Metabolic Changes in Chronic Fatigue Syndrome

Thesis submitted for

the degree of Doctor of Philosophy (Ph D) in the Faculty of Medicine

University of Glasgow

ABHIJIT CHAUDHURI
DM MD FACP FRCP(Glasg)
Diplomate of National Board (Medicine & Neurology)

Division of Clinical Neurosciences
Institute of Neurological Sciences
Glasgow

SPECIAL THANKS

I express my sincere gratitude to my supervisors, Professor Peter O Behan and Professor Ian Bone for their very kind help and advice. It will be evident from the work presented in this thesis that Professor Peter O Behan was a close collaborator in the research project and it would have been impossible to conceive and excute the experiments without his support and guidance. I am equally grateful to Professor Peter GE Kennedy, who is the Burton Chair of Neurology and the acting Director of the Division of Clinical Neurosciences in the University of Glasgow, for offering me every support and facilities within the Department of Neurology at the Institute of Neurological Sciences, Glasgow. Finally, I thank Mrs. Debjani Chaudhuri, my sister-in-law, for her immense help with the statistical analysis of the data and my brother, Dr. Surajit Chaudhuri, for persuading me to finish the work.

DEDICATION

This thesis is dedicated to my family and to my teachers

DECLARATION

I hereby declare that the work presented in this thesis is an original research that was conceived and conducted solely by the author, except where collaboration with others has been acknowledged.

Approval of the local Medical Research Ethics Committee was obtained for all prospective studies involving administration of new drugs or non-standard products given solely for experimental purposes, use of new methods of investigations and for procedures over and above the normal treatment of the patients. Informed consent was sought and obtained from all the participants before proceeding with the experiments.

Dated,
3rd July 2003, University of Glasgow

Abhijit Chaudhuri

CONTENTS

EXPERIMENTS (Cont'd)

DISCUSSION (Cont'd)

ACKNOWLEDGEMENT

I am deeply indebted to the David & Frederick Barclay Foundation for supporting my position and my research in chronic fatigue syndrome. I am very grateful to all our patients and the healthy volunteers for participating in my research study and acknowledge the support of the staff at the neurology, neuroradiology and nuclear medicine departments (Institute of Neurological Sciences and the South Glasgow University Hospitals NHS Trust, Glasgow). Part of the research work was also funded by the Cunningham Trust, the George John Livanos Trust and the MacConnell Charitable Trust whose support is gratefully acknowledged.

LIST OF RESEARCH COLLABORATORS AND THEIR AFFILIATIONS

Professor WMH Behan (Department of Pathology, West Glasgow University Hospitals NHS Trust)

Professor T Clausen (Institute of Physiology, Universitetsparken, Aarhus, Denmark)

Dr. B. Condon (Department of Neuroradiology, Institute of Neurological Sciences, South Glasgow University Hospitals NHS Trust)

Dr. A. Cruikshank (Department of Biochemistry, South Glasgow University Hospitals NHS Trust)

Dr. M. Fu (Wallenberg Laboratory, Sahlgren's Hospital, Sweden)

Dr. C. E. Gray (Department of Biochemistry, Glasgow Royal Infirmary, Glasgow)

Professor D M. Hadley (Department of Neuroradiology, Institute of Neurological Sciences and South Glasgow University Hospitals NHS Trust)

Ms. K. Kingstone (Gastrointestinal laboratory, Western General Hospitals NHS Trust, Edinburgh)

Dr. R. Logan (Department of Biochemistry, Royal Hospital for Sick Children, Yorkhill, Glasgow)

Dr. J. Patterson (Department of Neuroradiology, Institute of Neurological Sciences and South Glasgow University Hospital NHS Trust)

Dr. Lorna Paul (Department of Physiotherapy, Glasgow Caledonian University)

Dr. R. Rudel (Department of General Physiology, University of Ulm, Germany)

Dr. R. Spooner (Department of Biochemistry, Gartnavel General Hospital, West Glasgow University Hospitals NHS Trust, Glasgow)

Dr. R. Swaminathan (Department of Chemical Pathology, St. Thomas' Hospital, London).

Mrs. J. Veitch (Neuroimmunology laboratory, South Glasgow University Hospitals NHS Trust, Glasgow)

Professor A. Vincent (Neuroscience laboratory, Oxford Radcliffe Hospital, Oxford)

Dr. W. Watson (Department of Nuclear Medicine, South Glasgow University Hospitals NHS Trust)

List of Publications on Chronic fatigue syndrome

Monograph

1) Shepherd C, Chaudhuri A. *ME/CFS/PVFS: An exploration of the key clinical issues*. Essex, ME Association, 2001.

Chapters in book

1) Chaudhuri A, Watson WS, Behan PO. Arguments for a role of abnormal ionophore function in chronic fatigue syndrome. In: S Yehuda & D I Mostofsky (eds). *Chronic Fatigue Syndrome.* New York, Plenum Press 1997; 119-30.

2) WS Watson, Chaudhuri A, McCreath GT, Behan PO. A possible cell membrane defect in chronic fatigue syndrome and syndrome X . In: Kaski JC(eds). *Chest pain with normal coronary angiograms: Pathogenesis, diagnosis and management.* London, Kluwer Academic Publishers 1999; pp 143-49.

3) Chaudhuri A, Gow J, Behan PO. Systemic viral infections and chronic fatigue syndrome: current evidence and recent advances. In: Abramsky O, Compston DAS,Miller A, Said G (eds). *Therapeutic strategies for brain injury and repair.* London: Martin Dunitz 2002; pp 127-136.

Review Articles

1) Chaudhuri A, Behan PO. Chronic Fatigue Syndrome. *World Neurology* 1997; 12(2): 12.

2) Chaudhuri A, Behan WMH, Behan PO. Chronic fatigue syndrome. *Proc R Coll Physician Edinburgh*, 1998; 28: 150-163.

3) Chaudhuri A, Behan PO. Overlap syndromes of chronic fatigue. *CNS* 1998; 1(2): 16-20.

4) Chaudhuri A, Behan PO. Chronic fatigue syndrome is an acquired neurological channelopathy. *Human Psychopharmacology*, 1999; 14: 7-17.

5) Chaudhuri A, Behan PO. Neurological dysfunction in chronic fatigue syndrome. *J Chr Fatigue Synd* 2000; 6(3/4): 51-68.

6) Chaudhuri A, Behan PO. Fatigue and basal ganglia. *J Neurol Sci* 2000; 179: 34-42.

7) Chaudhuri A, Behan PO. Fatigue in neurological disorders. *Lancet* 2004 (*in press*).

Original Articles

1) Watson W S, McCreath GT, Chaudhuri A, Behan P O. Possible cell membrane transport defect in chronic fatigue syndrome? *J Chr Fatigue Synd* 1997; 3(3): 1-13.

2) Chaudhuri A, Majeed T, Dinan T, Behan PO. Chronic fatigue syndrome: A possible disorder of central cholinergic transmission. *J Chr Fatigue Synd* 1997; 3(1): 3-16.

3) Watson WS, McMillan DC, Chaudhuri A, Behan PO. Increased resting energy expenditure in chronic fatigue syndrome. *J Chr Fatigue Synd* 1998; 4(4): 3-14.

4) Chaudhuri A, Watson WS, Pearn J, Behan PO. Symptoms of chronic fatigue syndrome are due to abnormal ion channel function. *Medical Hypotheses* 2000; 54: 59-63.

5) Gow JW, Simpson K, Behan PO, Chaudhuri A, McKay I, Behan WMH. Antiviral pathway activation in patients with chronic fatigue syndrome and acute infection. *Clin Infect Dis* 2001; 33: 2080-1.

6) Chaudhuri A, Condon BR, Gow JW, Brennan D, Hadley DM. Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *NeuroReport* 2003; 14: 225-8.

Letters

1) Chaudhuri A. Lactose intolerance and neuromuscular symptoms. *Lancet* 2000; 356: 511-2.

2) Chaudhuri A. Cognitive behaviour therapy in chronic fatigue syndrome. *Lancet* 2001; 358: 238.

3) Chaudhuri A. Patient education to encourage graded exercise in chronic fatigue syndrome. Trial has too many shortcomings. *BMJ* 2001; 322:1545.

4) Chaudhuri A. Chronic fatigue syndrome and myalgic encephalomyelitis. *Lancet* 2002; 359: 1698-9

Abstracts

1) Chaudhuri A, Watson W, Behan PO. Factors influencing energy expenditure and therapeutic strategies in chronic fatigue syndrome. *J Neurol Sci* 1997; 150: S225.

2) Behan PO, Chaudhuri A, Watson WS, Pearn J. The symptoms of chronic fatigue syndrome are related to abnormal ion channel function. *J Chr Fatigue Synd* 1999; 5(3/4): 258-9.

3) Condon BR, Chaudhuri A, Hadley DM, Bennan D. Increased levels of choline-containing compounds in the basal ganglia of patients with chronic fatigue syndrome. *Proc Intl Soc Mag Reson Med* 2002; 10.

GLOSSARY OF TERMS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
AT	Anaerobic threshold
AVP	Arginine vasopressin
BBB	Blood brain barrier
BMI	Body Mass Index
Ca++	Calcium
CDC	Centers for Disease Control
CFS	Chronic fatigue syndrome
Cho	Choline
CK	Creatine Kinase
Cr	Creatine
CRH	Corticotrophin releasing hormone
CSF	Cerebrospinal fluid
DCA	Dichloroacetate
DSM	Diagnostic and Statistical manual
DXA	Dual Xray energy absorptiometry
FFST	Fat free soft tissue
GH	Growth hormone
GTT	Glucose Tolerance Test
HFE	Haemochromatosis, familial
HIV	Human immunodeficiency virus

HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
5HT	5-hydroxy tryptamine (serotonin)
IBZM	Iodobenzamide
IgA	Immunoglobulin A
K+	Potassium
ME	Myalgic encephalomyelitis
Mg++	Magnesium
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MVC	Maximum Voluntary Contraction
Na+	Sodium
Na+/K+-ATPase	Sodium-Potassium Adenosine Triphosphatase
NAA	N-acetyl aspartate
NMR	Nuclear Magnetic Resonance
OLS	Ouabain like substance
PIF	Prolactin inhibitory factor
POTS	Postural orthostatic tachycardia syndrome
PTSD	Post-traumatic stress disorder
QYNAD	A pentapeptide with the aminoacid sequence of Gln-Tyr-Asn-Ala-Asp
REE	Resting energy expenditure
SATET	Sub-anaerobic threshold exercise test

SEM	Standard error of the mean
SPECT	Single photon emission computed tomography
TBK	Total body potassium
TBW	Total body water
VGKC	Voltage-gated potassium channel
VO_2	Volume of oxygen consumption

Metabolic Changes in Chronic Fatigue Syndrome

SUMMARY

Chronic fatigue syndrome (CFS) is a complex disorder where neurological symptoms are common. Historically, this illness was first recognised in its epidemic form, often in proximity with an epidemic of polioclastic illness and was called myalgic encephalomyelitis (ME). Most cases of CFS/ME now occur sporadically. Patients usually present with a wide range of physical and neuropsychiatric problems without abnormal physical signs. Occasionally, fatigue, angina-like chest pain and sweating may be the presenting symptoms and these features are similar to that described in patients with cardiological syndrome X.

Although viral infections are known to precipitate CFS, persistence of known viruses does not explain the symptoms. In the initial studies of CFS patients, non-specific activation of the immune system was observed (Chronic Fatigue Immune Dysfunction Syndrome). There was also some evidence to suggest an impairment of the aerobic metabolism in the skeletal muscle of these patients. Muscle biopsy findings and isolated muscle cell culture studies were suggestive of subtle mitochondrial dysfunction. Multifocal perfusion defects were reported in the cerebral SPECT (Single Photon Emission Computed Tomography) scans of a small number of patients who were studied. In the neuroendocrine studies of CFS, impaired activation of the hypothalamic-pituitary-adrenal (HPA) axis and relative hypocortisolemia were observed. Despite these interesting research developments, attempts to treat fatigue in CFS pharmacologically have been largely unsuccessful. There is still no specific diagnostic marker for CFS.

Metabolic functions are one of the principal determinants of energy expenditure and are exquisitely susceptible to the effects of circulating hormones and chemical

changes. Consequently, clinical experiments based on energy expenditure and metabolic functions were considered to be valid approaches to the present research. Significant abnormalities were found in the proton magnetic resonance spectroscopy of basal ganglia in CFS patients. Autonomic cardiovascular responses to exercise are also impaired in a subset of CFS patients. Finally, plasma membrane injury appears to be a possible explanation for a range of observations made in this research.

Subjective fatigue is a complex symptom. It is the outcome of a variable combination of physiological and neuropsychological changes induced by the primary disease process. Downstream links between brain, neuromuscular and the cardiorespiratory functions are implicated in the neural control of force output during exercises in health and disease. Higher perceived fatigue in CFS is probably caused by the central mechanisms while the sensory input to these neural regulatory mechanisms may limit endurance to maximal and submaximal exercises. Based on these findings and more indirect evidence from other studies, changes in cell membrane properties affecting neuronal signalling in the basal ganglia seem to emerge as one of the likely pathophysiological mechanisms in CFS. There is also evidence of an imbalance of the central autonomic tone in a subset of CFS patients. Surely, research in CFS has the potential to unravel the biology of central fatigue and may bridge the gap that exists between the borderland of neurology and psychiatry.

1. INTRODUCTION

"It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of light, it was the season of darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us"

(Charles Dickens, *A tale of two cities*)

Chronic fatigue syndrome (CFS) is characterised by otherwise unexplained, overwhelming persistent or relapsing fatigue of new onset in variable combination with post-exertional malaise, unrefreshing sleep, self-reported impairment in short term memory, headache, muscle and joint pain (Table 1). For research purposes, case definition of CFS is based on the modified international criteria proposed by the Centers of Disease Control (CDC), Atlanta in 1994 [1]. CFS is recognised worldwide, is common and occurs in all age groups, usually affecting more women than men [2].

Because of its chronicity, lack of effective therapy and consequent disability in adults, a diagnosis of CFS has a significant socio-economic impact. Since CFS lacks any specific or sensitive biological marker, symptoms of chronic fatigue have been attributed in the psychiatric literature to the distorted perception of effort and avoidance of physical activity consequent to depression or somatisation. [3,4]. However, CFS patients can be distinguished from those with depression and indeed, fatigue in CFS does not respond to antidepressant therapy [2,5]. No more than 5% of CDC-defined CFS patients fulfil the current diagnostic criteria for somatisation disorder [6]. Recent clinical data also indicate that psychological co-morbidity in CFS is modest [7] being comparable to the psychological symptoms associated with chronic medical diseases such as diabetes and multiple sclerosis [8]. In a recent prospective study, it was shown that the pre-morbid psychiatric history did not predict future development of CDC-defined CFS after Epstein-Barr viral infection [9]. Thus, the psychiatric models of CFS [4,10] do not adequately explain the mechanism of fatigue and physical limitations to exertion in appropriately defined CFS patients.

Table 1. Modified Centers for Disease Control (CDC) criteria for the diagnosis of Chronic fatigue syndrome (CFS)

Patient must fulfil the major criteria and 4 or more minor criteria

Major Criteria

Clinically evaluated, unexplained, persistent or relapsing chronic fatigue that is of new or definite onset (has not been life long), is not the result of ongoing exertion; is not substantially relieved by rest; and results in substantial reduction in previous levels of occupational, educational, social or personal activities; and

Minor Criteria

The concurrent occurrence of four or more of the following symptoms, all of which must have persisted or recurred during six or more consecutive months of the illness and must not have predated the fatigue:

1. self-reported impairment in short-term memory or concentration severe enough to cause a substantial reduction in previous levels of occupational, educational, social or personal activities
2. sore throat
3. tender cervical or axillary lymph nodes
4. muscle pain
5. headaches of new type, pattern or severity
6. unrefreshing sleep
7. post-exertional malaise lasting more than 24 hours
8. multi-joint pain without joint swelling or redness

No specific treatment is currently considered effective in CFS and the claimed benefits of interventions such as cognitive behaviour therapy [11,12] and graded exercises in CFS [12,13] are debatable.

Typically, community-acquired common viral infections trigger CFS symptoms [5] in previously healthy and often, physically active individuals [14]. In the past century, epidemic outbreaks of CFS-type symptoms were recognised after viral infections ("atypical poliomyelitis") and were variously termed neuromyasthenia or epidemic myalgic encephalomyelitis (ME) [15]. These post-viral fatigue syndromes are considered to be neurologic diseases [16]. CFS-type fatigue is also common in post-polio syndrome [17], Guillain Barré syndrome [18] and multiple sclerosis [19], conditions that are triggered by viral infections as in CFS. There is little evidence however to suggest that fatigue symptoms in CFS, multiple sclerosis or post-polio syndrome are caused by persistent infection or reactivation of the offending viruses [20]. However, it is recognised from the laboratory experiments that viruses can affect more differentiated ("luxury") functions of the cells without causing cell death [21]. Changes in the release, transport or receptor activation by specific neurotransmitters and neurohormones are some of the possible functions that may be affected after viral infections.

In CFS research, it will be of considerable interest and importance if objective changes in normal physiological functions are identified that are of relevance to its symptoms. A substantial body of literature already exists on the neuroendocrine dysfunction and dysregulated hypothalamic-pituitary-adrenal axis in CFS [22]. A subpopulation of CFS patients has been shown to have neurally mediated hypotension

[23] and in particular, adolescent CFS patients are known to be highly sensitive to orthostatic challenge [24]. In the peripheral nervous system, chronic fatigue and acquired deficiency of myoadenylate deaminase deficiency have been documented after systemic viral infections [25]. A subgroup of CFS patients has been consistently shown to have altered skeletal muscle bioenergetics in the exercise studies and ^{31}P-NMR spectroscopy [26-28] and produce excess venous lactate after subanaerobic exercises [29]. However, it is not known if the some of the observed abnormalities in the exercise tests and cardiovascular function were confounded by physical deconditioning and the consequent lack of fitness in CFS patients. Because there is no specific or sensitive diagnostic test for CFS, the case definition selects a heterogeneous group. The heterogeneity of patient population in the research studies has also contributed to the conflicting results. The present research was designed to study the central and peripheral neurometabolic functions in CFS, to identify possible neurobiologic markers for fatigue and to make an attempt for clinical subgrouping of the CDC-defined CFS patients based on objective and reproducible criteria.

2. REVIEW OF THE LITERATURE: CLINICAL ASSESSMENT

"*Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing*"

Voltaire (1694-1778)

Definition of fatigue

Fatigue is common, yet one of the poorly understood symptoms. It is estimated that fatigue accounts for 10-15 million physician office visits in USA alone every year [30]. After pain, fatigue is probably the second commonest symptom of patients seeking medical attention. Because fatigue is common, it is frequently trivialised. To add to the difficulty, fatigue, like pain, is largely subjective and cannot be objectively quantified.

Fatigue has been defined in the dictionary of the English language as "*great tiredness*; *weakness or strain caused by use*". In the medical literature, the term fatigue was introduced to characterise the rate of decline in the peak contractile force of the muscle fibres in the isolated nerve-muscle preparation in the laboratory. Three types of motor units have been distinguished according to the rate at which the skeletal muscle fibres fatigue (fast *fatiguable*, slow *fatigue-resistant* and fast *fatigue-resistant*). All three types of motor units are found in most muscles, but in different proportions [31]. In clinical practice, however, the term fatigue was applied to designate low levels of motor activity without muscle paralysis or to convey a subjective sense of lethargy, profound tiredness, lack of energy or feeling of constant exhaustion. It was also recognised that fatigue is sufficiently distinct from motor weakness because fatigue was not a major problem in a number of neuromuscular diseases that actually weakened muscles. Although fatigue is common in depression, not all fatigued patients experience low self-esteem, despair, and feelings of hopelessness and guilt that are frequently associated with lethargy and reduced

motivation in depressive disorders. It is therefore clear that as a symptom, fatigue is not the same as muscle weakness or depression.

Subtypes of fatigue

It may be important to distinguish between types of fatigue. In muscle physiology, fatigue is considered to be an inability to sustain a specified force output or work rate during exercise due to suboptimal muscle contractility. This has been termed objective fatigue [32]. Objective fatigue is characteristic of diseases that primarily affect muscles, neuromuscular junctions or the peripheral nerves (*peripheral fatigue*). Broadly speaking, peripheral fatigue is a physiologic measure of muscle contractility and is associated with primary muscle diseases and disorders of neuromuscular transmission. Neuromuscular disorders like myasthenia gravis and metabolic myopathy are the best examples of this type of fatigue. These disorders maximally reduce force output without causing much motor weakness. Similar objective fatigue may also be experienced by patients with peripheral circulatory disorders, as also in ischemic heart disease and left ventricular failure because of a failure to augment blood flow to meet the requirements of the exercising muscles.

In contrast, some patients experience difficulty in enduring normal physical as well as mental activities. This is characteristic of a number of chronic fatiguing disorders of medical aetiology. In these, the subjective symptom of fatigue is characterised not only by physical, but also, by mental fatigue. This is non-neuromuscular fatigue or central fatigue and cannot be directly quantified directly by muscle electrophysiology unlike the physiological, objective fatigue [32].

Table 2.1 : Neurological disorders of central fatigue

Symptomatic:

Cerebral vasculitis and cerebrovascular diseases

Channelopathies

Developmental disorders (cerebral palsy, Chiari malformations)

Dysautonomic states

Encephalitis lethargica

Granulomatous disorders (neurosarcoid, Wegener's granulomatosis)

Hypothalamic and pituitary diseases

Intracranial infections (meningitis and encephalitis)

Metabolic encephalopathy and mitochondrial diseases

Migraine

Motor neuron disease

Multiple sclerosis

Multiple system atrophy

Myotonic dystrophy

Narcolepsy and related sleep disorders

Paraneoplastic (limbic encephalitis, opsoclonus-myoclonus)

Parkinson's disease and other Parkinsonian disorders

Posterior head injury

Post-Guillain Barré syndrome fatigue

Post-infective fatigue states (post-polio, -Lyme disease, -Q-fever and -viral fatigue)

Idiopathic: Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME)

Patients with symptoms of physical and mental fatigue have no profound weakness, persistent or progressive cognitive decline or failure of peripheral neuromuscular function. Since cognitive performance during the periods of attentional tasks is a measure of cerebral function, these patients can be considered to have a type of fatigue suggestive of a dysfunction in their central nervous system, i.e. a *central fatigue* as opposed to the peripheral neuromuscular fatigue [33]. Besides CFS, multiple sclerosis and Parkinson's disease would be the two typical examples of central-type fatigue. Other diseases producing similar symptoms of *central fatigue* are listed in Table 2.1.

There are several distinctive features of central fatigue that distinguish it from peripheral neuromuscular fatigue (Table 2.2). While peripheral fatigue appears rather exclusive to the neuromuscular and cardiorespiratory disorders, central fatigue is common in neurological disorders affecting both central and peripheral nervous systems, for example, mitochondrial cytopathy, B_{12} deficiency, post-polio fatigue, ciguatera fish poisoning, motor neurone disease and myotonic dystrophy. Central fatigue is also an important symptom of certain neurological channelopathies such as migraine, epilepsy and paroxysmal dyskinesias [33].

Fatigue symptoms in CFS are closely comparable to the fatiguing neurological disorders of central fatigue. For example, fluctuating fatigue, paroxysms of sweating, and increased resting energy expenditure are common to both Parkinson's disease and CFS and relapsing fatigue induced by stress and exertion in CFS is similar to that seen in multiple sclerosis [2]. Unlike the symptoms of the peripheral fatigue, which are usually short-lived and recover by rest, symptoms of central fatigue tend to be more

persistent and are incompletely relieved by rest and/sleep. Central fatigue is the dominating feature in the symptoms of persistent or relapsing fatigue in patients with a clinical diagnosis of CFS [33].

Chronic fatigue as a symptom

Chronic fatigue is a symptom, not a disease. There is a long list of medical and psychiatric diseases associated with chronic fatigue. The pathophysiology of chronic fatigue is usually explained on the basis of the knowledge of the medical diseases it is associated with. Inflammatory and immunological disorders like rheumatoid arthritis provide excellent examples where cytokines may be responsible for fatigue. When injected in experimental animals, anti-inflammatory cytokines induce slow wave sleep [34]. Cytokines given as drug therapy commonly produce fatigue and myalgia. In a smaller proportion of patients, cytokines also induce neuro-psychiatric symptoms and depression. Paroxetine, an anti-depressant that selectively inhibits serotonin reuptake, has been successfully used in patients for the primary prevention of depression induced by high dose interferon-alfa therapy [35]. In a number of diseases, fatigue similar to CFS may be the only symptom before other signs or symptoms declare themselves. On the other hand, CFS-like fatigue may be the only persistent and disabling symptom in patients with otherwise well-controlled and treated diseases. Multiple sclerosis, sarcoidosis and haemachromatosis are common examples where fatigue can antedate other symptoms or persist despite appropriate therapy directed to the primary disorder.

Table 2.2: Characteristics of central fatigue

- Lack of persistence of motor activity (easy fatigability)
- Lack of persistence of mental activity (easy distractibility, impaired concentration, indecision)
- Sleep disorder
- Anomia
- Personality changes (emotional lability and mood swings)
- Reduced motivation or lack of motivation
- Worsening of symptoms by physical or mental stress and chemicals (alcohol)

Cardiological syndrome X is an important overlap disorder of CFS. This is characterised by paroxysms of typical anginal chest pain with normal coronary angiogram. Syndrome X and CFS share many similarities including an identical clinical course, abnormal oxidative metabolism in skeletal muscle as found on ^{31} P nuclear magnetic resonance (NMR) spectroscopy, abnormal cerebral and cardiac Single Photon Emission Computed Tomographic (SPECT) scans, and increased lactate production [36].

Chronic fatigue is also a symptom of depression and is known in a number of psychiatric illnesses such as phobic anxiety and somatoform disorders. In the psychiatric literature, chronic fatigue is considered to be a "pseudoneurologic" symptom of somatisation. In this model, fatigue is the physical attribute of chronic depression and anxiety and chronic fatigue is a functional somatic disorder [4]. Despite overlap in some cases, however, neurogenic and somatising chronic fatigue may be distinguished clinically. This is usually possible on the basis of associated symptoms. Fatigue is seldom the first and the most important problem in psychogenic fatigue unlike in CFS where fatigue is the hallmark symptom. In addition, unlike CFS, somatising fatigue is more uniform and rarely fluctuates in severity on a day-to-day basis. Typically, asthenia in dissociative or somatoform disorders is frequently associated with generalised body and limb weakness where the strength of the voluntary movement is proportional or less than the resistance offered [37]. In addition, the severity of fatigue in functional disorders is significantly improved by rest or sleep and the post-exertional deterioration is maximum soon after activities whereas in appropriately defined CFS patients, fatigue remains unrelieved by rest or

sleep and post-exertional malaise is usually maximum after first 24 or 48 hours of physical activities.

Idiopathic chronic fatigue as a clinical entity

The concept of unexplained chronic fatigue for which there is no apparent medical cause has been recognised for centuries. Description of a disease complex with features similar to CFS exists in the papyrus Ebers and is dated circa 1400 B.C. [38]. The first epidemic of CFS to strike England probably dates back to the time of Henry VIII when one of his wives, Anne Boleyn, fell ill during this epidemic, called the "English Sweats" [39]. Charles Darwin was believed to suffer from CFS: a year after his return from the Beagle voyage, Darwin described how any form of stress completely disabled him; two years later he gave up going out to parties because he found them too tiring and three years after that, in 1842, he retired to his country village to lead a life of permanent invalid [40]. In 1854, the very active Florence Nightingale contacted an infectious disease in Crimea while organizing the field services for the British Army. She recovered briefly, but only to fall ill again, this time chronically, with chest pain, headaches and fatigue. Numerous heart specialists failed to find any fault with her. She was unable to walk any distance or concentrate when more than one person spoke to her [41].

In 1856, Finsen observed an epidemic of muscle rheumatism and chest pain in Iceland [42]. This was probably the first recorded coxsackie epidemic, later called Bornholm disease. The same epidemic repeated itself in a more serious fashion in the district of Ofjord in 1865 [42]. It was not until the full-blown poliomyelitis epidemic that swept

California in the summer of 1934 that CFS was considered as a separate epidemic illness. During that poliomyelitis epidemic, another epidemic but of different type, occurred among the personnel of the Los Angeles County General Hospital. [43] There were no deaths and 198 or more cases occurred among the doctors and other paramedical staff of the hospital. It is apparent that the large majority of the medical staff fell ill with this "new illness" after they were passively immunised with the prophylactic globulin prepared from the serum of patients who had fallen ill during this epidemic. Their symptoms were same as CFS: relapsing muscle weakness, unusual pain syndromes, personality changes, memory loss and anomia. The nurses in particular were all treated as having hysteria and several of the nurses affected in the 1934 epidemic underwent hysterectomy, which obviously did not help [44]. This first, carefully recorded epidemic disease came to be called "atypical poliomyelitis" [43].

In the following years, a number of epidemics of CFS occurred in close association with the epidemics of poliomyelitis. The 1936 Wisconsin epidemic of CFS occurred when a student nun returned to her cloistered school after spending sometime in Brooklyn with her friend who developed poliomyelitis at the same time when she fell ill with CFS [45]. The 1948 epidemic in Iceland started as a poliomyelitis epidemic and finished as a major epidemic of CFS involving 1,116 patients [46]. By 1959, fourteen major outbreaks of CFS were documented [45, 47], including the Royal Free Hospital epidemic where the attack rate was calculated as 18.3% for nurses and 8.3% in the community, much higher than usually seen in poliomyelitis [48]. In most epidemics, the attack rate was uniform at about 6% [47] and the symptoms of the acute illness were consistent in over sixty recorded epidemics recorded worldwide [15]. In the largest Scottish outbreak of the disease between 1980 and 1983 in

Ayrshire [49], twenty-two patients were seen who complained of characteristic symptoms of CFS and had severe exhaustion most obvious after exercise. The disease had started in every case after an acute or subacute illness, with symptoms of vertigo, hyperacusis, and tinnitus or with palpitations and chest pain. Of the symptomatic patients, 82% had increased neutralising antibody titres to Coxsackie B virus [50].

Terminology of idiopathic chronic fatigue

In the 19th century, neurasthenia was a popular but rather imprecise diagnosis applied to non-specific chronic fatigue that had a high prevalence in women [51]. However, the subjective nerve and muscle "fatigue" that followed the epidemic outbreaks of viral infections were fairly distinctive. The term neuromyasthenia has been used to characterise the latter syndrome since the early part of the 20th century. The clinical characteristics of the viral epidemic that triggered neuromyasthenia were often similar to non-paralytic poliomyelitis (atypical poliomyelitis)[52]. Epidemic neuromyasthenia had a distinctive clinical picture consisting of "headache, myalgia, myasthenia, encephalopathy, lymphadenopathy, morbidity and survivance" [53]. Residual symptoms years after included persistence of fatigue, muscle pain, nervousness and disturbance of cutaneous sensitivity (allodynia) [54]. Myalgic encephalomyelitis (ME) was the preferred name given to these patients during the 1960s [45]. This was considered inappropriate pathologically and hence many referred to these cases as having post-viral fatigue syndrome (PVFS) [5]. Many patients with a diagnosis of PVFS had a documented history of Epstein-Barr virus or enterovirus infections [5].

Late-onset fatigue was also the commonest sequel of the polio epidemic and the clinical characteristics of the fatigue and related symptoms were very similar in post-polio fatigue, epidemic neuromyasthenia, ME and PVFS.

The term ME has been criticised because of inadequate pathological evidence for inflammation in the brain and spinal cord of these patients [2]. Since 1988, chronic fatigue syndrome (CFS) became the official designation for otherwise unexplained, persistent or relapsing chronic fatigue of new onset [55].

Chronic fatigue syndrome (CFS)

For clinical and epidemiological purposes, patients with CFS are currently defined according to the modified CDC criteria which were developed by the International CFS Study Group and introduced in December, 1994 [1] replacing the first research case definition published six years' earlier [55]. According to these criteria (Table 1), a case of CFS is defined as follows: 1) clinically evaluated, unexplained, persistent or relapsing chronic fatigue that is of new or definite onset (has not been lifelong); is not the result of ongoing exertion; is not substantially alleviated by rest; and results in substantial reduction in previous levels of occupational, social or personal activities; and 2) concurrent occurrence of four or more of the following symptoms, all of which must have persisted or recurred during 6 or more consecutive months of illness and must not have predated the fatigue: (i) self-reported impairment in short-term memory or concentration severe enough to cause substantial reduction in previous levels of occupational, educational, social or personal activities; (ii) sore throat; (iii) tender cervical or axillary lymph nodes (iv) muscle pain (v) multijoint pain without

joint swelling or redness; (vi) headaches of a new type, pattern or severity; (vii) unrefreshing sleep; and (viii) post-exertional malaise lasting more than 24 hours.

Other definitions include *prolonged fatigue*, defined as self-reported persistent fatigue lasting 1 month or longer and chronic *fatigue*, defined as self-reported persistent or relapsing fatigue lasting 6 or more consecutive months. A case of *idiopathic chronic fatigue* is defined as clinically evaluated, unexplained chronic fatigue that fails to fulfil the above criteria for CFS. Presently, a diagnosis of CFS can only be made in exclusion of other causes since there is no specific or sensitive laboratory test for this condition.

Epidemiology

Current estimates of the CFS prevalence are 4 cases per 10,000 population [56]. It affects adults as well as children. Cases are approximately twice more common in women. The incidence in adolescents is lower than that in adults and most cases occur between the ages 11-15 years although younger children (5 years and above) may rarely be affected. The prevalence of paediatric CFS is not known; an approximate estimate is 0.07% [56]. Most cases of CFS now occur sporadically. Spontaneous and full recovery in adults is usually limited and occurs within the first 1-2 years; indeed very few symptomatic adults will experience spontaneous and substantial recovery at a later period [57]. Clinical outcome of CFS may take any of three courses; some patients recover completely, some follow a relapsing course, and some are permanently incapacitated [8]. Levels of disability in symptomatic patients vary

considerably but it has been estimated that at least 25% of all CFS cases experience high levels of disability.

Clinical assessment of CFS

Onset and Precipitating factors: CFS begins abruptly in nearly one third of cases and is of subacute or insidious onset in the remaining two-thirds [2]. The fatigue can appear over a few minutes or hours. Nearly 80% cases of CFS follow an antecedent respiratory, gastrointestinal or other acute infection with 'flu like symptoms [58]. In the epidemic form of CFS, it was calculated that the illness has an incubation period of 5 days (range 4-10 days) [45,47]. Other cases develop after emotional or physical trauma such as bereavement or surgery [59].

Environmental agents or toxin have been implicated in a number of chronic fatigue disorders. A syndrome comparable to CFS is seen in some patients after immunisation [60], food botulism [61] and chronic lead neurotoxicity [62,63]. A neurobehavioural syndrome identical to CFS has been observed after ciguatera fish poisoning [64] and after chronic exposure to low-dose organophosphate pesticides in farmers [65,66]. CFS-type symptoms have been described after a prolonged period of stress [67], in the immunised veterans of the first Gulf-War [68], after overtraining in athletes [69], in individuals with multiple chemical sensitivity [70] and in women after silicone breast implants [71].

