The Royal Society of Edinburgh / Wellcome Trust Workshop

New developments in the biology of Myalgic Encephalomyelitis / Chronic Fatigue Syndrome

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University of Dundee, Dundee, UK
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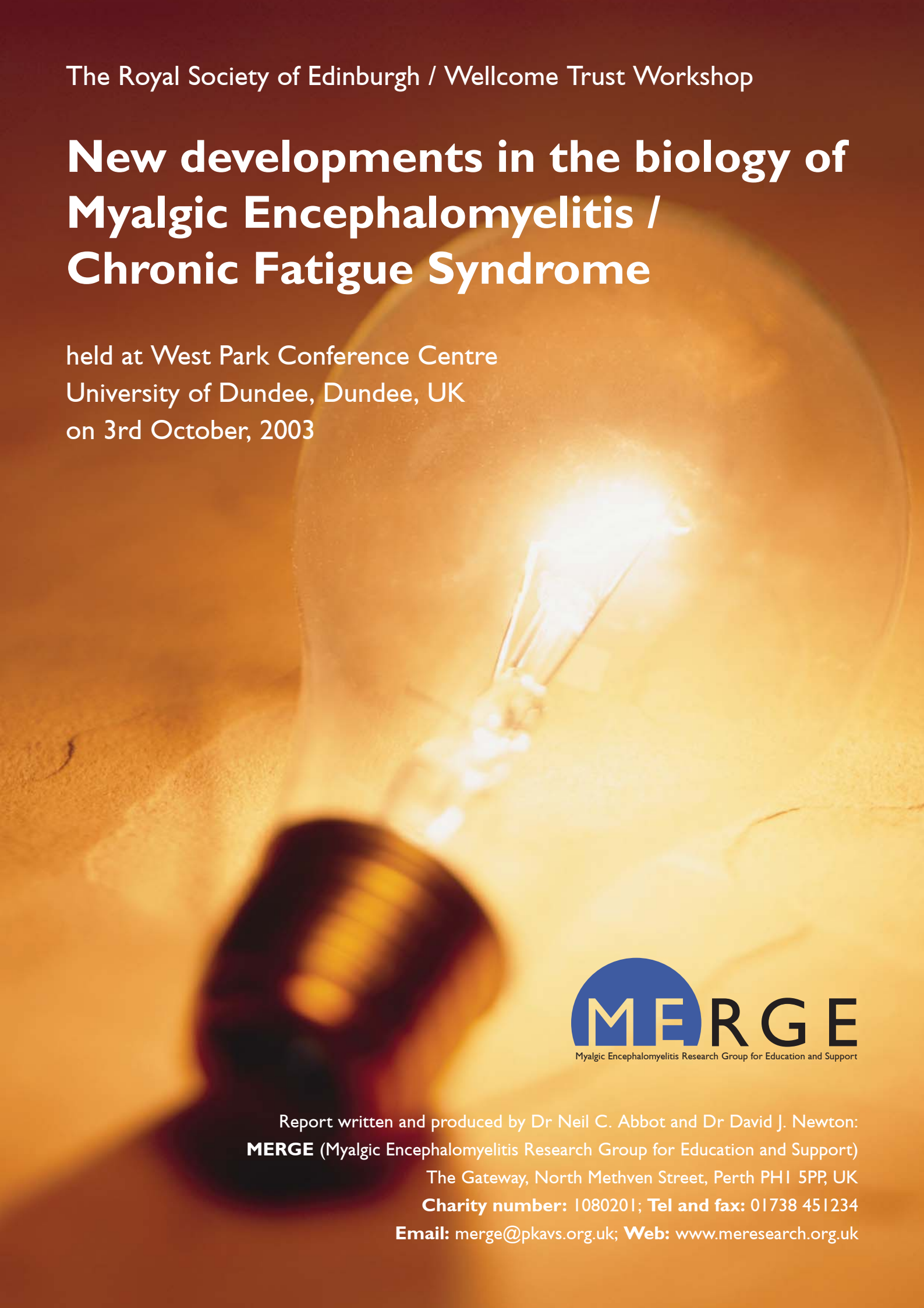

MERGE
Myalgic Encephalomyelitis Research Group for Education and Support

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Foreword

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It is my great pleasure to introduce this overview of the RSE/Wellcome Trust Research Workshop, written and produced by MERGE to energise ME research. Every day in the UK between 120,000 and 240,000 people waken with ME, a condition which principally involves debilitating malaise and pain. Studies tell us that around 50% are employed but struggling to maintain their lives, while another 40% exist on benefits, with considerable economic and social costs to the country. Despite the policy developments of recent years — including the report of the Chief Medical Officer of England, the short-life working group report to the Scottish Parliament and the Medical Research Council's ME/CFS research "strategy" — people with ME remain ill and largely ignored by mainsteam academic medicine.

The diagnosis of CFS which they are given is imperfect and lacks specificity, and "*is a significant complicating factor in understanding the dynamics of this illness…there are probably different types of illnesses now contained within the CFS construct*" (Jason et al., 1997).

In the past, most research effort has gone into the validation of psychosocial strategies designed to manage the illness. Indeed when MERGE conducted an overview of 139 completed or ongoing research projects listed in the UK's National Research Register (Abbot & Spence, 2002), we found that most concerted interest (and almost certainly funding) was directed towards researching psychosocial aspects of the illness.

It is widely recognised, however, that such psychosocial strategies have their practical difficulties and, importantly, that they are not *curative*, at least not in the sense in which ordinary people understand the term. Susan Sontag's words have a particular poignancy in the case of ME: "*Theories that diseases [illnesses] are caused by mental states...are always an index of how much is not understood about the physical terrain of a disease.*"

Something more has to be done: fundamental biomedical research on the subgroups of patients presently given the label "CFS". Illnesses are most easily accepted when they have a specific clinical or scientific thumbprint — a biochemical test, a cluster of specific symptoms or signs, etc. — that confers legitimacy in the eyes of healthcare professionals. Until then, patients are in a no-man's land between the living and the well, subject to a variety of quasi-therapeutic interventions. The discovery of a clinical or scientific thumbprint for ME, indicative of the physical terrain, would be the single transforming event in the lives of many thousands of people. MERGE hopes this research workshop, which has brought together practical scientists from a range of disciplines, will be one more step towards this goal.

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Welcome

Dr Gwen Kennedy, Postdoctoral Research Fellow, Vascular Diseases Research Unit, Department of Medicine, University of Dundee, Dundee, UK *(to whom the RSE grant for this workshop was awarded)*

On behalf of the Royal Society of Edinburgh, it is my pleasure to welcome you to this workshop, which has been awarded funding under the RSE/Wellcome Conference Scheme.

We are all here today because of our interest in and concern about the impact of ME/CFS on human health. According to the 2002 report of the Chief Medical Officer, ME/CFS affects 0.2 to 0.4% of the population, and a significant number of people with the illness are substantially functionally impaired.

The report goes on to stress that "*improvement of health and social care for people affected by the condition is an urgent challenge*," and to recommend that a programme of research on all aspects of the illness is required. It explicitly states that "*research is urgently needed to elucidate the aetiology and pathogenesis of CFS/ME*".

