times g$ for 20 minutes). The supernatant fluid was used to determine RNA by the dual wavelength method—at 260 nm and 232 nm, as

described by Munro and Fleck.¹³ Protein was estimated as a modification of the methods described by Lowry *et al*¹⁴ and Downs and Wilfinger (for DNA).¹⁵

WHOLE BODY POTASSIUM

Measurements of total body potassium were carried out to assess lean body mass, as described by Smith *et al*.¹⁶ Patients were laid supine in an NaI whole-body counter with an array of eight detectors, data from which were analysed by a 512-channel analyser. Counting was done for 3.3 hours and appropriate corrections were made for backgrounds. As lean body mass varies with age, sex, and height, patient data cannot be reliably presented as absolute values, but instead are compared with age, sex, and height matched controls—that is, as a percentage.¹⁶

SEROLOGY

Serum was assayed for VP1 polypeptide, using an enterovirus group specific monoclonal antibody (5-D8/1), as described by Yousef *et al*.¹⁷ The assay used the specific antibody 5-D8/1 which acts against a single enterovirus capsid protein—virus protein 1 (VP1). The antibody does not generally react with non-enteroviral proteins.¹⁷ Enterovirus group specific IgM antibodies were assayed using the method of McCartney *et al*.¹⁸ Assays to assess reactivation of Epstein-Barr virus antibody were done according to the method of Hotchin *et al*.⁹

SERUM ENZYMES

Serum carnosinase was determined by the fluorometric method, described by Duane and Peters.²⁰ Creatine kinase activities were assayed by routine clinical laboratory procedures.

STATISTICS

All data were the mean (SEM) ($n-1$ weighing) of five to 23 subjects in each group. Differences between means were assessed by Student's *t* test where variances were equal and when data were normally distributed. Data not normally distributed were analysed using the Wilcoxon rank sum test, as indicated in the relevant text. Statistical tests were performed using the package, Routine Statistics, version 6.3 (RS Cormack and GD Smith, Northwick Park Hospital and Clinical Research Centre, Harrow, Middlesex, England).

Results

In all patients haematological analyses, liver function tests, thyroid function, and blood biochemistry results were normal. Standard viral screens were also within normal limits (results not shown).

Table 2 shows data pertaining to body mass index, age, and length of illness. In general, the mean Quetelet's Index did not indicate any overt differences from the control patient range (20–25). One patient, however, had a Quetelet's Index of 18.4 (case 10)

Table 2 Clinical features of patients studied

Case No	Quetelet's Index	Age (y)	Length of history (y)	Antibody reaction against		
				VP1 polypeptide	Coxsackie B (IgM)	Epstein Barr Virus
1	25.7	38	2	+	+	-
2	ND	30	2	-	-	+
3	ND	23	4	-	-	+
4	ND	23	1	+	-	-
5	20.5	23	8	+	-	-
6	20.1	23	7	-	-	-
7	ND	65	1	+	-	-
8	ND	40	0.5	+	-	-
9	ND	50	1	+	-	-
10	18.4	37	3	+	-	-
11	ND	34	17	+	-	-
12	ND	27	6	+	-	-
13	ND	50	7	+	-	-
14	19.3	30	10	+	-	-
15	ND	20	3	+	-	-
16	24.6	29	1	-	-	-
17	21.5	32	7	+	-	-
18	22.8	50	1	+	-	-
19	19.9	40	10	-	-	-
20	27.3	21	3	+	+	-
21	ND	18	0.5	+	-	-
22	23.4	44	3	+	-	ND
23	19.7	41	8	+	-	ND
Mean(SEM)	21.9(0.8)	34(3)	4.6(0.9)	18/23	2/23	2/20
(n)	(12)	(23)	(23)			

Patients were diagnosed as having PVFs on the basis of their clinical histories. The normally accepted range of the Quetelet's Index is 20-25. Plasma was tested for IgM antibody (Coxsackie B 1 gM) and viral antigen for Epstein-Barr virus. + and - signs indicate positive and negative reactions, respectively. ND = not determined.

Table 3 Whole-body potassium and plasma enzyme activities in PVFS

Case number	Total body potassium (%)	Plasma carnosinase (IU/l)	Plasma creatine kinase
1	105	190	88
3	ND	ND	54
5	ND	ND	41
8	ND	ND	42
9	ND	ND	40
10	ND	85	43
14	105	132	ND
16	ND	ND	41
17	ND	ND	91
19	100	159	27
20	ND	ND	78
22	98	130	166
23	85	100	87
Mean (SEM)	99 (4)	133 (16)	67 (11)
Normal range	95-105	100-200	20-205

Data are shown as the mean (SEM) of samples taken from 20 patients or 22 controls. Significance for the RNA composition was assessed by Student's *t* test as data were normally distributed and variances were equal. The data for the protein composition were not normally distributed and the Wilcoxon rank sum test was used.

but she did not have any histological abnormalities in either type I or II fibres. Nevertheless, this patient did have a low plasma carnosinase activity. The data in table 2 show that 18 patients (80%) were positive when tested against the VP1 antigen polypeptide and two (10%) were also positive for enterovirus IgM. A separate 10% of patients had positive indications of Epstein-Barr virus reactivation (table 2).