Children with history of atopy (eczema or extrinsic allergic asthma) are more prone to develop symptoms of post-viral CFS [72] whereas new-onset asthma has been

observed in both adults and children symptomatic of CFS [59]. A proportion of male patients developed the illness following an attack of epididymo-orchitis [60]. There may be more than one precipitating factor or triggering agents. It has also been suggested that once an individual is exposed to a triggering agent, certain co-factors may play a role in increasing his susceptibility to the development of CFS symptoms [14]. Possible co-factors are cryptic gluten sensitivity [73], Gilbert's syndrome [58], atopy, prior physical exercise and preceding or concurrent history of stressful life events [2,14].

Like many patients with idiopathic epilepsy or migraine, patients who receive a diagnosis of CFS will have no objective abnormality on clinical examination. Research studies, however, have often revealed variable and subtle neurological findings in CFS [74]. The diagnosis of CFS is clinical. *Fatigue* remains the most important and the central symptom in CFS. It must fulfil the characteristics as defined in the CDC criteria, i.e. fatigue has to be of definite or new onset, not the result of on-going exertion, generally unrestored by rest and sleep and results in substantial reduction in overall performance. Typically, there is significant post-exertional worsening of fatigue in CFS lasting more than 24 hours and continuing for 48-72 hours.

Typically, a number of other neurological symptoms appear in combination with fatigue in CFS. *Myalgia*: Both diffuse muscle aches and pain as well as fibromyalgia are common symptoms in CFS. Similarly, patients often have joint pain in both small and large joints without any redness or swelling (*arthralgia*). *Weakness*: Weakness of sustained motor performance and skilful work are common symptoms. *Sleep*: Patients

are generally hypersomnolent at the beginning and can have prolonged periods of sleep, both during the day and night. Subsequently, these patients develop altered sleep rhythm with frequent, short periods of increased sleepiness during the daytime and have poor night time sleep with broken sleep pattern and vivid dreams. *Vertigo*: Acute vertiginous episodes are common, often lasting for a week to 10 days on average and then leaving the patient with a constant feeling of unsteadiness or dysequilibrium. Some CFS patients suffer from frequent spells of vertigo with gait disorder and dysequilibrium as their major physical symptom besides fatigue (*Pedersen's syndrome*) [75]. *Orthostatic intolerance*, tachycardia, light-headedness and simple faints are particularly common among young adults and women with CFS. *Visual symptoms*: Temporary visual obscuration is not uncommon and patients often report intense photophobia simulating meningism.

Many CFS patients also have symptoms outside the nervous system. Development of *asthma* or worsening of pre-existing asthma is also recognised among CFS patients. Patients also report *skin changes*, usually in the form of dry skin, the new onset of eczema or psoriaform rashes. A large number of patients have abdominal pain, distension and unsatisfactory bowel movements and suffer from typical *irritable bowel syndrome*. *Loss of libido* and sex drive is common symptoms in both sexes. Male sufferers of CFS may have *prostatism* and women have frequent problems with their menstrual periods, which may be irregular, with periods of *menorrhagia* or *oligomenorrhoea*. Women with CFS also have an interesting association with *idiopathic cyclic oedema*. They feel overweight, with swelling of legs and sensations of breast engorgement. *Weight changes*: Though some weight loss is common in the early phase of illness, the majority of patients eventually recover the lost weight and

may even continue to gain weight, usually attributed to their sedentary lifestyle. *Unusual food cravings*, especially to chocolates or sweets, are known to develop after acquiring CFS may also contribute to weight gain. *Alcohol intolerance*: CFS patients characteristically develop intolerance to alcohol and even small amounts of alcohol dramatically worsen symptoms of fatigue and orthostatic intolerance.

Paroxysmal attacks of *chest pain* and *sweating* are two common cardiac symptoms found in this condition. Some patients with CFS were originally referred by the cardiologists who diagnosed a condition called syndrome X (anginal chest pain with normal coronary arteries). Many of these patients eventually went on to develop other typical symptoms of CFS [76]. A sizeable proportion of CFS patients do have typical chest pain suggestive of angina but on further investigation, do not appear to have any underlying stenosis of their coronary arteries. Unexplained attacks of sweating, typically nocturnal, are common in these and other patients with CFS and often the sweating can be so profound as to necessitate a change of full clothing and bed sheets at night [2].

The most consistent neuropsychiatric findings in CFS are of *mild depression*, usually accompanied by chronic anxiety and intense introspection. Well-characterised cases invariably report cognitive deficits, usually *anomia*, *impaired short-term memory* and *concentration difficulties*. Self-reported *cognitive impairment*, particularly in tasks requiring sustained attention, as well as anomia are recognised in CFS. The degree of symptomatology may vary, but at its most severe, patients are forced to abandon all intellectual pursuits and children with CFS discontinue their schooling. Some patients do develop *hypergraphia*, i.e. keeping the most detailed records and long descriptions

of all their symptoms and come to the clinic with interminable notes. Every ache and pain is ascribed to the illness and carefully recorded. However, patients do not have profound anhedonia or suicidal symptoms of typical depressive illness although a pre-morbid psychiatric abnormality ("depression-proneness") is not uncommon [77]. In general, patients with CFS appear more than depressed at their inability to work at the level of their expectations and may develop mood swings and temper tantrums. *Emotional lability* and a tendency to tearfulness are less common and if present, these emotional changes are suggestive of depression that may be reactive. Overall, psychiatric co-morbidity in CFS is modest and is probably not in excess of, what is seen in other chronic medical disorders, for example, multiple sclerosis and diabetes mellitus [8].

Very occasionally, objective clinical signs may be present at the time of diagnosis in CFS. Cervical *lymphadenopathy*, if present, is one of the (minor) diagnostic criteria. *Deep reflexes* may appear pathologically (and symmetrically) brisk but plantar responses remain flexor. Postural fine hand tremors are, however, not uncommon. Occasionally, focal myokymia ("*benign fasciculations*") may be observed in some patients. Significant objective *motor weakness* is unusual, although CFS patients are unable to produce maximal voluntary effort in muscle contraction against resistance. Disorder of gait is usually present in patients with dysequilibrium symptoms (*Pedersen's syndrome*). Rare patients do develop mild *Parkinsonian features* (poor facial expression, hypomimia and minimal hypokinesia [74].

3. REVIEW OF THE LITERATURE: CFS RESEARCH

"Disease is very old and nothing about it has changed. It is we who change as we learn to recognize what was formerly imperceptible" (Jean Martin Charcot, 1887)

More than 4000 papers have been published in the peer-reviewed journals on CFS. In these, peripheral and central mechanisms of fatigue have been extensively explored. Changes in the hypothalamic-pituitary-adrenal axis and other neuroendocrine responses have been identified to have important implications in the pathogenesis of fatigue [78]. Abnormalities have been reported in the skeletal muscle metabolism, cardiovascular response, neuroendocrine function and in the nervous system [2]. CFS has also been attributed to physical deconditioning, abnormal illness behaviour, chronic multiple functional symptoms and due to chronic anxiety and depression in the psychiatric literature [4]. The wide divergence of opinion in the literature may have been influenced by the difference in the selection criteria applied to the patients receiving a diagnosis of CFS. It is generally accepted that prevalence of CFS is at least higher in the population when broader definitions (e.g. Oxford criteria) have been used (2-4%) [79]. Because by definition, CFS patients do not have any known fatiguing medical disease, broader definitions are likely to select a substantially higher proportion of patients with functional symptoms and medically unexplained chronic fatigue. The variability of case selection make it difficult to compare research findings and it is also likely that CFS patients do not conform to a single clinical entity [80].

Research on peripheral aspects of fatigue

Symptoms reported by many CFS patients relate to peripheral tissues, such as muscle, with prominent fatigue and pain at rest and on minimal exercise. It may be the case that these patients suffer from a predominantly peripheral type of fatigue. While some studies have been unable to confirm metabolic abnormalities [81], others found some CFS patients to have a moderate reduction in oxidative capacity, as measured by the

rate of recovery of phosphocreatine after submaximal exercise [26,28]. It has been proposed that the CFS patients can be classified into those with a high (and normal) oxidative capacity or those with a low (and abnormal) oxidative capacity. Recent work however indicates that autonomic dysregulation is common in CFS, with reports suggestive of abnormalities in both sympathetic and parasympathetic functions [82,83]. It has been proposed that the impairment in autonomic vasomotor control limits the increase in the blood flow to the exercising muscles and leads to reduced exercise capacity in CFS [84].

^{31}P nuclear magnetic resonance spectroscopy (NMR) provides an excellent method for continuous, in vivo, monitoring of intracellular energy metabolism in skeletal muscles. NMR studies have shown a significant reduction in the exercise capacity in CFS, accompanied by excessively early intracellular acidification [26-28, 85,86]. The first positive report of a single case [85] CFS was followed by work from the same group showing similar features in five of six cases [86] and then 12 out of 46 patients [27]: six had increased acidification relative to phosphocreatine (PCr) depletion and six, reduced acidification. Other workers have postulated that CFS patients may have relatively reduced concentrations of muscle ATP. A significant reduction in aerobic metabolism was noted in PCr recovery [28]. Reduced oxidative muscle metabolism in CFS patients has been shown in a study that compared 22 CFS patients with normal sedentary subjects before and for two days after a maximal treadmill test [26]. Muscle oxidative capacity was measured as the maximal rate of post-exercise PCr resynthesis in the calf muscles using ^{31}P NMR spectroscopy. The oxidative capacity (maximal rate of ATP synthesis) was reduced in CFS patients as opposed to controls. No further changes, however, were seen in the post-exercise period. Skeletal muscle NMR

studies in syndrome X have reported similar abnormalities, suggesting that CFS and syndrome X could share a common metabolic disorder [87].

Muscle biopsy: No consistent abnormality has been reported in the muscle biopsies of CFS patients. A variety of non-specific findings, however, were noted. In about 30%, scattered atrophic fibres were seen and in another 30%, there was type 2 fibre hypertrophy and predominance [88]. Type 2 fibre atrophy was also noted in occasional cases. Nearly half of the biopsies were entirely normal. Of the biopsies with fibre type changes, rare cases revealed evidence of myoadenylate deaminase deficiency. At present, there is no consensus on the implications of such a finding except that it has been reported after a viral infection and in association with myalgia [25]. It has also been seen in the muscle biopsy of patients with collagen vascular disease, myasthenia gravis and facioscapulohumeral muscular dystrophy, as well as in the normal subjects [89]. Unlike congenital myoadenylate deaminase deficiency where the enzyme deficiency is total, loss of enzyme in acquired myoadenylate deaminase deficiency is partial. On electron microscopy, muscle biopsies in CFS have revealed, on rare occasions, changes that might be interpreted as minor mitochondrial abnormalities [90]. However, these changes appear non-specific and secondary and in general, muscle pathology in CFS is not the primary or essential part of the clinical syndrome. Comparable changes have been reported in the muscle biopsies of patients with fibromyalgia and in the symptomatic veterans of the first Gulf-War [91]. Finally, significant loss of muscle protein synthetic potential (defined as mean muscle RNA composition - mg RNA/mg DNA) was noted in acute onset CFS cases by one research group [92].

Muscle cell culture (*in vitro*): Aerobic metabolism was studied in the myoblast cultures established from muscle biopsies of 16 typical cases of CFS compared to 10 normal controls. The lactate/pyruvate (L/P) ratio was determined by measurement of lactate and pyruvate production; respiratory chain function, cytochrome oxidase and lactate dehydrogenase were assayed. Culture from 10 of the 16 cases showed mild defects of aerobic metabolism. There was a statistically significant broader range of L/P ratios in the patients' myoblast cultures, compared to controls. Myoblasts from two patients had increased L/P ratios, suggestive of a defect in oxidative phosphorylation while eight had decreased ratios, consistent with a mild deficiency in pyruvate dehydrogenase. Respiratory function, cytochrome oxidase and lactic dehydrogenase activities were similar in two groups. Mitochondrial volume was not significantly increased and no mtDNA rearrangements were present. One of the patients with decreased L/P ratio had biopsy findings of a mild mitochondrial myopathy [93].

Exercise tests: Since by definition, CFS patients experience profound deterioration of their fatigue and muscle symptoms after exercises, a number of studies have looked at the exercise and post-exercise motor performance in these patients, [81, 95-99] often with contradictory results. In an incremental treadmill protocol (walking to exhaustion) [94], CFS patients showed a lower VO_2 max (maximum volume of oxygen consumed) as compared with controls in the absence of any cardiopulmonary abnormality. In another study [95], isometric strength testing revealed conspicuous disparity between the prominent subjective complaint of muscle fatigue by the patients and the relatively normal contractility of their muscles. However, a later study by the same group [96] showed that neither the relative torque produced by

voluntary or electrically stimulated contractions, or the perceived exertion differed significantly between patients and controls. Another group could not demonstrate any difference between patients or controls in exercise performance and fatigability though about a third of CFS cases had an abnormal lactate response and tachycardia [97]. In a study using symptom limited exercise treadmill test with on-line gas analysis and blood sampling found reduced aerobic work capacity in CFS patients as compared to normal subjects and patients with the irritable bowel syndrome [98]. While some studies provided support for a central origin of fatigue, [81,95, 98, 99] others argued for a peripheral, i.e. metabolic muscle cause [92, 96, 97]. Using a repetitive isometric quadriceps exercise (knee extension against fixed resistance) in a well-characterised group of CFS patients and comparing their performance with matched controls, no significant difference was observed between the two groups on the day of exercise itself, a marked and more rapid decline in quadriceps tension was evident in the patient group on the following day (after 24 hours) [100].

Overall, these findings are consistent with the view that mild defects in skeletal muscle metabolism may be present in some CFS patients although presence of these abnormalities are not an obligate part of the disorder. Measurements of oxidative metabolism are not only a function of oxygen delivery to the working muscles, but also of mitochondrial phosphorylation. Early intracellular acidosis during submaximal exercise in the CFS muscles may have several explanations, including accelerated glycolysis relative to oxidative metabolism, deficiency of mitochondrial rich type 1 muscle fibres, impaired autoregulation of muscle blood flow and secondary muscle changes consequent to physical deconditioning induced by chronic fatigue

Research on central fatigue in CFS

Changes in the central nervous system may account for fatigue and neuropsychiatric symptoms in CFS. One of the important observations supporting a dominant role of central fatigue in CFS comes from a study that showed CFS patients were unable to fully activate skeletal muscles during intense sustained exercise despite evidence of normal muscle function [81]. In other studies, patients demonstrated an increased perception of effort during exercise with an exercise-related decrease in central motor drive as measured by the motor evoked potential following transcranial magnetic stimulation [101,102]. Post-exercise facilitation of motor evoked potential was also delayed in CFS, suggestive of depressed cortical excitability [103]

Neuroimaging: Anatomical visualisation of neuroaxis has been always normal in CFS patients. No specific abnormality in the conventional CT and MRI scans is diagnostic of CFS. However, regional cerebral hypoperfusion has been noted in the Single Photon Emission Computed Tomographic (SPECT) scans of CFS patients [104-106] The hypoperfused areas were both cortical and subcortical, typically involving the temporal lobes in one study [104] while hypoperfusion of the brain stem was reported by others [105]. There has not been any consistent pattern but what is clear is that CFS patients, both adults [104] and children [106], may have multiple areas of regional cerebral hypoperfusion that are neither specific nor unifocal [107]. In the absence of any demonstrable ischemic lesions in the CT or MRI scans in any CFS patients, these hypoperfused areas in the SPECT images are likely to be a consequence of metabolic or vasomotor changes influencing regional cerebral blood flow. Positron emission tomographic (PET) scans have also been performed in

patients with CFS and compared to those with major depressive disorder, CFS patients have lower metabolic rates in the hippocampus and amygdala, as well as in the caudate nucleus, the premotor cortex and the anterior cerebellum [108, 109].

Hypothalamus and hypothalamic-pituitary-adrenal (HPA) axis in CFS: Hypothalamus is the seat of autonomic regulation, affecting both parasympathetic and sympathetic nervous system functions in health and diseases. A large body of evidence has been derived implicating the hypothalamus in the control systems which are critically involved in many other physiological, endocrine and behavioural processes. Among these are feeding and drinking, temperature, fatigue, reproduction and the regulation of sleep-wake cycle [110]. The pre-optic region of the hypothalamus is implicated in the neural control of endocrine functions and in the temporal organisation of a wide spectrum of biological rhythms. Hypothalamus also modifies both humoral and cell-mediated immune processes utilising an extensive network of endocrine, autonomic and related pathways [111].

Hypothalamic disorders are known to be associated with clinical symptoms of profound fatigue. Hypothalamic tumours may present with symptoms of fatigue, forgetfulness and irritability [112] but eventually these disorders give rise to a variety of endocrine abnormalities and intracranial pressure symptoms because of their anatomical locations. Overwhelming fatigue is also a common symptom after hypophysectomy. *Symptoms that suggest hypothalamic involvement in CFS* are (a) fatigue; (b) sleep disorders (somnolence and sleep-rhythm reversal); (c) abnormal sweating; (d) altered temperature; (e) poikilothermia (feeling hot and cold); (f) mood disorder; (g) changes in appetite and craving for certain foods; (h) weight changes and

idiopathic cyclic oedema; and (i) frequently reported abnormalities in menstrual function.

Abnormal hypothalamic arginine-vasopressin secretion and water metabolism have been previously documented in CFS. Secretion of arginine-vasopressin (AVP) was erratic in these patients as shown by lack of correlation between serum and urine osmolality and the corresponding plasma AVP levels. When compared with control subjects, patients with CFS as a group had significantly low baseline AVP levels and showed evidence of increased total body water content [113]. An abnormal water loading test in CFS has also been documented [114]. Changes in the hypothalmic-pituitary-adrenal axis in CFS have been confirmed by a number of workers. In the original study by Demirack and others [115], CFS patients were found to have a supersensitivity of cortisol response to exogenous adrenocorticotrophic hormone (ACTH) suggestive of a pituitary deficient hypocortisolic syndrome. All patients had normal levels of circulating ACTH but the evening plasma ACTH levels were higher in patients than controls. The pituitary ACTH release was blunted in response to the exogenous corticotrophin releasing hormone (CRH) in CFS patients [115].

CRH resistance of pituitary ACTH, however, is not unique to CFS since this has been found in related conditions associated with stress such as the overtrained athlete syndrome [70]. Presumably, this reflects a lack of adaptation of the HPA axis to chronic stress and implicates a relative dissociation of the normal harmony between the hypothalamic and pituitary control of endocrine functions. Both *physical stress* (such as operative trauma) and *emotional stress* (such as bereavement) is known to precipitate CFS [59]. Stress directly activates the HPA-axis and modifies the immune

system [116]. Chronic or repeated complex stress can be associated with a loss of diurnal rhythm and a relatively normal circulating concentration of ACTH and glucocorticoids [117]. A paradoxical fall in CRH mRNA in the paraventricular nucleus of the hypothalamus has been described in animal models exposed to chronic inflammatory stress, suggesting chronic stress (as opposed to acute stress) can depress HPA-axis, [118] a finding similar to that observed in CFS.

In summary, the HPA axis is hypoactive in CFS [119] but it is not entirely clear whether this is a primary or a secondary event in the syndrome. Therapeutic trials of corticosteroids (low dose hydrocortisone and/or fludrocortisone) have not been found to be effective in CFS; indeed, higher doses of exogenous steroids may lead to rapid suppression of HPA axis in these patients [120]. There is, however, an emerging view that low levels of tissue cortisol or relative hypocortisolemia may lead to the development of a chronic fatigue state under conditions of stress. An increase in the corticosteroid levels during acute illnesses, physical and psychological stress is a protective biological response that depends on the integrity of the HPA axis. It seems that low cortisol levels predict the development of post-traumatic stress disorder (PTSD) after serious physical and psychological trauma [121]. PTSD is characterised by chronic fatigue, sleep disturbance, poor concentration and depressive mood. In a recent study, patients with sepsis who were randomised to receive corticosteroids did significantly better as compared to the placebo-treated patients in terms of chronic fatigue and other neurobehavioural symptoms following recovery [121]. It may be speculated that a state of pre-existing relative hypocortisolemia sensitises the glucocorticoid receptors at a pivotal locus in the HPA axis and the stress response,

and this may precipitate the development of chronic fatigue after acute illness or serious traumatic life events.

Neurotransmitter abnormalities in CFS: Neuroendocrine axis assessment has traditionally been considered one of the best and safest approaches for assessing specific neurotransmitter functions in the central nervous system [122]. The release of prolactin from the anterior pituitary is under the inhibitory control of dopamine. Prolactin release is stimulated by serotonin and buspirone is an indirect serotonin agonist. Normally, buspirone would induce a serotonin-mediated release of prolactin, which, however, is blunted in major depression [123] An altered sensitivity of the central serotoninergic receptors has been postulated as a possible explanation of the painful symptoms, fibromyalgia and depressive features in CFS [124,125]. Serotonin (5-hydroxytryptamine, 5HT) has a very important role in the vasomotor control and hypothalamic release of CRH [126,127]. In addition, serotonin is an important neurotransmitter in the limbic and paralimbic areas of the brain and is implicated in migraine headache. In one study, basal levels of plasma 5-hydroxyindolacetic acid (5-HIAA), the breakdown product of 5-HT, were elevated in CFS patients as opposed to controls [128].

Buspirone challenge test has been developed as a paradigm to test the sensitivity of the serotonin receptors in CFS. After oral administration of buspirone, a selective $5HT\text{-}_{1A}$ receptor agonist, prolactin level was estimated in CFS patients and compared to the results in patients with major depression. A significant augmentation of prolactin release seen only in CFS, both in male and female patients, suggesting an upregulated hypothalamic serotoninergic receptors in CFS [124]. Plasma prolactin

levels were also higher in response to d-fenfluramine in CFS patients as compared to the depressed patients matched for age, weight, sex and stage of menstrual cycle [125].

The level of the aminergic tone in the central nervous system is also considered to influence the level of central fatigue. Chronic fatigue, comparable to CFS, is common in patients with autonomic failure, multiple system atrophy, Parkinson's disease and other Parkinsonian syndromes. Central stimulants like pemoline, modafinil and methylphenidate have been used to treat symptomatic central fatigue with modest success in multiple sclerosis (MS) and multiple system atrophy. Amantadine, an indirect dopamine agonist, is effective in approximately 40-50% of fatigued MS patients [129]. Downregulation of adrenergic receptors in CFS patients was considered to be a possibility on the basis of the desipramine challenge test [78]. Desipramine is a tricyclic antidepressant and acts by monoamine re-uptake inhibition. It indirectly stimulates the post-synaptic alpha$_2$ adrenoceptor in the anterior pituitary leading to the release of growth hormone (GH). Blunted GH release in response to desipramine challenge was observed in CFS patients but not in the healthy controls. These results may indicate post-synaptic adrenergic receptor subsensitivity in CFS, in keeping with a previous observation of lower levels of plasma 3-methoxy 4-hydroxyphenethyleneglycol (3,4 MHPG) in CFS patients [128]. Anterior pituitary GH release is also augmented by acetylcholine. Pyridostigmine has been used as a standard probe to stimulate acetylcholine-mediated GH release [130]. Both depressed [130] and CFS patients [78] demonstrate an exaggerated GH response to oral pyridostigmine.

In summary, the research evidence in the study of neurotransmitter abnormalities in CFS suggests supersensitivity *to* serotonin and *subsensitivity to monoamines* such as dopamine and norepinephrine. Since dopamine is also the hypothalamic prolactin inhibitory factor, functional deficiency of dopamine in the neural system could lead to relative hyperprolactinaemia as observed in some CFS patients, especially women with amenorrhoea or idiopathic cyclic oedema, and also in patients with post-polio fatigue [131]. From these observations, one may speculate that in CFS, the normal balance of the dopaminergic and serotoninergic tone in the central nervous system is shifted in favour of a functionally overactive serotoninergic state.

Other laboratory findings

General: Routine laboratory tests seldom reveal any specific abnormality in the chronic phase of the illness [88, 132]. Haematology: Relative lymphocytosis, neutropenia, and monocytosis have been reported in small numbers (<30% cases) in some cases. The ESR is generally within normal limits and indeed, any significant elevation of ESR would lead one to suspect an inflammatory muscle disease such as polymyositis as a possible cause of fatigue and myalgia. However, in a review of laboratory abnormalities in CFS, ESR was found to be elevated in 18% of patients [132]. Occasionally, abnormal red blood cell morphology has been observed where the erythrocytes lose their normal appearance as biconcave discs and develop both surface changes and "cup forms", becoming non-discocytic erythrocytes [133].

Biochemistry: Plasma glucose and renal functions are always normal but between 5-20% of patients will have elevated liver enzymes and creatinine kinase in serum,

usually raised to about two or threefold the normal value [134,135]. Deficiency in serum acylcarnitine has been reported in a series from Japan [136]. In a Glasgow study, levels of carnitine (total and free) in both peripheral blood lymphocytes (PBL) and sera were measured in 27 CFS patients and matched healthy controls. Though the serum levels of carnitine were comparable in both groups, the concentrations of total and free carnitine in PBLs were significantly lower in the CFS patients as compared to the healthy controls, suggestive of an intracellular carnitine deficiency [137].

Micronutrient assays: Though one study suggested that patients with CFS suffer from magnesium deficiency [138], in another study, patients were found to have normal red cell magnesium concentration and normal response to magnesium loading test indicating no evidence of magnesium deficiency in CFS [139].

Immunological findings

Non-specific immunological abnormalities have been widely reported in this condition. Tests for heterophile antibody was found to be positive in an average of 15% patients in a pooled data [132]. No difference has been recorded in the serology testing for Lyme disease (B. burgdorferi antibodies) between patients and controls [14].

Autoantibodies: Certain circulating autoantibodies may be found more frequently in CFS patients than in healthy individuals [88,132]. Positive anti-thyroid and anti-reticulin (or antigliadin) antibody are the two common autoantibodies that have been reported in CFS, being present between 10-30% of patients. Low concentrations of

anti-nuclear antibodies and rheumatoid factor could be seen in some cases without other evidence of SLE or rheumatoid arthritis. Studies have also reported occasional presence of antibodies to smooth muscle, gastric parietal cells, insulin and insulin receptors; elevated levels of cryoglobulins, cold-agglutinins and false positive syphilis serology in 0-8% of cases [132,135]. A variety of subtle immunological abnormalities have been reported in CFS [140,141] but these do not appear to be specific or sensitive.

Cellular mechanism of fatigue in CFS:

Nearly 5% of victims of acute ciguatera poisoning develop a chronic disabling syndrome with persistent fatigability and weakness similar to CFS [142]. Ciguatoxins(CTX) are heat stable, non-protein, lipophilic toxins produced by a dinoflagellate (Gamberdiscus toxicus) which is associated with dead coral and algae and can be passed up the food chain to result in sporadic human outbreaks. These toxins bind sodium channel receptors in the open mode in both somatic and autonomic nerves, thus producing prolonged sodium channel activation. The association of ciguatoxicosis and CFS-like syndrome stimulated an interest in the possibility that abnormal ion channel function may underlie CFS symptoms [143]. This hypothesis is considered compatible with the clinical observations in CFS patients that symptoms are worsened by certain drugs, alcohol and local anaesthetics, chemicals that are known to alter cell membrane ion channel function [2]. Although the pathogenesis of CFS is still unknown, one of the most characteristic features of the illness is the fluctuation in symptoms, which can be induced by physical and/or

mental stress. In this respect, CFS bears some resemblance with other neurological conditions caused by abnormal ion channels in the cell membrane [144]. These conditions are known as neurological channelopathies [144] and include both genetically determined channelopathies, e.g. hypokalemic periodic paralysis, familial hemiplegic migraine (FHM), episodic ataxia type 2 and acquired conditions such as neuromyotonia (Isaac's syndrome) and certain forms of epilepsy.

Syndrome X and CFS: Myocarditis, with or without Bornholm-type was a common symptom in an analysis of 1,000 patients with CFS who were seen in Glasgow over the past 20 years [5]. A number of patients who were eventually diagnosed to have CFS had initially experienced a severe chest pain that was considered to be a form of angina or atypical non-cardiac chest pain by the cardiologists. This association between patients who developed CFS and previously had chest pain resembling an acute coronary event was striking. All these patients were initially admitted to the medical units and had had extensive cardiological investigations, including electrocardiogram (ECG), cardiac enzyme assay and coronary angiogram. Other than minor ECG changes, all cardiac investigations were essentially normal or negative. During subsequent clinical follow-up of patients with this type of presentation, termed syndrome X, [145,146] a significant proportion progressed to develop symptoms of chronic fatigue that were indistinguishable from patients who had CFS following a typical viral infection. Patients with cardiological syndrome X closely resembled CFS in their clinical course. Like CFS, syndrome X is more common in women.

Following their extensive research in this condition, Waldenström and his colleagues proposed that there could be abnormal ion channels (ionophores) in the skeletal

muscle and cardiac cells of patients with syndrome X [147]. These ionophores are capable of altering the net transport of ions across the cell membrane. This would eventually force the cell to upregulate its ATPase activity in order to maintain the transmembrane ionic equilibrium. Eventually, this leads to a change of the adenine nucleotide pool inside the cell. Waldenström and colleagues expressed this abnormality by using the equation

$$\text{Energy Charge (EC)} = \frac{ATP + 0.5ADP}{ATP + ADP + AMP}.$$

This ratio, i.e. EC, was very stable when measured from most muscle tissues biopsied and its value is constant at 0.9 (except uterine myometrium) but fell to values between 0.48 and 0.70 in myocardiac and skeletal muscle biopsies in patients with syndrome X and abnormal thallium cardiac scans [147, 148]. Similar findings were observed in mouse myocardium after experimental infection with Coxsackie B3 virus [149]. These findings suggested low intracellular ATP levels consequent to an upregulated ATPase activity in syndrome X.

Thallium cardiac scans in CFS: In their study of the cardiac thallium-^{201} SPECT scans on a series of syndrome X patients, Waldenström and colleagues detected perfusion abnormalities in a significant proportion [148]. Cardiac thallium-^{201} SPECT scanning involves the intravenous injection of radioactive thallium-^{201} which is rapidly accumulated intracellularly in a fashion similar to potassium. The perfusion abnormalities seen in the thallium cardiac scans in syndrome X were not due to any major coronary stenosis because all these patients had angiographically normal coronary systems. Waldenström proposed that the defects in the uptake of thallium-^{201}

could be due to a change in the cell metabolism resulting from abnormal ion leakage from the cells and not due to any microvascular ischemia [148].

Very similar changes in the cardiac thallium-^{201} SPECT scans were observed in a small group of well-characterised CFS patients with or without chest pain [150]. Image analysis revealed moderate perfusion defects in the left ventricles of 7 out of the 10 patients studied [150].

Resting energy expenditure in CFS: Resting energy expenditure (REE) is the energy expended by an awake, alert subject in the post-absorptive state [151]. REE accounts for between 60% and 90% of the total energy expenditure and any increases in REE, in the absence of compensatory increase in diet, should result in there being less energy available for other physical activities.

In a pilot study, REE of female patients with CFS was measured and compared to the values in healthy sedentary controls [152]. REE varies with body size; therefore several body compartments that are potentially important in the prediction of the expected REE were also measured. These compartments were total body potassium (TBK), total body water, extracellular water and intracellular water.

Eleven female patients with CFS were studied together with 11 healthy female control subjects in the same height, weight and age range. Since gross indices of body mass, such as height and weight are relatively poor predictors of the expected REE, TBK was chosen as the predictor of the expected REE in deference to the previous observations [153]. Using *TBK as a predictor of the expected REE*, a significant rise

in REE was observed in 5 of the 11 CFS patients as compared to controls [152]. In theory, it is possible that this transmembrane potassium leak leads to an overactive membrane membrane pump (Na+/K+- ATPase) that balances the transmembrane potassium and sodium fluxes essential to the function of excitable cells. The reduced TBK and marginally low intracellular water values as found in CFS patients in this study [152] support the possibility of "leaky" cell membranes, an assumption originally made to explain hyperbilirubinemia in patients with Gilbert's disease where chronic fatigue is common [154].

Genetics of chronic fatigue

Like any other complex disorder, it is likely that genetic influences may modify clinical expressions of CFS. Several mitochondrial DNA (mtDNA) deletions were reported in a typical case of CFS and common $mtDNA^{4977}$ deletion in two of eight cases was observed [155]. In a classic twin study, concordance rates for CFS-defining idiopathic chronic fatigue were 55% in monozygotic twins and 19% in dizygotic twins. The twin study data are supportive of the familial aggregation of CFS and suggest that genes may play a role in the predisposition to the development of CFS under appropriate environmental trigger.

Summary of CFS research review

In summary, a review of published literature in CFS will clearly draw attention to two major paradigms of this disorder. A broad (and relatively non-specific) paradigm is

based on the psychiatric model and assumes CFS to be a functional somatic disorder and comparable to psychogenic illnesses like globus hystericus. From a neurological perspective, this model cannot be objectively researched because there is no biological template that corresponds to somatisation. The alternative neurobiologic paradigm is based on a more strictly defined population of patients with medically or psychiatrically unexplained chronic fatigue. In this paradigm, fatigue has been attributed to the functional changes in the peripheral, autonomic and /or central nervous system. None of these changes may be specific or sensitive for CFS, but some of the observations on neuroendocrine functions (hypoactive HPA axis and exaggerated prolactin response to oral buspirone) and muscle physiology (subgroup of patients showing delayed post-exertional recovery) have been reproduced by different workers. The psychosocial and neurobiological models of CFS are summarised in Box 1. No doubt the diverse and, at times, contradictory conclusions reached in many of the research publications make it difficult to identify specific pathophysiological mechanisms in CFS.

Box 1. *Chronic fatigue syndrome: current hypotheses*

(1) *The psychiatric hypothesis*: CFS is a functional somatic syndrome and/or an illness behaviour linked to the depressive disorders. In this view, it is held that CFS develops when a psychologically vulnerable individual acquires an infection and perceives himself/herself to be physically ill even after recovery from this disease. The illness is perpetuated by a combination of depression, dysthymia, physical deconditioning, avoidance behaviour for physical activities and somatisation. The relative contributions of the individual components of these perpetuating factors may vary among patients. This hypothesis has been claimed to have been supported by the outcome of the intervention studies of graded exercises and cognitive behaviour therapy in CFS.

(2) *The neurobiologic hypothesis*: CFS is primarily considered to be a disorder of the central nervous system (central fatigue) and the symptomatology reflects the outcome of a complex interaction between the cognitive, physical and neuropsychological changes induced by the triggering disease process (commonly a viral infection, rarely a toxin like ciguatera fish poison). The physical changes may affect the immune system, neuroendocrine regulation, ion channels, autonomic responses and the cerebral perfusion. Studies have consistently shown that patients with CFS do have some of these changes although the relative contributions of the individual components of these changes may differ in patients and at present, there is no sensitive or specific disease marker for CFS.

