Papers read to Section of Psychiatry, 10 March 1987

Transmissible disease and psychiatry

Magnetic resonance muscle studies: implications for psychiatry

R P Yonge DPhil *Clinical Magnetic Resonance Laboratory, Radcliffe Infirmary, Oxford*

Keywords: magnetic resonance; postviral fatigue; schizophrenia; exercise

Introduction

Physiologists have been struggling with the problem of understanding muscle fatigue for many years[1,2]. Locating the site of fatigue, whether central or peripheral in the nervous system, in neuromuscular transmission, in muscle depolarization and repolarization, or in the biochemistry of muscle contraction, has presented the major problem. If this is a problem for the physiologist it is an even greater one for the patho-physiologist. Many conditions involve impaired muscle function which may range from acute pain in individual muscles to a general malaise accentuated by physical exertion.

Where the fatigue is associated with a psychiatric disorder there is the additional problem of distinguishing true symptoms of reduced muscle performance and psychosomatic symptoms. It is easy to assume that the symptoms of fatigue of a psychiatric patient are purely a result of the psychiatric disorder causing a perception of fatigue rather than there being any abnormal function of the neuromuscular system. Even if it is thought that there is some underlying organic cause for the fatigue it is very hard to demonstrate it clearly using conventional techniques.

In other cases fatigue may precede psychological symptoms such as depression. Some patients complain of both physical and mental fatigue but there appears to be no clear cause of their symptoms. They may be unable to perform their normal work, walk around or climb stairs without severe physical stress. This physical stress may be accompanied by a feeling of unsteadiness and a confusional state. And yet they may be told by their doctor that there is nothing physically wrong since available tests are of limited diagnostic use. It may be suggested or left to inference that the fatigue is all in the patient's mind. Anxiety and depression are a common result of these circumstances and tend to confirm for the doctor and relatives that the symptoms are indeed in the mind of the patient, which itself may cause further anxiety.

Conventional electrophysiology and histochemistry have proved limited in their ability to shed light on the possible organic basis for some of these fatigue syndromes. Using Magnetic Resonance Spectroscopy it is now possible to study the biochemical activity of intact human muscle during rest and exercise and show up certain abnormalities of its metabolism. This technique has recently been applied to the study of muscle fatigue with psychiatric associations in order to determine whether the fatigue is in the mind or in the muscle.

Magnetic resonance muscle studies

Phosphorus magnet resonance was first used to study cell metabolism by Moon and Richards[3] and has been subsequently used to study skeletal muscle[4] and many other isolated tissues[5]. More recently, the technique has been extended for examination of muscle metabolism in human limbs[6,7] and is now used routinely for the examination of patients with a wide variety of metabolic muscle disorders[8,9]. It is also used to study the muscle metabolism of patients who do not have a primary muscle disorder, but have secondary fatigue symptoms as in heart failure[10,11].

The technique is based on the principle that certain atomic nuclei when placed in a magnetic field respond to radio frequency radiation of a particular frequency. In effect this radiation is absorbed and re-emitted by the nuclei but when re-emitted the frequency will be affected by the chemical environment of each nucleus. This is known as the chemical shift. Thus if the correct frequency is transmitted for 31 phosphorus in a magnetic field of 2 Tesla (around 32 MHz) the signal collected from a human muscle will contain different frequencies corresponding to the chemical shifts of phosphocreatine, inorganic phosphate and of each of the three phosphorus atoms in ATP. In addition the amount of signal received at each frequency will be proportional to the concentration of the compound giving that chemical shift.

In this way phosphorus magnetic resonance spectroscopy gives a quantitative measure of intracellular ATP, phosphocreatine (PCr), inorganic phosphate (Pi) as well as intracellular pH in human muscle[3]. In addition, by assuming that creatine kinase is in equilibrium it is possible to calculate the level of ADP. This quantitative information is obtained non-invasively which means that many sequential observations can be made in the same human subject in a single experiment. Also the study can be repeated as often as required without causing the subject discomfort or damaging the muscle.