At present, biomedical research into this illness wordwide is sparse given the extent of the problem. Therefore the principal aim of this unique workshop is to focus medical and scientific minds on the need for fundamental research. One of the difficulties faced by researchers is that investigation of the biology of ME/CFS is at an embryonic stage, and so obtaining funding — especially central funding — is still very difficult. Accordingly, we hope that by bringing together scientists from various biomedical backgrounds, a critical level of interest can be generated. We can then begin to consider making joint applications for future funding from major bodies such as the Medical Research Council.

The workshop will be divided into two sections dealing respectively with vascular and biochemical aspects. The reason for the former topic is that for several years now the Vascular Diseases Research Unit at the University of Dundee has been exploring vascular aspects of ME/CFS under the auspices of Professor Jill Belch, Dr Vance Spence, Dr Faisel Khan and myself. Accordingly, Professor Belch will describe the vascular abnormalities we have found. Following this, Professor Julian Stewart will review biomedical aspects of orthostasis, focusing on the type of hypotension and tachycardia that is relevant to the symptoms experienced by ME/CFS patients when they stand up.

Biochemical aspects will be discussed by Professor Kenny De Meirleir, one of the most widely-respected academic researchers in the field of ME/CFS who has experience of examining some 8,000 patients, and by Professor Grahame Hardie, who will describe his own work on the energy of the cell. After each session a discussion will take place.

There are two reasons why we are likely to have a stimulating meeting. First, we may be at the start of a new era in which problems with the umbrella-term "CFS" are being recognised, allowing better targeted research to occur on well-defined sub-groups of patients. Second, the multidisciplinary nature of this audience means that there can be fruitful and valuable communications between specialists, all working towards the common goal of understanding the biomedical basis of ME/CFS.

Royal Society of Edinburgh Workshop on ME/CFS, Dundee, 2003

ME/CFS: a research and clinical conundrum

Dr Vance Spence, Senior Research Fellow, Vascular Diseases Research Unit, Department of Medicine, University of Dundee, Dundee, UK; and Chairman of Trustees, MERGE, The Gateway, Perth

Welcome to everyone attending this research workshop on ME/CFS. As far as we know, this is the first time that the Royal Society of Edinburgh has funded a workshop on the biomedical aspects of this illness. As Dr Kennedy has discussed, the day is divided into two sections, dealing with vascular aspects in the morning and new biochemical developments in the afternoon.

However, my role is to provide an overview of the difficulties surrounding the illness, especially for those of you who are coming fresh to the topic from other scientific areas and specialties. One of our aims is to bring together experts from a variety of disciplines, some with little or no experience of ME/CFS, as we attempt to energise research into this condition with new ideas and novel approaches to solving its inherent problems.

The most widely-used definition of "Chronic Fatigue Syndrome" is that developed in 1994 by a consensus conference: the CDC-1994 (Fukuda et al., 1994) definition. This was developed in response to criticisms that previous definitions (including the CDC-1988) were too restrictive. It requires the presence of chronic fatigue of six months duration which is persistent or relapsing, of new or definite onset, not substantially alleviated by rest, not the result of ongoing exertion, resulting in a substantial reduction in activities, and leading to substantial functional impairment.

In addition, at least four of the following are required: sore throat, cognitive symptoms, tender lymph nodes, muscle pain, multi-joint pain, headaches, unfreshing sleep and post-exertional malaise. Cognitive or neuropsychiatric symptoms may be present, but the definition excludes clinically important medical conditions such as melancholic depression, substance abuse, bipolar disorder, psychosis and eating disorders.

Some would argue that I could just mention this definition and sit down again; but in fact it is part of the problem, and it is worth examining why that is so.

As you can see, the definition relies on "fatigue" as its major criterion. For that reason many patients who fall under this diagnostic label hate the name — they call it the F-word — since for many of them "fatigue" *per se* is not the major problem, and does not best represent how they would explain their condition. Thus, this CDC-1994 definition is now widely recognised to have a number of limitations. These include the fact that symptoms are mainly self-reported (e.g., the clinical signs required in the CDC-1988 definition have been removed); the terminological criteria are vague (e.g., "fatigue", "malaise", "unrefreshing sleep", etc.); the specificity of the definition is poor, allowing heterogeneous groups of patients (e.g., those with somatoform disorders, fibromyalgia syndrome, etc.) to coexist under the one umbrella term (Salit, 1996; Jason et al., 1999); and it makes no attempt to differentiate patients on the basis of severity of illness or level of functional disability.

Indeed, there is a growing realisation that the current CDC-1994-defined "CFS" term is an impossibly inclusive diagnostic construct, begging Simon Loblay (1995) to ask the ontological question: "*Is CFS a recognisable disease entity with a unique pathophysiology, or is it a ragbag of common non-specific symptoms with many causes, mistakenly labelled as a syndrome?*"

As an example, our work in Dundee has compared three groups of patients each fulfilling the CDC-1994 criteria: patients with ME, those with Gulf War Syndrome and patients with a definite history of exposure to organophosphate pesticides. We showed clear differences between the groups in terms of measured parameters, including muscle pain, and physical and mental status (Kennedy et al., 2004). Importantly, a high proportion of people in each group had measurable signs of muscle weakness in arms or legs, indicating that clinical signs can, in fact, be found in these patients if physicians take care to do a full physical examination. Future work will explore such important findings.

There have been other definitions apart from the CDC-1994 Fukuda one (see Figure). The most recent attempt to revise the definition (Carruthers et al., 2003) is based on clinical experiences with very large numbers of patients. It will, however, be some time before this new "Canadian" description of ME/CFS replaces the CDC-1994 definition in clinical and research practice.

When comparing scientific studies, it is important to bear in mind that different definitions of ME/CFS may have been used, and this complicates interpretation and comparison of data. It can also be seen from the Figure below that there have been several attempts in the past decade to define diagnostic criteria for the illness. Each definition has been problematic, reflecting in part the special interest of the author, and taking little account of the extensive literature prior to 1988 (see Figure) that made the case for myalgic encephalomyelitis as a distinct clinical entity based on reports of epidemic and endemic cases.

Diagnostic criteria (adults) for "CFS-like" illness 1988–2003

"Canadian" Expert Consensus Clinical Case Definition for ME/CFS, 2003
US Centers for Disease Control and Preventation (*Fukuda et al., 1994*) *CFS*
World Health Organisation, 1994 (non-clinical)
"Oxford Criteria", UK (*Sharpe et al., 1991*) *CFS*
Australia (*Lloyd et al., 1990*) *CFS*
London (*Dowsett et al., 1990*) *ME*
US Centers for Disease Control and Prevention (*Holmes et al., 1988*) *CFS*

Previous literature

Epidemic Neuromyasthenia (*Parish, 1978*)
Myalgic Encephalomyelitis (*Acheson, 1959*)
Epidemic Neuromyasthenia (*Henderson & Shelokov, 1959*)

What was this condition "Myalgic Encephalomyelitis" that existed before 1988, when it was subsumed within the "CFS" construct, and which is still referred to by patients in the lay literature as "ME"? Myalgic encephalomyelitis was first defined by Acheson (1959). It had been found to occur in epidemic and sporadic forms, and was believed to result from a continuing or persisting viral infection. It has been defined as a systemic illness, characterised by marked muscle fatigability (not just weakness); muscle pain, tenderness and swelling; variable involvement of the central nervous system (ataxia and cranial nerve involvement); muscle weakness and/or sensory changes due to neuronal damage; impairment of memory; sleep disorders, etc.; vascular involvement (orthostatic tachycardia, pallor); reticulo-endothelial dysfunction; and recurrences of flu-like symptoms with myalgia.