4.1 OBJECTIVES

CFS is a complex illness the pathogenesis of which is not clearly known. While it is reasonable to assume that like any other chronic medical and psychiatric syndromes, CFS will have both organic and psychosocial aetiologies, identification of reliable and objective disease markers are considered important for several reasons. Firstly, specific biological markers are considered to be the signatures of a medical disease. Depending on the sensitivity and specificity of the marker, a disease can be reliably distinguished from other disorders sharing similar symptoms. Thirdly, biological markers reveal the disease mechanism and may lead to more effective treatment. Finally, biological markers may have prognostic values in the natural history of the disease.

The objectives of the present research were to develop a neurobiological paradigm for CFS and make a search for possible biological markers that may be associated with CFS. Because the level of fatigue in health or in diseases is sensitive to the changes in metabolic functions, this research was focussed on the metabolic changes in patients with CDC-defined CFS. Secondary objectives in this research were sub-categorisation of CFS patients based on reproducible tests and developing new paradigms of neuroimaging that may identify metabolic and functional brain changes in CFS patients.

4.2 AIMS OF RESEARCH

The aims of this research were to answer the following questions:

- Is there any significant alteration of intermediate metabolism in CFS?
- Is there any change of energy utilisation and expenditure in CFS?
- Is there any alteration in the muscle physiology of CFS patients?
- What is the response of CFS patients to standardised, symptom-limited exercises?
- How does the dopaminergic system in CFS respond to external pharmacological challenge?
- Is there any evidence of altered regional brain metabolism in CFS?

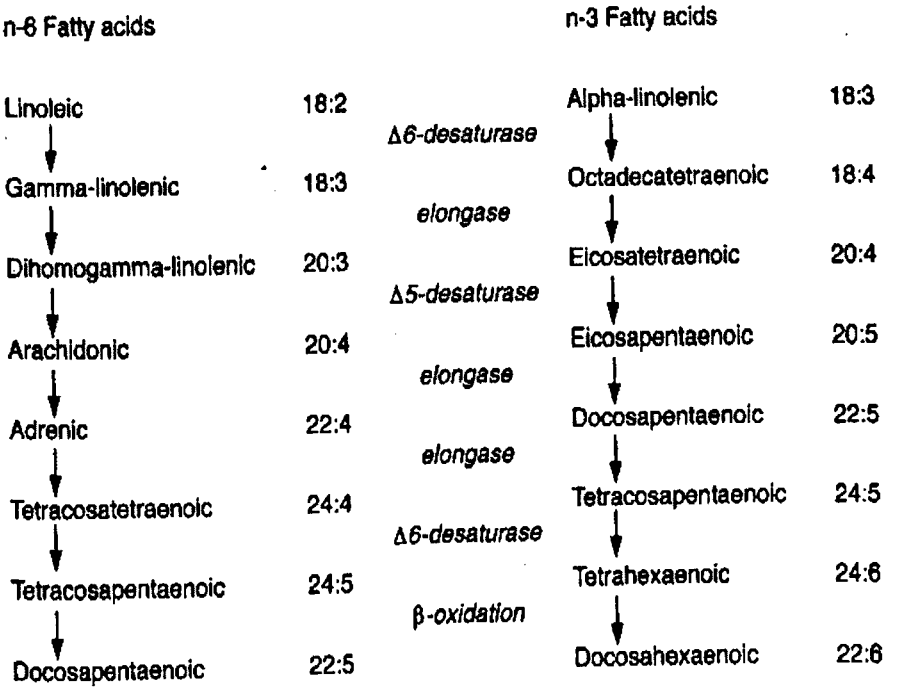
5. EXPERIMENTS

5.1 Blood and urinary metabolic markers in CFS

Background

Symptoms of peripheral and central fatigue are common in metabolic diseases. Fatty acids and other charged single chain lipids are considered to act as messenger molecules that target certain types of K+ channels [157]. After glucose, free fatty acids are the major source of energy in the tissues. Estimates suggest that the free fatty acids supply about 25-50% of the energy requirement in the fasting state [158]. The long chain unsaturated fatty acids of metabolic significance can be divided into non-essential and essential, the only source of the nutritionally essential fatty acid is diet. Palmitoleic acid (C16:1) and oleic acid (C18:1) are non-essential fatty acids. Linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic acid (C20:4) are the essential fatty acids. Other C20 and C22 polyeonic fatty acids are derived from linoleic and linolenic acids by chain elongation [158]. The synthesis of essential fatty acids of the n-6 and n-3 series from the dietary precursors is shown in the adjoining diagram (Flow Chart 1) [159]. Linoleic acid and alpha-linolenic acid constitute the dietary precursors of the n-6 and the n-3 series. Although previous research has not identified specific metabolic disorders in CFS patients, lipid metabolism in CFS has not been studied in any detail. Because liver diseases affect fatty acid metabolism, liver function tests were also performed in these patients.

Flow Chart 1. Summary of the synthesis of essential fatty acids from the dietary precursors, linoleic acid of the n-6 series and α-linolenic acid of the n-3 series. In mass and also in functional terms, arachidonic acid (AA) and docosahexanoic acid (DHA) are the most important fatty acids. Dihomo-γ-linolenic acid and eicosapentaeonoic acid are important for cell-signalling and enzyme regulating molecules [Ref. 159].



Methods

Baseline blood and urine samples were collected in the morning from fulfilling the modified CDC definition of CFS patients after overnight fast. Clear supernatant was separated in each blood sample by centrifugation immediately after collection and was refrigerated at -70° C prior to analysis. 20ml of the voided urinary sample was taken from each patient and was deep frozen until the time of biochemical analysis.

Fasting plasma samples were biochemically analysed for the following liver function markers: glutathione-S-transferase (GST), total protein (TP), albumin (Alb), globulin (Glb), bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartarte aminotransferase (AST), gamma-glutamyl transpeptidase (GGT). Serum creatine kinase (CK), plasma lactic acid (lactate), free and total plasma carnitine were also measured. For evaluating the lipid profile, following biochemical markers were analysed in the fasting venous blood sample: total cholesterol (TC), total triglyceride (TGL), very low density lipoprotein-cholesterol (VLDL-C), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C).

Plasma fatty acids were assayed as follows (Dr. R. Logan, Department of Biochemistry, Yorkhill Hospital). After initial solvent extraction and derivatisation to fatty acid methyl esters, the fatty acids were analysed by gas chromatography and mass spectroscopy. Individual fatty acids were reported as weight percent of the total fatty acids. The individual fatty acids included the following medium and long chain fatty acids: C14:0, C16:0, C:18:0, C18:1, C18:2 (n-6), C18:3(n-3), C20:3 (n-6), C20:4(n-6), C20:5(n-3) and C22:6(n-3).

Urine samples were tested for pH, urinary creatine and urinary orotic acid. Chromatographic analysis were performed for the following urinary metabolites and organic acids: lactate, p-cresol, 3-hydroxybutyrate, acetoacetate, 4-deoxytetronate, cis-aconitate, homovallinate, hippurate, isocitrate and citrate.

Results

Thirty-five CDC-defined CFS patients completed the study. There were 17 men and 18 women. Pooled results of the blood biochemical markers were compared with the standardised and existing reference ranges of the laboratory for the normal population. These data are shown in Tables 5.1.1 and 5.1.2 and graphs (Figures 5.1.1-5.1.6). Liver function tests were found to be normal in the CFS patients. Mean plasma bilirubin was 4.4 μmol/l (range: 1- 17) and no patient had a high level of fasting plasma bilirubin consistent with Gilbert's syndrome. Mean serum creatine kinase was 64.97 i.u./L and all patient values were within normal reference range. Mean free and total plasma carnitine levels were 32.2 μmol/L (patients'range: 16-50; normal range 19-60) and 46.8 μmol/L (patients' range: 20-74; normal range 30-73) respectively. Only one patient had low free plasma carnitine (16 μmol/L) and total plasma carnitine (20 μmol/L).

Table 5.1.1 Blood biochemical profile of CFS patients (n= 35)

Biochemical marker (units)	*Reference range (normal)*	*Range in the CFS patients*	*Mean*	*Median*	*SD*	*SEM*
GST (u/l)	0- 15	1.5- 23	5.1	4.5	5.05	0. 85
TP(g/l)	55-80	55-70	66.3	67	4.72	0.80
Alb(g/l)	35-55	33-48	43.9	44	3.32	0.56
Glb(g/l)	20-35	18-27	22.4	23	3.01	0.51
Bilirubin (μmol/l)	5-17	1-17	4.4	3	3.12	0.53
ALP (u/l)	50-150	84-224	144.9	142	38.50	6.51
AST(u/l)	0- 35	10-47	18.9	17	7.23	1.22
ALT (u/l)	0-35	9-57	21.4	14	14.38	2.43
GGT (u/l)	0-50	7-295	29.7	14	50.94	8.61
CK (u/l)	30-150	20-142	64.9	60	35.84	6.24
Free Carn (μmol/l)	19-60	16-50	32.2	32	8.28	1.40
Total Carn (μmol/l)	30-73	20-74	46.8	46	10.87	1.84
Lactate (mmol/l)	0-2	1-2.6	1.9	1.8	0.50	0.09
TC(mmol/l)	<6.5	2.7-7.3	4.7	4.7	0.98	0.17
TGL(mmol/l)	<3.0	0.5-2.6	1.2	0.9	0.71	0.12
VLDL-C (mmol/l)	<1.2	0.2-1	0.48	0.4	0.34	0.06
LDL-C (mmol/l)	<4.5	1.7-5.6	2.9	2.9	0.82	0.14
HDL-C (mmol/l)	>1.0	0.9-1.9	1.32	1.3	0.26	0.04

Table 5.1.2 Plasma fatty acids in CFS patients (n=35)

Fatty acid expressed as % of total fatty acids	*Reference Range (normative)*	*Range in the CFS patients*	*Mean*	*Median*	*SD*	*SEM*
Myristic (C14:0)	0.67 - 2.05	0.55 - 2.44	1.08	0.89	0.48	0.81
Palmitic (C16:0)	19.10- 27.36	20.55 -27.39	23.99	23.76	1.46	0.25
Palmitoleic (C16:1)	1.09 - 4.05	0.97-7.25	2.34	2.2	1.17	0.20
Stearic (C18:0)	7.53 - 12.59	6.96-10.32	8.08	8.0	0.87	0.36
Oleic (C18:1)	18.01-26.39	20.9-30.46	24.53	24.25	2.14	0.36
Linoleic (C18:2)	21.18-32.96	26.84-37.0	30.99	30.88	4.34	0.73
Linolenic (C18:3)	0.17-0.73	0.001-1.19	0.43	0.34	0.25	0.04
Dihomogamma-linolenic (C20:3)	1.12 - 2.45	0.33- 2.26	1.38	1.35	0.36	0.06
Arachidonic (C20:4)	6.19-10.41	3.64 - 8.3	5.71	5.76	1.18	0.20
Eicosapentaenoic (C20:5)	0.09-0.89	0.001 -1.23	0.41	0.41	0.25	0.04
Docosahexaenoic (C22:6)	1.71-4.05	0.55-2.22	1.32	1.28	0.41	0.07

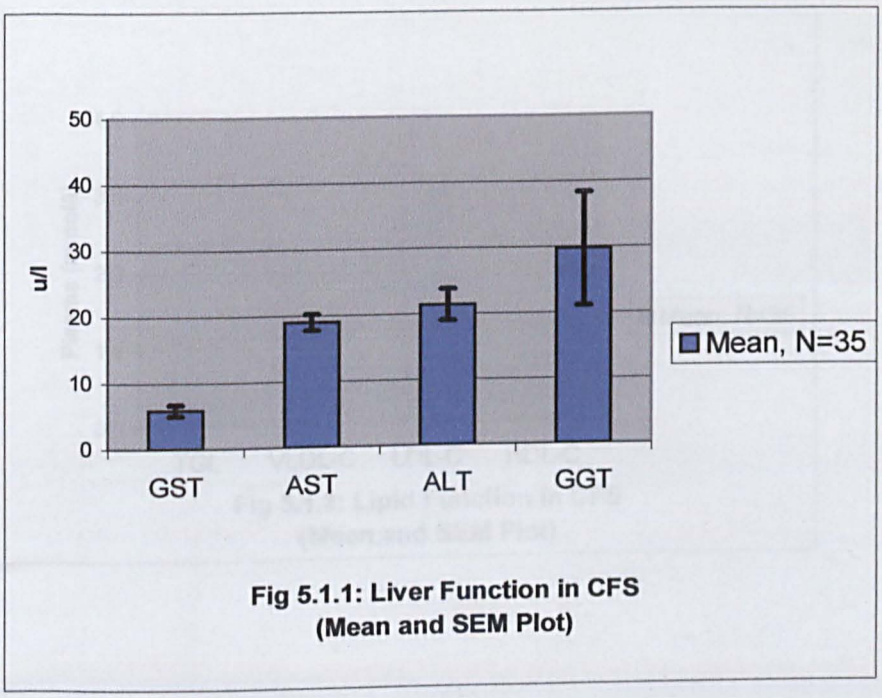


Fig 5.1.1: Liver Function in CFS (Mean and SEM Plot)

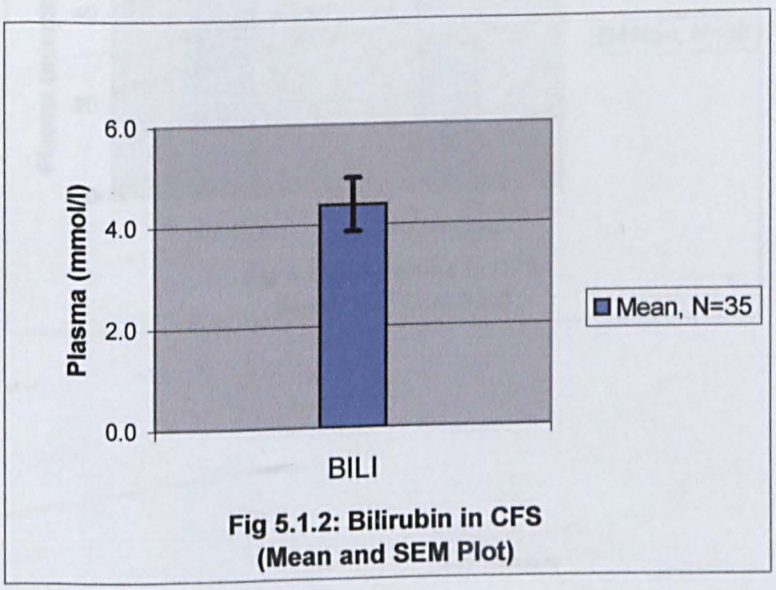


Fig 5.1.2: Bilirubin in CFS (Mean and SEM Plot)

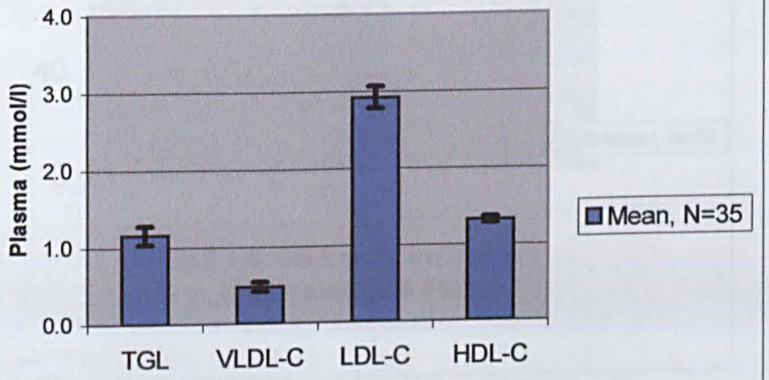


Fig 5.1.3: Lipid Function in CFS (Mean and SEM Plot)

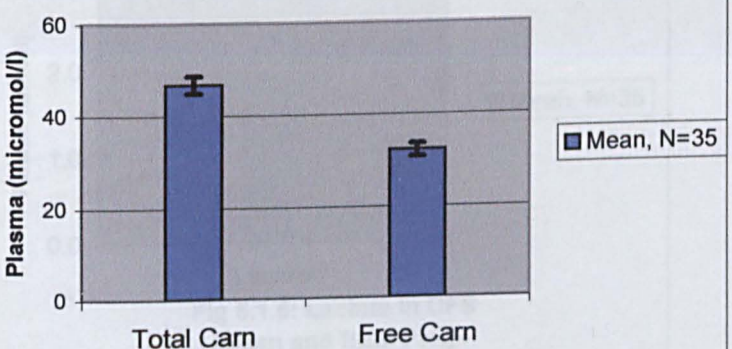


Fig 5.1.4: Carnitine in CFS (Mean and SEM Plot)

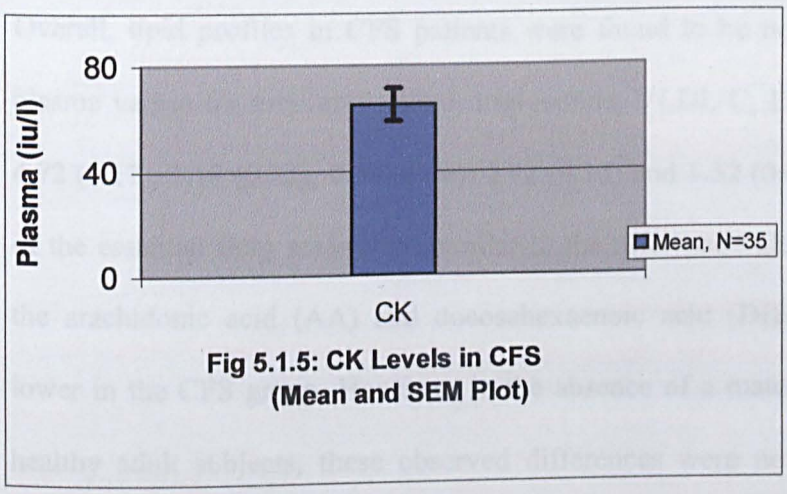


Fig 5.1.5: CK Levels in CFS (Mean and SEM Plot)

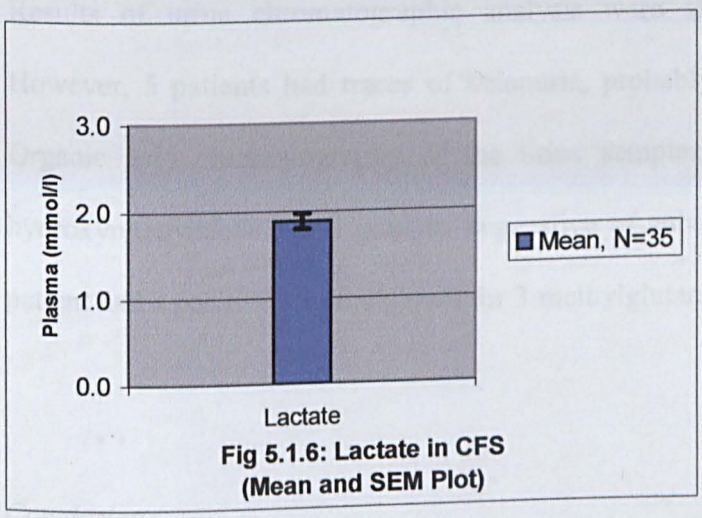


Fig 5.1.6: Lactate in CFS (Mean and SEM Plot)

Overall, lipid profiles in CFS patients were found to be normal. Mean (± S.E.M.) plasma values for total cholesterol, triglyceride, VLDL-C, LDL-C and HDL-C were 4.72 (0.17), 1.12 (0.12), 0.48 (0.06), 2.92 (0.14) and 1.32 (0.04) mmol/L. The ranges of the essential fatty acids were similar to the reference value with the exception of the arachidonic acid (AA) and docosahexaenoic acid (DHA) which were slightly lower in the CFS group. However, in the absence of a matched controlled group of healthy adult subjects, these observed differences were not suitable for statistical analysis.

Results of urine chromatographic analysis were also normal in CFS patients. However, 5 patients had traces of ketonuria, probably due to the starvation effect. Organic acid chromatography of the urine samples showed minor traces of p-hydroxyphenyllactate in 2 patients suggestive of subtle liver cell dysfunction. One patient had a positive chromatogram for 3-methylglutarate and 3-methylgluconate.

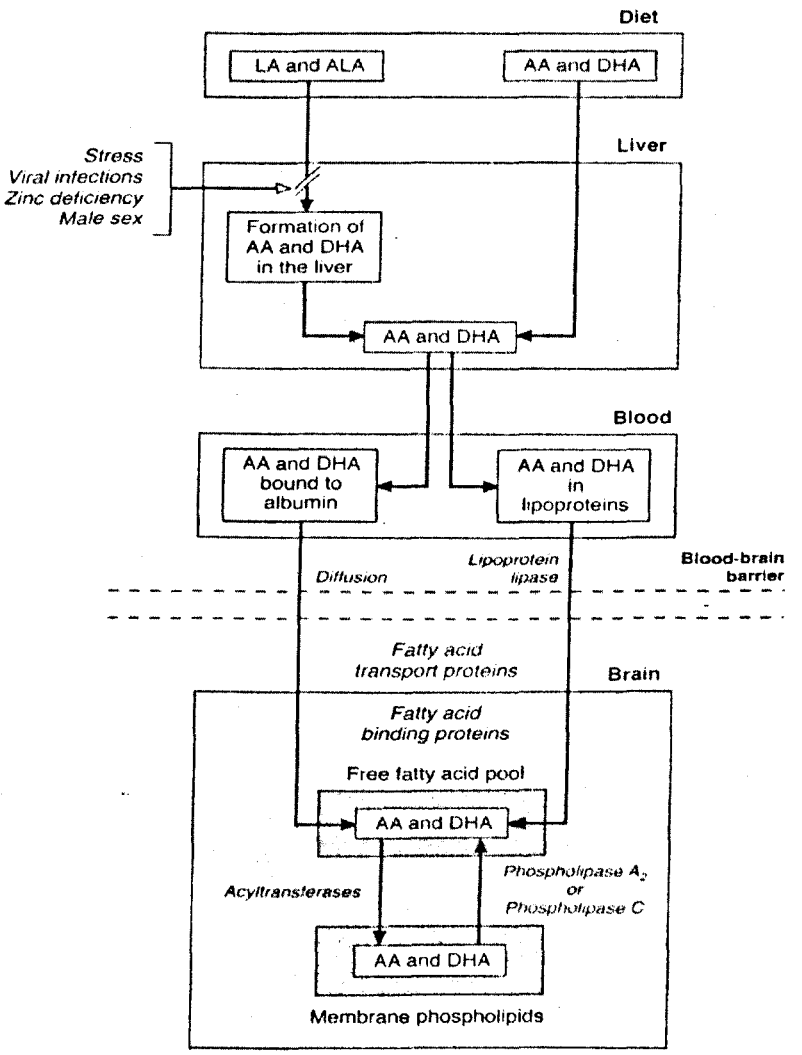
Conclusions

As compared to the established range of normative data in the control population, there were no significant abnormalities in the values for blood and urinary biochemical markers in the study population as a group. Results of minor liver cell dysfunction (e.g. traces of p-phenyllactate in 2 patients) were interpreted to reflect recent use of analgesics (paracetamol). Presence of rare metabolites (3-methylglutarate and 3-methylgluconate) in trace amounts in the urine sample of a single case could not be explained. When excreted at much higher levels, these

metabolites are associated with 3-methylgluataconic aminoaciduria, a disorder caused by the deficiency of 3-methylglutaconyl CoA hydratase in the branched chain amino acid metabolism.

Lipid metabolism was generally normal in the CFS patients. There was a trend towards lower proportions of AA and DHA in the plasma levels of the essential fatty acids in CFS but there was no significance of this observation due to the lack of a control group. Furthermore, plasma levels of fatty acids only reflect recent dietary intake whereas red cell membrane fatty acids are more likely to represent longer term changes related to hepatic synthesis and metabolic handling. In functional terms, however, AA and DHA are the most important fatty acids required for brain phospholipid function (Flow Chart 2). The formation of AA and DHA in the liver is blocked by stress and viral infections, both of which have been implicated in the development of CFS [159].

Flow Chart 2. The routes whereby essential fatty acids require for brain function reach the brain phospholipids [Ref 159].



5.2 Relationship between plasma glucose and insulin in CFS

Background

Maintenance of plasma glucose concentrations within narrow limits is an essential metabolic requirement in life. Glucose is also the most important source of energy. Glucose tolerance test has been used extensively in the diagnosis of diabetes and in patients with impaired glycaemic control. In normal subjects, insulin levels rise with increases in plasma glucose concentration. Conversely, insulin release from the pancreatic beta cells is inhibited when plasma glucose levels fall during the fasting state. In interpreting plasma insulin concentration at a given time point, absolute plasma insulin levels are not considered very helpful and plasma insulin levels are interpreted in the light of a simultaneously determined plasma glucose value. This is because a "normal" plasma insulin level may be abnormal in a hypoglycaemic state whereas a "high" plasma insulin level is appropriate if plasma glucose concentration is elevated. In an attempt to relate these two parameters, the concept of insulin:glucose ratio was developed [160] and was originally expressed as:

$$\textit{insulin}\text{: }\textit{glucose}\text{ (I/G) ratio} = \frac{\text{plasma insulin (microunits/ml)}}{\text{plasma glucose (mg/dl)}} = 0.4 \text{ or less}$$

The ratio (expressed in the conventional units) is usually 0.4 or less. This observation is based on *in vitro* models of pancreatic beta cell function where insulin secretion ceases if plasma glucose concentration falls below 90 mg/dl (5 mmol/L).

Adam, King and Schwartz defined the dynamic relationship between plasma insulin and glucose levels in a study where glucose solution was infused in the normal subjects intravenously at different rates with measurement of plasma insulin concentrations when a steady state had been reached [160]. Based on their data, normal relationship between plasma insulin and plasma glucose is expressed by a linear equation:

$$y = 0.41x - 34$$

where y = plasma insulin in microunits/ml, and x = plasma glucose in mg/dl.

The intercept of the x-axis in this equation for plasma glucose is 83 mg/dl (approximately 4.6 mmol/L) when pancreatic insulin release is expected to cease in normal health.

Methods

An extended glucose tolerance test was performed as part of the metabolic screening in the same cohort of patients as in the previous experiment (5.1). Thirty-five CFS patients M=17, F=18) were given of oral glucose solution (equivalent to 75g glucose) in a condition of overnight fasting. We measured plasma insulin levels simultaneously with plasma glucose every 30 minutes for 2.5 hours. All CFS patients (M=17, F=18) successfully completed the study protocol (extended glucose tolerance test). Blood samples for plasma glucose and plasma insulin were collected from a cannulated arm vein. All samples were flash frozen after plasma separation by centrifugation and thawed samples were assayed biochemically in an autoanalyser at the Department of Biochemistry, Glasgow Royal Infirmary (Dr. C. E. Gray).

Results

Analysis of plasma glucose values in CFS patients for the duration of extended glucose tolerance test revealed no fasting hyperglycaemia or impaired glucose tolerance (defined as venous plasma glucose concentration > or = 11.1 mmol/L at 2 hours and at least on one occasion during the first 2 hours of the test). None of the patients had any symptoms of hypoglycaemia during the test and did not have inappropriately high plasma insulin values at any of the pre-selected time points (baseline, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes).

The relationship between simultaneous plasma glucose and insulin values was found to be essentially linear in the CFS patients but with a different slope. This relationship was best defined by the following regression equation:

$$y = 1.33\,x - 87.75$$

As compared to the normal equation, the intercept of the x-axis in this equation for plasma glucose is 66 mg/dl (3.6 mmol/L).

The statistical analysis of the results is shown in Table 5.2. As compared to the temporal relationship between plasma glucose and insulin after glucose challenge in the normative controls, the observed relationship in the CFS patients was statistically significant. This significance is more relevant given that the individual plasma ratios of insulin: glucose at different time points were always normal (<0.4) in the CFS patients. The regression equation of the insulin : glucose relationship in CFS patients

as compared to the normative equation is plotted in Figure 5.2.1. Figure 5.2.2 shows the mean (±S.E.M.) changes in the plasma insulin levels of CFS patients at different time points.

Conclusions

CFS patients do not have evidence of impaired glucose tolerance, fasting hypoglycaemia or hyperinsulinemia. However, it appears that CFS patients as a group may have pancreatic insulin release occurring at a relatively lower value of plasma glucose (about 1 mmol/L lower than normal). The differential rates of absorption of oral glucose solution in the patients (as compared to the intravenous glucose in the historic controls) might explain the apparent differences in the plasma glucose: plasma insulin relationship seen in this study. An alternative explanation, however, is that the insulin release in CFS patients continues at a lower range of plasma glucose but without any inappropriate hyperinsulinemia.

Table 5.2: Statistical analysis of the plasma insulin: glucose relationship (n=35)

Regression Statistics					
Multiple R	0.907				
R Square	0.823				
Adjusted R Square	0.778				
Standard Error	11.645				
Observations	6				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2515.380	2515.380	18.551	0.013
Residual	4	542.384	135.596		
Total	5	3057.763			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	
Intercept	-87.752	29.929	-2.932	0.043	
Glucose(X)	1.333	0.309	4.307	0.013	

The fitted linear model is: y=-87.752+1.33x. The slope of this model is significantly different from the slope (0.41) of the normative model (p=0.0411 at .05 level of significance, t stat=2.97 is greater than 2.776, the t-critical value with n-2 = 4 degrees of freedom).

Fig 5.2.1:Analysis of Plasma Glucose:Insulin

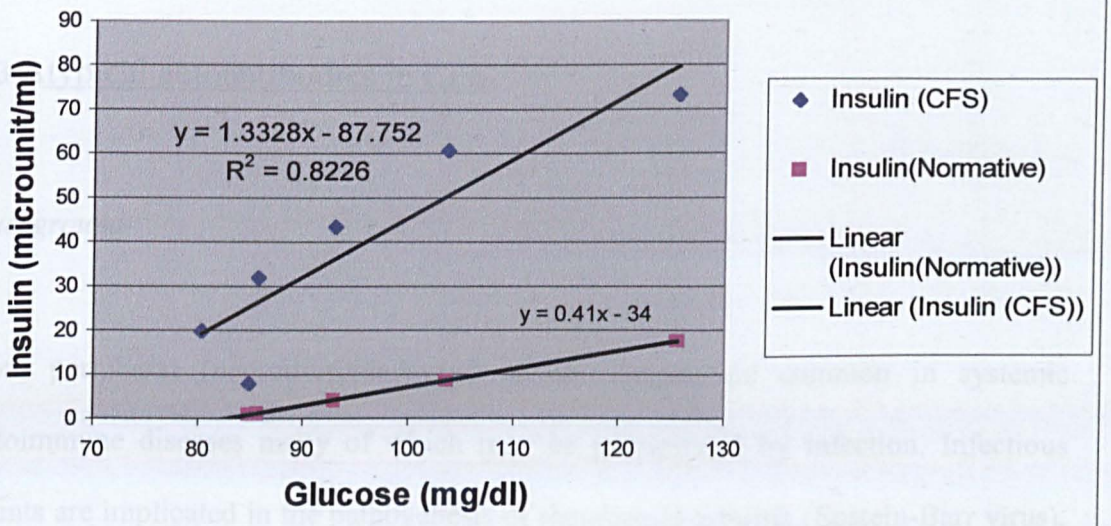
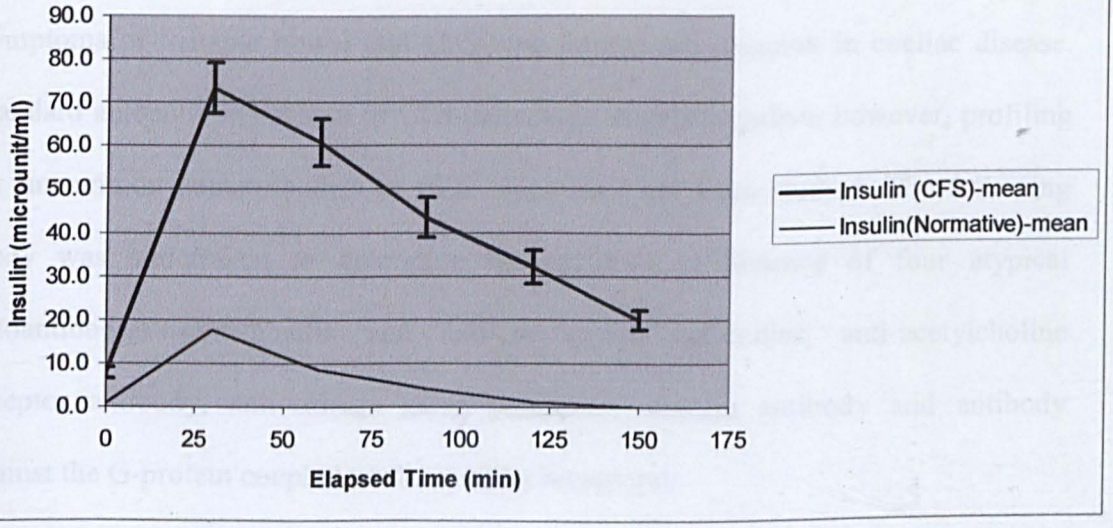


Fig 5.2.2: Change in Mean Insulin Levels (CFS vs Normative)



5.3 Atypical autoantibodies in CFS

Background

Both peripheral (neuromuscular) and central fatigue are common in systemic autoimmune diseases many of which may be precipitated by infection. Infectious agents are implicated in the pathogenesis of rheumatoid arthritis (Epstein-Barr virus), diabetes (Coxsackie B4), and multiple sclerosis (human herpes virus type 6 and measles virus), Guillain-Barré syndrome (Campylobacter jejuni). CFS has been associated with preceding infections due to Epstein-Barr virus, Coxsackie B virus and human herpes virus-6. Many characteristics of CFS are comparable to the symptoms of autoimmune diseases (muscle and multi-joint pain, enlarged lymph nodes, fatigue, cognitive impairment). CFS patients also experience irritable bowel syndrome. Symptoms of irritable bowel and CFS-type fatigue are common in coeliac disease. Standard autoantibody screen in CFS patients is usually negative; however, profiling for uncommon autoantibodies in CFS cases have not been studied. The following study was undertaken to determine the presence or absence of four atypical autoantibodies (anti-gliadin and anti-endomysial antibodies, anti-acetylcholine receptor antibody, anti-voltage gated potassium channel antibody and antibody against the G-protein coupled cardiovascular receptors).

Methods

Serum samples collected from CFS patients were tested for the chosen antibodies: anti-gliadin and anti-endomysial IgA antibody, anti- acetylcholine receptor (AChR) antibody, Anti-voltage gated potassium channel (VGKC) antibody, and antibody against G-protein coupled cardiovascular receptors (adrenergic and muscarinic).

Serum antibody levels were measured in the regional reference laboratories employing in-house technologies. The samples were assayed blindly. The results were interpreted in comparison with the appropriate laboratory negative and positive controls.