In this study, the patients and healthy control subjects had their forearm muscles (flexor digitorum superficialis) examined at rest and during exercise using a 1.9T superconducting magnet. The exercise protocol has been used in this laboratory for more than 7 years to investigate a wide range of healthy control subjects and patients with muscle problems[12]. The subjects squeeze a rubber sphygmomanometer bulb set to a pressure of 100 mmHg 22 times per min for 5 min. The pressure is then increased to 300 mmHg for a further 2½ min. Phosphorus spectra are collected at rest before the start of exercise and then at 1 min intervals throughout exercise. During the first 2 min of the recovery period they are collected every 30 sec and then every minute until the 10th minute.

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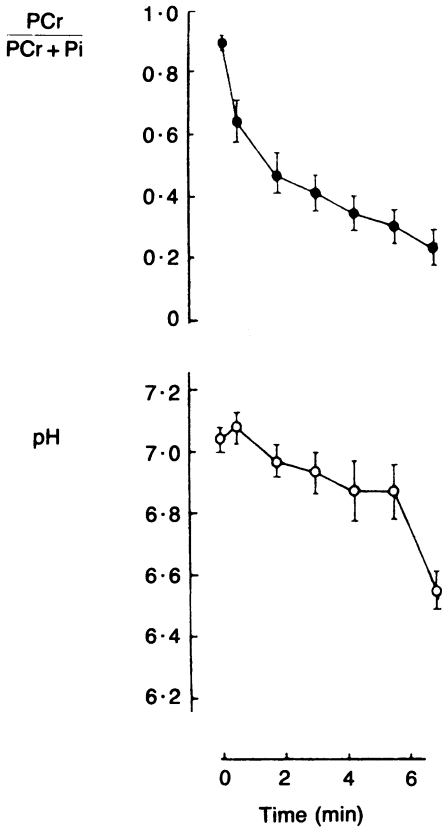

Figure 1. Phosphocreatine, inorganic phosphate and pH changes in healthy subjects during exercise

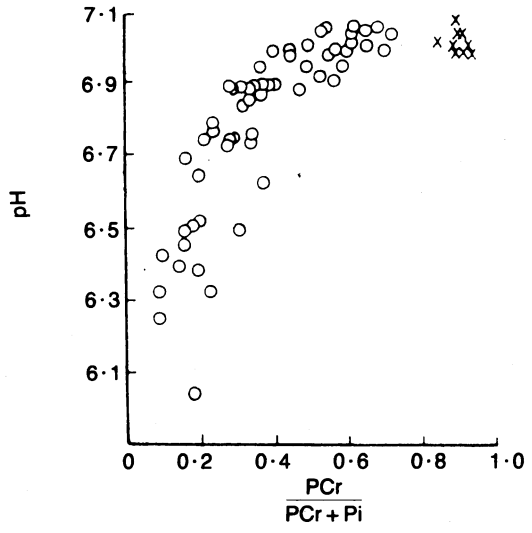

Figure 2. The relationship of pH and PCr changes at rest (crosses) and during exercise (circles) in healthy subjects

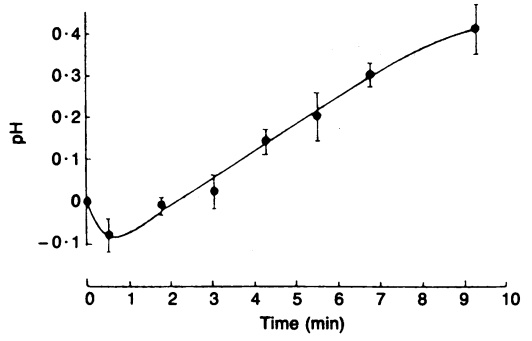

Figure 3. Intracellular pH during recovery from exercise in healthy subjects

During exercise in healthy subjects of all ages there is a characteristic pattern of fall in PCr, rise in Pi and fall in pH (Figures 1 and 2). There are also characteristic rates of recovery of PCr and Pi which approximate to exponentials with half times of 64 sec and 30 sec respectively, and also of pH[13] (Figure 3). The patterns of these changes reflect the relative contributions to the energy supply of exercise of glycolytic and oxidative metabolic pathways. In patients with certain metabolic abnormalities such as McArdle's syndrome or mitochondrial myopathies, these patterns are altered as the contributions of the different biochemical pathways are altered[8,9].