In all 23 patients light microscopic examination of sections of the muscle biopsy specimens showed that most of the muscle fibres were of normal appearance. Two cases showed occasional split fibres and these were the two cases which had the highest atrophy factors. Two further cases showed a modest amount of centronuclear chains but were normal on histomorphometric analysis. There was no evidence of fibre necrosis, phagocytosis, or of any inflammatory infiltrate in any of the biopsy specimens. Histomorphometric analysis indicated eight patients with atrophy or hypertrophy factors outside the normal range (table 1). These were three patients with hypertrophy of both type I and type II fibres, one patient with hypertrophy of just type I fibres, one patient with hypertrophy of type II fibres and three patients with atrophy of type II fibres (table 1). It must be emphasised that these were needle biopsy specimens and so, inevitably, are subject to some artefact. A biopsy specimen from case 15, which had the highest hypertrophy factor, appeared particularly disrupted and this may well have produced artefactually large fibres. It is also recognised that some fibres in a needle biopsy specimen may undergo hypercontraction before freezing, and so the samples showing mildly increased hypertrophy factors were probably normal.

Table 3 shows that whole-body potassium values in the five patients studied were within the control range. In general, plasma carnosinase and creatine kinase activities were also within the normal ranges, but one patient had a slightly reduced carnosinase activity.

The figure shows that RNA concentrations were reduced in patients with PVFS, by about 15%, though the concomitant decrease in protein:DNA did not reach significance.

Discussion

The pathogenetic mechanisms responsible for the disorder commonly known as post-viral fatigue syndrome or myalgic encephalomyelitis are unknown. A metabolic disorder of muscle caused by persistent viral infection has been suggested.^{2,3,21} In one study 46% of patients and 25% of controls had IgM antibodies to Coxsackie B virus, though in this study only 10% of patients had Coxsackie B IgM antibodies.²¹ Nevertheless, 80% of patients had serological indications of current or ongoing (VP1 positive) enterovirus infection. A positive VP1 antibody titre is a measure of enterovirus group specific antigen, which is both free and complexed. There has to be a persistent enterovirus infection to pro-

duce the response; it does not rely on the body's development of antibody. Other recent studies, using molecular biology techniques, have also shown enteroviral RNA sequences in significant numbers of patients with PVFS.²²⁻²⁴ How a persistent viral infection might mediate the disorder has not been fully explained, though various theories have been forwarded.²⁵ Such persistent infections might interfere with the balance of protein synthesis and degradation, as has been shown to occur in acute sepsis.²⁶ This would imply that PVFS is characterised by a generalised reduction in the relative amount of skeletal muscle protein, to account for the muscle weakness. In general, however, neither the Quetelet's Index, whole-body potassium (a direct estimate of muscle mass), nor plasma carnosinase activities (an indirect estimate of muscle mass) were changed in patients with PVFS.

It is therefore necessary to speculate whether groups of proteins, such as those pertaining to the contractile apparatus (the myofibrillary proteins), are reduced in PVFS. Although there is no evidence to support this suggestion, there was a small reduction in the protein:DNA ratio in patients with PVFS. Furthermore, skeletal muscle RNA was significantly reduced. This reflects a reduced capacity or potential to synthesise proteins.²⁷ Disturbed protein turnover has indeed been shown by Pacy *et al.*²⁸ Using primed constant infusions of L-[1-¹³C]leucine they showed that fractional rates of skeletal muscle protein synthesis were significantly reduced in patients with PVFS.²⁸ By implication, muscle protein breakdown may also be reduced to account for the maintenance of the tissue protein content.

Histochemical studies by Byrne *et al.* showed striking type II fibre atrophy.⁴ Their investigations, however, were carried out in only two patients. Our data showed that only three of 23 patients had relatively mild type II fibre atrophy and these were considered to have managed their disease poorly. The study of Byrne and our results together imply that there is a subgroup of patients with PVFS in which symptoms of skeletal muscle weakness may be related to proximal myopathy. In this regard more detailed investigative studies have been made into muscle strength, such as endurance and fatigability. Lloyd *et al.* examined a relatively large patient population (20 controls and 20 patients) and determined that, while maximal isometric strength was not affected by the disease process, there was a noticeable inhibition recovery from the peak torque after endurance exercise.²⁹ These authors concluded that the contrasting data of subjective muscle weakness and objective state of normal muscle function may have been due to altered patient perception of achieved force and exertion.²⁹ The disease definitions of their patients were, however, different from ours. To a certain extent, these conclusions, are similar to those of Stokes *et al.* who studied patients with effort syndrome.³⁰ Not all their patients had histories of

viral infections before the onset of symptoms. It is appropriate to speculate whether numerous reports in this field do, in fact, examine heterogeneous groups of patients. And there is a fundamental need for a concise and universally accepted medical definition of PVFS.³¹

More recently, direct evidence has been obtained for a subcellular metabolic defect in the muscle per se.³² These studies indicate that up to 80% of patients with PVFS have identifiable mitochondrial abnormalities.³² Thus deficiencies in energy generation may in some way be related to defects in the turnover of muscle RNA or proteins, which are energy dependent processes.²⁷ Alternatively, inactivity, or lack of exercise may be precipitating factors for perturbations in protein metabolism in these patients.

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