Results

Anti-gliadin antibody and anti-endomysial IgA antibody: This was carried out in 33 patients with symptoms of chronic fatigue by using a standardised enzyme linked immunoabsorbent assay (Ms. K. Kingstone, Gastrointestinal laboratory, Western General Hospitals NHS Trust, Edinburgh). There were 27 patients with CFS (M=9, F=18). Six patients had other neurological diseases with chronic fatigue. All 33 patients had negative IgA anti-endomysial antibody and normal levels of antigliadin IgA antibody (<30 units/ml).

Anti-AChR antibody: Serum antibody levels were measured by radioimmunoassay (Mrs. J. Veitch, Neuroimmunology laboratory, South Glasgow University Hospitals NHS Trust). All 17 CFS patients (M=7, F=10) had antibody titres less than 0.1 nmoles/l (negative).

Anti-VGKC antibody: The serum antibody levels were measured by immunoprecipitation of ^{125}I -alpha dendrotoxin labelled VGKC extracted from human foetal cortex. A total of 45 samples were assayed (Prof. A. Vincent, Neuroscience laboratory, Oxford Radcliffe Hospital) from 18 male and 27 female CFS patients. In 4 patients (2 M, 2F), the results were equivocal (antibody level 100-200 pmol/l); when the tests were repeated in three (1M, 2F) the results were negative. It was not possible to repeat the test in the fourth (M). In the remaining 42 samples, the assay for anti-VGKC antibody was negative (titre <100 pmol/l) in each case. Overall, transient/low positivity of serum anti-VGKC antibody was detected in 8.88% (4/45) of the CFS samples affecting both sexes equally (2M, 2 F).

Antibodies against G protein coupled cardiovascular receptors: Using an enzyme-linked immunoabsorbent assay (ELISA) previously described [161], antibodies to the G-protein coupled cardiovascular receptors was measured in the serum samples of 16 patients with CFS (M=8, F=8). The results were compared with 9 control serum samples (Dr. M. Fu, Wallenberg Laboratory, Sahlgren's Hospital, Sweden). Two CFS patients (1M, 1F) were found to have positive antibody levels (12.5%; 2/16).

Conclusions

In this study, we found no evidence of coeliac disease or acquired neuromuscular junction disorder due to autoimmune myasthenia gravis in CFS patients. However, a small minority of CFS patients had positive VGKC antibody and G protein coupled adrenergic receptor antibody. The significance of these low positive antibody titres in CFS is unclear but these antibodies do not appear essential in the pathogenesis of CFS.

5.4 Serum immunossay for ouabain like substance (OLS)

Background

An endogenous, digitalis-like sodium pump inhibitor in plasma has been recently identified. This circulating factor is a specific inhibitor of the human erythrocyte, ouabain-sensitive sodium pump and is called "ouabain-like substance"(OLS). OLS leads to an excessive accumulation of intracellular sodium that has been implicated as the basic pathology in both essential hypertension and depression [162,163].

From a pilot study of the resting energy expenditure (REE), it was concluded that a proportion of CFS patients had increased levels of resting energy expenditure with respect to their total body potassium (REE_{TBK}). It was hypothesised that raised REE_{TBK} might reflect increased energy expenditure to maintain the transmembrane potassium gradient [152]. This gradient is maintained by the membrane sodium pump (Na+/K+ ATPase) and the membrane pump is sensitive to circulating OLS.

Methods

Thirty-eight serum samples were assayed for the OLS level. These samples were collected from 17 CFS patients and 16 neurological patients without chronic fatigue and 5 patients with multiple sclerosis and fatigue. Serum OLS level was measured by in-house radioimmunoassay at the Department of Chemical Pathology, St. Thomas'

Hospital, London (Dr. R. Swaminathan). Annonymised serum samples of healthy blood donors obtained from the Regional Transfusion Service in Glasgow were used to derive the normal reference range for this experiment.

Results

In this radioimmunoassay, the laboratory reference value for mean OLS concentration in healthy individuals was 0.70 ±0.05 nmol/L (mean ± S.E.M). In the CFS patients, the mean OLS was 0.80 nmol/L (range 1.15 - 0.49 nmol/L, S.E.M. 0.07); the corresponding value in the healthy blood donors was 0.73 nmol/L (range 0. 46 - 0.93 nmol/L, S.E.M. 0.05). In 5 patients with multiple sclerosis, the mean OLS level was 0.87 nmol/L (range 1.17 - 0.67 nmol/L, S.E.M. 0.09).

The statistical analysis of these results is shown in Table 5.4. Individual group differences calculated by one-sided ANOVA were not found to be significant for a p value <0.05. Figure 5.4 shows the results of serum OLS in the three groups.

Conclusions

The mean serum OLS levels in CFS patients, healthy controls and other neurological patients were similar and the observed variations were not statistically significant. It is unlikely that CFS symptoms are related to the changes in the serum OLS levels.

Table 5.4: ANOVA -Single factor analysis to test equality of mean serum OLS levels in CFS, NC and MS patients

Anova Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS	17	13.53	0.80	0.08	0.07	
NC	16	11.64	0.73	0.03	0.05	
MS	5	4.37	0.87	0.04	0.09	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.092	2	0.046	0.869	0.428	3.267
Within Groups	1.859	35	0.053			
Total	1.952	37				

The differences among means are not significant, p=0.428

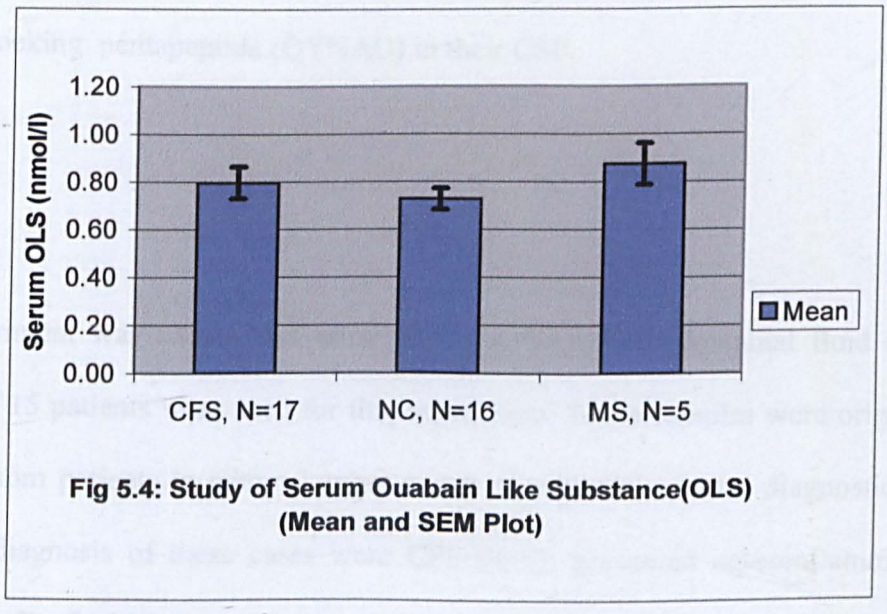


Fig 5.4: Study of Serum Ouabain Like Substance(OLS) (Mean and SEM Plot)

5.5 Cerebrospinal fluid levels of an endogenous pentapeptide (QYNAD)

Background

CFS patients have experienced deterioration of symptoms after local anaesthetics given for minor procedures or surgery [2]. Lignocaine (lidocaine) has been shown to unmask demyelinating symptoms in multiple sclerosis and a local-anaesthetic like factor in the cerebrospinal fluid (CSF) was previously identified in patients with multiple sclerosis and Guillain-Barré syndrome where fatigue is common [164]. This pentapeptide with the sequence Gln-Tyr-Asn-Ala-Asp (QYNAD) exerts its blocking by shifting the steady state inactivation curve of the membrane sodium channels to more negative potentials, like most local anaesthetics [165]. The purpose of the experiment was to determine if CFS patients may have a higher concentration of this sodium blocking pentapeptide (QYNAD) in their CSF.

Methods

This experiment was carried out retrospectively. Stored cerebrospinal fluid (CSF) samples of 15 patients were used for this experiment. These samples were originally collected from patients in whom lumbar puncture was advised as a diagnostic test. The final diagnosis of these cases were CFS (n=5); presumed neuroinflammatory diseases (n=5) of which two were found to have multiple sclerosis, two had isolated spinal cord demyelination (transverse myelitis) and in one case, a diagnosis of post-encephalitic Parkinsonism was made. All five patients in the second group had

positive oligoclonal bands in the CSF indicative of a breakdown in their blood-brain-barrier (BBB). The remaining 5 cases had headache syndromes due to non-inflammatory neurological disorders. Oligoclonal bands were absent in the CSF samples of patients in the first (CFS) and the third (benign headache) groups. The putative pentapeptide (QYNAD) was assayed by the liquid chromatography as previously described in the Department of General Physiology, University of Ulm, Germany (Dr. R. Rudel).

Results

The mean values of micromolar QYNAD in the CSF samples were 8.4 μmol/l in the CSF group (range: 4.8-11.2 μmol/l; S.E.M. 1.39); 8.02 μmol/l in the neuro-inflammatory group (BBB) of neurological patients (range: 4.3-11.2 μmol/l; S.E.M. 1.11) and 6.58 μmol/l (range: 3.9-11.0 μmol/l; S.E.M. 1.42) in patients with benign headache syndrome.

The statistical analysis is shown in Table 5.5. Individual group differences were not found to be statistically significant for $p<0.05$. Figure 5.5 shows the CSF levels of the pentapeptide (QYNAD) in the three groups (CFS, BBB and headache patients). It was of interest that the levels of the pentapeptide did not differ among these patients.

Conclusions

Based on this experiment in a relatively small number of patients, it appears that the cerebrospinal fluid levels of this putative endogenous pentapeptide is not increased in CFS patients as compared to patients with other neurological disorders.

Table 5.5: ANOVA -Single Factor Analysis to test equality of mean values of micro-molar QYNAD in CSF samples of CFS, BBB and headache patients

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS	5	42.0	8.40	9.66	1.39	
BBB	5	40.1	8.02	6.15	1.11	
Headache	5	32.9	6.58	10.05	1.42	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.217	2	4.609	0.535	0.599	3.885
Within Groups	103.416	12	8.618			
Total	112.633	14				

The differences among means are not significant, p=0.599.

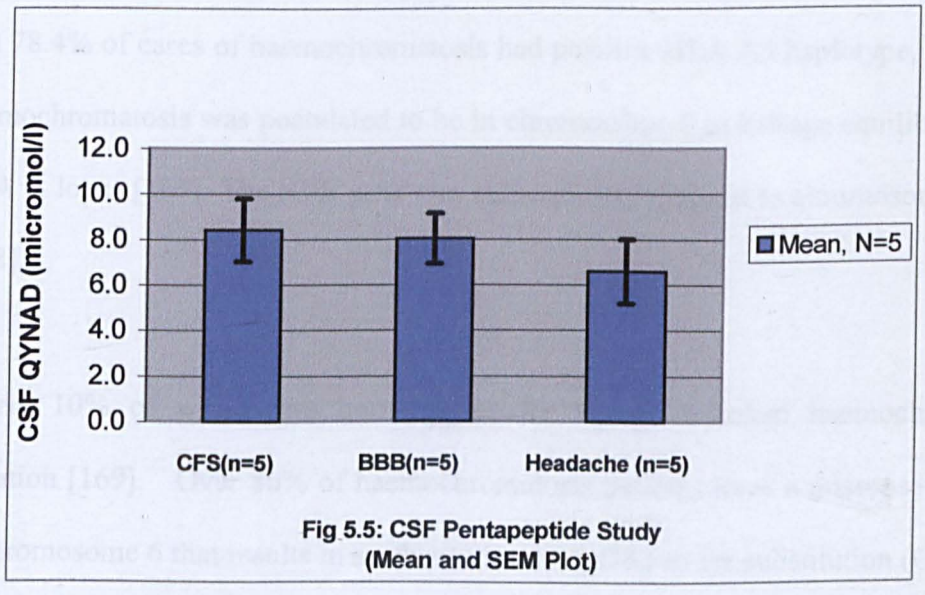


Fig 5.5: CSF Pentapeptide Study (Mean and SEM Plot)

5.6 Screening for familial haemochromatosis gene (HFE) mutation

Background

Haemochromatosis is a metabolic disease characterised by chronic fatigue, joint pain, endocrine insufficiency, diabetes, cutaneous hypermelanosis, diabetes and heart failure. However, at least four different types of disease expression have been associated with hereditary haemochromatosis linked to the haemochromatosis gene (HFE) [166]. Different phenotypic expressions of haemochromatosis are correlated with variabilities in the serum biochemical tests (total body iron stores, transferrin saturation and serum ferritin levels), age of onset and clinical forms. For example, juvenile haemochromatosis as a more severe clinical course characterised by an earlier onset of cardiac symptoms and does not show a male preponderance. Fatigue is a common symptom in all the clinical phenotypes of HFE that is inherited as an autosomal recessive disease with tight linkage to HLA-A. Based on the observation that 78.4% of cases of haemochromatosis had positive HLA A3 haplotype, a gene for haemochromatosis was postulated to be in chromosome 6 in linkage equilibrium with HLA A locus [167]. The HFE gene was subsequently mapped to chromosome 6p21.3 [168].

Nearly 10% of whites are homozygous for the HLA-linked haemochromatosis mutation [169]. Over 80% of haemochromatosis patients have a missense mutation in chromosome 6 that results in a substitution of cys282-to-tyr substitution (C282Y) at nucleotide 845. HFE is relatively common in the Caucasians, affecting more than 1 in 300 individuals of northern European origin. It also has a strong founder effect. In

European populations, the frequency of C282Y mutation is highest (6.88%) among Celtics (Scottish/Irish) and Australian patients have been traced to a common Celtic ancestry. The carrier frequency of this mutation is around 6% in the Northern Europe with the highest frequency reported in Ireland (14%) [168]. Given the fact that fatigue in HFE does not correlate the severity of clinical forms of the disease, we wanted to test if CFS patients may have a higher frequency of HFE mutation.

Methods

Using polymerase chain reaction and restriction-enzyme digestion, peripheral leukocytes from the anticoagulated blood sample of 19 CFS patients (M=10 F=9) were screened for cys-282-tyr (C282Y) mutation (Dr. R. Spooner, Department of Biochemistry, West Glasgow University Hospitals NHS Trust). Additionally, full haematology screen was performed and serum ferritin level was estimated in all cases.

Results

17 CFS patients had normal HFE genotype. 2 CFS patients (M=1, F=1) were heterozygous for the C282Y mutation. Haematological indices were normal in all 19 patients; 3 female CFS patients however had low serum ferritin levels. The carrier frequency of the HFE gene mutation in the CFS patients was found to be 2/19 (10.5%) in this study. This was interpreted as the expected carrier frequency expected in the normal Scottish population.

Conclusions

The frequency of HFE gene mutation in the Scottish CFS patients is not increased as compared to the normal frequency in the population. However, this was a small study and because the number of CFS patients was not large enough, a significant difference might have been missed (Type 2 error). Some female CFS patients may have low serum ferritin levels without the haematological manifestations of iron deficiency anaemia. Iron supplementation may be helpful in such a situation.

5.7 Skeletal muscle histology in CFS

Background

Early onset of fatigue in physical activities and post-exertional malaise are the key defining symptoms of CFS. In muscle physiology, exertional fatigue is considered to be a cardinal feature of impaired oxidative phosphorylation. Abnormalities of muscle oxidative phosphorylation are known to be present in a subgroup of CFS patients. In this study, muscle biopsies from CFS patients were analysed for changes in histochemistry and fibre morphology.

Methods

Under local anaesthesia, three cores of muscle biopsy were obtained from the right or left vastus lateralis muscle from 14 CFS patients (M= 7, F=7). Routine microscopy and ultrastructural studies were performed at the University Department of Pathology, West Glasgow University Hospitals NHS Trust (Prof. W.M.H. Behan).

Results

The male patients were 32-56 years of age with an average duration of symptoms of CFS for 4.5 years (range: 1-14 years). Female CFS patients were between 24-50 years of age with a mean duration of illness of 6.2 years (range: 1-11 years). In one patient,

examination of the biopsied muscle was found to be entirely normal. Minor, non-specific abnormalities were present in the 13 biopsies (M=6, F=7). Mild fibre atrophy was noted in 6 samples (M= 2, F=4). In an additional female patient (age: 24 years, CFS duration: 3 years), fibre atropy was more marked. Fibre atrophy in the muscles was generally paralleled by an increase in muscle lipid. The morphology and distribution of mitochondria, however, were normal; mild increases in mitochondrial number and cristae polymorphism was commented on in 8 patients (M=5, F=3). In ultrastructural studies, myofibrillary damage was seen in 6 biopsies some of which contained lysosomal debries.

Conclusions

Histological changes in the muscle biopsy were non-specific. The fibre type atrophy and lipid accumulation in half of the CFS cases were suggestive of changes due to physical deconditioning. This is likely to be the consequence of the physical inactivity imposed by the persistent CFS symptoms. There was a definite excess of female CFS patients with these changes in the muscle biopsies (F:M = 7:2). Our observations do not support the view that physical deconditioning itself causes CFS symptoms in which case atrophic changes should have been present in nearly every biopsy sample.

5.8 Intramuscular electrolyte distribution in CFS

Background

As an extension of the previous study, muscle biopsy samples from CFS patients were chemically analysed to determine the intramuscular concentrations sodium, potassium and the membrane sodium-potassium pump (Na+/K+-ATPase). The experiment was designed to test whether intramuscular electrolyte composition in CFS might differ from the normal individuals.

Methods

A total of 18 patient samples (M=9, F=9) were used in this experiment, including ten cryopreserved muscle biopsies from CFS cases in the previous study. For comparison, histologically normal muscle biopsy samples from 10 normal subjects (all male) were used as controls. The concentrations of intramuscular sodium (Na+), potassium (K+) and magnesium (Mg++) were analysed in the solution of muscle homogenates extracted with trichloroacetic acid. The concentration of the sodium-potassium pump (Na+/K+-ATPase) was quantified as the total capacity of tritiated (^{3}H)-ouabain binding by using methods previously described [170]. This phase of the study was carried out at the laboratory of the Institute of Physiology, University of Aarhus in Denmark in collaboration with Prof. T. Clausen and his colleagues. The laboratory experimenters were kept blinded to the clinical identities of the biopsy material. No participant had any known electrolyte disorders and none of them were on diuretic

treatment. The intramuscular concentrations of Na+, K+ and Mg++ were expressed as micromol/g of wet muscle tissue and the concentration of ATPase was expressed as picomol/g of wet muscle tissue. It was possible to measure intramuscular Mg++ concentration in 12 and 4 biopsy samples taken from patients and controls respectively.

Results

Mean (±S.E.M.) intramuscular concentrations of Na+ and K+ were 27.6 (1.79) μomol/g and 96.5 (2.42) μmol/g respectively in CFS patients and 30.4 (1.80) μmol/g and 94 (2.80) μomol/g in control subjects. Mean intramuscular concentration of Mg++ was found to be 9.2 μmol/g in CFS patients and 8.9 μmol/g in controls. The mean (±S.E.M) intramuscular concentrations of the membrane pump (Na+/K+ ATPase) were 285.3 (10.7) picomol/g in CFS patients and 306 (15.2) picomol/g in the control subjects.

The statistical analysis is shown in Tables 5.8.1- 5.8.3. The intramuscular concentrations of Na+, K+ and Na+/K+-ATPases in CFS patients and the control subjects are shown in the Figures 5.8.1-5.8.2. No statistically significant difference was noted in the intramuscular concentrations of sodium, potassium, magnesium and membrane pump (Na+/K+- ATPase) between patients and the control groups.

Table 5.8.1: ANOVA -Single Factor Analysis to test equality of means of intramuscular concentrations of sodium in CFS and control subjects.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
Na (CFS)	18	495.88	27.55	57.80	1.79	
Na (Controls)	12	364.99	30.42	38.85	1.80	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	59.179	1	59.179	1.175	0.288	4.196
Within Groups	1409.976	28	50.356			
Total	1469.155	29				

The differences among means are not significant, p=0.288.

Table 5.8.2: ANOVA -Single Factor Analysis to test equality of means of intramuscular concentrations of potassium in CFS and control subjects

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
K (CFS)	18	1737.51	96.53	105.59	2.42	
K (Controls)	12	1127.64	93.97	94.05	2.80	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	47.125	1	47.125	0.466	0.500	4.196
Within Groups	2829.578	28	101.056			
Total	2876.702	29				

The differences among means are not significant, p=0.5.

Table 5.8.3: ANOVA -Single Factor Analysis to test equality of means of intramuscular concentrations of sodium-potassium ATPase in CFS and control subjects

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
ATPase (CFS)	18	5135	285.28	2059.39	10.70	
ATPase (Controls)	12	3672	306.00	2753.82	15.15	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3091.756	1	3091.756	1.326	0.259	4.196
Within Groups	65301.611	28	2332.200			
Total	68393.367	29				

The differences among means are not significant, p=0.259.

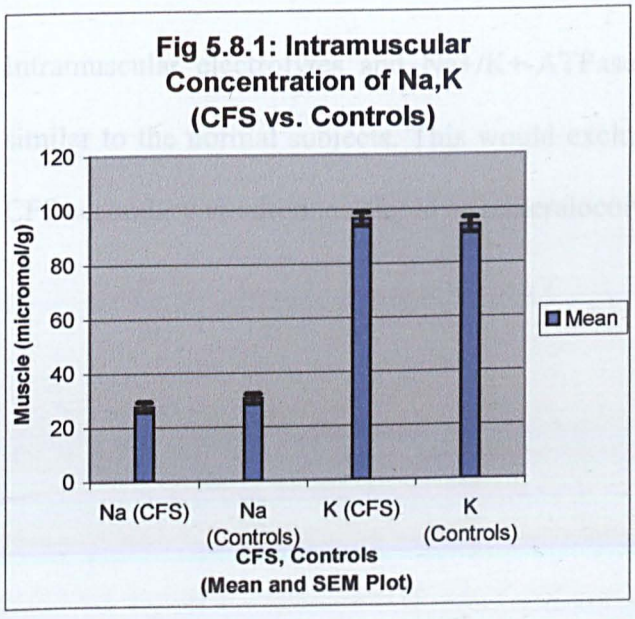


Fig 5.8.1: Intramuscular Concentration of Na,K (CFS vs. Controls)
Muscle (micromol/g)
120
100
80
60
40
20
0
Na (CFS)
Na (Controls)
K (CFS)
K (Controls)
Mean
CFS, Controls
(Mean and SEM Plot)

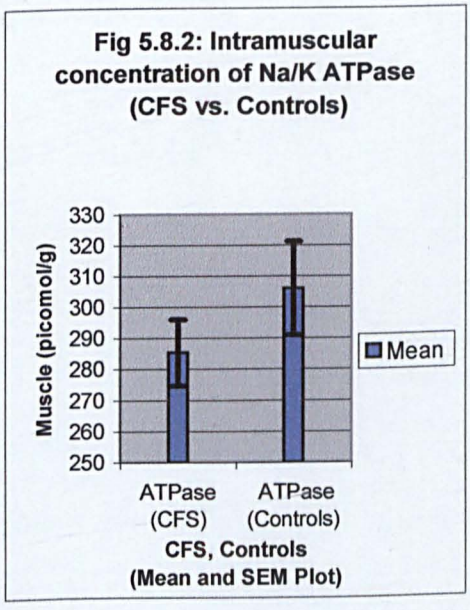


Fig 5.8.2: Intramuscular concentration of Na/K ATPase (CFS vs. Controls)
Muscle (picomol/g)
330
320
310
300
290
280
270
260
250
ATPase (CFS)
ATPase (Controls)
Mean
CFS, Controls
(Mean and SEM Plot)

Conclusions

Intramuscular electrolytes and Na+/K+-ATPase concentration in CFS patients are similar to the normal subjects. This would exclude any muscle metabolic changes in CFS secondary to adrenocorticoid or mineralocorticoid insufficiency.

5.9 A randomised, double blind, placebo controlled trial of dichloroacetate in CFS

Background

A proportion of CFS patients produce excess lactate after incremental exercise in a cycle ergometer to the anaerobic threshold [29]. ^{31}P nuclear magnetic resonance spectroscopy of the exercising muscles in CFS patients have shown evidence of early intracellular acidosis [85,86] and in an *in vitro* study of oxidative metabolism in myoblast cultures established from skeletal muscle biopsies, 10 of the 16 CFS samples showed mild defects of aerobic metabolism as compared to the normal controls [93]. These results are consistent with the view that impaired oxidative muscle metabolism in CFS patients might contribute to exercise intolerance.

Dichloroacetate (DCA) exerts multiple effects on pathways of intermediate metabolism. By stimulating the activity of pyruvate dehydrogenase, DCA facilitates tissue oxidation of lactate [171]. When used in patients with lactic acidosis due to mitochondrial disorders, short term DCA treatment was found to improve indices of cerebral metabolism [172]. In addition, DCA stimulates peripheral glucose utilisation, inhibits gluconeogenesis and improves left ventricular mechanical efficiency under ischemic conditions. The outcomes of both acquired and congenital forms of lactic acidosis are improved by DCA [171].

Because DCA reduces lactate accumulation and improves muscle oxidative metabolism, we wanted to assess the effect of DCA on fatiguing muscle exercise in

CFS. 14 patients participated in the study (M=6, F=8), The age range of the study group was 27-66 years with a mean age of 44.5 years. Only CFS patients with a retained level of functional activity compatible with their ability to participate in the exercise protocol were recruited in this trial that was approved by the local Medical Research Ethics Committee of the Southern General Hospital NHS Trust, Glasgow.

Methods

The design of the study was randomised, double blind and placebo-controlled. Selected patients were randomly assigned to receive either the active drug (DCA) or placebo. All assessors were blind to the randomisation. Patients in the active arm were treated with DCA (25 mg/kg/day). The placebo group received identical gelatine capsules. Both DCA and the gelatine capsules were taken by patients three times daily for 14 days followed by a ten week washout period to remove any residual treatment effects.

The fatiguing exercise consisted of repetitive, sustained contractions in the right quadriceps muscle in a previously established protocol of focal muscle fatiguability [100]. The protocol required repetitive, sustained (10 seconds) contraction of the muscle. Because sustained muscle contractions are known to result in lactic acid accumulation within the muscle, it was felt appropriate to use this protocol to test the therapeutic effect of DCA on muscle fatiguability and overall fatigue in CFS patients.

Muscle fatigue and recovery were assessed at three time points:

i. one day before commencement of DCA (or placebo);

ii. a day after the 14 day treatment; and

iii. finally, after the 10 week washout period to remove any long-term effects of drug therapy.

On each of these occasions, all participants were required to produce 18 maximum voluntary contractions (MVC) of the right quadriceps muscle in succession. This was then followed by single MVCs at 5,10, 15, 20, 35, 50,65, 80,110,140,170 and 200 minutes into the recovery period and further three MVCs after 24 hours. The exercise protocol was administered in collaboration with the Department of Physiothearapy at the Therapy Centre of the Southern General Hospital (Dr. Lorna Paul, Glasgow Caledonian University).

The effect of treatment on overall fatigue was assessed during the follow up visits by using Clinician's Global Impression (CGI) score (Table 5.9.1). A multi-dimensional fatigue scale was also developed (CFS Rating Scale, Appendix I). Patients were randomised to receive DCA or placebo. Both the patients and the investigators were blinded to the randomisation that was carried out by a computer-generated code in the hospital pharmacy. The randomisation code was broken only after all the participants had completed their final assessments.

Table 5.9.1. Clinicians Global Impression (CGI) : 7 point scale

Compared to the patient's condition at the end of the RANDOMISATION VISIT, how much has the patient changed:

- ☐ Very much improved
- ☐ Much improved
- ☐ Minimally improved
- ☐ No change
- ☐ Minimally worse
- ☐ Much worse
- ☐ Very much worse

Table 5.9.2: Participating subjects in the DCA trial :all patients fulfilled modified CDC criteria for the diagnosis of CFS (M=male, F= female)

Patient Number	Sex	Age (years)
1	M	59
2	F	44
3	F	42
4	M	26
5	F	62
6	M	46
7	F	47
8	M	36
9	F	35
10	F	49
11	F	52
12	M	27
13	F	32
14	M	66

Results

14 CFS patients participated in this trial (Table 5.9.2). At baseline, there was no difference between the two arms in terms of the level of physical activity and the duration of CFS symptoms. Testing of the right quadriceps muscle group was undertaken using a KinCom dynamometer (Chatteex Corporation, Chattanooga Group Inc. Huxon, Tennesse, USA). Because randomisation was based on the order of entry of patients into the study and the assessors were blind to the nature of randomisation, the two groups were not matched for sex. Six of the 7 CFS patients in the DCA arm were male; all patients in the placebo arm were female.

The CGI scores remained "unchanged" for all the participants in the study who were able to complete the treatment trial and all the assessments. One patient in the DCA group could not complete the duration of treatment for 14 days as he felt generally unwell and was allowed to withdraw. Two other patients in this group had mild gastrointestinal symptoms (nausea) but were able to complete the protocol. None of the patients in the placebo arm had any adverse symptom attributed to the intervention. Three patients, one in the DCA and two in the placebo arm, did not reach quadriceps muscle fatiguability on the recommended exercise protocol of the experiment. Their exercise data were not included in the analysis. Therefore, the clinical data of this experiment were available from 13 patients whereas completed exercise data on quadriceps muscle fatiguiability were available in 5 patients from each of the DCA and placebo arm for the trial (Table 5.9.2). The two groups did not match for sex but were of similar age and weight.

Table 5.9.3: Randomisation data of the participants

DCA 25/mg/kg daily x 14 days (n=7)			Placebo x 14 days (n =7)		
Sex	Age	Weight (kg)	Sex	Age	Weight (kg)
M[a]	59	64	F	44	68
M	26	52	F	42	50
M	46	82	F[b]	62	81
M	36	76	F	47	56
M	35	65	F	49	59
M[b]	66	92	F[b]	52	70
F	27	66	F	32	51

a = withdrew because of feeling generally unwell in week 1

b = excercise data were not analysed due to failure to fatigue during the exercise period

The maximum voluntary contractions (MVC) of the right quadriceps muscle were more powerful in the treatment arm at baseline and after washout, as would be expected in view of the fact that all males have a larger muscle bulk and therefore were able to generate greater force. However, the rate of decline of force during repetitive contractions (fatigue) and the pattern of recovery after exercise were similar between these two groups. After adjustment for the gender effect on the initial MVCs, no difference was noted in the two groups with respect to the patterns of muscle fatigue and recovery at 14 days and following ten-week washout period. The MVC data of the partcipants who had completed the exercise protocol are shown in Table 5.9.3.

Unpaired t tests were used to compare the mean force (in Newtons) generated by the quadriceps MVC in the two treatment groups after 14 days of therapy. A difference in the p value of > 0.05 was interpreted as non-significant. Despite a higher baseline quadriceps MVC in the male-dominated DCA arm, there was no statistical difference between the two groups after 14 days of treatment in terms of the force generated either during repetitive contractions or after 24 hours (during recovery). This was certainly against any therapeutic effect of DCA in CFS. Indeed, the mean force generated during repetitive contraction of the right quadriceps muscle was statistically lowered in the treatment group after 14 days of DCA therapy as compared to the baseline value (paired t test, $p= 0.01$). However, this difference might not be real as the muscle foce during the recovery period (after 24 hours) in the treatment arm was similar to the baseline value.

Table 5.9.3 MVC data of the participants (mean force in Newtons ± S.E.M.)

Assessment point	DCA group (n =5, 4 M, 1 F)	Placebo group (n=5, 5F, 0 M)
Before treatment:		
repetitive contractions	544.4 (±86.9) N	361.0 (±58.7) N
recovery period	337.3 (±25.3) N	256.5 (±22.5) N
After 14 days treatment		
repetitive contractions	434.4 (±89. 3) N	301.6 (±51.4) N
recovery period	406.2 (±52.0) N	239.5 (± 19.0) N
After washout period		
repetitive contractions	449.0 (±77.0) N	277.2 (±42.8) N
recovery period	322.0 (±44.0) N	257.9 (±27.6) N

Conclusions

We concluded from this randomised, double blind controlled trial that treatment with DCA (25 mg/kg/day) does not improve quadriceps muscle contractility or fatigue in CFS.

5.10 Subanaerobic threshold exercise test, body composition and resting energy expenditure studies in CFS

Background

This study was designed primarily to address the issue of heterogeneity in the CFS population by developing both reproducible and objective data that may sub-categorise patients into specific subgroups. Since fatigue following trivial exertion is a major symptom in CFS, response to a standardised sub-anaerobic threshold exercise test (SATET) [29] was selected as the objective tool to stratify the patient population at baseline. Additional physiological measures such as resting energy expenditure (REE) and body composition studies were applied to further characterise these patients. Total body potassium (TBK) measurement was also included because low TBK was previously reported in CFS [173]. Regional fat-free soft tissue mass (FFST) and body fat mass were measured by dual X-ray energy absorptiometry (DXA), offering the possibility of objectively assessing limb muscle wasting. All results from CFS patients were compared with normal healthy subjects (controls).

The study was supported by a Research Grant from the Cunningham Trust, Scotland (UK) and was carried out in collaboration with the Department of Nuclear Medicine, South Glasgow University Hospitals NHS Trust (Dr. W.S. Watson). The research protocol was approved by the local Medical Research Ethics Committee and informed consent was obtained from all the participants.

Methods

Case selection: 16 CFS females and 13 CFS males took part in this experiment. All CFS patients fulfilled the current CDC criteria and were first assessed clinically to exclude other fatiguing disorders. CFS patients who were clinically depressed were excluded. The control population consisted of a group of physically active, healthy volunteers (15 female controls and 14 male controls). All women subjects (CFS and controls) were studied during the non-menstrual phase of their ovarian cycle. Other than the replacement hormones and contraceptives, the study subjects were free from any medication for 24 hours before their tests.

Baseline assessments: Patients and control subjects had the following tests: haematology (FBC, ESR), blood biochemistry (U&Es, LFTs, TSH), serum C-reactive protein (CRP) and urine analysis (including 24 hour collection for electrolytes, creatinine and nitrogen).

Exercise study: A modified sub-anaerobic threshold exercise test (SATET) on a bicycle ergometer was offered to the CFS patients and control subjects. In this test originally developed by Nashef and Lane [174], the level of exercise was set to achieve 90% of the pre-calculated anaerobic threshold as predicted from body weight, age and gender. Pulmonary gas exchange, respiratory rate, blood pressure and heart rate were continuously monitored for all the exercising subjects. All participating subjects had the option to withdraw at any stage before completing the test. Blood samples were drawn for plasma electrolytes, serum lactate and serum creatine kinase (CK) pre- and post-exercise. A short period of warm-up exercise (for 3 minutes) was

set for all the participants immediately before the 15 minute SATET in order to familiarise them with the equipment. Although the workload was adjusted for the body weight, age and gender of each subject, it was recognised that different degrees of fitness would result in widely different intra-group maximum end-exercise heart rates and end-exercise lactate responses. In order to compensate for differing fitness levels, multiple regression of end-exercise lactate against maximum end-exercise heart rate (expressed as a percentage of predicted age–adjusted maximum and gender (female=0, male =1) was established for the control subjects. An end-exercise lactate was then considered abnormally high only if it was greater than the upper 95% confidence limit predicted for the subject's maximum end-exercise heart rate and gender. In other words, only lactate responses that were inappropriately high for the subject's gender and heart rate response to exercise were considered abnormal. Following the exercise test, participants were asked to score their perceived effort in the scale of 1-10 as originally developed by Borg [175].