This technique is usually applied to detect metabolic muscle disorders, but it can also be used to study the muscles of patients with fatigue not based on a primary muscle disorder but where there is also a disturbance of the normal metabolic response to exercise as in heart failure or renal failure[10,11].

Postviral fatigue

Many patients complain that they suffer from muscle fatigue for which there is no obvious cause. In some cases they have never fully recovered from the fatigue associated with a viral illness. In others there is a less clear association with a previous viral infection. Normal clinical investigation of these patients usually fails to provide an explanation of their symptoms, although there have been suggestions of electrophysiological and immune system abnormalities[14].

Patients diagnosed as having postviral fatigue syndrome have been investigated by nuclear magnetic resonance spectroscopy (NMR) to try to determine whether there is some underlying biochemical cause for their symptoms. In 1984 the first case of postviral syndrome to be investigated by NMR was reported in the *Lancet*[15]. The study showed a consistent metabolic abnormality in the biochemical response of the patient to the exercise protocol described above. The patient, who had suffered the fatigue since a bout of chicken pox two years earlier, showed a much greater acidification of his muscles during exercise than is seen in normal healthy subjects. In addition the fall in intracellular pH was disproportionate for the rate of breakdown of phosphocreatine (giving a right shift in Figure 2). This indicates that the rate of glycolytic (acid producing) metabolism was much greater than would be expected for the level of work being performed or for the rate of oxidative (non-acid producing) metabolism. This was confirmed by observing the rates of recovery of PCr and Pi which are dependent upon the rate of oxidative metabolism. Both were significantly reduced in the patient, confirming that the rate of oxidative phosphorylation was less than in the control subjects. This also means that it is unlikely that the abnormal acidification was a result of a change in intracellular buffering capacity and the normal rate of recovery of pH after exercise indicates that there was no problem with the extrusion of acid from the muscle during exercise, these being the main alternative explanations for the observation of excessive acidification in exercising muscle.

This was the first time that it was possible to show unequivocally that there was an organic basis for the fatigue experienced by a patient diagnosed as having postviral syndrome. It did not, however, show how the viral infection caused the metabolic defect or indeed whether it did. The test itself is non-specific and cannot show a clear causal link between a viral

infection and a particular biochemical abnormality in the patient's muscles. It might be suggested that the virus had a direct effect on the activity levels of the enzymes involved in oxidative phosphorylation and glycolysis. But which ones and in what way could not be investigated simply in a human subject. It would need a very much more complex biochemical analysis of the activities of a large number of enzymes from a biopsy sample as well as a means of demonstrating that the virus had indeed got into the muscle cells. Even then it would not be possible to prove causality or explain the mode of action.

The most immediate effect on this first patient of the NMR examination and its demonstration of a clear metabolic defect was that of reassurance. As has been described above, one of the serious side-effects of these sorts of unexplained episodes of muscle weakness and fatigue especially if accompanied by mental tiredness is depression. The patient has very clear and distressing symptoms for which there is no observable physical cause and it may be suggested that it is all in the mind. To be told after all that it is possible to demonstrate something wrong with the function of the muscles comes as a great relief to the patient. And in more practical terms, such NMR findings may allow the patient to receive state or other disability benefits.