From 1934–90 there were at least sixty-three outbreaks of epidemic proportions, all well-documented, distributed geographically in North America (29 outbreaks), the UK (16), the rest of Europe (11), Australasia (4), Africa (2) and Asia (1). One of the most studied, and possibly the most controversial, of these outbreaks occurred at the Royal Free Hospital, London, in 1955, during which 292 people were affected. Indeed, outbreaks may still be occurring, and some of the patients who currently come under the CDC-1994 CFS definition have clinical features similar to the classical description of post-infectious ME patients defined above.

Physiological and biochemical abnormalities found in "CFS" cohorts

Biochemical
Oxidative stress *(e.g., Richards et al., 2000; Manuel et al., 2001; Pall & Scatterle, 2001; Kennedy et al., 2003; Vecchiet et al., 2003)*
Anti-viral dysregulation *(Suhadolnik et al., 1994; De Meirleir et al., 2000; Shetzline SE et al., 2002; Tiev et al., 2003)*

Vascular
Endothelial dysregulation *(Spence et al., 2000; Khan et al., 2003; Khan et al., 2004)*
Brain perfusion *(Schwartz et al, 1994; Costa et al, 1995)*
Orthostatic Hypotension *(Streeten et al., 2000; Stewart, 2003)*

Brain
Metabolic abnormalities (*Tomoda et al., 2000; Puri et al., 2002; Chaudhuri et al., 2003*)

Muscle
Metabolism *(e.g., Fulle et al., 2000; Vecchiet et al., 2003)*
Abnormal recovery after exercise *(e.g., Paul et al., 1999; McCully & Natelson, 1999)*
Enteroviral sequences in muscle *(Lane et al., 2003)*

The fact that we are still aware of these details is in no small measure due to Dr J. Gordon Parish who is attending this workshop today. Dr Parish has over many years collected reports of these outbreaks of ME (Parish, 1978; Shelokov & Parish, 1989), and has a complete archive of the relevant literature.

A complete listing of these references can be found on the MERGE web site (www.meresearch.org.uk).

Given the heterogeneous nature of the term CFS, and the different ways of defining it, it is probably no surprise that many of the biomedical studies conducted into the illness — a relatively small number given the scale of the problem — have had inconclusive results. Despite this, however, a range of abnormalities have been found by a number of different research groups, and these are summarised in the Figure above.

Today's workshop will concentrate on the vascular and biochemical aspects of ME/CFS, but MERGE intends to facilitate further workshops concentrating on other aspects of ME/CFS pathophysiology, such as muscle metabolism and function, and neuro-imaging and brain function.

Royal Society of Edinburgh Workshop on ME/CFS, Dundee, 2003

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Vascular abnormalities in ME/CFS

Prof Jill J.F. Belch, Professor of Vascular Medicine
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Many of the symptoms associated with ME/CFS, such as fatigue, muscular pain, intestinal problems and neural disorders, can be linked to abnormalities in the regulation of acetylcholine (ACh). Furthermore, in our experience with ME/CFS patients in Dundee, we have encountered a variety of symptoms which include the triad of Raynaud's phenomenon (spasm of the fingers), migraine and irritable bowel syndrome: symptoms which potentially involve abnormalities in vascular reactivity. These combined observations led us to hypothesize that patients with ME/CFS may have inappropriately enhanced cholinergic vasodilator activity.

Our microvascular research laboratory, run by Dr Faisel Khan, has developed a number of methods whereby vascular reactivity can be assessed non-invasively, and we decided to apply some of these latest techniques to this illness. The advantage of these techniques lies in the fact that they are safe, painless, sensitive and specific. One recent technical advance is iontophoresis which allows us to pass chemicals, including vasoactive substances, across intact skin and into the cutaneous microcirculation. The effects of these substances on the local vasculature can be monitored and quantified using laser Doppler imaging. This detects the velocity of red cells from the Doppler shift in frequency that they impart on incident laser light. An image of blood flow within a defined region of interest can then be constructed (see Figure), and each image analysed to provide quantitative information about blood flow in that area.

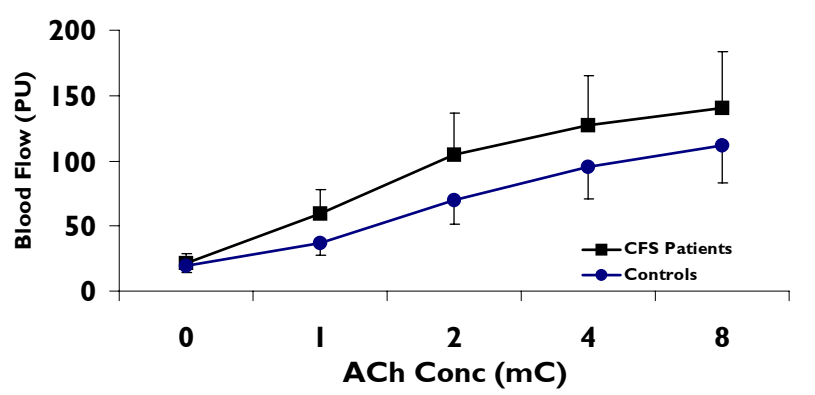
In our first study on ME/CFS patients, we assessed the vascular responses to the endothelium-dependent vasodilator ACh and the endothelium-independent vasodilator nitric oxide (NO), using the NO donor sodium nitroprusside (SNP). ACh causes vasodilatation by activating muscarinic receptors on the endothelial lining of the vessel wall. This, in turn, induces relaxation of the vascular smooth muscle via the effects of NO generated within the endothelial cell from the action of calcium and endothelial nitric oxide synthase on L-arginine. SNP, acting as an NO donor, does exactly the same thing; however, it has a direct effect on smooth muscle and is not dependent on signaling mechanisms on or within the endothelium. Separate applications of ACh and SNP are very useful because they help in locating specific abnormalities within the cells that constitute the blood vessel wall. The effects of ACh are also time-limited by the action of the enzyme acetylcholinesterase (AChE) which breaks down the ACh attached to endothelial receptors.

In patients with a wide variety of vascular disorders, and in those with specific risk factors (such as diabetes and high cholesterol) for developing cardiovascular disease, the blood flow response to ACh is normally blunted; i.e., the blood vessels open less than in normal people in response to a cumulative dose of ACh. When we investigated people with CDC-1994-defined CFS, however, we observed an increased response to ACh (see Figure), while the vasodilatory response to SNP was normal. These results indicated to us that the smooth muscle of the microcirculation in ME/CFS patients was normal, but that the pathway producing NO from ACh, within the endothelium, was abnormally sensitive. We concluded (Spence et al., 2000) that these patients might have:

a) increased sensitivity of ACh-muscarinic receptors on blood vessels,
b) some abnormality in post-receptor signaling, or
c) under-expression of AChE in vascular endothelial cells.