Energy expenditure study: Resting energy expenditure (REE) was measured on two consecutive mornings by the indirect calorimetry as previously described [152] using a Sensormedics Vmax 29n (Sensormedics Corporation, USA) or a Deltatrac II (Sensormedics Corporation, USA) calorimeter. The following parameters were recorded for each subject: Body Mass Index (BMI) (kg/m^2), body temperature (°C), O_2 consumption (ml/min) and CO_2 production (ml/min). The participants (patients and volunteers) fasted overnight before this test. REE was expressed as the percentage of the predicted REE derived from the Harris-Benedict equation [176]. All REE measurements were made in the fasting state prior to any exercise or venepuncture. No smoking was permitted prior to these measurements.

Body composition: This was assessed by the following methods:

Dual Energy X-ray Absorptiometry (DXA) for measuring fat mass and regional fat-free soft tissue mass (FFST) [177]. In order to reduce the effect of body size, age and gender from these results, the specific mass for the control subjects, e.g. fat, lower limb FFST was stepwise regressed against height, weight, age and gender. In each case, a predictive equation based on weight and gender was obtained. Results were then expressed as a percentage of the values predicted from these equations. This was done for fat mass, upper limb FFST, lower limb FFST and non-appendicular FFST.

Intravenous tritiated (^3H_2O) water dilution at 4 hours post-injection was measured for *total body water* (TBW) [178] and intravenous stable bromine dilution at 18 hours was measured for *extracellular water* (ECW) as previously described [177].

Total body potassium (TBK) was assessed by whole body counting of the naturally occurring radioisotope (^{40}K) [179]. Intracellular potassium concentration (ICKC) measured indirectly as ICKC ≅ TBK/(TBW-ECW) where (TBW-ECW) = ICW, i.e. intracellular water which contains over 95% of body potassium.

TBW, ECW, ICW and TBK were expressed as percentages of predicted values as previously described for the body water composition [180] and TBK [181]. In this way, differences due to body size, gender and age are eliminated as possibly confounding factors.

Physical Activity Questionnaire: All patients and control subjects were asked to complete a modified Baecke Physical Activity Index for self-assessment of work, sport and home leisure activities [182].

All the assessments in patients and control subjects were carried out over one and a half consecutive working days. During the data analysis, non-parametric statistical tests such as Mann-Whitney U test and Kruskal-Wallis ANOVA (with correction for multiple comparisons by the method of Dunn) were used as appropriate (Unistat v5.0, Unistat Ltd, UK).

Results

CFS patients and control subjects were first subcategorised on the basis of their objective performance in the bicycle ergometer SATET. There were four groups:

Controls (n=29, 14M, 15F):- All of the control subjects completed the 15 minute exercise test and none had a significant end-exercise lactate rise.

CFS patients Group 1 (9, 4M, 5F: 31%):- CFS patients who completed the 15 minute exercise test without any significant end-exercise lactate rise.

CFS patients Group 2 (7, 4M, 3F: 24%):- CFS patients who completed the 15 minute exercise test but had significant end-exercise lactate increase.

CFS patients Group 3 (13, 5M, 8F: 45%):- CFS patients who could not complete the 15 minute exercise test.

The demographic details of these four groups are presented in Table 5.10.1. The distributions of the end-exercise plasma lactate levels are shown in Figure 5.10.1.

Summary of results from the exercise test:

Perceived exertion (Borg Scale): Results are shown in Figure 5.10.2 where it can be seen that CFS patients as a group, had a significantly higher rate of perceived effort as compared to the control subjects ($p<0.001$). There were also significant differences between the control group and CFS Groups 1 and 3 (($p<0.01$) and $p<0.001$ respectively), but not between the control group and CFS Group 2. There were no statistically significant differences between the three CFS sub-groups.

Modified Baecke physical activity index: The results are shown in Figure 5.10.3. There was a significant decrease in physical activity when controls were compared with the total CFS group ($p<0.001$). As with perceived exertion, there were significant differences between the control group and CFS Groups 1 and 3 ($p<0.05$ and $p<0.001$ respectively), but not between the control group and CFS Group 2. There were no significant differences between the CFS sub-groups.

End exercise serum CK: There was no statistically significant difference between the control subjects and the CFS patients in any of the three groups.

End exercise VO_2 (% of predicted VO_2 at anaerobic threshold): The lactate-producing CFS patients (Group 2) had a 20% higher end-exercise VO_2 compared with controls although this failed to reach statistical significance. CFS Group 3, who failed to

complete the exercise, had significantly lower end-exercise VO_2 relative to CFS Group 2 ($p<0.01$; Figure 5.10.4).

Cardiovascular (heart rate and blood pressure) responses during exercise:

The heart rate during exercise was expressed as a percentage of the maximum heart rate, i.e. 220-age(years). As the data was recorded breath-by-breath, in order to examine the heart rate response at fixed time intervals, ie every 15 seconds, the heart rate versus time (breath-by-breath data) was interpolated using a curve-fitting utility [CurveExpert V1.37 (Hyams, USA)]. When response of heart rate with exercise time was inspected for controls, there was a gender difference in the response with control females recording a higher heart rate than control males. In order to eliminate this gender bias, all results at each time interval were expressed as T-scores defined as [Heart rate (%max)-mean Control Heart rate(%max)/ Control Standard Deviation] where the control mean and standard deviation results for males and females were used as appropriate. Any deviation of heart rate response for the CFS groups with time relative to the control group will result in positive T-scores if the group heart rate response is higher than that of the controls while the T-scores will be negative if the heart rate response is subnormal.

From the results shown in Figure 5.10.5, it can be seen that the heart rate response for Group 1 is consistently lower than that for controls ($p<0.01$) while the response for Group 2 is consistently higher than the control response ($p<0.01$). The results for Group 3 are complicated by the fact that the patients stopped exercising at different times during the study. The single most significant finding in Group 3 patients was that the heart rate at the start of the exercise (0-30 seconds) was significantly higher

than normal. There were no significant differences between the three groups in terms of systolic or diastolic blood pressure throughout the exercise period.

Results from the body composition studies:

FFST in limbs (DXA study): Only the CFS patients who could not complete the exercise (Group 3) had a significant decrease of FFST compared to the controls in arms ($p<0.04$) and legs ($p<0.02$); no inter-group difference was noted for the non-appendicular FFST (Figure 5.10.6). In terms of body fat, only Group 3 had significantly increased fat relative to the controls ($p<0.001$) indicative of physical underactivity.

Total body potassium (% predicted): CFS patients have lower TBK as compared to the control subjects. This difference is statistically significant in both Group 2 ($p<0.05$) and Group 3 ($p<0.04$) patients but not in Group 1. However, intracellular potassium concentration (TBK/ICW) was largely similar among all the three groups of CFS patients and the control subjects. No significant inter-group differences were seen for TBW, ECW or ICW.

Resting Energy Expenditure (REE): CFS patients in Group 3 have raised REE residuals as compared with patients in Group 2 ($p<0.03$) but not relative to the controls or Group 1 patients (Figure 5.10.7).

Relative to the controls, Group 3 patients had raised lymphocyte counts ($p< 0.03$).

Figure 5.10.1: End exercise serum lactic acid in controls and CFS patients after SATET

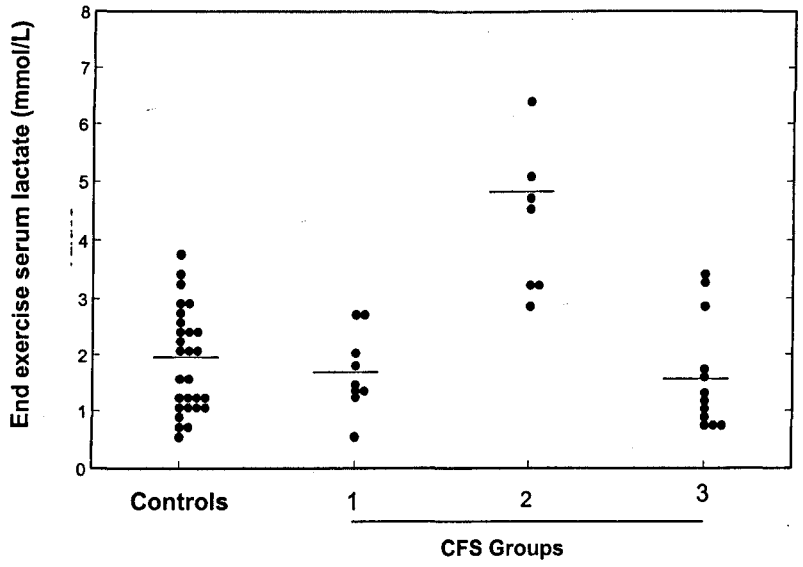


Figure 5.10.2: Borg Scale scores of controls and CFS patients after exercise

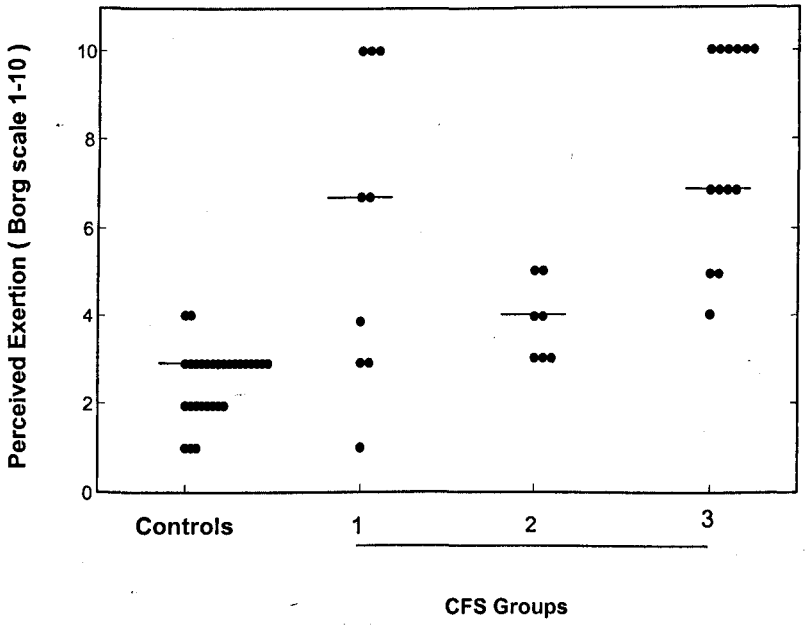


Figure 5.10.3: Baecke Physical Activity Index of controls and CFS patients

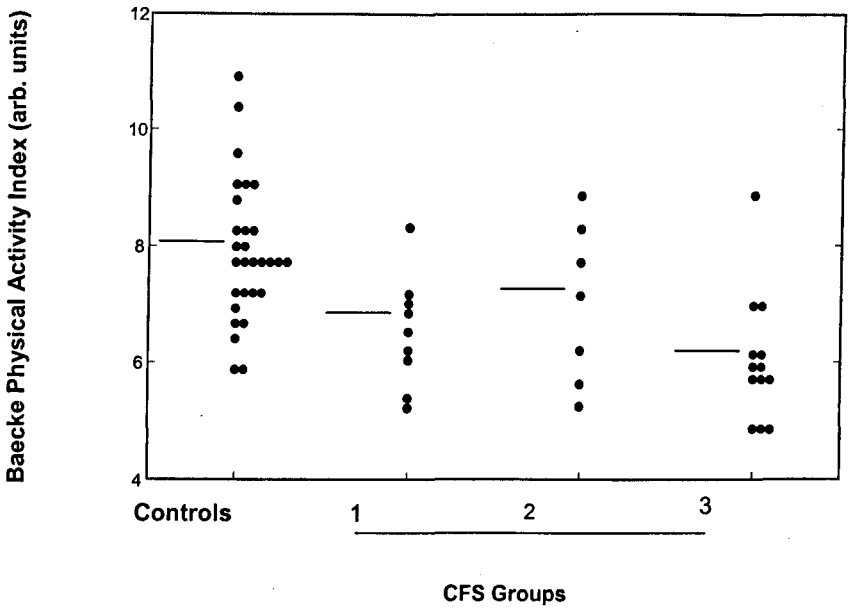


Figure 5.10.4: End-exercise VO_2 as % of predicted anaerobic threshold value in controls and CFS patients

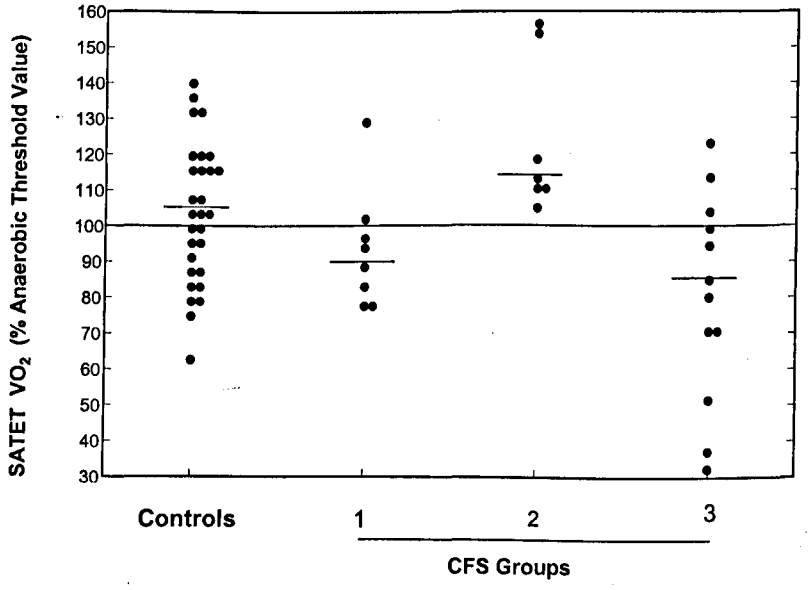


Figure 5.10.5: Heart rate T score in controls and CFS patients during exercise (SATET)

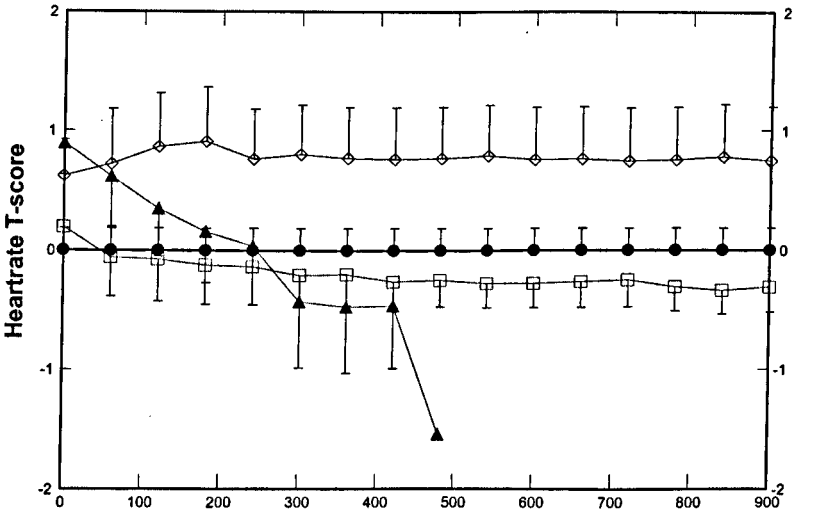


Figure 5.10.6: Lower limb fat free soft-tissue mass in controls and CFS patients

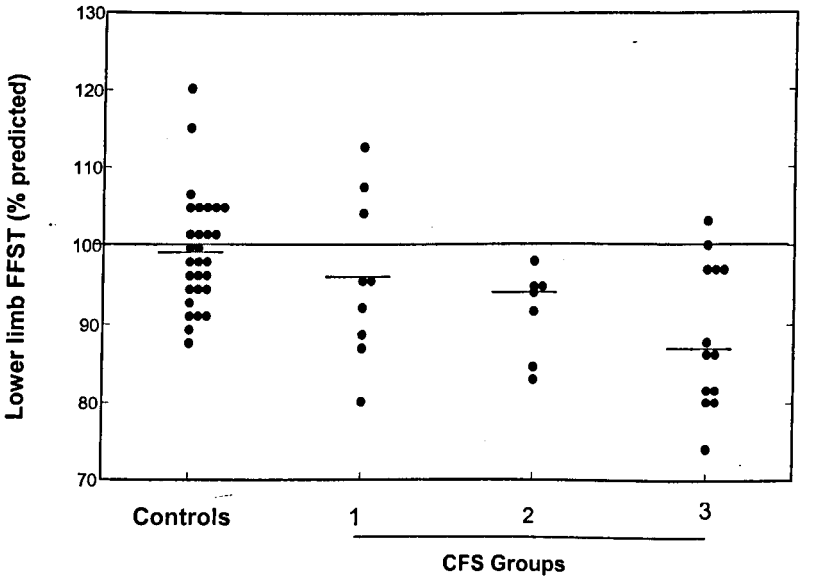


Figure 5.10.7: Resting energy expenditure in controls and CFS patients as% of predicted values

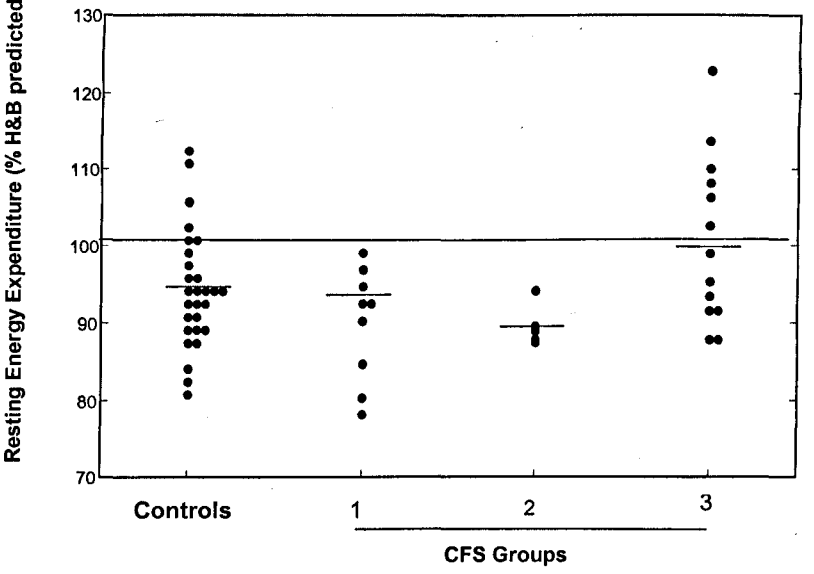


Table 5.10.1: Summary of patients' data

Measures	Controls n=29(14M/15F)		CFS group 1 n=9 (4M/5F)		CFS group 2 n=7 (4M/3F)		CFS group 3 n=13 (5M/8F)	
	Median	Range	Median	Range	Median	Range	Median	Range
Age (y)	29	19 - 59	39	29 - 53	42	25 - 47	43	18 - 60
BMI	23.91	18.76 – 32.35	24.21	18.39 – 37.55	26.78	21.01 – 33.11	24.07	18.40 – 32.93
Borg Perceived Exertion Score (1-10)	3	1 - 4	7	1 - 10	4	3 - 5	7	4 - 10
Baecke Physical Activity (arb units)	7.9	5.9 – 10.9	6.6	5.2 – 8.3	7.2	5.2 – 8.9	6.0	4.9 – 8.9
Workload (watts)	63	38 - 86	61	47 - 77	72	44 - 80	49	0 - 96
End-exercise lactate (mmol/L)	1.84	0.54 – 0.77	1.57	0.54 – 2.72	4.65	2.86 – 6.4	1.35	0.74 – 3.41
End-exercise VO_2(% pred AT VO_2)	105	63 - 140	93	77 - 129	115	105 - 156	86	33 - 123
Arm FFST (% predicted)	101	76 - 132	99	81 - 123	89	79 - 109	88	40 – 121
Leg FFST (% predicted)	99	88 - 120	96	80 - 113	95	83 - 98	87	74 – 103
Non-appendicular FFST (% predicted)	102	86 - 111	98	88 - 120	95	86 – 103	93	86 – 116
Body Fat (% predicted)	101	50 - 163	112	59 - 188	118	105 - 143	120	90 - 158
TBK (% predicted)	101.6	78 – 114	99	86 – 106	88	75 – 100	89	66.4 – 102
Lymphocyte count (10^9/L)	1.8	1.15 – 3.15	1.76	1.25 – 2.5	1.9	1.13 – 2.65	2.88	1.29 – 3.45
REE (% H&B predicted value)	95	81 - 112	93	78 - 99	89	88 - 94	100	88 - 123

Table 5.10.2 Characterisation of the CFS Groups

Characteristics	CFS group 1	CFS group 2	CFS group 3
Percentage	31%	24%	45%
CFS CDC criteria	yes	yes	Yes
DSM-IV criteria for depression	no	no	No
Physical activity (Baecke index)	low	low	Low
Exercise (SATET)	completed	completed	did not finish
End-exercise serum lactate	normal	high	Normal
Perceived effort (Borg score)	high	normal	High
VO_2(L/min)	normal	high	Low
Heart rate (bpm)	normal	high	high (at rest)
Limb FFST (DXA)	normal	normal	Reduced
TBK	normal	low	low
REE	normal	normal	high
Possible pathophysiology	parasympathetic overactivity/limbic -basal ganglia integration failure	reduced blood flow to the exercising muscles	sympathetic (norepinephrine) overactivity
Categorisation	central fatigue	metabolic fatigue	exercise intolerant fatigue

Conclusions

On the basis of their performance in the SATET, CFS patients could be categorised into three distinct subgroups (*central fatigue*, *metabolic fatigue* and *fatigue with exercise intolerance*). Patients in these three subgroups did not differ significantly in the quality of their symptoms, duration of fatigue, physical activity index, quality of life and psychological co-morbidity. (Table 5.12.2) A reduced heart rate response to exercise was typical of CFS patients with central fatigue (31%) while those with presumed metabolic fatigue (24%) produced higher plasma lactate at the end of the exercise protocol (SATET). Exercise intolerant CFS patients (45%) were more likely to be women with an overactive sympathetic system, increased resting energy expenditure and muscle wasting. Autonomic response of CFS patients to symptom-limited exercise is variable. CFS patients had a higher perceived effort to exercise and Group 3 patients were significantly physically deconditioned in the DXA measures.

The specificity and the sensitivity of the proposed classification of the CDC-defined CFS cannot be estimated without a prospective study of a larger number of patients. However, sub-categorising CFS patients with objective physiologic tests like SATET and DXA may be of considerable value to individualise therapeutic interventions and plan future research in this complex disorder.

5.11 Proton magnetic resonance spectroscopy of basal ganglia in CFS

Background

A single study of proton magnetic resonance spectroscopy (^{1}H MRS) in CFS had shown a reduction of N-acetyl aspartate (NAA) in the right hippocampus [183]. Reduction of neuronal NAA has also been reported in the basal ganglia and brainstem among symptomatic Gulf-War veterans who experience symptoms of chronic fatigue [184]. Because central fatigue is postulated to be due to a failure of the integrative striatal function [33], ^{1}H MRS study of the basal ganglia was carried out in 8 CFS patients and the results were compared to a matched group of healthy controls. The objective of the study was to identify possible changes in the basal ganglia metabolites in CFS with special reference to the levels of NAA since a reduced NAA is generally considered to be a marker of neuronal loss or injury. The research protocol was approved by the local Medical Research Ethics Committee.

Methods

Patients and Controls: CFS patients strictly fulfilling the modified Centers for Disease Control (CDC) diagnostic criteria [1] were selected for this study. There were 8 patients (M:F = 1:7) between the ages of 32 and 54 years (median: 42 years) with a duration of CFS symptoms between 2-14 years (median:4.5 years). None of these

patients was depressed. Sex and closely age-matched group of 8 healthy volunteers between the ages of 28 and 59 years (median: 42.5 years) were chosen as controls.

^{1}H MRS: This was performed on a 1.5T Siemens Magnetom using single voxel MRS. Spin-echo (SE) of 1500/135 ms sequence was used and spectra were acquired with (256 averages) and without (1 average) water suppression (Dr. B. Condon, Department of Neuroradiology, Institute of Neurological Sciences) . The volume of interest (VOI) was positioned using three orthogonal T_1 (SE 500/140) weighted scans. It was placed on the left basal ganglia in all the subjects. Its dimensions were 20X40X20 (sagittal, coronal, axial) mm^3. Baseline and phase corrections were applied manually to the Fourier transform of the Fourier induction decay and a single observer (MRI physicist) defined the peak areas without having the knowledge of the participants (patients or controls). Areas of the N-acetyl aspartate (NAA), choline containing (Cho) and creatinine-containing (Cr) compounds were calculated under the curves. The area of the unsuppressed water peak was also calculated as a reference value to which these metabolite areas could be compared. An independent neuroradiologist unaware of the diagnosis of patients or control subjects blindly interpreted the results. He visually examined and scored the Cho peaks in comparison to the Cr peaks using a simple two point scale, namely Cho>Cr, or Cho< = Cr.

Results

^{1}H MRS values of the peak areas for the individual metabolites (absolute values and the ratios) for each of the patients and her/his best-matched control are shown in

Table 9. Percentage differences in the peak ratios of CFS patients compared with controls are depicted in Table 5.11.1. These results showed a low NAA/Cho ratio and high Cho/Cr ratio consistent with a higher Cho peaks in the CFS patients as compared to the healthy subjects matched for age and sex (Figures 5.11.1 and 5.11.2). These values were highly significant statistically when compared to the healthy controls ($p<0.001$). The blinded radiologist visually scored all volunteers as Cho = Cr but only two CFS patients; the other six CFS patients were scored as Cho >Cr, the difference between the two groups being significant at $p<0.001$.

Conclusions

Our observations clearly confirm an underlying neurobiological process in CFS [185]. Although no significant differences were found in the N-acetyl aspartate concentration between patients and the controls, a highly significant increase in the spectra from choline-containing compounds was seen in the CFS patient group ($p<0.001$). In the absence of regional structural or inflammatory pathology, increased basal ganglia choline resonance in CFS is likely to be an indicator of higher cell membrane turnover due to gliosis or altered intramembrane signalling. The exquisite susceptibility of the basal ganglia to the viral infections and inflammatory processes [33] might explain the localisation of the choline peaks in the ^{1}H MRS of the CFS patients.

Table 5.11.1 Unsuppressed water and metabolite peaks in the ^{1}H MRS

(H_2O = water, NAA = N-acetyl aspartate, Cho = choline, Cr = creatine, CFS = patient with chronic fatigue syndrome, HC = healthy control)

Case	H_2O	NAA	Cho	Cr	NAA/ Cr	NAA/ Cho	Cho/ Cr	NAA/ H_2O	Cr/ H_2O	Cho/ H_2O
CFS1	501	0.9	0.41	0.45	2.0	2.195	0.911	1.796	0.898	0.818
HC1	436	0.76	0.28	0.34	2.229	2.717	0.821	1.739	0.779	0.639
CFS2	417	0.69	0.47	0.29	2.379	1.468	1.621	1.655	0.695	1.127
HC2	396	0.76	0.32	0.33	2.296	2.346	0.979	1.919	0.836	0.818
CFS3	554	0.89	0.4	0.37	2.405	2.225	1.081	1.606	0.668	0.722
HC3	614	0.9	0.4	0.44	2.05	2.249	0.911	1.469	0.717	0.653
CFS4	442	0.88	0.5	0.37	2.378	1.76	1.351	1.991	0.837	1.131
HC4	466	0.67	0.31	0.36	1.893	2.154	0.879	1.442	0.762	0.669
CFS5	358	0.58	0.33	0.3	1.933	1.758	1.1	1.62	0.838	0.921
HC5	438	0.82	0.29	0.4	2.025	2.810	0.720	1.868	0.922	0.664
CFS6	488	0.87	0.55	0.45	1.933	1.582	1.222	1.783	0.922	1.127
HC6	266	0.62	0.22	0.3	2.066	2.902	0.712	2.346	1.135	0.808
CFS7	442	0.8	0.43	0.36	2.222	1.860	1.194	1.809	0.814	0.973
HC7	384	0.66	0.27	0.38	1.741	2.464	0.711	1.469	0.717	0.695
CFS8	499	0.66	0.31	0.28	2.357	2.129	1.107	1.323	0.561	0.621
HC8	351	0.67	0.25	0.34	1.988	2.720	0.731	1.915	0.963	0.704

Table 5.11.2 : Percentage difference in the peak ratios of CFS group compared to HC group (ns = not statistically significant)

Peak ratios	% difference	p value
[NAA]/[Cr]	8.1	ns
[NAA]/[Cho]	-26.5	$p<0.001$
[Cho]/[Cr]	48.5	$p<0.001$
[NAA]/[H_2O]	-5.7	ns
[Cr]/[H_2O]	-12.2	ns
[Cho]/[H_2O]	31.7	$p<0.01$

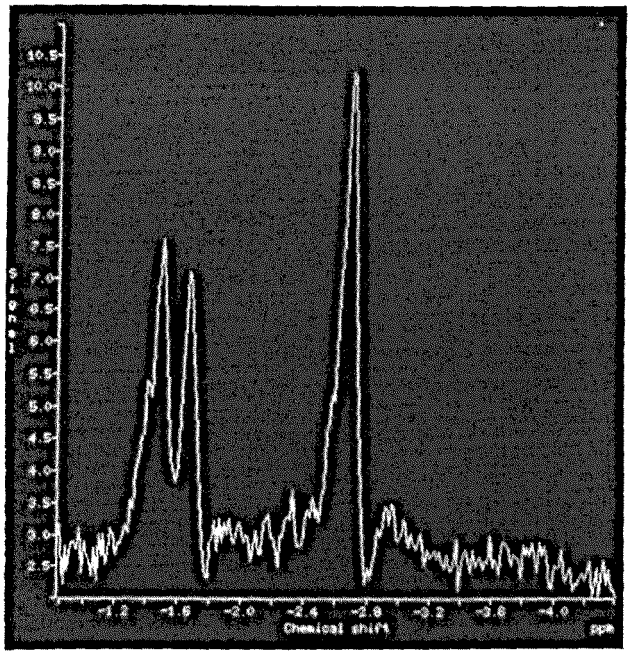


Figure 5.11.1: ^{1}HMRS of left basal ganglia in a CFS patient

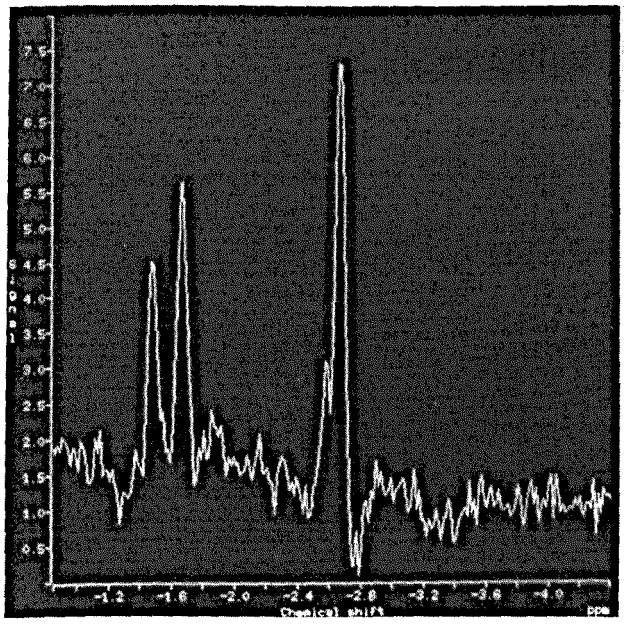


Figure 5.11.2. ^{1}HMRS of left basal ganglia in a healthy control

5.12 Pramipexole induced prolactin suppression in CFS

Background

Pramipexole is a novel, non-ergoline dopamine agonist with specific affinity for dopamine D_2 receptors of D_3 subfamily. By stimulating the D_2 receptors of the lactotrophs, dopamine tonically inhibits prolactin release from the anterior pituitary and is considered to be the hypothalamic prolactin inhibitory factor (PIF). In animal experiments, dopamine has an established role in arousal, self-stimulation and reward seeking behaviours [186]. Pharmacological dopamine blockade is frequently associated with fatigue, poor concentration and lack of motivation. Fatigue is also common in disease states characterised by dopamine deficiency [33]. Few studies have directly addressed the sensitivity of dopamine receptors in CFS patients. However, when compared to controls, CFS patients show exaggerated prolactin response to oral buspirone that principally acts as a serotonin agonist but also has weak dopamine antagonist effect. Higher levels of plasma prolactin have been reported in patients with post-polio fatigue who experience symptoms of fatigue very similar to CFS [131].

This study was designed to evaluate the sensitivity of the dopamine D_2 receptors in CFS by receptor stimulation with pramipexole, a directly acting dopamine agonist. Pramipexole-induced prolactin suppression test was used as a novel probe to test the

sensitivity of the dopamine D_2 receptors. The results in CFS patients were compared with a healthy control population.

Methods

After overnight fasting, 0.25 mg of pramipexole (equivalent to 0.18 mg of Mirapexin) was taken by mouth by the CFS patients and the control subjects. Female patients were matched with control women with respect to their menstrual week in the reproductive cycles. Blood samples were drawn by using an indwelling cannula inserted at an arm vein immediately prior to the oral administration of pramipexole. Blood samples were collected in the lithium heparin tubes. Samples were drawn at baseline (0 minute) and then for every 60 minutes for first 4 hours (at 60 minute, 120 minute, 180 minute and 240 minute). All the patients and the control subjects were rested during the period of the test while kept under observation in the medical ward. None of the patients or control subjects was taking any pro-dopaminergic or dopamine-antagonist medications. .

All blood samples were collected in lithium heparinised tubes. Plasma was separated by centrifugation and stored at -70°C until biochemical tests were arranged. Thawed plasma samples were transported to the Department of Biochemistry, Southern General Hospital, for measurement of plasma prolactin by standard radioimmunometric assay.

Results

The patient group consisted of 9 patients (M=5, F=4) in the age range of 26-56 years; the control group consisted of 9 healthy volunteers (M=5, F=4) in the similar age group. Baseline plasma prolactin levels showed no significant difference between patients and controls. Mean (±S.E.M) plama prolactin was 151.78 (15.49) mU/L in patients and 148.89 (22.21) mU/L in controls. In both groups, plasma prolactin fell from baseline values at 60 minutes and was maximally suppressed between 60-120 minutes. The lowest mean (±S.E.M) plasma prolactin in CFS patients was 58.3 (6.99) mU/L at 120 minutes and the prolactin level remained suppressed at 180 minutes. In the control subjects, lowest mean (±S.E.M) plasma prolactin was 59.89 (9.53) mU/L at 180 minutes. No patient reported any adverse symptom during the test. The statistical analysis of the results and the graphs are shown in Tables 5.12.1-5.12.4 and Figures 5.12.a and 5.12.b.