Subsequently a larger study[16] was carried out on a group of 6 patients diagnosed as having postviral syndrome and selected on the basis of having symptoms and immune responses similar to the first patient. They were studied at rest and during exercise in the same way as has been described above. Five of the 6 patients showed an abnormally large acidification early in exercise like the first patient, however the acidification only appeared to be disproportionate for the fall in PCr (right shift in Figure 2) in two cases.

The 6 patients examined were a fairly homogeneous group as regards their symptoms and yet they did show some variations in their metabolic response to exercise as studied by NMR. The wider population of patients who have been diagnosed as having postviral syndrome is very much more diverse and it is likely that they would have a much more ambiguous set of results. Indeed in a retrospective review of 74 patients with a wide range of disorders examined by NMR, 18 showed early acidification and three showed disproportionate acidification. Two of these patients had unexplained symptoms of muscle weakness and fatigue[16]. However, many more of the 56 patients showing no abnormality of acidification also had unexplained muscle weakness and some had even been diagnosed as having postviral syndrome.

Although NMR has been able to demonstrate a hitherto undemonstrable metabolic abnormality in the muscles of some patients diagnosed as having postviral syndrome it has its limitations as a test. The differences between individuals are hard to explain and there is no clear causal link between a viral infection and the metabolic changes. It is not possible to use NMR as a diagnostic test for this syndrome and a clear diagnosis is the first problem in understanding this condition.

Schizophrenia

Some schizophrenics, though by no means all, suffer from muscle fatigue. It is very difficult for the clinician to distinguish between a psychosomatic sensation of fatigue and physiological fatigue in these patients. So it is hard to know whether to attempt to treat the muscle symptoms separately from the other symptoms of schizophrenia, since they may or may not be caused by some metabolic abnormality.

It has been long suggested that there is a viral component in the aetiology of schizophrenia. Meninger postulated a link between influenza and schizophrenia after the 1919 epidemic in Boston[17] and Jeliffe noted links between schizophrenia and epidemic encephalitis[18]. This might imply that the muscle symptoms experienced by some schizophrenic patients are similar in origin to those experienced in postviral syndrome. If this were the case we might expect similar metabolic changes in the muscles of these patients during exercise, reflecting a disturbance in the relative contributions of oxidative and glycolytic metabolism. And, of course, we would expect to be able to pick up such a change in enzyme activities by the MNR exercise test showing disproportionate acidification followed by slow PCr recovery and normal pH recovery.

Conversely it has been suggested by Crayton and others[19,20] that the muscle symptoms of schizophrenics are related to endplate abnormalities which he has observed in some patients. If these abnormalities are the only neuromuscular consequence of schizophrenia then we would expect that during exercise there would be a reduced exercise capacity. However for a given work load we would expect the neural activity to be greater than normal but the metabolic response of the muscle to be normal. Thus an NMR study should reveal a normal pattern of PCr, Pi and pH changes during exercise and recovery, as the enzyme activities would be unaltered so glycolysis and oxidative phosphorylation should proceed in their normal proportions for a given level of work. Any weakness would merely be reflected in a greater effort required to produce a particular force of contraction as compared with normal control subjects.

It should be added that even if the major muscle defect from schizophrenia were an endplate abnormality this might itself lead to other metabolic changes. Such a chronic 'semi-denervated' state might be the cause of a disturbance of the normal metabolism of the muscle cell. So NMR abnormalities would not necessarily preclude the possibility of endplate changes being the primary factor in the weakness and fatigue associated with schizophrenia.

In a preliminary study, three schizophrenic patients, all of whom had muscle symptoms, were investigated using NMR by the method described above. It was a heterogeneous group of subjects in terms of their other symptoms, duration of the disease and treatment.

There were no differences in the metabolite concentrations at rest between the patients and normal control subjects and there were no changes in ATP concentration during exercise as is normal. Two of the three patients gave normal PCr and pH responses to the exercise test but the third displayed similar changes to those found in postviral syndrome. He produced an excessive and disproportionate acidification and a slow rate of PCr recovery indicating an over-dependence on glycolytic metabolism for the resynthesis of ATP in the muscles.