In the previous presentation, Dr Spence highlighted the problems associated with the CDC-1994 definition of CFS, especially the heterogeneity of the patients included under the diagnostic category "CFS", and we can confirm that, on examination, a great diversity of symptoms can be observed among patients (Kennedy et al., 2004). Indeed, some of the variation in outcomes between biomedical research studies probably result from the fact that different subgroups of patients receive the same insensitive "diagnosis". In three of our groups of patients, each fulfilling the CDC-1994 criteria (patients with ME/CFS, those with Gulf War Syndrome and those with a definite history of exposure to organophosphate pesticides), clear differences between the groups, in terms of measured parameters including muscle pain, and physical and mental status, can be observed (Kennedy et al., 2004).

Blood flow responses to acetylcholine (ACh) in CFS patients versus controls



Spence VA et al. AJM 2000; 108: 736–9

Why should the idiopathic ME/CFS patients have this increased blood vessel sensitivity to ACh? One explanation is that there might be problem with the breakdown of ACh by the enzyme AChE, and this hypothesis found some support in subsequent experiments. In these investigations, we monitored the blood vessel response to ACh throughout its complete time-course (i.e., from baseline to peak flow and back to baseline), and we did this in thirty ME/CFS patients and thirty matched control subjects. We found that the time taken for blood flow to return to normal following ACh stimulation was significantly longer than normal in ME/CFS patients (Khan et al., 2003), and this provided additional support for the hypothesis that the breakdown of ACh is impaired.

Of course, one straightforward explanation for the findings would be that AChE was deficient as a consequence of exposure to a cholinesterase-inhibiting agent, such as those contained in organophosphate insecticides. To test this hypothesis, we studied three groups of patients, all of whom fulfilled the CDC-1994 criteria. In addition to fifty classic ME/CFS patients, we recruited two groups of patients who had evidence of a definite history of exposure to AChE-inhibiting agents: 25 farmers exposed to sheep dip (OP), and 25 people with Gulf War Syndrome (GWS) who had taken nerve-agent protection tablets containing the cholinesterase inhibitor pyridostigmine bromide. In these experiments, we tested the various patient responses to ACh and also to methacholine (MCh). MCh is very like ACh with the exception that it is not broken down so readily by AChE.

The results were very interesting. We confirmed our initial findings that patients with ME/CFS are unusually sensitive to ACh, but also found that their response to MCh was the same as that for ACh, in contrast to normal controls whose MCh response was significantly greater than that for ACh. Finally, we discovered that the ACh and MCh responses in both the OP and GWS cohorts were exactly like their own matched controls (Khan et al., 2004). This is the first time that anyone has demonstrated significant differences in biomedical measures between these three groups of patients who all fulfill specific CDC-1994 criteria for CFS. These results are very exciting and may well help us to explain some of the unusual symptoms that these ME/CFS patients experience, as I mentioned earlier.

It is also important to recognise, however, that these tests are not diagnostic markers. The endothelium is influenced by many factors, and all that we can say is that the vascular response to ACh in ME/CFS patients is very unusual and unlike that seen in any other disease we have encountered. We are currently formulating new hypotheses and designing new experiments in order to unravel the significance of ACh sensitivity in ME/CFS patients.

There is also mounting evidence that oxidative stress (Richards et al., 2000; Pall & Scatterle, 2001; Manuel et al., 2001; Logan & Wong, 2001; Vecchiet et al., 2003) and, more specifically, lipid peroxidation (Pall, 2000) contribute to disease progression and to the presentation of symptoms in many ME/CFS patients. Free radicals are central to this process. They are extremely active molecules with important physiological effects, some beneficial (such as bacteriolysis). However, free radicals are also prothrombotic and proatherogenic, both directly and indirectly. Importantly, they interact within the arachidonic cascade with the net effects of vasoconstriction and platelet aggregation. In addition, they have a deleterious effect on lipids, and increased markers of their generation are a feature of all vascular diseases.

While free radicals may generate tissue oxidative injury, it is also evident that other oxidative byproducts, especially peroxidised lipids such as 8-iso-prostaglandin $F2_{\alpha}$, may be even more pivotal in the pathological process. This substance is a member of the F2-isoprostane family and can exert potent biological activity such as platelet activation. It can also act as a powerful vasoconstrictor on the peripheral vasculature (Sametz et al., 1999; Fontana et al., 2001). Such effects may be instrumental in the development of some of the vascular symptoms that characterise ME/CFS (Naschitz et al., 2003). The *in vivo* consequences of increased lipid peroxidation would be higher levels of oxidised low-density lipoproteins (oxLDL) accompanied by low levels of high-density lipoproteins (HDL), which are associated with the development of atherosclerosis (Nordin-Fredrikson et al., 2003), and stiffening of the arterial wall (Wilt et al., 1997).

As part of the same experiment discussed above, looking at ACh and MCh responses in ME/CFS , OP and GWS patients, we also measured oxLDL, HDL, 8-iso-prostaglandin $F2_{\alpha}$ and the antioxidant scavenger glutathione (GSH) in these three patient groups. The results once again surprised us. We found a pattern of significant oxidative stress — increased oxLDL and 8-iso-prostaglandin $F2_{\alpha}$, with decreased HDL and GSH — only in the ME/CFS group (Kennedy et al., 2003a).

It is important to note that, as mentioned previously, isoprostanes also act as vasoconstrictors, and their effects will impact detrimentally on the already compromised circulation of patients with various cardiovascular-linked disorders. For ME/CFS patients, however, the presence of these isoprostane compounds, in association with additional free radicals accompanying exercise, may give rise to what is termed "reperfusion injury", a state where the vasodilatation that is necessary for the delivery of nutrients is compromised. Furthermore, the combination of these "reperfusion" free radicals with nitric oxide can form hypoxyperoxynitrite, which may be responsible for some of the symptoms (such as pain) seen in ME/CFS after exercise. Of course, everyone generates free radicals during exercise, and these are usually scavenged by antioxidant systems. It is also true that exercise training improves antioxidant defences, and there is, therefore, a rationale for advocating exercise therapy as a treatment strategy in ME/CFS patients. Graded exercise therapy is not universally successful in ME/CFS patients, however, and so research into this area is urgently required.

To recap, we have measured an enhanced response to the endothelium-dependent vasodilator ACh and increased oxidative stress in patients with ME/CFS; we see symptoms relating to both of these in the clinical situation, although in different vascular beds. However, is there a case for suggesting that ME/CFS is an inflammatory disorder, albeit an unusual one? We have recently developed an assay for measurement of C-reactive protein (CRP), which is recognized as a robust marker of the inflammatory process. Elevated CRP is also highly predictive of future cardiovascular events. We found that CRP levels, measured using a high sensitivity assay, were significantly

increased in ME/CFS patients (although no differences were observed using conventional CRP assays).

We also have new data that provides novel evidence that ME/CFS patients may have detectable abnormalities in their neutrophils (Kennedy et al., 2003b). We found that neutrophils from these patients had a larger proportion of apoptotic cells than healthy subjects, consistent with an activated inflammatory process, which is possibly the consequence of a past or present infection. These same neutrophils also expressed a higher percentage of the death receptor tumour necrosis factor-receptor I, and had increased binding of annexin V (which is indicative of phosphatidylserine exposure), and consequently early cell death. Accompanying these markers of neutrophil apoptosis, we found that platelet-poor plasma levels of transforming growth factor beta-1 (TGF-1) were significantly elevated in the ME/CFS patients. An increase in TGF-1 in conjunction with neutrophil apoptosis is an important process in the down-regulation of cytokines and eicosanoid production during the chronic inflammatory process (Fadok et al., 1998).