Conclusions

The results of this preliminary study of pramipexole-induced prolactin suppression indicate that as a group, CFS patients do not significantly differ from the healthy controls in their sensitivity to dopaminergic (D_2) receptor stimulation as compared to the healthy controls.

Table 5.12.1: ANOVA -Single Factor Analysis to test equality of mean values of CFS and control subjects at 0 minutes in pramipexole-induced prolactin suppression study.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS, 0 min	9	1366.0	151.78	2158.44	15.49	
Controls, 0 min	9	1340.0	148.89	4441.36	22.21	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	37.556	1	37.556	0.011	0.916	4.494
Within Groups	52798.444	16	3299.903			
Total	52836	17				

The differences among means are not significant at 0 minutes, p=0.916.

Table 5.12.2: ANOVA -Single Factor Analysis to test equality of mean values of CFS and control subjects at 60 minutes in pramipexole-induced prolactin suppression study.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS, 60 min	9	849	94.33	812.75	9.50	
Controls, 60 min	9	807	89.67	2330.50	16.09	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98	1	98.000	0.062	0.806	4.494
Within Groups	25146	16	1571.625			
Total	25244	17				

The differences among means are not significant at 60 minutes, p=0.806.

Table 5.12.3: ANOVA -Single Factor Analysis to test equality of mean values of CFS and control subjects at 120 minutes in pramipexole-induced prolactin suppression study.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS, 120 min	9	525.00	58.33	439.50	6.99	
Controls, 120 min	9	567.00	63.00	1243.00	11.75	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98	1	98.000	0.116	0.737	4.494
Within Groups	13460	16	841.250			
Total	13558	17				

The differences among means are not significant at 120 minutes, p=0.737.

Table 5.12.4: ANOVA -Single Factor Analysis to test equality of mean values of CFS and control subjects at 180 minutes in pramipexole-induced prolactin suppression study.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*	*SEM*	
CFS, 180 min	9	541	60.11	1075.11	10.93	
Controls,180 min	9	539	59.89	817.61	9.53	
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.222	1	0.222	0.000	0.988	4.494
Within Groups	15141.778	16	946.361			
Total	15142	17				

The differences among means are not significant at 180 minutes, p=0.988.

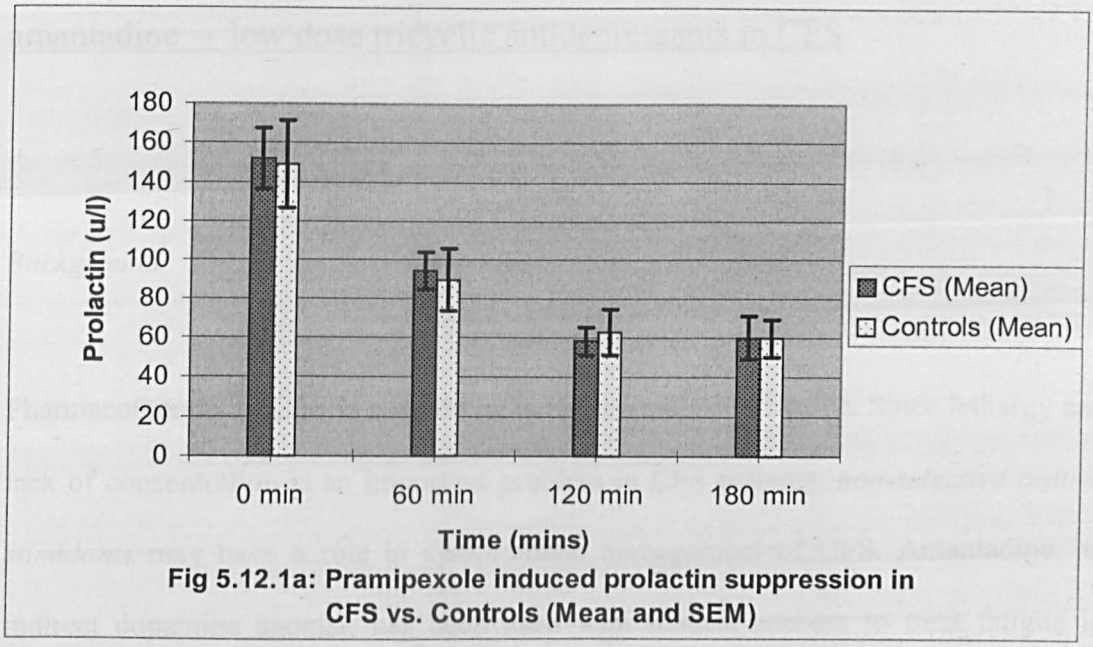


Fig 5.12.1a: Pramipexole induced prolactin suppression in CFS vs. Controls (Mean and SEM)

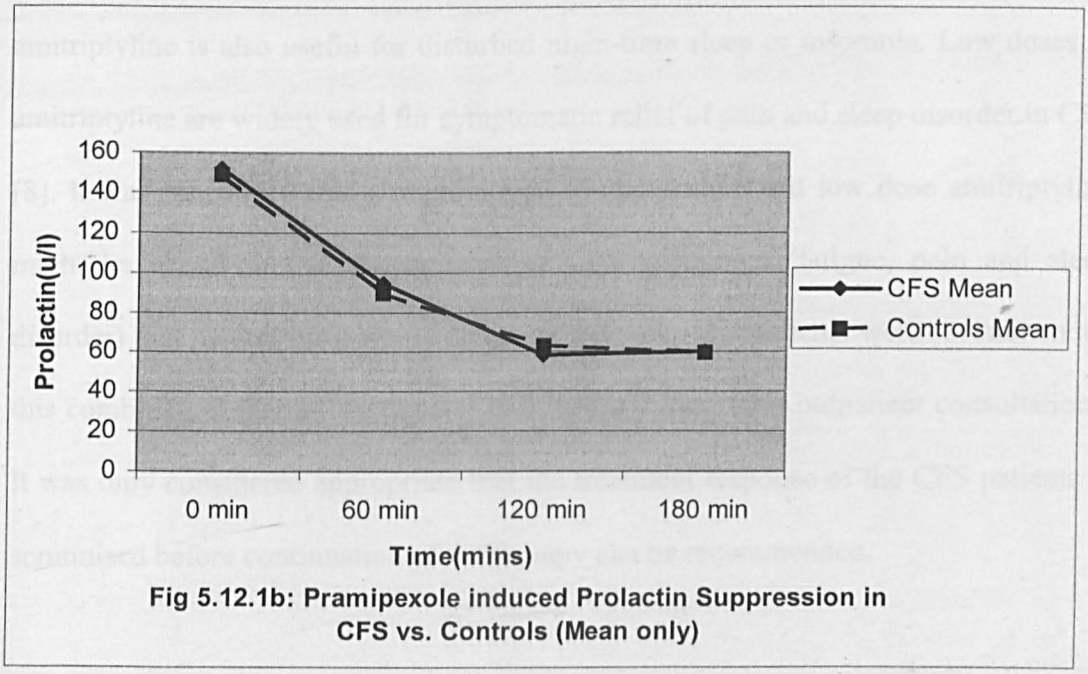


Fig 5.12.1b: Pramipexole induced Prolactin Suppression in CFS vs. Controls (Mean only)

5.13 A retrospective clinical analysis of pharmacotherapy with amantadine ± low dose tricyclic antidepressants in CFS

Background

Pharmacotherapy in CFS is considered to be generally ineffective. Since lethargy and lack of concentration is an important problem in CFS patients, *non-selective central stimulants* may have a role in symptomatic management of CFS. Amantadine, an indirect dopamine agonist, has been used with modest success to treat fatigue in multiple sclerosis [129]. Amitriptyline, a tricyclic antidepressant, is widely used in neurological practice for migraine prophylaxis and pain relief. The sedative effect of amitriptyline is also useful for disturbed night-time sleep or insomnia. Low doses of amitriptyline are widely used for symptomatic relief of pain and sleep disorder in CFS [8]. It was speculated that a combination of amantadine and low dose amitriptyline might be useful in the management of CFS symptoms (fatigue, pain and sleep disorder) that neither drug would achieve on its own and patients were recommended this combination therapy on clinical indications during their outpatient consultations. It was only considered appropriate that the treatment response of the CFS patients is scrutinised before continuation of the therapy can be recommended.

Methods

Twenty-one CFS patients (M=10, F=11) were included in a retrospective analysis of amantadine and amitriptyline therapy in CFS patients. Amantadine on its own was given to 6 patients (M=2, F=4). Amantadine and amitriptyline was prescribed to 13 patients; 2 patients (M=1, F=1) were treated with a combination of amantadine and another tricyclic antideprssant (venlafaxine). In all cases, amantadine was commenced in a once daily dose of 100 mg and amitriptyline was given at bed-time in a single dose of 10 mg. The doses were increased to a maximum of 200 mg and 20 mg respectively between the third and fourth weeks of the trial period. Dose of venlafaxine was 37.5 mg twice daily (unchanged dose).

Results

The retrospective assessment was carried out between 6-8 weeks after the therapy was initiated. The principal outcome measure was a self-reported change in the severity of fatigue. Patients were asked whether their fatigue symptom had "*improved*", showed "*no change*" or "*worsened*". Improvement in fatigue was reported by 3 patients (M=2, F=1), one of whom was only on amantadine monotherapy. Fatigue was reported as unchanged in 7 patients (M=3, F=4) on a combination of amantadine and amitriptyline though one patient reported an improvement in her musculoskeletal symptoms. Two patients were unsure about the outcome, one of whom felt that his sleep and painful symptoms were better. Four patients (M=2, F=2) felt that their fatigue symptoms had worsened on treatment (one was only on amantadine

monotherapy). Two patients did not take the treatment and three other patients declined to rate their treatment response.

Conclusions

Retrospective analysis of pharmacotherapy amantadine with or without amitriptyline (venlafaxine in one case) did not provide sufficient evidence of any short-term treatment effect on fatigue in the CDC-defined CFS population.

5.14 Buspirone-augmented ^{123}I-IBZM cerebral SPECT scan

Background

Buspirone is a serotonin agoinst (5 HT_{1A}) with very weak dopamine D_2 antagonist property. Previous studies have consistently shown that when compared to normal healthy controls or depressed individuals, buspirone-induced release of prolactin is exaggerated in CFS patients (both in men and women). The mechanism of hyperprolactinemic response to buspirone in CFS is not clear [124,125]. Dopamine is the hypothalamic prolactin inhibitory factor and it tonically inhibits prolactin release from the anterior pituitary. An exaggerated prolactin response to buspirone may indicate a reduced dopaminergic tone in CFS. Since prolactin release is considered to be a direct function of the dopamine D_2 receptors, it is possible that CFS patients may have a reduction in the distribution or density of the dopamine D_2 receptors.

Radioiodine (^{123}I) tagged iodobenzamide (^{123}I-IBZM) cerebral single photon emission computed tomography (SPECT) is an established neuroimaging tool for assessing dopamine D_2 receptor function in the basal ganglia [187]. ^{123}I-IBZM binding to basal ganglia is a reliable indicator of free (i.e. unoccupied) and functional postsynaptic dopamine D_2 receptors. Neuroleptics induce a lower binding of ^{123}I-IBZM to the basal ganglia due to its higher affinity for postsynaptic dopamine D_2 receptor occupancy [188]. Because buspirone stimulation in CFS patients of both sexes produces an exaggerated prolactin release response [124], it was considered possible that buspirone pre-treatment modifies post-synaptic D_2 receptor function in CFS patients in a way different from the controls.

This experiment was based on the hypothesis that CFS patients are more sensitive to the dopamine D_2 receptor blocking effect of buspirone and show an exaggerated prolactin response as a result. We sought to measure D_2 receptor occupancy in CFS patients and control subjects of both sexes by ^{123}I-IBZM cerebral SPECT scan after a single oral dose of buspirone (45 mg). The study protocol was approved by the local Medical Research Ethics Committee and Administration of Radioactive Substances Advisory Committee (ARSAC), Oxon, UK. Informed consent was obtained from all participants before proceeding with this study.

Methods

All patients fulfilled the CDC definition of CFS. For all female participants (patients as well as controls) of child- bearing age, urinary pregnancy test was performed and the study was not undertaken during the pre-menstrual and menstrual phases of their reproductive cycles. Participants were free from all medications known to interfere with dopamine receptor sensitivity for at least one week prior to the study. Persons with known sensitivities to buspirone, iodine and/or potassium iodide were excluded.

After a light breakfast, all participating patients and control subjects were given 45 mg of oral buspirone by mouth in the late morning. A thyroid-blocking dose of potassium iodide (120 mg) was also administered by mouth simultaneously to prevent any irradiating effect of the radioactive iodine in the tracer (^{123}I-IBZM) on the thyroid gland. One hour later, a standard dose of ^{123}I-IBZM (approximate radiation dose of

110Mbq) was injected intravenously. Cerebral SPECT imaging was performed with a dedicated Phillips head scanner linked to a computer system for image processing and analysis. Image acquisition was performed at 60 minutes and 120 minutes after intravenous injections of ^{123}I-IBZM according to a standardised protocol [189]. The images were analysed in collaboration with Dr. J. Patterson (Department of Neuroradiology, Institute of Neurological Sciences).

Attenuation correction and reconstruction of the images were performed prior to the analysis of ^{123}I-IBZM binding. In each case, the ratio of striatal to non-specific binding was calculated in the right and left striatum and for anterior as well as posterior striatum on each side. Based on the standard region of interest template of basal ganglia, binding was also calculated for right and left basal ganglia. Mean uptake ratio values were calculated for each of these areas for male CFS patients, female CFS patients, healthy male and female controls. Values were compared with normal ^{123}I-IBZM uptake ratios (i.e, unaugmented scans) for right and left striatum as well as basal ganglia. Finally, mean D_2 receptor occupancy due to buspirone was calculated as a percentage of the mean uptake ratios in these areas.

Results

A total number of 10 CFS patients (M=5, F=5) and 4 healthy controls (M=2, F=2) had buspirone-augmented ^{123}I-IBZM cerebral SPECT scans. The mean age of the male CFS patients was 47.4 (age range 40-55 years) with duration of CFS symptoms ranging between 2-12 years prior to the study. The mean age of the female CFS

patients was 46.2 years with duration of CFS symptoms between 3-10 years. The average age of the male controls was 36 years (31 and 41 years) and 46.5 years (45 and 48 years) for the female controls. The mean group ages of the CFS patients and control subjects were 46.8 years and 41.3 years. Two CFS patients of both sexes each had mild postural hand tremors but no other features suggestive of Parkinsonism.

The study was originally designed with 8 patients and 8 controls since it was estimated that this number of subjects would be required to detect a difference in IBZM specific/non-specific ratio of 0.1 uptake units with a statistical power of 80%. The experiment was also designed to idsentify any difference between the CFS patients and controls during buspirone stimulation, the assumption being that the CFS patients and controls (as groups) do not differ in the non-stimulated level of D_2-receptor density.

Two region-of-interest (ROI) methods were used to measure uptake in the basal ganglia (BG) from the I^{123}-IBZM SPECT scans in CFS patients and control subjects:

i. a set of regions, representing the caudate (anterior striatum), putamen (posterior striatum) and occipital reference regions were derived from a large number of normal scans in previously studied subjects. These selected structures represented the average shape and size of the ROI.

ii. a much larger ROI encompassing all of the uptake in the basal ganglia (caudate, putamen, pallidum). The uptake was corrected for the average size of the striatum. The advantage of this region is that it allows for changes in shape of the individual components of the striatum but includes some IBZM activity from surrounding structures (e.g. pallidum)

Uptake in each region was expressed as specific uptake/non-specific region. The mean uptake of ^{123}I-IBZM as a ratio of total: non-specific uptake between patients and controls with or without adjustment for sex is shown in Table 5.14.1. The present results show no significant differences between the two groups, for any of the calculated values (including BG Right and BG Left). With only 4 controls, a significant difference might not have been expected.

Receptor Occupancy (RO) values were calculated on the assumption that all subjects have exactly the same non-stimulated, normal ^{123}I-IBZM uptake value (1.72) in the ROI relative to the non-specific region, i.e., occipital cortex. These were not true RO values, which would have required a non-augmented IBZM scan in each individual subject. Rather, this was simply an attempt at estimating "average" occupancy to show that buspirone does not have the same levels of D_2 receptor occupancy as, for example, the neuroleptics. Recalculating the data in terms of RO (Table 5.14.2) does not improve significance (or lack of it) in this experiment.

Conclusions

There was no diagnostic pattern of ^{123}I-IBZM uptake after oral buspirone in CFS patients as compared to the control group.

Table 5.14.1. Buspirone ^{123}IBZM SPECT Scans

BG = Basal Ganglia, AS= Anterior Striatum, PS= Posterior Striatum;
CFS: Chronic fatigue syndrome; HC: Healthy controls

Ratio of total: non-specific uptakes

Subjects	Sex	Striatum Right	Striatum Left	BG Right	BG Left	AS Right	AS Left	PS Right	PS Left
CFS1	M	1.66	1.67	1.82	1.96	1.64	1.62	1.76	1.74
CFS2	M	1.60	1.79	1.87	2.13	1.59	1.81	1.64	1.84
CFS3	M	1.65	1.57	1.83	1.86	1. 73	1.70	1.67	1.56
CFS4	M	1.61	1.56	1.86	1.87	1.51	1.63	1.74	1.58
CFS5	M	1.48	1.47	1.74	1.72	1.44	1.46	1.53	1.59
CFS6	F	1.67	1.70	1.95	2.06	1.69	1.73	1.72	1.77
CFS7	F	1.64	1.64	1.90	1.88	1.56	1.56	1.71	1.73
CFS8	F	1.55	1.61	1.76	1.77	1.71	1.60	1.52	1.67
CFS9	F	1.69	1.68	1.93	1.91	1.64	1.74	1.79	1.72
CFS10	F	1.62	1.60	1.90	1.80	1.63	1.69	1.64	1.59
HC1	M	1.64	1.72	2.05	2.16	1.66	1.59	1.68	1.85
HC2	M	1.58	1.64	1.83	1.88	1.56	1.70	1.61	1.67
HC3	F	1.52	1.58	1.84	1.89	1.62	1.51	1.52	1.72
HC4	F	1.71	1.69	2.12	2.08	1.71	1.74	1.85	1.80

Table 5.14.2: Buspirone ^{123}IBZM SPECT Scans

BG = Basal Ganglia, AS= Anterior Striatum, PS= Posterior Striatum;
CFS: Chronic fatigue syndrome; HC: Healthy controls

Mean Uptake Ratio Values

Subjects	Striatum Right	Striatum Left	BG Right	BG Left	AS Right	AS Left	PS Right	PS Left
Male CFS (n=5)	1.60	1.61	1.82	1.91	1.58	1.64	1.67	1.66
Female CFS (n=5)	1.63	1.64	1.89	1.88	1.65	1.66	1.68	1.70
Controls (n=4)	1.61	1.66	1.96	2.00	1.64	1.64	1.67	1.76
All CFS (n=10)	1.62	1.63	1.86	1.90	1.61	1.65	1.67	1.68
Normal IBZM	1.72	1.72	1.96	1.96				

Receptor Occupancy (RO)%

Subjects	Striatum Right	Striatum Left	BG Right	BG Left
Male CFS (n=5)	16.49	14.65	14.07	5.19
Female CFS (n=5)	12.28	10.50	7.29	8.12
Controls (n=4)	15.22	8.64	-0.01	-4.51
All patients (n=10)	14.39	12.57	10.68	6.66

6. DISCUSSION

"*If a man will begin with certainties, he shall end in doubts, but if he will be content to begin with doubts, he shall end in certainties*"

(Francis Bacon: *Of the Proficience of Advancement of Learning Divine and Humane*, 1605)

A diagnosis of CFS is essentially made on the clinical grounds and by excluding other medical and psychiatric causes of persistent fatigue. The precise aetiology of CFS and the pathogenesis of its symptoms are unknown. There is, as yet, no specific biological marker or a diagnostic test in CFS. New onset fatigue and limitation in physical endurance are the two most important and defining characteristics of CFS for neither of which there is any clear explanation. No specific pharmacological or psychological intervention is known to cure CFS and medical management in most cases is symptomatic and rehabilitative. Despite these obstacles, there is a growing recognition that service provisions are necessary for patients with CFS because of its potential morbidity, long- term disability and consequent socio-economic impact [56].

Appropriate diagnosis and effective intervention strategies are essential for the success of any health care system for patients with CFS/ME. In the absence of consistent physical signs or disease-specific biological marker, the present case definition of CFS [1] essentially selects a heterogeneous group of patients. In the review of research literature, this heterogeneity has been considered to be one of the explanations for the variability in the research findings on CFS. In addition, patient heterogeneity reduces the cost-effectiveness of any intervention that may be useful for a subgroup of CFS patients but not for all. However, a fundamental issue regarding CFS is its recognition and understanding as a clinical condition. Many physicians and psychiatrists still do not believe CFS to be a distinct clinical disorder [3]. Psychiatry based research has proposed that idiopathic chronic fatigue is not be a "physical" disorder and is more likely to be a symptom of somatisation due to chronic anxiety or depression [4]. In the psychiatric explanation, chronic fatigue and its associated

symptoms are considered to be a functional somatic syndrome developing in response to non-specific stressors (illness behaviour), interpersonal conflicts, depression or anxiety [3]. Traditionally, psychiatric diseases have been characterised by complex symptoms in the absence of a diagnostic marker and the lack of a specific or sensitive test for CFS has been held against an organic, biomedical model of this disorder. Given that fatigue is a ubiquitous symptom, it therefore become necessary to develop a biological concept of fatigue before making any attempt to rationalise the symptoms of fatigue in CFS patients.

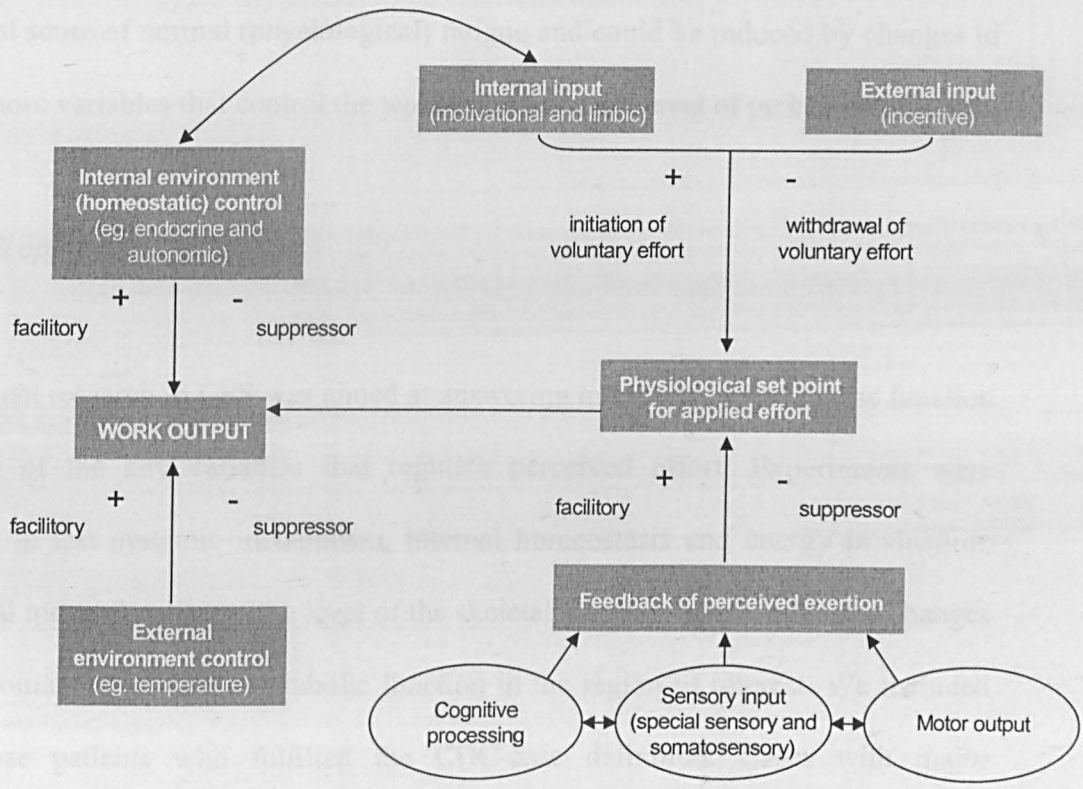
Concept of fatigue

Fatigue is not only common but in many cases, also an entirely subjective symptom that is part of normal day-to-day life. As an isolated symptom, fatigue is usually transient and self-limiting. It is only when fatigue is also associated with other illness symptoms then it draws attention to itself. Fatigue is reported by patients with infections, nutritional deficiency, autoimmune diseases, cancer, anxiety, depression, diseases of heart, liver, respiratory and endocrine systems and as a side-effect of a wide variety of medications. In addition, persistent, relapsing or fluctuating fatigue is common in many neurological diseases like multiple sclerosis or central dysautonomia (multiple system atrophy).

There is no exact definition of fatigue because of the overlap between the lay perception of tiredness and the clinically significant symptom of fatigue. For operational purposes, fatigue is best defined as the difficulty in initiating or sustaining voluntary activities. A normal sense of fatigue ("physiological fatigue") is

experienced after strenuous physical labour as well as after intense mental activity. The precise neurological substrate of this physiological fatigue is not known. Physiological fatigue may be pleasurable, especially when the activity is linked to a reward (for example, winning a race). In normal health, muscle aches and pain after exercise are due to a combination of lactic acidosis and minor muscle fibre injury. Rest and sleep relieve physiological fatigue. When fatigue occurs within normal limits of daily activities in association with other disease symptoms or illnesses, then it is considered to be abnormal (pathological fatigue).

The interplay between the various physiological control systems that regulate work output is shown in a schematic diagram (Flow Chart 3). To understand fatigue, it is helpful to consider voluntary effort as a controlled variable under the influence of multiple control systems. Perceived exertion is an important feedback control for the level of the applied effort. It has to be remembered that normal physical activity depends on the structural and functional integrity of the sensory and motor systems [190]. Sensory signals from the skin, cardiorespiratory systems, muscles and joints as well as from the special senses (skin, eyes and ears) provide the afferent input that ascend through the spinal cord and brainstem to be relayed to the thalamus before reaching the sensory cortex. This information is processed by the association areas in the cerebral cortex (cognitive input) for the activation of the primary motor cortex that sends signals to the brainstem motor nuclei and the anterior horn cells in the spinal cord (efferent pathway). The signal from the lower motor neurons is carried forward by the peripheral nerves that proceed through a synaptic relay (neuromuscular junction) to excite the muscle fibres. Within the muscle, a series of metabolic events then provides the chemical energy for contraction. Any interruption to this complex



Flow Chart 3. Interplay of the physiological variables that control work output. In this model, work output is a dependent variable of the applied effort that is controlled by motivational input (internal and external) and feedback from the motor, sensory and cognitive systems that determines the level of the perceived exertion. Additional control systems that regulate work output are environmental factors like temperature and internal mileu (homeostasis and autonomic function). Fatigue is a failure to sustain work output due to a shift in the physiological set point for applied effort. This shift may occur in neurological conditions associated with a higher level of perceived exertion and/or a reduced level of motivational input for the applied physical effort.

chain of events will have the potential to influence the level of applied effort and perceived exertion. Using this model, pathological fatigue is best understood as an amplified sense of normal (physiological) fatigue and could be induced by changes in one or more variables that control the work output and the level of perceived effort.

Research approach to CFS

The present research in CFS was aimed at answering questions regarding the function of some of the key variables that regulate perceived effort. Experiments were designed to test systemic metabolism, internal homeostasis and energy production, peripheral motor function at the level of the skeletal muscles, neuroendocrine changes and neuroimaging of brain metabolic function in the region of interest. We included only those patients who fulfilled the CDC-case definition. Cases with major psychiatric problems, history of drug and substance abuse and eating disorders, previous psychiatric history and somatoform disorders were excluded (Box 2). Only adult men and non-pregnant women were included. All patients were neurologically examined and appropriate screening investigations were reviewed to make sure that other medical and neurological disorders were excluded. Where indicated and considered necessary, additional investigations were undertaken to exclude other diseases. Same cohort of patients was studied in the first two experiments (5.1 and 5.2) and an identical cohort was used for the experiments on muscle biopsy (5.7 and 5.8). Other than these, all other experiments in this research were conducted on a different population of patients on each occasion.

Box 2. Case definition of Chronic Fatigue Syndrome used in this research

Essential requirements:

- fulfils CDC criteria
- minimum symptom duration of 6 months
- substantial functional impairment must be present
- cognitive or neuropsychiatric symptoms may be present
- fatigue is the major symptom
- four other symptoms required in addition to fatigue (Table 1)
- symptoms must be of new onset

Essential exclusions:

Medical : all clinically important diseases

Psychiatric: melancholic (severe, major) or psychotic depression

bipolar disorders

substance abuse

eating disorder

schizophrenia

dementia

delusional disorders

somatisation (somatoform) disorders

The experiments that were designed for the purpose of this research fall into three broad groups:

1. Assessment of the metabolic and humoral factors that may be predisposing to, or perpetuating the symptoms of fatigue in CFS. Experiments carried out in this area were general metabolic evaluation, genetic screening for haemochromatosis, tests for uncommon antibodies, circulating ouabain-like factor, and the cerebrospinal fluid and serum levels of endogenous pentapeptide (QYNAD).

2. Evaluation of the skeletal motor function and peripheral fatigue in CFS. Tests on skeletal muscle biopsy (histological studies and measurements of the intramuscular concentrations of Na+, K+ and the membrane pump (Na+/K+- ATPase), a randomised, double blind, placebo-controlled trial of dichloroacetate and subanaerobic threshold exercise tests (SATET) were the chosen experiments in this area.

3. Assessment of central fatigue and the functional role of basal ganglia. Experiments that were specifically undertaken in this aspect were neuroendocrine response to a novel dopamine agoinst pramipexole, proton magnetic resonance spectroscopy of basal ganglia and buspirone-augmented ^{123}I-IBZM cerebral SPECT scans in CFS patients.

Interpretation of research findings: studies on metabolic and humoral factors

Overall, results of systemic metabolic screening in CFS patients did not differ from the normal control population. In particular, no abnormality was detected in the intermediate metabolism of fatty acids, liver functions and urinary profiles of organic

acids and amino acids. Serum CK levels were measured in a number of different experiments and in all CFS patients, serum CK values have been well within the normal reference range. Elevations in serum CK appear to be uncharacteristic of CFS. In a patient with exertional fatigue and raised serum CK, a primary muscle disease is a more likely diagnosis than CFS.

A possible marker of CFS susceptibility is considered to be Gilbert's syndrome. The prevalence of Gilbert's syndrome is estimated to be 6% in population in some studies [191]. A significant proportion of patients with Gilbert's syndrome experience fatigue symptoms that is very similar to CFS. In this research, we were unable to identify and association between hyperbilirubinemia (fasting plasma bilirubin >20 mmol/L) and CFS. It therefore seems unlikely that as compared to the general population, the frequency of Gilbert's disease is higher among the CFS patients.

Extended glucose tolerance test (GTT) was essentially normal in CFS. This excluded any propensity for diabetes (pre-diabetes) in CFS patients. Fatigue is, of course, a recognised symptom in diabetes and this possibility needs to be excluded before a diagnosis of CFS is considered. What appeared to be of interest in this experiment is that the insulin release in CFS patients continued at a lower value of plasma glucose. This is clearly shown in the regression equation that was obtained for CFS patients. In addition, compared to the normative data, insulin level was relatively higher (but within physiologic range) for the corresponding values of plasma glucose at different time points during the glucose tolerance test.

There are a number of possible explanations for this observation. Firstly, the change in the slope of the line representing the relationship between plasma insulin and glucose in our patients may just be a normal variation due to the difference in the population characteristics of patients. Secondly, it may represent differential rates of glucose absorption in our patients. Only if these two possibilities are excluded, a third explanation is that there is a true change in the pattern of insulin release in CFS. The regression equation for plasma insulin and glucose in patients clearly draws attention to the fact that for any given range of plasma glucose, the corresponding insulin levels are higher, but not inappropriately high enough to cause hyperinsulinemic hypoglycaemia in CFS patients. This is suggestive of a "peripheral overutilisation" of glucose [192]. Fasting hypoglycaemia due to peripheral overutililisation of glucose may occur due to a number of conditions. These are usually metabolic diseases (systemic carnitine deficiency, deficiency in enzymes of fat oxidation, 3-hydroxy-3-methylglutaryl CoA lyase deficiency) where insulin levels are "normal", or conditions where insulin levels are inappropriately increased (insulinoma, exogenous insulin, sepsis and certain drugs like quinine). However, none of our patients had fasting hypoglycemia, carnitine deficiency or defective fat oxidation (Experiment 5.1), evidence of infection or history of treatment with hypoglycemic agents. One of the mechanisms for higher levels of plasma insulin in the absence of fasting hypoglycemia or hyperinsulinemic hypoglycemia is "insulin leak" from the pancreatic beta cells. This phenomenon was first observed with pentamidine, a drug that induces beta cell cytolysis [192]. It is, however, not known if "insulin leak" may occur due to less severe beta cell membrane injury although it is recognised that certain viral infections can cause beta cell inflammation ("insulinitis") leading to the development of autoimmune diabetes mellitus (type 1). Viruses, particularly Coxsackie B virus has

been associated both with the development type 1 diabetes mellitus [193] and CFS [194].

Studies on genetic susceptibility

Like most other multifactorial and acquired diseases, genetic susceptibility will play a role in the selection of individuals developing CFS after an appropriate environmental trigger. Previous studies have consistently failed to show any specific pattern of HLA haplotype in CFS patients other than an association with HLA DR2 in patients with hypersomnolence [60]. Since haemochromatosis shares many features of CFS including fatigue, myalgia, arthralgia, endocrine dysfunction and cardiac abnormality (cardiomyopathy), a possible association of familial haemochromatosis gene (HFE) was explored in CFS patients. The carrier frequency of HFE gene was not increased in the CFS population and therefore it seems unlikely that the HFE gene is a risk factor for the development of CFS in the Scottish population.

Screening for atypical autoantibodies

Because fatigue is common in systemic autoimmune inflammatory disorders, immune based research has been one of the major research interests in CFS. CFS typically follows local or systemic infections and a number of pathogens, viruses as well as bacterial, have been implicated in the autoimmune diseases. For example, beta-haemolytic streptococci have been implicated in rheumatic fever and both cytomegalovirus and Coxsackie B4 virus have been associated with autoimmune (type 1) diabetes mellitus. Coeliac disease is associated with fatigue and focal

neurological symptoms and cryptic gluten sensitivity has been postulated to play a role in neurological illnesses. Many CFS patients have bowel symptoms (abdominal pain, diarrhoea) usually attributed to irritable bowel and a proportion of CFS patients may have low levels of serum ferritin (Experiment 5.6) and/ serum folate [195]. However, our study failed to show any significant association of cryptic coeliac disease with CFS. None of the tested patients were hypo-gammaglobulinemic and it is unlikely that an undetected deficiency of IgA was responsible for the negative results in the anti-IgA endomysial antibody tests. The implication of this observation is that the findings of positive anti-gliadin and anti-IgA antibody in a patient with chronic fatigue and symptoms of irritable bowel are more likely to be due to coeliac disease rather than due to CFS.