As we have already seen, such a finding is not at all conclusive. The test is non-specific and could have a number of explanations. It is, though, a very exciting and encouraging first study. This may be an important finding in the understanding both of

fatigue syndromes generally and schizophrenia in particular. Clearly, a follow-up is needed to ascertain the differences between those patients showing a metabolic defect and those not.

In the mind or in the muscle?

Nuclear magnetic resonance is a very exciting new tool to aid our understanding of muscle metabolism including the physiology and pathophysiology of fatigue. However, it only gives a limited amount of information about a few of the important metabolites involved in the process of energy conversion in the muscle. Despite its limitations it has shown a metabolic basis to the fatigue experienced by some patients diagnosed as suffering from postviral syndrome and given an indication of similar problems in schizophrenia.

It is not clear from these studies how the viral infection responsible for postviral syndrome might cause the metabolic abnormalities and why it occurs in some patients and not in others. It might be a direct effect of the virus affecting the production of one or a number of enzymes in the muscle. The virus might affect the availability of one of the co-enzymes involved in glycolysis or oxidative phosphorylation. Indeed it is not known at the moment how precisely the muscle controls the relative rates of these two energetic processes so we cannot understand the possible modes of action of the virus.

There is a very wide range of symptoms associated with patients who have been diagnosed as having post-viral syndrome. Indeed, there is no clearly diagnosed condition and many doctors do not believe the condition exists at all. This makes its investigation by NMR and the interpretation of results very difficult. Certainly thus far there is no clear pattern emerging that relates NMR abnormalities to symptoms or the definition of the disease. And without any certainty that there really is a viral cause in all cases it is impossible to understand the cellular mechanism of the fatigue.

Schizophrenia is also a condition where diagnosis is not clear cut and the muscle symptoms that have been investigated here are often not present at all. Whether this means that patients have varying degrees of the same disease or a number of different diseases is far from clear. The aetiology of schizophrenia may be heterogeneous and certainly there is a wide range of treatments used in this condition. This might be sufficient to explain the range of NMR-visible metabolic responses. Until patient groups with similar disease histories can be studied and compared, it is difficult to use NMR to explain the muscle symptoms sometimes associated with the disease. These first results were none the less very encouraging and do indicate that there is something here worth further investigation.

We have shown in a few cases that muscle fatigue and weakness for which there had previously been no explanation is indeed in the muscle rather than in the mind. And that is in itself an important first step. But NMR studies alone cannot answer all our questions about muscle fatigue in these conditions. They need to be used in conjunction with other tests of neuromuscular function. In this way it may be possible to detect where precisely the fatigue is taking place: in the nervous system, in neuromuscular transmission or in the biochemistry of the muscle itself. And if it is in the cell metabolism, whether it is in the cytosol or the mitochondria. In addition there is a need for rigorous selection of patient groups that are to be investigated. The definitions of postviral syndrome and schizophrenia may be difficult but patients can be selected on the basis of their medical histories, detailed symptoms and treatments and not simply on a very broadly defined diagnosis. Unless this is done such tests cannot help with understanding the problems of diagnosis itself let alone in explaining the possible contributory factors and mechanisms of fatigue.

Acknowledgment: I would like to thank Professor George Radda and the other members of his research group, especially Dr Doris Taylor, for their help in preparing this paper. The experiments described here were performed with the support of the Medical Research Council and the British Heart Foundation.