In conclusion, we have carried out a number of experiments relating to the vascular biology of ME/CFS. In the cutaneous microcirculation we have discovered an enhanced ACh response. ACh is a potent vasodilator in all vascular beds, large vessels as well as small, but we cannot be certain if what we have found in the small vessels necessarily applies to the arterial circulation in general. More work is now ongoing to resolve the underlying mechanisms involved in this unusual sensitivity. As far as the general circulation is concerned, we have established that oxidative stress and the production of vasoconstrictor and prothrombotic byproducts are central to the pathophysiology of ME/CFS. In addition to increased lipid peroxidation, there is increased white blood cell apoptosis accompanied by raised markers of inflammation, such as CRP and TGF-1. These abnormalities may account for some of the symptoms of ME/CFS, and might also indicate areas for potential therapeutic intervention. However, it is important to remember that these abnormalities are not consistent across all vascular beds, and that treating one aspect may worsen another. Another question to consider is whether, in the long term, these patients are at risk of developing cardiovascular events, and whether we should be looking aggressively at patient welfare from this point of view.

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Orthostatic intolerance in ME/CFS

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Orthostasis is the ability to stand upright, and orthostatic intol erance (OI) may be defined as "the development of symptoms during upright standing relieved by recumbency". Orthostatic intolerance, therefore, encompasses disorders of blood flow, heart rate and BP regulation that are most easily demonstrable during orthostatic stress, yet are present in all positions. There is evidence from the literature that OI is a substantial problem in ME/CFS patients. My own work is exclusively with adolescents, and as a paediatric specialist I see the "subset" of ME/CFS patients who could be thought of as having predominantly "neurovascular syndrome-type" rather than "pain syndrome-type" ME/CFS.

A variety of evolutionary compensatory mechanisms exist to enable people to maintain the upright position. These include physical forces (e.g., interstitial compression via the skeletal muscle pump), mechanisms relating to blood and blood volume, and a variety of vascular control mechanisms. The latter can be grouped into long distance control (including autonomic nervous system and neurohumoral factors) and local control mechanisms (such as autacoids, inflammatory mediators, etc.). Defects in any or all compensatory mechanisms for orthostasis may produce OI, and these defects may also be evident in the supine position. The traditional symptoms of OI are lightheadedness, headache, fatigue, neurocognitive/sleep disorders, exercise intolerance, weakness, tremulousness, nausea/abdominal pain, sweating and anxiety/palpitations, and there are corresponding signs that accompany these.

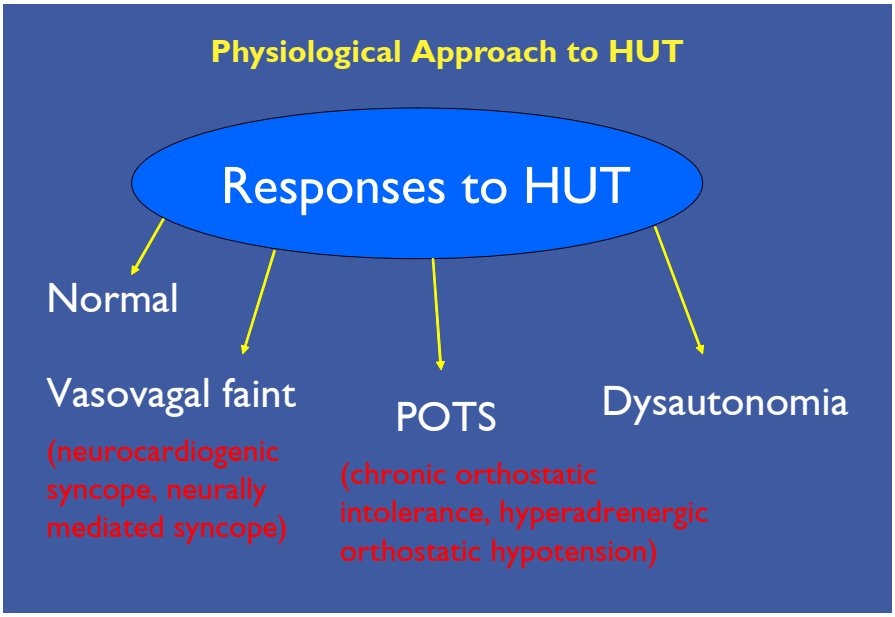
However, it is important to note that the term "orthostatic intolerance" may be a misnomer, since tolerance of upright stance encapsulates disorders of blood flow, heart rate and blood pressure regulation which, though most easily demonstrable during orthostatic stress, are always present. Thus, OI may be the most outstanding finding, but it is a manifestation of more widespread impaired integrative physiology.

Early observations of orthostatic intolerance in ME/CFS, made by Rowe et al. (1995) and Bou-Holaigah et al. (1995), found symptoms and signs of OI. Indeed the symptoms of OI include many of the symptoms of ME/CFS, but not the pain symptoms. Other researchers have also found increased incidence of OI, but these results were highly variable, in part because of varying orthostatic stress test definitions and methods.

The first investigations of OI in ME/CFS were aimed at identifying abnormalities of the autonomic nervous system, based on the highly restrictive assumption that orthostatic intolerance is an (exclusively) "dysautonomic" response. However, most of these studies found essentially normal autonomic function, except for vagal withdrawal-based tachycardia. For example, Freeman & Komaroff (1997) saw a slight increase in supine heart rate (which most investigators still see) and an increase in sitting, standing and upright tilt heart rate. In common with most earlier studies, the authors' patient group included the full spectrum of ME/CFS cases without any attempt to subdivide into "phenotypes". Nevertheless, their findings (in patients and controls, respectively) for tilt table maximum heart rate (100.2±3.5 vs 80.4±2.3) and tilt table heart rate increase (31.8±3.4 vs 18.6±2.1) indicate that approximately 50% of patients fulfilled the criteria for postural tachycardia syndrome (POTS).

Patterns of orthostatic intolerance are best illustrated through orthostatic stress testing: to impose upright stress in a controlled fashion and to monitor physiologic response in detail. The three standard forms of orthostatic testing are lower body negative pressure (LBNP), standing and head-upright tilt (HUT) table. Test techniques typically involve a motionless patient, to negate the effects of the skeletal muscle pump. Most investigators study the neurovascular and neurohumoral responses, and all three tests have a related function as research tools to evoke the orthostatic response, a complex interplay between arterial baroreflex, vasculature, local factors and the CNS.

In the physiological paradigm (see below), the outcome of orthostatic stress can be a "normal" heart rate and blood pressure response, a fainting or vasovagal response, a dysautonomic response, or a postural orthostatic response.