Approximately 9% of CFS patients in this study were found to be positive for voltage gated potassium channel (VGKC) and 12.5% had positive antibody to G-protein coupled adrenergic receptors. Anti-VGKCs are considered to be good candidates as pathogenic antibodies. This is because VGKCs are located in the plasma membranes and are key determinants of neuronal excitability [196]. Point mutations in the human VGKC Kv1.1 gene can cause episodic ataxia, myokymia and epilepsy [197]. The radioimmunassay used for quantitating serum VGKC binds to Kv1.1, 1.2 and 1.6 subunits that are expressed neuronally in the cerebral cortex [198]. However, anti-VGKC antibodies are present in varying proportions throughout the brain and the peripheral nervous system [199]. These antibodies are positive in patients with peripherally restricted neuromuscular disorder (neuromyotonia), cases with autoimmune limbic encephalitis affecting brain and are also found to be positive in Morvan's syndrome and are thought to be pathogenic. Morvan's syndrome is

characterised by a combination of central and peripheral neurological symptoms. Morvan first described this syndrome in 1890 [200] comprising of benign muscle fasciculations (myokymia), muscle pain, excessive sweat, saliva, tears and bronchial secretion in association with sleep disorder (insomnia) and neuropsychiatric symptoms (hallucinations and altered behaviour). The neuromuscular feature of Morvan's syndrome ("*chorea fibrillaire*") is considered to be an example of continuous muscle fibre activity (neuromyotonia) and the central symptoms share similarities with limbic encephalitis. Anti-VGKC antibodies are pathogenic of Morvan's syndrome [201]. None of the CFS patients, including the antibody positive cases, had clinical features of neuromyotonia, Morvan's syndrome or limbic encephalitis.

In the context of CFS, transient and low levels of positive autoantibody to VGKC make it less likely that a primary autoimmune process targeted to the plasma membrane potassium channels is a specific pathogenic mechanism. In general, serum antibodies are of relatively large molecular weight and are thought not to penetrate blood brain barrier. An explanation for the observation that anti-VGKC antibodies have low positivity in CFS is that the potassium channel subunits in the brain and/or peripheral nervous system have been exposed for immunological sensitisation due to plasma cell membrane injury. A history of infection is often present in anti-VGKC antibody associated autoimmune limbic encephalitis (Prof. A. Vincent, personal communication). It may be a possibility that plasma membrane injury from infection exposes a limited number of voltage gated potassium channels for immune sensitisation at the early phase of the illness in CFS and these antibodies rapidly disappear because the sensitisation is neither specific nor sustained.

Like the voltage gated potassium channels, G-protein coupled cardiovascular receptors are also expressed at the plasma membranes. Antibodies to G-protein coupled adrenergic and muscarinic receptors have been observed in dilated cardiomyopathy [161]. None of our patients with CFS had clinical features to suggest cardiomyopathy. The interpretation of positive autoantibodies in 12.5% of patients in CFS would be similar to that of anti-VGKC antibody in that the plasma membrane sites of the G-protein coupled cardiovascular receptors may be affected in a proportion of CFS patients from cell membrane injury early in the course of the illness.

The lack of significant results in the experiments on serum ouabain-like substance (OLS) and cerebrospinal fluid endogenous pentapeptide (QYNAD) excludes these candidates as important players in the pathogenesis of CFS symptoms.

Interpretation of research findings: neuromuscular studies

In the second group of experiments, studies on muscle histology, histochemistry and electron microscopy did not reveal any new information. Fibre atrophy of muscles with increase in lipid may indicate physical deconditioning (replacement of muscle fibre by lipids) and was observed in nearly half of all the patients tested for muscle biopsy (53.9%; 7/13). It has to be noted however that the histological changes of deconditioning did not correlate with the duration of CFS symptoms, being equally

present in patients both with the shortest (1 year) and the longest duration of illness (14 years).

The experiment on muscle electrolytes and ATPase was considered in view of a previous study that had shown increases in the calcium ATPase activity in skeletal muscles of CFS patients [202]. Intramuscular sodium, potassium, magnesium and Na+/K+-ATPase concentrations were not significantly altered in the quadriceps muscle of CFS patients. These observations reinforce the view that muscle pathology is not a primary or essential feature of CFS. Failure of CFS patients to respond to a trial of dichloroacetate is, therefore, not entirely unexpected given the fact that neither biochemical nor histological features of mitochondrial myopathy were observed in other CFS patients.

Muscle tissue electrolyte concentration is considered to be more sensitive than plasma electrolyte changes. Typically, chronic use of diuretics depletes intramuscular potassium in spite of potassium supplementation. The close correlation between muscle magnesium and potassium concentrations in patients on long-term diuretic therapy indicates that potassium deficiency was also related to or caused by magnesium deficiency. In addition, these patients have a reduction in Na+/K+-ATPase activity due to the loss of intramuscular potassium [170]. In CFS patients, however, normal distributions of intramuscular sodium, potassium and Na+/K+-ATPases exclude primary sarcolemmal membrane injury in CFS. Because tissue electrolytes are more sensitive than serum electrolytes in chronic disorders [170], normal results in this experiment reliably exclude other metabolic muscle diseases

associated with chronic dysregulation of plasma and tissue electrolytes due to the deficiency of glucocorticoid or mineralocorticoid hormones.

Subanaerobic threshold exercise test and resting energy expenditure

The purpose of the sunanaerobic threshold exercise test (SATET) was to define more homogenous subgroups of CFS population and also to identify, if possible, any underlying pathophysiological processes specific to the identified subgroups. CFS Patients selected for this study were sub-categorised on the basis of SATET because it is objective, provides a reliable measure of the physical endurance in unselected CFS patients and has been validated in other studies. The resting energy expenditure (REE) study was an extension of the previous study where we had found some evidence of elevated REE in a proportion of female patients (5 out of 12) with CFS [152] who also had reduced total body potassium (TBK). Since muscle is the richest source of potassium which is predominantly intracellular, it was unclear whether the reduced TBK (and REE corrected for TBK) could have been caused by physical deconditioning and reduced muscle mass. Body composition studies provide indirect measures of physical deconditioning and have not been previously studied in the CFS population in any detail.

Objectively, the first CFS subgroup (Group 1) differed from the control subjects in the level of perceived exertion, consistently lower heart rate response to submaximal exercise and the level of physical activity. These patients did not show any obvious changes in their muscle metabolism in response to exercise, had normal REE and were not physically deconditioned. It is likely that the origin of fatigue in this group

of patients is in the central nervous system (*central fatigue subgroup*). A number of possible changes in the central nervous system have already been identified in CFS patients. These include impaired regional cerebral -blood flow in the SPECT studies, reduced excitability of the motor cortex and altered neuroendocrine responses. It is important to emphasise that none of these patients was depressed or had any other underlying neurological disease associated with chronic fatigue either at the time of their assessment or during their follow up. In the absence of prior ethical approval, no additional neuroimaging (anatomic or functional) was undertaken in this group that might have been helpful. It is noteworthy that patients in this group had a slightly lower heart rate response during the exercise despite being physically less active than the controls. A reduced heart rate response to an exercise that was perceived to be demanding by these patients excludes physical deconditioning as the cause of exercise intolerance because deconditioned subjects characteristically show a higher heart rate response to unaccustomed exercise. These patients clearly had a central-type fatigue and we postulate a possible overactivity of the parasympathetic system (i.e. cholinergic) to account for their reduced heart rate response during the exercise. Cholinergic supersensitivity is known to occur in some CFS patients [66, 203] and in theory, central fatigue is attributed to a failure of the autonomic and limbic integration of the volitional activities at the level of the basal ganglia [33]. Unlike antidepressants, interpersonal psychotherapy in depression has been shown to be effective in reversing limbic hypometabolism [204]. This may explain why some CFS patients benefit from cognitive behaviour therapy when administered by the trained therapists.

The results obtained in Group 2 suggested that metabolic dysfunction of the exercising muscles is a possible explanation for the elevated lactate response and consequent changes in the end-exercise heart rate observed in these patients (*metabolic fatigue*). None of these patients had any family history of muscle disease and did not have the common symptoms of metabolic myopathy (e.g. exercise cramps, myoglobinuria). Their core symptoms (muscle pain and fatigue unrelieved by rest or sleep) were no different from other CFS patients (Groups 1 and 3). In the absence of prior ethical approval, we were unable to perform muscle biopsies in all these patients as a part of this study which might have been useful to detect metabolic muscle diseases like unsuspected mitochondrial myopathy. Clearly, an identifiable cause of metabolic myopathy would exclude these patients from the diagnosis of CDC-defined CFS. This subgroup of CFS patients did not experience significantly higher perceived fatigue than the physically active and healthy control population. The relatively higher lactate response in this subgroup is consistent with the previous observations by Lane and others. [29] Lane's recent data from the magnetic resonance spectroscopy of muscles also confirm that CFS is a heterogeneous disorder and CFS patients who produce abnormal lactate response to exercise (SATET+ve) is likely to have a peripheral component to their fatigue [205]. In metabolic disorders like Addison's disease, muscle fatigue is associated with both decreased endurance (a central effect) and altered muscle contractile function [206]. However, end-exercise serum lactate increase is fairly non-specific as a marker of metabolic muscle enzyme defect and similar changes may be observed if oxygen delivery to the exercising muscles is impaired due to the abnormal autonomic control of blood flow reported in CFS [207]. Elevated resting blood lactate levels may provide indirect evidence of a mitochondrial defect [208], but this was not present in any of these patients. Increased

levels of lactate after exercise may indicate poor tissue perfusion and both exercise and previous diet can result in elevated levels of lactate in normal subjects [209].

The results of our patients in Group 3 (*fatigue with exercise intolerance*) were more difficult to explain. These patients were the most severely affected and could not complete a customised, moderate intensity exercise for 15 minutes (SATET). Relative changes in the proportion of their lower limb muscle mass and fat are suggestive of significant physical inactivity imposed by more severe fatigue in these patients. There were more women were in this group as compared to the other two groups. Given the fact that CFS is commoner in women than in men, it is possible that this subgroup constitutes a substantial proportion of CFS cases. Since the level of physical activity (as measured by the Baecke index) in this subgroup was comparable to the CFS patients in the other two subgroups and yet only these patients were exercise intolerant and had significant evidence of decreased limb muscle mass in DXA, it is more likely that in these patients, exercise intolerance was the cause, rather than the effect, of physical deconditioning.

There may be several possible explanations for the characteristics of Group 3 patients. Firstly, some of these patients may a pro-inflammatory disorder as would be expected, for example, in patients with rheumatoid arthritis. Cytokines are known to induce fatigue, increase REE and initiate catabolism of muscle proteins [210]. This pattern would also be consistent with the known effects of a hypoactive hypothalamic-pituitary-adrenal axis in CFS leading to relative tissue hypocortisolemia and raised levels of pro-inflammatory cytokines [119]. The second possibility is that the exercise intolerant CFS patients (more likely to be women) suffer from orthostatic intolerance

and postural tachycardia due to an exaggerated noradrenergic response [211]. This is supported by their haemodynamic responses during the attempted exercise (Figure 5.10.5). Patients in this subgroup had postural tachycardia, lowest physical activity index and raised REE. Sympathetic nervous system also plays an important part in regulating metabolic rate and norepinephrine increases REE due to its effect on the non-muscular tissues [212]. The highest rates of REE, lowest physical activity index and a higher resting heart rate before commencing the exercise and the similarity with POTS may suggest that CFS Group 3 patients could have had sympathetic overactivity as a result of acquired changes in the autonomic function. Another explanation is that the patients in this subgroup experienced unpleasant sensations (e.g. pain) while exercising from very early on and that the central nociceptive input might have limited their exercise tolerance. This mechanism has been proposed in women with muscle and soft tissue pain due to fibromyalgia where a reduced central threshold for nociceptive stimuli is considered to play a role [213]. Varicella-zoster virus infection has been implicated in CFS and it may be of interest that in a recent *in vitro* model, infection with VZV was shown to confer increased norepinephrine sensitivity to the sensory neurons of the rat dorsal root ganglia [214]. Beta-blockers with or without drugs that modulate central pain perception (e.g. carbamazepine and low doses of tricyclic antidepressants) may be useful in improving orthostatic function and exercise tolerance in Group 3 patients who appear to be at a high risk of progressive physical deconditioning and disability.

Analysis of results of this experiment (5.10) identifies three subgroups within the CDC-defined CFS population. The relative distribution of patients in the three CFS subgroups (31%, 24% and 45% in Groups 1, 2 and 3 respectively) might have differed

if a larger CFS population was studied. It is important to emphasise that the fatigue symptoms and the limitation in physical activities as measured by the Baecke physical activity index were very similar among patients in the three subgroups. In addition, there was no difference in the DXA measures relating to physical deconditioning (limb FFST) for Groups 1 and 2 patients. Patients who were unable to complete the SATET (Group 3) had some evidence of reduced muscle mass in their limbs when compared to the physically active control subjects. The three subgroups did not differ in their age, duration of CFS, range of physical symptoms, subjective severity of fatigue, range of self-reported cognitive impairment and psychological co-morbidity. Proven or presumed viral infection was the single common precipitant of CFS in patients across all subgroups. Dysregulation of the sympathetic autonomic nervous system as the likely explanation of the observed changes in Group 3 patients is consistent with the established view that sympathetic activity stimulates nociceptors even if they are not obviously damaged and that under some circumstances, sympathetic nervous system plays an active role in inflammation [215]. Interestingly, "vegetative neuritis" was earlier proposed to emphasise the frequent affection of the autonomic nervous system in CFS (cited by Holt, 1965 [53])

Neuroendocrine response: Dopaminergic function

Dopaminergic system has been implicated in migraine, post-polio fatigue and CFS where central fatigue is common [33]. Growth hormone release from anterior pituitary is stimulated by dopamine. Bromocriptine is a dopamine agoinst with affinity for both dopamine D_1 and D_2 receptors. A previous study of bromocriptine-stimulated GH-response in CFS could not differentiate patients from healthy controls [78]. Dopamine

is also the hypothalamic PIF and tonically inhibits prolactin release by the lactotrophs in anterior pituitary. Plasma prolactin response to pharmacologic probes has been traditionally accepted as a reliable and direct measure of dopamine D_2 receptor function in the brain. Pramipexole is a synthetic dopamine agoinst with special affinity for D_2 family of dopamine receptors. Pramipexole-induced suppression of prolactin response may be considered to be an indirect way to assess the sensitivity of the central dopamine D_2 receptor system. This formed the basis of the study where we evaluated neuroendocrine response (inhibition of prolactin release) to pramipexole in CFS patients and compared the results with normal healthy controls.

Like the bromocriptine-induced GH response [78], no significant statistical difference emerged from this study between the two groups (CFS and controls). There was no evidence for a reduced tone of pituitary dopaminergic D_2 receptors in CFS. However, even in Parkinson' s disease, hypothalamic dopamine sensitivity to GH and prolactin is normal when patients are challenged by apomorphine, a dopamine agonist [216]. This may indicate that neuroendocrine response to dopamine agonists may not be a sensitive test to identify patients who may have a more selective loss of dopaminergic function outside the pituitary-hypothalamic axis. Determination of GH response to dopaminergic stimulation with pramipexole may also have been useful.

Buspirone-augmented ^{123}I-IBZM cerebral SPECT scan

^{123}I-IBZM cerebral SPECT scan is widely used for nigrostriatal dopaminergic system and offers a reliable measure of post-synaptic D_2 receptor function particularly in the striatum and generally, in the basal ganglia. A reduction in the normal striatal uptake

of the radiotracer (^{123}I-IBZM) is characteristically associated with a reduction of functional post-synaptic D_2 receptors due to neurodegeneration or receptor occupation by dopamine blocking drugs. In the absence of drug-induced dopamine receptor blockade, reduced striatal uptake of ^{123}I-IBZM in the cerebral SPECT scan is associated with neurodegenerative disorders affecting basal ganglia, which CFS is not. It is rather unlikely that a significant loss of post-synaptic D_2 receptors will occur without clinical symptoms in CFS. In addition, findings of an abnormal ^{123}I-IBZM cerebral SPECT scan in a patient with chronic fatigue will probably suggest a fatiguing Parkinsonian disorder rather than CFS.

It was, however, considered possible that the functional characteristics of post-synaptic dopamine D_2 receptors may be sufficiently altered in CFS to alter receptor sensitivity and affinity to certain neuroendocrine probes but without causing obvious clinical signs of basal ganglia disease. A weak dopamine receptor antagonist will be expected to produce longer receptor occupancy in a situation like this. Buspirone is a $5HT_{1A}$ agoinst but also has a weak dopamine antagonist property [217]. In contrast to patients with depression, CFS patients of both sexes show an exaggerated release of prolactin in response to buspirone and prolactin release is considered to be a reliable marker of D_2 receptor occupation [111].

Buspirone augmented ^{123}I-IBZM cerebral SPECT scans is a novel form of functional brain imaging that has not been tested before. Oral buspirone is rapidly absorbed and pharmacokinetic studies indicate that peak plasma level of buspirone is reached at 60 minutes [218]. The traditional dose for oral buspirone in neuroendocrine studies is 60 mg but we used a single dose of 45 mg (rather than 60 mg) for reasons of safety

because it is the recommended maximum daily therapeutic dose of buspirone in clinical practice [218]. Because of the instructions from the regulatory authorities for research studies using radioactive substance (ARSAC), only patients aged 30 and above were included in this neuroimaging protocol. On the assumption that peak plasma levels of buspirone will be attained at 60 minutes after oral administration, intravenous injection of the radiotracer (^{123}I-IBZM) was delayed until this point. Image acquisition and analysis were based on a computerised system attached to the SPECT scanner and results were compared independently between both sexes of patients and control subjects.

Irrespective of the sex distribution, there was no statistically significant difference in the striatal uptake of ^{123}I-IBZM in CFS patients and controls. However, mean uptake ratio values were low both in patients than controls when compared to the reference ratio for normal ^{123}I-IBZM uptake (i.e. without buspirone pre-treatment). The weak D_2 blocking effect of buspirone was also confirmed in the calculated dopamine D_2 receptor occupancy of patients and controls. Analysis for the uptake ratios and dopamine D_2 receptor occupancy in the buspirone augmented ^{123}I-IBZM SPECT scans between CFS patients and controls showed a lower mean uptake ratio values in control subjects; calculation for statistical significance was not possible because of the difference in the number of subjects that could be studied (10 CFS patients and 4 healthy controls). The calculated percentage of dopamine D_2 receptor occupancy in the striatum was similar between patients and controls. It is possible that because of unequal sizes of the patient and control groups, the results of this experiment failed to reach the anticipated level of significance.

Proton magnetic resonance spectroscopy of basal ganglia

Proton magnetic resonance spectroscopy (^{1}H MRS) is a relatively new tool for imaging metabolic brain functions. Typically, ^{1}H MRS measures regional brain metabolite levels for N-acetyl aspartate (NAA), choline-containing compounds (Cho) and creatine-containing compounds (Cr). While NAA levels broadly correlate with the functional neuronal mass, Cr is generally considered to be an unvarying metabolic marker of brain function in the ^{1}H MRS. In contrast, Cho peak is considered originate from the cell membrane lipids. ^{1}H MRS of brain tumours shows high peaks of Cho reflecting mobile brain lipids due to the tissue degradation and necrosis [219]. Choline is acutely elevated in relapsing multiple sclerosis where lipid breakdown follows myelin injury [220]. However, elevated Cho is also observed in the areas of reactive or repairative gliosis [221] probably as a result of increased membrane turnover. Increments in Cho/Cr ratios in the basal ganglia were recently reported in patients with mild hepatitis C (but not hepatitis B) virus infection [222] and human immunodeficiency virus (HIV) infected patients [223]. Elevations in Cho may occur before [NAA] decrements in seropositive HIV patients [223] who experience symptoms of chronic fatigue very similar to CFS [2].

We chose basal ganglia as the region of interest in our study primarily because of the theoretical reasons [33] and also due to the fact that changes in the basal ganglia ^{1}H MRS were reported in the study of Gulf-War veterans symptomatic of fatigue [184]. CFS cases were selected carefully using strict criteria to identify the neurologically defined post-viral fatigue patients. Unlike the symptomatic Gulf War veterans [184], there was no evidence of significant loss of functional neuronal mass (reflected by

reduced levels of NAA and low NAA/Cr ratio) in the basal ganglia of our CFS patients. However, whether referenced to water or other metabolites, the peak of the Cho-containing compounds in the ^{1}H MRS were uniformly increased in all CFS patients irrespective of their age or the duration of illness. The statistical strength of this association was extremely high ($p<0.001$). In the only other ^{1}H MRS study of the basal ganglia in three CFS children (ages 11, 12 and 13 years), a remarkable elevation of Cho/Cr ratio was similarly observed [225]. None of these patients (ours and the three paediatric cases) had focal structural abnormalities of the basal ganglia in the MRI. In another study in adults, relative increases in the Cho peaks were observed in the ^{1}H MRS of the occipital cortex of non-psychiatric CFS patients as opposed to controls [225].

In general, choline peaks in the ^{1}H MRS are influenced by specific structural changes (tumour and demyelination), certain viral infections (e.g. hepatitis C and HIV), and higher rates of cell turnover (as in repairative gliosis). From this study, it appears that irrespective of the age of the patient or the duration of the symptoms, choline-containing compounds are elevated in the basal ganglia of the CFS patients. However, CFS patients do not have any MRI evidence of structural changes in the basal ganglia nor serological evidence of chronic hepatitis or HIV infection. Although persistent Epstein Barr, enteroviral or human herpesvirus infection was proposed from time to time as a model for CFS, current opinion does not favour this theory [20]. A continuing repairative gliosis is a possibility although the precise stimulus for this activity in CFS is unknown at present. Because systemic metabolic studies on lipid and peroxisomal functions in CFS patients did not reveal any abnormality (Experiment 5.1), it is more likely that the increased choline spectroscopic peaks in

two different brain areas reported independently by us [185] and the other workers [225] indicate altered neural cell membrane metabolism and function in CFS.

It has been previously hypothesised that sustained changes in cell membrane function may follow exposure to infections and specific neurotoxins leading to the CFS symptoms. [143] Ciguatera toxin is probably one of the best examples where delayed symptoms of chronic fatigue may be related to the alteration of the membrane ion channels (ciguatera toxin irreversibly inactivates sodium channels in an open mode). [142] It is also known that the viral membrane glycoprotein and viroporin molecules induce changes in the host cell membrane permeability, leading to the activation of phospholipases with consequent release of a number of phospholipid moieties including choline [226]. A common route of cellular entry shared by many bacteria, viruses and parasites involves lipid rich microdomains (caveolae) of the cell membrane. A number of microbes or their exotoxins (in the case of extracellular infections) co-opt the features of the host cell caveolae probably because their cognate receptors are localised within the lipid-rich plasmalemma of host cells [227]. Subsequent adaptations of the host cells to these pathogens and toxin could modify cell membrane functions that may persist long after symptomatic recovery in some patients.

Therapeutic interventions in CFS

Two therapeutic trials were part of this research project. The first trial, a prospective, randomised double blind use of dichloroacetate (DCA) in CFS was without any benefit. There was neither an objective improvement in isometric muscle contraction

nor any subjective improvement of fatigue as reported by patients. The second trial was an open labelled retrospective observational study. Combination of amantadine and amitriptyline in low doses appeared to be of little benefit in terms of self-reported improvement of fatigue. The negative results from the prospective DCA trial reinforce the view that fatigue in CFS has a mechanism that is distinct from the muscle pathology. This is supported by the observations in a recent study of blood flow and muscle metabolism in CFS [228].

From the results of the SATET, energy expenditure and body composition studies, a subgroup of CFS patients (Group 3) was characterised by orthostatic intolerance, tachycardia, muscle wasting and raised energy expenditure probably due to an overactive sympathetic system. Beta-blockers may have a potential role in these cases. In order to minimise the adverse effects of beta-blockers (fatigue, depression and peripheral circulatory compromise), a centrally active beta-blocker with intrinsic sympatho-mimetic activity (for example, pindolol) would be a suitable choice. Beta-blocker may also be a possible treatment for those CFS patients with a supersensitive response to clonidine (*CFS type 3*).

From the ^{1}H-MRS study, increases in the regional brain levels of choline-containing compounds suggest that membrane stabilising treatment may have a place in CFS pharmacotherapy. Clearly, the composition of the chosen product, bioavailability, side effect profile and target therapeutic doses are some of the key considerations for any likely pharamacological effect on central fatigue. Additionally, basal ganglia proton magnetic resonance spectroscopy has the potential to be used as an objective outcome measure in the research studies of therapeutic interventions in CFS.

A summary of positive and important negative findings

Taken together, the research data have a number of implications. It is clear that in general, CFS patients do not have a defined disorder of systemic or muscle metabolic functions and do not have higher susceptibility to haemochromatosis or Gilbert's syndrome. CFS is not an autoimmune disease and we found no evidence for a higher prevalence of coeliac disease in the CFS population. A minority of patients may have transient, low levels of anti-VGKC antibodies that do not appear to play a pathogenic role in this disorder. There is no predisposition to diabetes or hyperinsulinemia although pancreatic insulin release appears to continue at relatively lower values of plasma glucose. Serum levels of ouabain-like substance and cerebrospinal fluid levels of a putative endogenous pentapeptide with local anaesthetic (sodium channel blocking) effect were not increased in the CFS population.

Results from the exercise study (SATET) indicate that as a group, CFS patients have considerable variability in their autonomic and cardiovascular responses to symptom limited, sub-anaerobic exercise. Some patients have a relative overactivity of the parasympatheic tone and fatigue in these patients is primarily central. Nearly half of those patients who were able to complete the exercise protocol had increased plasma lactate. Although this observation may be interpreted as an evidence of metabolic oxidative defects in the exercising muscles, there are a number of caveats to this conclusion. Post-exercise rises in plasma lactate levels are fairly non-specific and may be influenced by diet, peripheral circulatory return and autonomic regulation of blood flow to the exercising muscles. Patients who fall into the category of the exercise

intolerant subgroup have objective evidence of muscle wasting due to physical deconditioning. These patients are more likely to be women and have a higher risk of progressive disability.

Neuroendocrine tests did not reveal any specific or diagnostic abnormalities affecting the dopamine D_2 receptor systems. Analysis of buspirone augmented ^{123}I-IBZM cerebral SPECT scans indicates that dopamine D_2 receptor affinity is similar in CFS and healthy controls, although the smaller number of the control subjects marginalises any meaningful interpretation of the experiment.

Preliminary result from a novel form of metabolic neuroimaging has been a particularly rewarding aspect of this research. ^{1}H-MRS of left basal ganglia in CFS patient has shown unequivocal evidence of increased levels of choline containing compounds without any loss of functional neuronal mass. Because choline resonance in ^{1}H-MRS is derived from membrane phospholipids and CFS patients do not have evidence of abnormal lipid metabolism, it is likely that the spectroscopic increases in choline reflect remodelling or rapid turnover of neural cell membranes. It appears that irrespective of the age of the CFS patients or the duration of their symptoms, choline-containing compounds are elevated in the basal ganglia. Taken together, recent ^{1}H-MRS observations in appropriately defined, non-psychiatric CFS population objectively confirm an underlying neurobiological process affecting neural cell membrane function. ^{1}H MRS observations in this research emphasise the need to explore long-term effects of viral-cell interactions on membrane functions. Minimal and non-specific pallidal changes were the only consistent pathological abnormality observed in encephalitis lethargica [229].

Developing a neurobiological paradigm in CFS

One of the main objectives of our research was to develop a neurobiological paradigm of fatigue in CFS and account for some of the observed heterogeneity in the research outcome as previously reviewed. The research findings are consistent with the view that the clinical heterogeneity is a function of biological variables. Changes in the relative balance of the autonomic nervous system appear to be an important predictor with respect to the symptom-limited exercise endurance in CFS. It is possible that these changes are determined by the inherent genetic predisposition, the nature of the precipitant or trigger of the illness, acquired dysfunction of the brainstem or limbic autonomic functions and changes in synaptic inactivation of norepinephrine. This research has drawn attention to a neuroanatomic pathway that may be relevant to the genesis of central fatigue. ^{1}H MRS clearly suggests abnormal membrane metabolism in basal ganglia and the following section briefly reviews the role of basal ganglia in central fatigue [33].

From Denny-Brown's experimental work on caudate ablation in monkeys, it became clear that damage to the caudate nuclei led to a condition in which animals had difficulty formulating internally driven responses [230]. Under normal circumstances, initiation and sequential performance of a task requires an internally driven mechanism integrated at the level of basal ganglia to prepare the emotive, motor and sensory apparatus ("cues") responsible for next and the subsequent set of responses. It is known that some striatal units maybe highly specific and organised to carry information about the predictive value of stimuli (the preparatory cues). Disruption of normal basal ganglia derived algorithm of the sequential task processing mechanism

would not only delay the initiation, but also prevent the smooth execution of the intended task, a feature that is typical of patients with *central fatigue.*

Observations have confirmed that parts of the basal ganglia circuitry functionally involved in the non-motor and emotional process are linked to the frontal lobes [231]. Stahl originally proposed that the basal ganglia may be divided into a neurologist's, a psychologist's, and a psychiatrist's portion, responsible for motor, cognitive and emotional or behavioural disorders respectively [232]. In his plan, the circuitry for the extrapyramidal motor disorders was putamen based, while the caudate-dorsolateral prefrontal circuit and the ventral striatopallidal system, especially nucleus accumbens, accounted for the cognitive and behavioural syndromes respectively.

The dorsolateral prefrontal circuit in the caudate (psychologist's basal ganglia) is involved in the dopamine loss seen in Parkinson's disease where *central fatigue* is common. Disruption of this circuitry at the level of the striatum impedes the execution of appropriate tasks. The ventral striopallidal system (the psychiatrist's basal ganglia) has a more complex relationship involving the shell of nucleus accumbens (the ventral striatum) and the extended amygdala, incorporating connections with olfactory tubercle, hypothalamus and brain stem. While amygdala is anatomically organised to encode the emotional significance of sensory events [233], the lateral and medial orbitofrontal and cingulate circuits from ventral striatum may be involved in the evaluation of relevance of external events.

The ventral striatum is richly innervated with mesolimbic dopaminergic inputs (motivational/ reinforcement pathways) as well as with the converging inputs from

the orbitofrontal (affective/environmental associations), anterior cingulate (affective response) and the amygdaloid complex (emotional reactivity). Ventral striatum also exerts a global regulatory influence on the dopaminergic neurotransmission in the entire striatum, and is considered to represent the anatomic substrate for motivational anchoring and reinforcement of successful experience (rewarding or self-stimulatory behaviour).

Voluntary action is controlled both by motivation and cognition; basal ganglia may be the site where these kind of information meet. The striopallidal system and the striothalamo-cortical loop may be an important common neuroanatomic pathway for *central fatigue*. The data from ^{1}H MRS certainly point to this direction.

Cell membrane function and fatigue

In this research, a common explanation for a number of observations has been cell membrane injury. Ion channels and receptors are normally expressed on the cell membranes of the excitable tissues. Depending on the functional characteristics of these cells and their localisation both within and outside the nervous system, a range of symptoms may be experienced that will both variable in severity and inducible under conditions of physiological as well as psychological stress. Even if basal ganglia and their interconnecting pathways are central to the genesis of fatigue as a brain-derived symptom, one still has to explain what triggers dysfunction in this neural network in the first place. From the ^{1}H-MRS data, it appears that cell membrane remodelling, due to gliosis or increased cell membrane turnover may explain the increases in choline peaks in the basal ganglia of CFS patients. The

statistical strength of this observation was particularly high ($p<0.001$) even though the number of cases included in this study was small (8 patients and 8 controls).

Long term changes in the neural cell membrane may be induced by viral proteins. In the known models of chronic viral infections (for example, HIV), membrane bound phospholipases are activated by viral protein. Activated phospholipases split choline off the membrane phospholipids (phosphatidylcholine and phosphoglycerocholine). While idiopathic CFS is not due to any known chronic viral infections, it begs the question whether those who become symptomatic of CFS after a non-specific infection may have altered cell membrane function sustained as a result of complex interplay between genetic factors, infective agent and the subsequent adaptive response of the host. This paradigm is certainly supported by the CFS-like syndrome developing in a proportion of patients with ciguatera fish poisoning where a specific neurotoxin (ciguatoxin) modifies host cell function by permanent inactivation of the membrane sodium channels. Fatigue is recognised symptom of diseases and drugs that affect cell membrane and ion channel functions.

Limitations of the present research

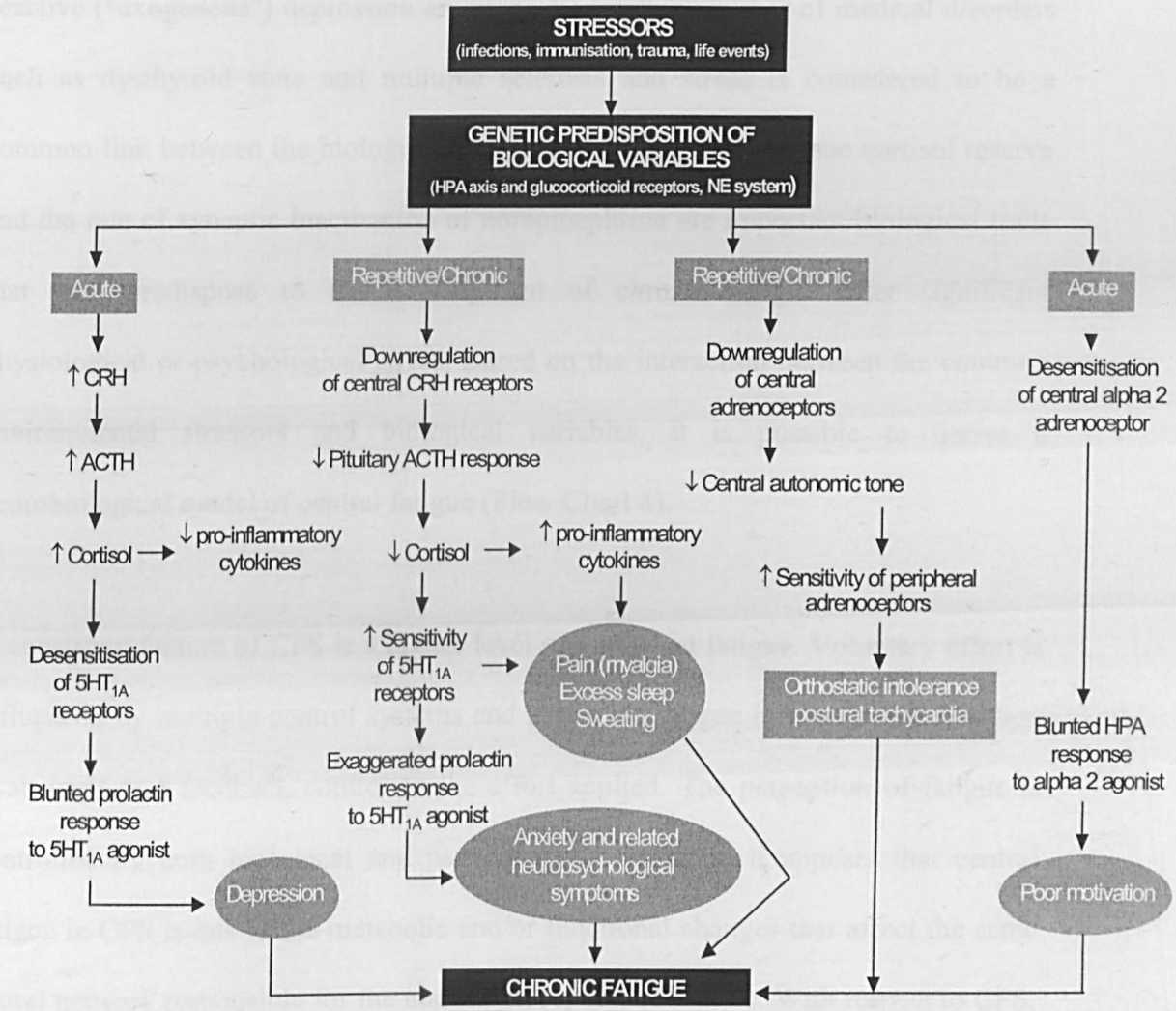
A number of limitations may, however, apply to this work. For the majority of experiments undertaken in this research, different cohorts of CFS patients were recruited. The inherent heterogeneity in the clinical diagnosis of CFS, biological variability of the different groups of patients and controls might contribute to the inconsistency of findings. The choice of controls in the experiments was mostly

healthy (but not necessarily physically active) individuals. Ideally, one should have included patients with psychogenic fatigue (clinical depression) as a third control group. Inclusion of physically deconditioned healthy subjects as controls might have been ideal. The small number of cases in some of our experiments might account for findings of significance (positive G-protein coupled receptor antibody) or lack of significance (normal frequency of HFE gene mutation). For experiments based on off-site laboratory analysis, preservation and transportation of samples could have affected their qualities and final results.