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(Accepted 23 July 1987)

Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatine kinase

L C Archard PhD **N E Bowles** BSc
Department of Biochemistry, Charing Cross and Westminster Medical School, London
P O Behan MD FRCP *Department of Neurology, Glasgow University*
E J Bell PhD MRCPath *Enterovirus Reference Laboratory, Ruchill Hospital, Glasgow*
D Doyle MD *Institute of Neurological Sciences, Southern General Hospital, Glasgow*

Keywords: myalgic encephalomyelitis; enterovirus; molecular hybridization

Summary
Enterovirus-specific probes have been prepared by reverse transcription of conserved sequences in purified Coxsackie B2 virus genomic RNA and molecular cloning techniques. These probes were used in quantitative slot blot hybridizations to test for the presence of enterovirus-specific RNA in skeletal muscle biopsy specimens from 96 patients who had suffered from the postviral fatigue syndrome myalgic encephalomyelitis for up to 20 years. Biopsy specimens from 20 patients were positive for the presence of virus-specific RNA with hybridization signals more than three standard deviations greater than the mean of the normal muscle controls. Biopsies from the remaining 76 patients were indistinguishable from the controls.

These data show that enterovirus RNA is present in skeletal muscle of some patients with postviral fatigue syndrome up to 20 years after onset of disease and suggest that a persistent virus infection has an aetiological role.

Introduction
The genus Enterovirus of the family Picornaviridae consists of more than 70 serotypes including the 6 members of the group B Coxsackieviruses and the 3 serotypes of polioviruses. The enterovirus group has been associated with a number of diseases ranging from aseptic meningitis and minor upper respiratory tract infections to paralytic poliomyelitis, myocarditis, hepatitis and fulminating multisystem infection of the neonate[1]. Recently there has been interest in a link between persistent enterovirus infection and the postviral fatigue syndrome myalgic encephalomyelitis[2,3]. This syndrome is characterized by many symptoms, but particularly by severe exhaustion and muscle fatiguability following an apparent viral infection. More than half of the patients also suffer myalgia and many complain of dysequilibria, sleep disturbances and psychogenic symptoms[3]. The syndrome has been observed worldwide for the past 50 years, in epidemics and as sporadic cases. The observation that the syndrome is diagnosed particularly frequently in medical or hospital service staff and the failure to demonstrate objective clinical signs led to the proposition of a hysterical origin[4].

Improved criteria for diagnosis and the employment of additional laboratory technologies have revealed organic lesions[5]. Electromyographical (EMG) investigations have shown that many patients give abnormal responses, particularly in single fibre EMG where some patients exhibit prolonged jitter values[6] a feature previously observed in patients with acute viral infections[7]. Nuclear magnetic resonance spectroscopy (NMR) has shown that muscle of some of these patients undergoes premature intracellular acidosis during exercise and has a prolonged recovery period[8], indicating dysfunction of respiratory metabolism. Investigations of lymphocyte populations of patients ill for up to 20 years reveal that the helper/suppressor T-cell ratio is low compared with the normal population[5], a characteristic previously observed in virus-infected subjects[9]. Serological studies have shown that about 30% of postviral fatigue patients are positive for Coxsackie B virus-specific IgM compared with 9% of normal controls in the same study and that these responses persisted in some patients for at least one year[10].

Persistent enterovirus infection, particularly with the group B Coxsackieviruses, has been implicated in a number of disorders by such serological investigations. However, attempts to isolate virus or demonstrate the presence of virus-specific antigens in the affected tissue have generally been unsuccessful in chronic disorders. Despite these observations, we have demonstrated previously that enterovirus RNA (probably Coxsackie B virus) is present in the affected tissue in inflammatory and chronic myopathies of cardiac or skeletal muscle by molecular hybridization of an enterovirus-specific cDNA probe to RNA isolated from biopsy specimens[11,12]. We now report analogous data demonstrating the persistence of virus RNA in skeletal muscle biopsies from patients suffering from postviral fatigue syndrome (PFS).

Methods
Muscle biopsy specimens from the quadriceps of 96 patients diagnosed clinically as suffering from PFS, together with muscle from 4 normal controls, were investigated. Total nucleic acids were isolated from portions of these specimens (<10 mg), blotted onto nylon membranes and hybridized with an enterovirus

0141-0768/88/060326-04/$02.00/0