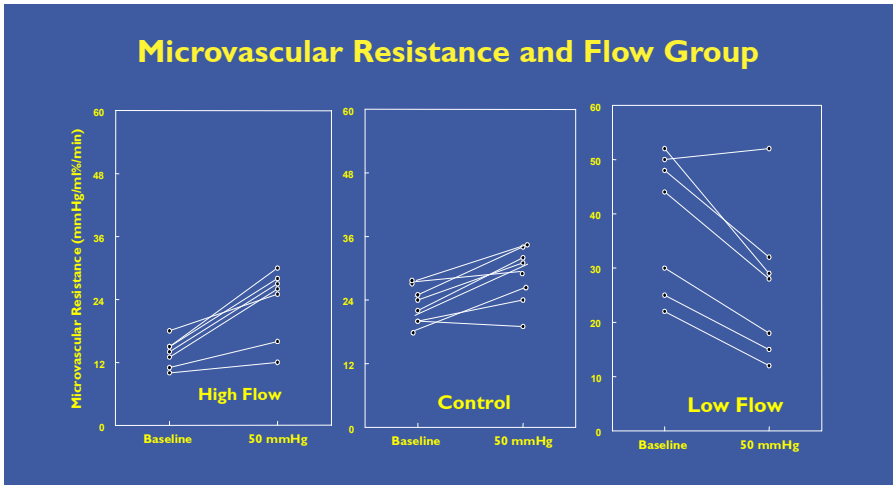


Clinically, OI has two general variants, acute and chronic, which may be roughly divided based on patient history. The acute occurs as syncope (fainting) where cerebral malperfusion results in a transient loss of consciousness and postural tone, or as presyncope without full loss of consciousness. Not all syncope is orthostatic, and there is no consensus about the mechanism. However, it is the chronic form that is of particular importance in the context of ME/CFS.

With chronic orthostatic intolerance, patients are ill for a long time. Chronic orthostatic intolerance may be confused with syncope because chronic illness is sometimes punctuated by acute syncopal episodes. The physician must rely heavily on the patient's history to determine whether chronic illness exists. Defining symptoms of chronic orthostatic intolerance include day-to-day dizziness in all patients, with a high incidence of altered vision (blurred, "white-outs", blackouts), fatigue, nausea, neurocognitive deficits, sleep problems, heat and palpitations. Headache, tremulousness, difficulty breathing or swallowing, sweating, and pallor may also be present.

POTS, the most common reason for referral of adults with orthostatic intolerance, is an emerging form of orthostatic intolerance in children. It is most often observed in young women, and was first reported in the paediatric population by my laboratory. Unlike patients with simple faint, patients with POTS often have day-to-day disability. It is a chronic disease which often waxes and wanes but is always present to some extent. Results of traditional autonomic function tests are often normal in these patients, although reports of some degree of dysautonomia exist. Patients are often unable to hold jobs or attend schools. David Robertson of Vanderbilt University has stated that this is the most common form of chronic orthostatic disability, and is present in virtually every patient with day-to-day orthostatic intolerance.

An operational definition of POTS includes a range of symptoms (such as fatigue, light-headedness, nausea, vomiting, headache, palpitations and tremulousness) and orthostatic intolerance, associated with an increase in heart rate exceeding 30 bpm or a heart rate greater than 120 bpm, within 10 minutes of HUT, as originally described by Schondorf & Low (1993). Onset of symptoms often follows



an infectious disease and may be related to inflammatory mediators. Some adult and paediatric patients with POTS fulfil the CDC-1994 criteria for "CFS" while others do not; however, most of the patients with non-CFS POTS have symptoms that mimic those of ME/CFS to a lesser extent. It is likely that some patients with non-CFS POTS have been classified as having CFS. The clinical course of non-CFS POTS is frequently short-lived, but may easily exceed six months in some patients. Many of the patients with short-lived POTS have little fatigue or exercise intolerance, and some patients remain competitive athletes. Others have a more prolonged course, although without the extent of disability present in many patients with ME/CFS.

POTS is common, affecting patients mostly aged 12–50 years, and mostly female (approximately 80%). Symptoms of orthostatic intolerance in POTS read almost as a litany for CFS, including light-headedness, fatigue, headache, sleep disorders and neurocognitive difficulties. Preliminary data suggest that autonomic findings may be related to circulatory abnormalities at rest and during orthostasis. Thus, ME/CFS may represent a severe form of POTS in adolescents (Stewart et al., 1999a and 1999b).

Patients with POTS or ME/CFS frequently display acrocyanosis and swelling (pooling) in their lower extremities. The literature contains a number of potential explanations for abnormal venous pooling and fluid collection in POTS, including impaired innervation of the veins or in their response to sympathetic stimulation. These potential explanations include an autonomic neuropathy that predominantly affects the lower extremities, resulting in alpha1-adrenergic denervation hypersensitivity, decreased beta1-receptor sensitivity, alpha1-receptor supersensitivity, altered venoconstriction and increased capillary filtration.

My own work has shown that blood pooling in some patients with POTS and ME/CFS is caused by a defect in arterial vasoconstriction that may be baroreflex-sensitive. Increased venous filling and enhanced microvascular filtration during orthostasis result in pooling (Stewart et al., 2003a). Blood is redistributed peripherally and redistribution is enhanced during orthostasis, producing increased microvascular filtration and dependent oedema. Central hypovolaemia causes reflex tachycardia. POTS results in a circulation at higher risk for simple fainting because of an underfilled thoracic vascular bed. The physiology resembles haemorrhage or hypovolaemia in many ways because the tachycardia and malperfusion are noted first but can proceed to hypotension, loss of consciousness or both.

POTS is heterogeneous, however, and not all patients have the vasoconstrictor defect. Current thinking asserts the central importance of disturbed flow physiology, and subscribes to groups of POTS patients ("high-flow", "low-flow" and "normal-flow" POTS; Stewart et al., 2003b) distinguished by altered peripheral blood flow and arterial resistance rather than venous pressure (see Figure):

1. A high flow, low resistance group with decreased Pv and normal limb capacitance ("high-flow POTS").
2. A low blood flow, high arterial resistance, high venous resistance, high Pv, low limb capacitance group ("low-flow POTS"). These have defects in local blood flow regulatory mechanisms (Stewart et al., 2003c).

3. A normal blood flow, normal arterial resistance group with normal Pv and normal limb capacitance ("normal-flow POTS").

It is likely that POTS has many aetiologies. Jacob et al. (2000) provide strong support for a defect in adrenergic vasoconstriction, and recently demonstrated reduced norepinephrine spillover in patients with POTS, consistent with defective innervation of the periphery. Again, the chronic elaboration of cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor alpha, with potent vasoactive consequences, is a potential link between altered vasoreactivity and antecedent inflammatory disease (Patarca-Montero et al., 2001). Such a link seems established in the type of ME/CFS in which POTS and orthostatic intolerance frequently occur.

Medical and non-medical treatments for POTS exist, but they are rarely curative and often incompletely palliative. Agents that expand blood volume, such as fludrocortisone and erythropoietin, are sometimes useful by reducing the degree of thoracic hypovolaemia. Vasoconstrictive agents, such as midodrine or phenylpropanolamine (recalled from the US market), are sometimes useful (Jacob et al., 1997; Gordon et al., 2000). Selective serotonin re-uptake inhibitors have met with some success in treating ME/CFS and orthostatic intolerance (Grubb et al., 1994). Recently, rapid ingestion of water has been advocated as a benign and temporarily effective means to raise BP (Shannon et al., 2002). Support hose and physical manoeuvers can reduce pooling. Exercises to add bulk to the leg muscles have a similar effect, but results have not been systematically applied or reported. Raising the foot of the bed at night can increase blood volume. Treatment success is sporadic because these treatments do not directly address the pathophysiology. As always, further research is needed to clarify these issues.

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Biochemical and cellular aspects of ME/CFS

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Let me state at the beginning that the group of researchers with which I am involved consider ME/CFS to be a disorder of the innate immune system. Indeed, in an ideal world the disorder would be renamed CIID: chronic immune innate dysfunction disease.

I have been working in this field since 1989 when, as an internist working in exercise physiology, I was asked by a psychiatrist to evaluate a patient with "depression" in whom all the depression scales were normal. I noticed that this patient's exercise capacity was only 50% of the expected value for his age and sex, and I subsequently diagnosed him with "CFS". I then began to receive similar patients as referrals. Fourteen years later, the collaborative group to which I belong consists of around thirty-five people worldwide, and includes Prof. Robert Suhadolnik in Philadelphia, and Dr Dan Petersen who has been conducting epidemiological research over many years on a group of patients from the Lake Tahoe outbreak. Thus, the work I shall describe represents the efforts of various dedicated groups which, in the past five years alone, performed over four thousand *in vitro* experiments. Let me just add that one of the most heartening recent developments has been the volume of work undertaken by researchers in Japan. Indeed, much of their work was presented at a conference in 2002 in Sweden, and will be published in a special issue of the Journal of Chronic Fatigue Syndrome in 2004.

In Brussels, we have specialised in antiviral pathways. We do not believe, however, that every case of ME/CFS has a viral origin, although a large proportion of patients do indeed report an infectious episode at the onset of their illness (no single agent has been conclusively associated with the disease, although several candidates have been proposed, including human T-cell lymphotrophic virus-1, human herpesvirus-6, enterovirus and mycoplasma; e.g., Nasralla et al., 1999; Ablashi et al., 2000). However, we do think — after much scientific investigation — that all ME/CFS patients have one of three permutations of immune dysregulation involving ds-RNA-activated protein kinase (PKR) and RNase L. These two proteins are released when infectious agents, mainly viruses,

invade a cell, inducing the production of interferons which trigger development of a defence response which is part of the innate system. In ME/CFS patients we can see:

a) PKR dysfunction without RNase L dysfunction,

b) PKR dysfunction *and* an RNase L dysfunction, or

c) RNase L dysfunction with a minimal PKR dysfunction.

It is important to note that dysregulation of RNase L and PKR is also found in various autoimmune diseases; so ME/CFS is not unique in this respect.

RNase L is the terminal enzyme in the 2',5' oligoadenylate (2,5A) synthetase/RNase L antiviral pathway, and plays an essential role in the elimination of viral mRNAs (for review: see Bastide et al., 2002). Deregulation of the (2,5A) synthetase RNase L antiviral pathway in subsets of CFS patients has been reported extensively in the scientific literature (e.g., Suhadolnik et al., 1999; Martinand et al., 1999; De Meirleir et al., 2000). As a consequence, elastases and calpain cleave high molecular weight RNase L (83 kDa) into a truncated low molecular weight RNase L of 37 kDa. By measuring and calculating the amount of low molecular weight protein relative to high molecular weight protein, we are able to quantify the deregulation of the antiviral pathway.

In ME/CFS patients we see a wide spectrum of cleavage of RNase L (a phenomenon also seen in MS patients), and such altered RNase L activity profoundly affects cellular physiology, including apoptosis (Fremont et al., 2002). Cellular RNase L abnormalities are found in monocytes but not in T-cells, and this provides clues leading to the Th2/Th1 cytokine imbalance reported in ME/CFS (e.g., Patarca-Montero et al., 2000; Patarca-Montero et al., 2001). Overall, an upregulated RNase L pathway in ME/CFS is consistent with an activated immune state and a role for persistent viral infection in the pathogenesis of the disorder. It is tempting to speculate that the severity of the symptoms of ME/CFS are related in some way to the presence of the 37 kDa form of RNase L, and work is continuing on the elucidation of this biochemical phenomenon (e.g., Suhadolnik et al., 1999).

The other element of immune dysregulation which we have observed is PKR upregulation. The ds-RNA-activated protein kinase (PKR) is a 68 kDa enzyme present in most cells and induced by interferon alpha. It can be activated by a range of stimuli, and seems to be important for controlling cellular growth and for tumour regulation. The protein is involved in the same pathways and regulated by the same mechanisms as RNase L, and in ME/CFS probably has its major function in the regulation and control of apoptosis.

The relationship between these immune dysregulations and clinical manifestations of the illness is probably of most interest to patients, and there is now some published evidence that RNase L levels can be related to symptom clusters and symptoms of

MCFS (e.g., De Becker et al., 2002; Snell et al., 2002). An acquired dysregulation of ion channels in ME/CFS patients — a "channelopathy" — involving RNase L and PKR is a very possible explanation for the symptoms experienced by patients. At the cellular level, ion channel regulation requires such fine-tuning that any dysfunction could have widespread consequences which, in ME/CFS, could range from autonomic dysfunction and oxidative stress alterations to modulations of the immune system. At present, the validity of this hypothesis is under intense investigation.

As researchers, we are compelled to use the CDC-1994 criteria which was the creation of a "consensus" conference in 1994, and was not based on hard data. Many of the symptoms are common to other diseases, complicating a diagnosis that still relies on extensive clinical testing to exclude other pathologies. There is growing agreement that this 1994 definition contains heterogeneous groups of disorders. For this reason, within our group in Brussels a great deal of work has gone into the more accurate characterisation of patients, both for therapeutic and diagnostic purposes (e.g., De Becker et al., 2001). Of particular relevance is the new Canadian definition for ME/CFS, which is based on the minor criteria from the Holmes et al. (1988) definition plus ten empirically-identified symptoms (identified from a factor analysis of over 2,500 of our Belgian patients) which include paralysis, new sensitivity to food and drugs, cold extremities, gastrointestinal symptoms, difficulties with words, and muscle fasciculations (for a full listing of symptoms see Carruthers et al., 2003).

Both an understanding of these abnormalities at the molecular level and sub-stratification of patients is essential for the development and identification of effective therapeutic strategies. It is now clear to our group that immunotherapy, immunomodulation, and antibiotic and antiviral therapies will have important future roles in such strategies.

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Management of cellular energy by AMPK

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I should start by saying that I do not currently work on ME/CFS, and do not know a great deal about it. My expertise lies in the mechanisms by which the energy status in individual cells is sensed, and in the responses that result when cellular energy status is compromised. Although it is not clear yet whether there is any connection between what I am going to talk about and ME/CFS, it certainly seems possible and worth investigating. I also greatly sympathise with the drive to uncover the underlying basis of ME/CFS, particularly the biochemical defects which must exist.

I will discuss the AMP-activated protein kinase (AMPK) system, since investigation of this system in ME/CFS may provide important information in the future. Prof. De Meirleir has spoken about PKR, which is another protein kinase, and I intend to discuss the AMPK system which (I would argue) is the key sensor of cellular energy status. AMPK gets switched on when the cell is "fatigued", but how relevant this is to whole body fatigue remains to be seen.

One of the most fundamental parameters that all living cells must maintain is a high ratio of ATP to ADP. In animal cells ADP and phosphate are converted to ATP (equivalent to charging a battery) by catabolic reactions; i.e., glycolysis and oxidative phosphorylation. Almost every other cellular process requires energy and converts ATP to ADP and phosphate (or, in a few cases, to AMP and pyrophosphate), thus discharging the battery. The fact that the cellular ATP:ADP ratio is usually maintained within very narrow limits indicates that the rates of these energy-requiring processes in the cell are balanced almost perfectly by the rate of catabolism. This balance is achieved by sophisticated regulatory systems in cells, and the AMPK system is a key player in this process.

AMPK exists as heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits. Homologues of these three subunits are found in all eukaryotic species where genome sequences have been completed. In humans, each subunit is encoded by either two or three distinct genes (α1, α2, α1, β2, γ1, γ2, γ3) so that there are twelve possible αβγ combinations (even excluding splice variants). As its name suggests, mammalian AMPK is allosterically activated by 5'-AMP. However, the complex is inactive unless it has been phosphorylated by upstream kinases. The precise details of the regulatory mechanisms are presented in a recent review (Hardie et al., 2003). The γ subunits of AMPK contain N-terminal regions which, in mammals, are very variable in both size and sequence, followed by four tandem repeats of a motif known as a CBS domain. Point mutations in CBS domains in other proteins cause a variety of human hereditary diseases, while six different point mutations occurring in the CBS domains of the AMPK γ2 subunit cause cardiac arrhythmias that can lead to sudden death. We have recently found that tandem pairs of CBS domains act together to bind adenosine-containing ligands, which in the case of AMPK can be AMP (activator) or ATP (inhibitor), and that mutations causing cardiac arrhythmias interfere with this ligand binding (Scott et al., 2004).

In vivo and in intact cells the control of the AMPK system is highly regulated. Because of its reciprocal regulation by AMP and ATP, mammalian AMPK is activated in intact cells by any stress that either increases ATP consumption or inhibits ATP production. These include heat shock, metabolic inhibitors such as arsenite or oligomycin, hypoxia or ischaemia, and glucose deprivation. These can all be regarded as pathological stresses, but more recently a number of more physiological stimuli have been found to activate AMPK, including exercise and contraction in skeletal muscle. The fact that the system is activated by exercise, and by "adipocytokines" involved in control of appetite, body weight and insulin sensitivity, points to its potential importance in the treatment of the current worldwide epidemic of obesity and type 2 diabetes. Indeed, AMPK is activated in intact cells by two of the main classes of drug currently used to treat type 2 diabetes; i.e., the biguanides (e.g., metformin) and the thiazolidinediones (e.g., rosiglitazone).

Identification of targets for the system *in vivo* has required the development of methods to manipulate the activity of the system. For instance, one method has involved the incubation of cells with the nucleoside analogue 5-aminoimidazole-4-carboxamide (AICA) riboside, which is taken up by cells and converted to the respective monophosphate ZMP, an analogue of AMP. Another method involves incubation of cells with the widely-used

anti-diabetic drug metformin to activate the kinase in intact cells. Metformin, derived from a plant (French lilac) used in medieval times as a herbal remedy for diabetes, does not appear to deplete ATP, and the mechanism by which it activates AMPK remains unknown. To date there are no readily-available specific pharmacological inhibitors of AMPK. Another approach to studying physiological functions *in vivo* is the use of transgenic mice, although this is complicated by the fact that a knockout of both genes encoding the catalytic subunit isoforms (α1 and α2) gives an embryonic lethal phenotype.

Regarding the clinical situation, the physiological role of AMPK as an energy sensor is best understood in the context of muscle. In a recent study we measured AMPK activity in skeletal muscle biopsies from eight athletes at rest, during cycling exercise for one hour at 70% peak oxygen consumption, and one hour into recovery (Wojtaszewski et al., 2003). This was done with the subjects in both glycogen-loaded and glycogen-depleted states. The results showed that AMPK was activated during exercise in skeletal muscle, but that this was much higher in the glycogen-depleted than in the glycogen-loaded state. This might be related to previous findings that the activation of AMPK in response to the same level of exercise in both rats and humans was reduced after a regime of endurance training, which also causes increases in muscle glycogen content. To investigate further the role of glycogen in regulation of skeletal muscle AMPK during exercise, we examined patients with McArdle's disease, who cannot break down glycogen rapidly due to a hereditary lack of phosphorylase (Nielsen et al., 2002). These patients have chronic high muscle glycogen stores and deficient glycogenolysis, and have a typical history of exercise intolerance and myoglobinuria. When the McArdle's disease patients were compared with control subjects during exercise, the main finding was that exercise caused a much larger activation of AMPK. This is, of course, consistent with the fact that they cannot use endogenous glycogen and must use exogenous fuel from the blood instead, a process which requires AMPK.

Other studies from my laboratory show that AMPK is involved in many aspects of the regulation of skeletal muscle metabolism during exercise; for example, stimulating glucose and fatty acid uptake and oxidation, and inhibiting glycogen synthesis. Again, looking at the role of AMPK activation in the heart during exercise (Coven et al., 2003), our experiments have shown that cardiac AMPK activity increases progressively with exercise intensity, supporting the hypothesis that AMPK also has a physiological role in the heart.

Other recently identified targets of AMPK include the cystic fibrosis transmembrane conductance regulator (the product of the gene that is mutated in cystic fibrosis) and the endothelial and neuronal isoforms of nitric oxide synthase (eNOS and nNOS). The latter may be of particular interest given the earlier presentations at this workshop. Phosphorylation of eNOS has been shown to occur in heart muscle during ischaemia, and of nNOS in skeletal muscle during exercise. There is evidence that NOS activity is required for the effects of AMPK on glucose uptake in muscle and, since the classical effect of nitric oxide is to increase blood flow by relaxing vascular smooth muscle, another effect of eNOS phosphorylation might be to increase local blood flow to hypoxic tissues.

Finally, as well as the more short-term acute effects which are due to direct phosphorylation of target proteins, AMPK has more long-term effects by regulating the expression of numerous genes. In muscle, AMPK activation had been shown to up-regulate expression of GLUT4 and hexokinase, and to stimulate mitochondrial biogenesis. Similar changes in expression of these genes are seen in response to endurance training, or in response to chronic ATP depletion in muscle by feeding rodents the creatine analogue β-guanidinopropionic acid.

In liver cells, activation of AMPK decreases the expression of enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase), both in culture cells and *in vivo*. Since elevated gluconeogenesis is a major cause of the high blood glucose in type 2 diabetes, this effect is probably a major factor in the success of AMPK-activating drugs in treating this condition.

To sum up, the AMP-activated protein kinase is a sensor of cellular energy status found in all eukaryotic cells. It is activated by rising AMP and falling ATP via a complex mechanism that results in an ultrasensitive response. Once activated by depletion of cellular energy reserves, the kinase switches on ATP-producing catabolic pathways and switches off ATP-consuming processes, both via direct phosphorylation of regulatory proteins and via indirect effects on gene expression. AMPK activation reverses many of the defects of the "metabolic syndrome", and AMPK-activating drugs are already used to treat type 2 diabetes. Studies on muscle, including work with McArdle's disease patients who have a typical history of exercise intolerance and myoglobinuria, have shown that dysregulation of the pathways in which AMPK is involved may be a contributing factor in muscle fatigue. While it is too early to postulate a direct role for AMPK dysregulation in the pathogenesis of ME/CFS, researchers into this illness should be encouraged to consider this possibility.

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