The research may also be criticised for not analysing power in the therapeutic trials and experiments. We consider all our experiments and the two therapeutic trials to be pilot studies of testing hypotheses rather than formal validation of any existing or proven theories. The interpretation of the present research data needs to be cautious and the evidence presented should be considered to provide only limited, but in some way useful, information for the next phase of neurobiological research in CFS.

Neurobiological research in CFS

Because of the overlap of CFS with depression and chronic anxiety, many physicians prefer to invoke a psychiatric explanation for fatigue when patient's symptoms do not fit a neurological diagnostic category. This is particularly true for CFS. Anxiety and



Flow Chart 4. A biological model of chronic fatigue based on neuroendocrine functions. In susceptible individuals, environmental stressors induce changes in the neuroendocrine axis primarily through the hypothalamic pituitary (HPA) axis and the norepinephrine (NE) system. Depending on the type of stressor (acute vs. repetitive/ chronic), responses may move in the opposite direction. Changes in synaptic sensitivities of the corticotrophin releasing hormone (CRH), serotonin (5 HT_{1A}) and $alpha_2$ adrenergic receptors determine both the nature and the severity of fatigue-associated symptoms (e.g. muscle pain, sleep disorder, orthostatic intolerance and anxiety). Both in men and women, prolactin is secreted from the anterior pituitary under conditions of stress. Prolactin secretion from pituitary is tonically suppressed by dopamine which is considered to be the hypothalamic prolactin-inhibitory factor. Prolactin release under stress is independent of, and probably occurs earlier than, the CRH-induced release of the pituitary adrenocorticotrophic hormone (ACTH) .

reactive ("exogenous") depression are associated with a number of medical disorders such as dysthyroid state and multiple sclerosis and stress is considered to be a common link between the biological and psychosocial events. Tissue cortisol reserve and the rate of synaptic inactivation of norepinephrine are important biological traits that may predispose to the development of chronic fatigue after significant physiological or psychological stress. Based on the interaction between the common environmental stressors and biological variables, it is possible to derive a neurobiological model of central fatigue (Flow Chart 4).

A consistent feature of CFS is a higher level of perceived fatigue. Voluntary effort is influenced by multiple control systems and perceived fatigue in response to exertion is an important feedback control to the effort applied. The perception of fatigue is controlled by both biological and psychological variables. It appears that central fatigue in CFS is due to the metabolic and/or functional changes that affect the same neural network responsible for the normal perception of fatigue. With respect to CFS, higher levels of perceived fatigue may be induced by alterations in the internal environment either due to neuroendocrine changes or a lack of limbic integration of voluntary motor activities at the level of the basal ganglia.

We had a number of aims in this research. With respect to the questions asked, we found no significant alteration of intermediate metabolism in CFS. There is also imited evidence in favour of energy utilisation and expenditure in CFS. Skeletal muscle physiology is normal in CFS patients. Three distinct subgroups of the CDC-defined CFS patients can be identified by standardised, symptom-limited exercises. The response of the aminergic system in CFS to external pharmacological challenge is

variable but the neuroendocrine tests do not show any alteration in the hypothalamic-pituitary dopaminergic system. There is unequivocal evidence of altered regional brain metabolism in CFS, characterised by increased peaks of choline in the neural membranes of the basal ganglia. An increased choline level in the ^{1}H MRS of basal ganglia is a possible neurobiological marker of the central fatigue in CFS.

Further research will hopefully define the possible pathophysiologic mechanism(s) within these individual subgroups and outline the interaction between the various physical and psychological changes. Ideally, this research should take the form of a prospective study, following several patients from their initial visits to the primary care physicians with a complaint of ill-health, right through to the diagnosis of chronic fatigue, CFS, and beyond. It also needs to be explored whether individual predispositions to the host-viral interaction may determine the consequent changes in the functions of the specific neurotransmitters, receptors, ion channels and/or enzymes at the different neuroanatomic levels of the autonomic nervous system in CFS. This appears to be the only way forward to disentangle CFS from the controversy that currently surrounds its existence and care.

Many physicians prefer to invoke a psychiatric explanation for fatigue when patients' symptoms do not fit a common medical or neurological diagnostic category. The classical descriptions of the major psychiatric disorders have been fairly consistent over the years and when a patient with fatigue perfectly fits the criteria for a psychiatric disorder outlined in DSM-IV [234], one can certainly have confidence in the diagnosis. However, it is when patient's symptoms or clinical course do not fit or fit the diagnostic criteria poorly, one needs to be cautious. There is good evidence at

present that the brain-derived, central fatigue is related to the circuits that connect basal ganglia, amydgala, thalamus and frontal cortex. Thalamus is an important centre for afferent input from the reticular activating system and sensory pathways. Basal ganglia, through its link with amygdala, limbic system and the neocortex, especially frontal lobe, act as an important integrator of motivational activities. While fatigue is likely to follow structural lesions in this circuit, fatigue due to metabolic changes or neurotransmitter disturbance affecting these pathways are more common and may occur in the absence of obvious structural injury. Common neuropsychological consequences of central fatigue are reduced speed of information processing and attention deficit, generating a sense of excessive mental fatigues and interfering with performance rather than competence in dealing with the task. Symptoms like muscle pain, sleep disorder and co-morbid anxiety in fatigued patients may be partly explained by changes in the sensitivity of post-synaptic receptors that are triggered by the interactions between environmental stressors, genetic predisposition and the HPA axis responsiveness (Flow Chart 4).

In summary, the perception of fatigue is controlled by both biological and psychological variables. This review underscores the importance of interpreting central fatigue on the basis of changes in cerebral metabolism and/or neurotransmitter function affecting subcortical-cortical network. It would be both incorrect and inappropriate to imply fatigue to be a medically unexplained, non-organic symptom in patients who do not have a primary psychiatric diagnosis. This likely mechanism of fatigue in CFS is central and while muscle metabolic abnormalities may be present in a few patients, there is an overall lack of evidence to suggest a peripheral (neuromuscular) role. Cell membrane receptors and ion channels may emerge as

candidate genes for future research in this area. Future research in CFS should ideally explore the central component of fatigue by using a combination of functional neuroimaging and neuroendocrine probes. To be sure, advances in modern neuroimaging and molecular neurosciences will offer better definition of the anatomical network and molecular pathophysiology of fatigue. Future development of drugs that act directly on the HPA axis, hypothalamic neuropeptides and the central glucocorticoid receptors will be important in our understanding of central fatigue and its cognitive and affective overlap. As in other complex medical problems, only the open minded, who are willing to consider observations and explanations at many different levels, are likely to succeed in offering the right solutions [235].

7. CONCLUSIONS

"*Life is the art of drawing sufficient conclusions from insufficient premises*" (Samuel Butler, 1912)

The findings in this research support the view that the primary origin of fatigue in CFS is in the central nervous system, i.e. fatigue is central in CFS. The research data also raise the possibility that the central fatigue in CFS correlates with altered signalling in the neuronal network that is connected to the basal ganglia. This alteration in neuronal signalling may be caused, among other possibilities, by an acquired dysfunction of the cell membranes with increased levels of membrane lipid breakdown. Central autonomic functions are also affected in CFS, probably by a mechanism affecting cell membrane receptor responses to the neurotransmitters and circulating hormones. Metabolic influences, anaesthetics and pharmacological agents that target membrane lipids can cause further dysregulation of transmembrane ion traffic and worsen fatigue. There is no evidence that specific neuronal loss is a feature of CFS. There is also no evidence that CFS is a muscle disease or primary muscle pathology is essential for CFS symptoms. The implications of the present research are summarised in Box 3.

Understandably, therapeutic options for correcting cell membrane lipid breakdown are going to be difficult. Drugs that stabilise cell membranes, such as HUFA, centrally active beta-blockers, antiepileptics like carbamazepine may have some effect but individual responses could be highly variable and the efficacy of any intervention in CFS will be difficult to interpret without a randomised trial. It is also possible that what we define today as CFS consists of heterogeneous patients with different pathophysiological mechanisms awaiting better definition and segregation into subgroups. Once this is achieved, there may possibly be diagnostic markers and therapeutic options for each of the defined subgroups of the CFS patients.

Box 3. What this research shows

- CFS patients do not have any deficiency of essential fatty acids or carnitine. CFS patients are not pre-diabetic or hyperinsulinemic but do show a degree of peripheral overutilisation of glucose possibly due to beta cell "insulin leak".
- CFS patients do not have a genetic predisposition to haemochromatosis.
- CFS patients do not have a specific or sensitive serum marker for autoantibody or ouabain-like substance. Cerebrospinal fluid levels of Na+-channel blocking endogenous pentapeptide (QYNAD) are similar in CFS patients as compared to patients with neuroinflammatory disorder and benign headache syndromes.
- Muscle histology in CFS patients is normal.
- Intramuscular concentrations of Na+, K+ and Na+/K+-ATPases are normal in CFS.
- Exercise response to CFS patients is influenced by the autonomic tone. Based on their physiological and metabolic responses to a standardised symptom-limited exercise, CFS patients can be divided into three groups:(central fatigue, metabolic fatigue and exercise intolerant fatigue). Patients with exercise intolerant fatigue are at high risk of progressive disability.
- Neuroendocrine response to dopaminergic stimulation is normal in CFS. Buspirone-augmented ^{123}I-IBZM SPECT scans do not suggest significant changes in the pallidal dopamine D_2 receptor binding to buspirone in CFS.
- ^{1}H MRS of basal ganglia is abnormal in non-psychiatrically defined adult cases with CFS. Significantly increased levels of choline in the basal ganglia ^{1}H MRS may indicate a pathology that affects the structure and/composition of the neural cell membranes in CFS patients.

In clinical practice, CFS must not be viewed in complete isolation from fatigue due to other medical or neurological disorders. Stereotyping CFS patients as depressed or somatising individuals is probably inaccurate and might jeopardise an effective therapeutic partnership developing between the patient and his/her physician. Failure to recognise fatigue or its importance in a patient may undermine the physician-patient relationship that is vital for any successful therapeutic partnership. It is easy to dismiss subjective fatigue as a largely psychological phenomenon though it may be open to debate what precisely constitutes this psychological process. From a neurologist's viewpoint, fatigue may be accepted as a psychological problem provided "psyche" is defined as the collective force of integrative cortical function driven by the physiological principles of neuroscience. On the other hand, if the psychological process in fatigue is equated with the unsubstantiated hypotheses of illusory perception of effort, wrong body image, avoidance behaviour for physical activity or functional somatic syndrome, then it is a tragic fallibility of science. Lest we forget, Charcot, one of the founders of modern neurology and psychiatry, had emphasised more than a century ago, " When a patient calls on you, he is under no obligation to have a simple disease just to please you". Nothing provides a better example than CFS. No doubt, improving the understanding of the basic mechanism of fatigue and its treatment in CFS will have significant connotations for neurological patients with disorders of central fatigue.

8. REFERENCES

1. Fukuda K, Strauss SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to case definition and study. *Ann Intern Med* 1994; 121: 953-59.

2. Chaudhuri A, Behan WMH, Behan PO. Chronic fatigue syndrome. *Proc R Coll Physician Edinb 1998*; 28: 150-63

3. Wessely S, Hotopf M, Sharpe M. *Chronic fatigue and its syndromes.* Oxford: Oxford University Press, 1998.

4. 4.Wessely S, Nimnuan C, Sharpe M. Functional somatic syndromes: one or many? *Lancet* 1999; 354:936-9

5. Behan PO, Bakheit AMO. Clinical spectrum of post-viral fatigue syndrome. *Br Med Bull* 1991; 47: 793-808

6. 6.Johnson SK, DeLuca J, Natelson BH. Assessing somatization disorder in the chronic fatigue syndrome. *Psychosom Med* 1996; 58: 50-57

7. Deale A, Wessely S. Diagnosis of psychiatric disorder in clinical evaluation of chronic fatigue syndrome. *J Royal Soc Med* 2000; 93:310-2.

8. Shepherd C, Chaudhuri A. *ME/CFS/PVFS: An exploration of the key clinical issues*. Essex: ME Association 2001.

9. Buchwald DS, Rea DS, Kanton WJ, Russo JE, Ashley RL. Acute infectious mononucleosis: characteristics of patients who report failure to recover. *Am J Med* 2000; 109: 531-37.

10. Prins JB, Bleijenberg G, Bazelmans E, et al. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre, randomised controlled trial. *Lancet* 2001; 357: 841-47.

11. Sharpe M, Hawton K, Simkin S, et al. Cognitive behaviour therapy for the chronic fatigue syndrome: a randomised controlled trial. *BMJ* 1996; 312: 22-26

12.Fulcher KY, White PD. Randomised controlled trial of graded exercise in patients with chronic fatigue syndrome. *BMJ* 1997; 314: 1647-52.

13.Powell P, Bentall RP, Nye FJ, Edwards RHT. Randomised controlled trial of patient education to encourage graded exercise in chronic fatigue syndrome. *BMJ* 2001; 322: 387-90.

14.MacDonald KL, Osterholm MT, LeDell KH, et al. A case control study to assess possible triggers and cofactors in chronic fatigue syndrome. *Am J Med* 1996; 100: 548-54.

15.Ramsay AM. *Myalgic encephalomyelitis and postviral fatigue states.* London:Gower Medical Publishing 1988.

16.World Health Organization. *The International Classification of Diseases.* Geneva: WHO 1992.

17.Bruno RL, Crenage SJ, Fick NM. Parallels between post-polio fatigue and chronic fatigue syndrome: a common pathopysiology? *Am J Med* 1998; 105(3A): 66S-73S

18.Merkies ISJ, Scwartz PIM, Samiju JPA, van der Meche FGA van Doorn PA.. Fatigue in immune mediated neuropathies. *Neurology* 1999; 53:1648-54.

19.Krupp LB, Alvarez LA, LaRocca NG, et al. Fatigue in multiple sclerosis. *Arch Neurol* 1988; 45: 435-37.

20.Chaudhuri A, Gow J, Behan PO. Systemic viral infections and chronic fatigue syndrome: current evidence and recent advances. In: Abramsky O, Compston DAS, Miller A, Said G (eds).*Brain disease: therapeutic strategies and repair.* London: Martin Dunitz 2002; pp127-135.

21.de la Torre JC, Borrow P, Oldstone MBA. Viral persistence and disease: cytopathology in the absence of cytolysis. *Br Med Bull* 1991; 47: 838-51.

22.Scott LV, Dinan TG. The neuroendocrinology of chronic fatigue syndrome: focus on the hypothalamic-pituitary-adrenal axis. *Funct Neurol* 1999; 14:3-11.

23.Bou-Holaigh I, Rowe PC, Kan JS, Calkins H. The relationship between neurally mediated hypotension and chronic fatigue syndrome. *JAMA* 1995; 274: 961-967.

24.Stewart JM, Gewitz MH, Weldon A, et al. Orthostatic intolerance in adolescent chronic fatigue syndrome. *Pediatrics* 1999; 103: 116-21.

25.Shumate JB, Katnik R, Ruiz M, et al. Myoadenylate deaminase deficiency. *Muscle Nerve* 1979; 2:213-6.

26.McCully KK, Natelson BH, Iotti S, Sisto S, Leigh JS Jr. Reduced oxidative muscle metabolism in chronic fatigue syndrome. *Muscle Nerve* 1996; 19: 621-5

27.Barnes PRJ, Taylor DJ, Kemp GJ, Radda GK. Skeletal muscle bioenergetics in the chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1993; 56: 679-83

28.Wong R, Lopaschuk G, Zhu G et al. Skeletal muscle metabolism in chronic fatigue syndrome. In vivo assessment by ^{31}P nuclear magnetic resonance spectroscopy. *Chest* 1992; 102: 1716-22

29.Lane RJM, Woodrow D, Archard LC. Lactate responses to exercise in chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1994; 57: 375-6.

30.Sharma OP. Fatigue and sarcoidosis. *Eur Respir J* 1999; 13:713-4.

31.Kandel ER, Schwartz JH, Jessell TM. Muscles and their receptors. In: *Essentials of neural science and behavior.* Connecticut: Appleton and Lange 1995; pp 501-14.

32.Layzer RB. Asthenia and chronic fatigue syndrome. *Muscle Nerve* 1998; 21: 1609-11.

33.Chaudhuri A, Behan PO. Fatigue and basal ganglia. *J Neurol Sci* 2000; 179:34-42.

34. Krupp LB, Pollina DA. Mechanisms and management of fatigue in progressive neurological disorders. *Current Opinion in Neurology* 1996; 9:456-60

35.Musselman DL, Lawson DH, Gumnick JF, et al. Paroxetine for the prevention of depression induced by high dose interferon-alfa. *N Engl J Med* 2001; 344: 961-6

36. Watson WS, Chaudhuri A, McCreath GT, Behan PO. A possible cell membrane defect in chronic fatigue syndrome and syndrome X. In: Kaski JC (eds). *Chest pain with normal coronary angiograms: Pathogenesis, diagnosis and management.* London: Kluwer Academic Publishers 1999; pp143-49.

37. Chaudhuri A. The role of neurodiagnostics in functional disorders. In Zasler ND, Marteli MF (eds). *Physical Medicine and Rehabilitation* (State of the Art Review). Philadelphia: Hanley and Belfus Inc. 2002; pp 63-76.

38. *The Papyrus Ebers: the greatest Egyptian medical document.* (Translated by B. Ebbell). Copenhagen: Levin & Munksgaard 1937; pp 108-13.

39. Sylvest E. *Epidemic myalgia: Bornholm disease.* Oxford: Oxford University Press 1934.

40. Smith F. Charles Darwin's ill health. *Journal of the History of Biology* 1990; 23: 443-459.

41. Cook ET. *Life of Florence Nightingale.* (Vols 1&2) London: MacMillan & Co. 1913.

42. Finsen J. *Uagttagekser angaaende Sygdomsforholdene i Island.* Copenhagen, 1874.

43. Public Health Bulletin No.240. *The epidemiological study of an epidemic, diagnosed as poliomyelitis, occurring among the personnel of the Los Angeles County General Hospital during the summer of 1934.* United States of America,

44. Marinacci A. *Applied electromyography.* Philadelphia: Lea & Febiger 1968; p 91.

45.Acheson ED. The clinical syndrome variously called benign myalgic encephalomyelitis, Iceland disease and epidemic neuromyasthenia. *Am J Med* 1959; 26: 569-595

46. Sigurdsson B, Sigurjonsson J, Sigurdsson JH, Thorkelsson J, Gudmundsson KR. A disease epidemic in Iceland simulating poliomyelitis. *Am J Hyg* 1950; 52: 222-238.

47.Henderson DA, Shelokov A. Epidemic neuromyasthenia - clinical syndrome. *N Engl J Med* 1959; 260: 757-764

48.The Medical Staff of the Royal Free Hospital,. An outbreak of encephalomyelitis in the Royal Free Hospital group, London, in 1955. *BMJ* 1957; 2: 895-904.

49.Fegan KG, Behan PO, Bell EJ. Myalgic encephalomyelitis - report of an epidemic. *J R Coll Gen Pract* 1983; 33:335-339.

50. Bell EJ, Irvine KG, Gardiner AJS, Rodger JC. Coxsackie B infection in a general medical unit. *Scottish Medical Journal* 1983; 28: 157-9.

51.Gowers WR. Neurasthenia. In: *A manual of diseases of the nervous system.* London: J&A Churchill, 1893. Volume II, pp1045-50

52.Ramsay AM, O'Sullivan E. Encephalomyelitis simulating poliomyelitis. *Lancet* 1956; 1: 761-4

53. Holt GW. Epidemic neuromyasthenia: the sporadic form. *Am J Med Sci* 1965; 249: 98-112.

54. Sigurdsson B, Gudmundsson KR. Clinical findings six years after the outbreak of Akureyri disease. *Lancet* 1956; 1: 766-67.

55.Holmes GP, Kaplan JE, Glantz NM, et al. Chronic fatigue syndrome: a working case definition. *Ann Intern Med* 1988; 108:387-9

56. Scottish Short Life Working Group. *Report on Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME)*. Development of services for Scotland. Edinburgh: Scottish Executive, December 2002.

57.Vercoulen JHMM, Swanink CMA, Fennis JFM, et al. Prognosis in chronic fatigue syndrome: a prospective study on the natural course. J *Neurol Neurosurg Psychiatary* 1996; 60: 489-494.

58. Majeed T, Behan PO. Clinical overview of chronic fatigue syndrome. *EOS- J Immunol Immunopharmacol* 1995; 15: 8-14.

59. National Institute of Allergy and Infectious Diseases, National Institute of Health. *Chronic fatigue syndrome: information for physicians*. Bethesda, 1996.

60. Behan PO, Behan WMH. Postviral fatigue syndrome. *CRC Crit Rev Neurobiol* 1988; 4(2): 157-78.

61.Critchley EM. Botulism and Gulf War syndrome. *Lancet* 1996; 347: 1561.

62. Parnis FX, Sage RE. Lead poisoning: a family study. *Med J Aust* 1991; 155: 750-2.

63. Mesch U, Lowenthal RM, Coleman D. Lead poisoning masquerading as chronic fatigue syndrome. *Lancet* 1996; 347: 1193.

64.Gillepsie NC, Lewis RJ, Pearn JH et al. Ciguatera in Australia: occurrence, clinical features, pathophysiology and management. Med J Aust 1986; 145: 584-590.

65.Behan PO, Haniffah BAG. Chronic fatigue syndrome: a possible delayed hazard of pesticide exposure. *Clin Infect Dis*. 1994; 18(suppl 1); S54.

66. Chaudhuri A, Majeed T, Dinan T, Behan PO. Chronic fatigue syndrome: a disorder of central cholinergic transmission. *Journal of Chronic Fatigue Syndrome* 1997; 3(1): 3-16.

67. Friedberg F. The stress/fatigue link in chronic fatigue syndrome. In: Klimas N, Patarca R (eds). *Clinical management of chronic fatigue syndrome.* Haworth Medical Press, New York 1996; 147-152.

68. Hotopf M, David A, Hull L, et al. Role of vaccination as risk factor for ill health in veterans of the Gulf War: cross sectional study. *BMJ* 2000; 320: 1363-7.

69.Miller CS. White Paper. Chemical sensitivity: history and phenomenology. *Toxicol Ind Health* 1994; 10: 253-276.

70.Barron JL, Noakes TD, Levy W, Smith C. Hypothalamic dysfunction in overtrained athletes. *J Clin Endocrinal Metab* 1985; 60: 803-806.

71.Solomon G, Espizona L, Silverman S. Breast implants and connective tissue diseases. *N Engl J Med* 1994; 331:1231

72. Bell KM, Cookfair D, Bell DS, Resse P, Cooper L. Risk factors associated with chronic fatigue syndrome in a cluster of paediatric cases. *Rev Inf Dis* 1991; 13 (S1): S32-38.

73. Hadjivassiliou M, Gibson A, Davies-Jones GAB et al. Does cryptic gluten sensitivity play a part in neurological illness? *Lancet* 1996; 347: 369-71

74. Chaudhuri A, Behan PO. Neurological dysfunction in chronic fatigue syndrome. *J Chr Fatigue Synd* 2000; 6(3/4): 51-68.

75. Pedersen E. Epidemic vertigo. *Brain* 1959; 82: 566-80.

76. Chaudhuri A, Watson WS, Behan PO. Arguments for a role of abnormal ionophore function in chronic fatigue syndrome. In: Yehuda S, Mostofsky DI (eds). *Chronic fatigue syndrome.* New York: Plenum Press 1997; pp 119-30.

77. Imboden JB, Canter A, Cluff LE. Convalescence from influenza: a study of psychological and clinical determinants. *Arch Intern Med* 1961; 108: 393-399.

78. Majeed T. Neuroendocrine alterations in chronic fatigue syndrome. (*PhD Thesis*).University of Glasgow, 1996.

79. Sharpe M, Archard LC, Banatvala JE, et al. A report. Chronic fatigue syndrome: guidelines for research. *J R Soc Med* 1991; 84:118-21

80. Swartz MN. The chronic fatigue syndrome - one entity or many? (Editorial). *N Engl J Med* 1988; 319: 1726-1728.

81. Kent-Braun JA, Sharma KR, Weiner MW, Massie B, Miller RG. Central basis of muscle fatigue in chronic fatigue syndrome. *Neurology* 1993; 43: 125-131.

82. Freeman, R., Komaroff, A. Does the chronic fatigue syndrome involve the autonomic nervous system? *Am J Med* 1997; 102: 357-364.

83. Cordero, D., Sisto, S., Tapp, W., LaManca, J., Pareja, J., Natelson, B. Decreased vagal power during treadmill walking in patients with chronic fatigue syndrome. *Clin. Auton. Res.* 1996; 6: 329-333.

84. McCully KK, Natelson BH. Impaired oxygen delivery to muscle in chronic fatigue syndrome. *Clinical Science* 1999; 97: 603-8.

85. Arnold DL, Bore PJ, Radda GK, Styles P, Taylor, D.J. Excessive acidosis of skeletal muscle on exercise in the post-viral exhaustion/fatigue syndrome: A ^{31}P NMR Study. *Lancet,* 1984; 1: 1367-1369.

86. Arnold DL, Bore PJ, Radda GK, Styles P, Taylor DJ. Excessive intracellular acidosis of skeletal muscle on exercise in the post-viral exhaustion/fatigue syndrome: a ^{31}P NMR study. Proc. 3rd Annual Meeting of the Society for Magnetic Resonance in Medicine, New York 1984; 12-13

87. Soussi B, Schersten T, Waldenstorm A, Ronquist G. Phosphocreatine turnover and pH balance in forearm muscles of patients with syndrome X. *Lancet* 1993; 341: 829-830

88. Behan PO, Behan WMH, Bell EJ. The postviral fatigue syndrome - an analysis of findings in 50 cases. *J Infect* 1985; 10: 211-222.

89. Kar NC, Pearson CM. Myoadenylate deaminase deficiency. Report of 6 new cases. *Arch Neurol* 1981; 38: 279-81.

90.Behan WMH, More IAR, Behan PO. Mitochondrial abnormalities in the postviral fatigue syndrome. *Acta Neuropathol* 1991; 83: 61-65

91. Amato AA, McVey A, Cha C, et al. Evaluation of neuromuscular symptoms in the veterans of the Persian gulf war. *Neurology* 1997; 48: 4-13.

92. Preedy VR, Smith DG, Salisbury JR, Peters TJ. Biochemical and muscle studies in patients with acute onset post-viral fatigue syndrome. *J Clin Pathol* 1993; 46: 722-726.

93. Behan WMH, Holt J, Kay DH, Moonie P. In vitro study of muscle aerobic metabolism in chronic fatigue syndrome. *J Chr Fatigue Synd* 1999; 5(1): 3-16.

94. Sisto SA, LaManca J, Cordero DL et al. Metabolic and cardiovascular effects of a progressive exercise test in patients with chronic fatigue syndrome. *Am J Med* 1996; 100: 634-640.

95.Lloyd AR, Harles JP, Gandevia SC. Muscle strength, endurance and recovery in the post-infectious fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1988; 51: 1316-1322.

96.Lloyd AR, Gandevia SC, Hales JP. Muscle performance, voluntary activation, twitch properties and perceived effort in normal subjects and patients with the chronic fatigue syndrome. *Brain* 1991; 114: 85-98.

97. Lane RJM, Burgess AP, Flint J, Riccio M, Archard LM. Exercise responses and psychiatric disorder in chronic fatigue syndrome. *BMJ* 1995; 311: 544-45.

98. Riley MS, O'Brien CJ, McCluskey DR, Bell NP, Nicholls DP. Aerobic work capacity in patients with chronic fatigue syndrome. *BMJ* 1990; 301:953-56.

99. Stokes MJ, Cooper RG, Edwards RHT. Normal muscle strength and fatiguability in patients with effort syndromes. *BMJ* 1988; 297: 1014-17.

100. Paul L, Wood L, Behan WMH, McLaren WM. Demonstration of delayed recovery from fatiguing exercise in chronic fatigue syndrome. *Eur J Neurol* 1999; 6: 63-9.

101. Sacco P, Hopec PAJ, Thickbrooma GW, Byrnesa ML, Mastaglia FL. Corticomotor excitability and perception of effort during sustained exercise in chronic fatigue syndrome. *Clinical Neurophysiology* 1999; 110: 1883-91.

102. Gibson H, Carroll N, Clague JE, Edwards RHT. Exercise performance and fatiguability in patients with chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1993; 56: 993-98.

103. Samii A, Wassermann EM, Ikoma K, et al. Decreased post-exercise facilitation of motor evoked potentials in patients with chronic fatigue syndrome or depression. *Neurology* 1996; 47: 1410-14.

104. Patterson J, Aitchison F, Wyper DJ, et al. SPECT brain imaging in chronic fatigue syndrome. *EOS- J Immunol Immunopharmacol* 1995; 15: 53-58

105. Costa DC, Brostoff J, Ell PJ. Brain stem hypoperfusion in patients with myalgic encephalomyelitis-chronic fatigue syndrome. (abstr) *Eur J Nucl Med* 1992; 19: 773

106. Goldberg M, Meena I, Darcourt J. NeuroSPECT findings in children with chronic fatigue syndrome. *Proceedings AACFS Conference* 1994; 79.

107.Schwartz RB, Komaroff AL, Garada BM et al. SPECT imaging of the brain: comparison of findings in patients with chronic fatigue syndrome, AIDS dementia complex and major unipolar depression. *AJR* 1994; 162: 943-951.

108. Tirelli U, Cherichetti F, Tavio M, et al. Brain positron emission tomography in chronic fatigue syndrome: preliminary data. *Am J Med* 1998; 105(3A): 54S-58S.

109. Siessmeier T, Nix WA., Hardt J, et al. Observer independent analysis of cerebral glucose metabolism in patients with chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 2003; 74: 922-28.

110.Martin JB, Riskind PN. Neurologic manifestations of hypothalamic disease. In: Swaab DF, Hofman MA, Mirmiran M, Ravid R, van Leeuween FW(eds.) *Progress in Brain Research.* Amsterdam: Elsvier Science Publications B.V. 1992; pp 31-41.

111.Martin JB, Reichlin S. *Clinical endocriology.* Philadelphia: F A Davis, 1987.

112. Case Records of the Massachussets General Hospital: a 30 year old man with polydipsia, hypopituitarism and a mediastinal mass. *N Eng J Med* 1991; 324: 677-87.

113.Bakheit AMO, Behan PO, Watson WS, Morton JJ. Abnormal arginine-vasopressin secretion and water metabolism in patients with postviral fatigue syndrome. *Acta Neurol Scand* 1993; 87: 234-238

114.Levy MS, Power MH, Kepler EJ. The specificity of the "water test" as a diagnostic procedure in Addison's disease. *Journal of Clinical Endocrinology* 1946; 6: 607-632.

115.Demitrack MA, Dale JK, Strauss SE, et al. Evidence for impaired activation of HPA axis in patients with chronic fatigue syndrome. *J Clin Endocrin Metab* 1991; 73: 1124-1134

116.Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995; 332: 1351-1362.

117.Harbuz MS, Lightman SL. Stress and hypothalamo-pituitary-adrenal axis: acute and chronic immunological activation. *Journal of Endocrinology* 1992; 134: 327-329.

118.Harbuz MS, Rees RG, Eckland D et al. Paradoxical responses of hypothalamic CRF mRNA and CRF-41 peptide and adenohypophyseal POMC mRNA during chronic inflammatory stress. *Endocrinology* 1992; 130: 1394-1400.

119.McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998; 338: 171-9.

120. McKenzie R, O'Fallon A, Dale J, et al. Low dose hydrocortisone for treatment of chronic fatigue syndrome. A randomised controlled trial. *JAMA* 1998; 280: 1061-66.

121.Yehuda R. Current status of cortisol findings in post-traumatic stress disorder. *Psychiatr Clin North Am.* 2002; 25:341-68.

122. Dinan TG. Studying brain receptor function: a neuroendocrine approach. *Irish Journal of Psychological Medicine* 1993; 10: 4-5.

123. O'Keane V, Dinan TG. Prolactin and cortisol responses to d-fenfluramine in major depression: evidence for diminished responsivity of central serotoninergic function. *Am J Psychiatry* 1991; 148: 1009-1015.

124.Bakheit AMO, Behan PO, Dinan TG et al. Possible upregulation of hypothalamic 5-hydroxytryptamine receptors in patients with postviral fatigue syndrome. *BMJ* 1992; 304: 1010-12.

125.Cleare AJ, Bearn J, Allain T et al. Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. Journal of Affective Disorders 1995; 34: 283-289.

126.Hillhouse EW, Milton NGN. Effect of acetylcholine and 5-hydroxytryptamine on the secretion of corticotrophin releasing factor-41 and arginine vasopressin from rat hypothalamus *in vitro*. Journal of Endocrinology 1989; 122: 713-718.

127.Hu SB, Lightman SL, Tannahill LA. 5-hydroxytryptamine stimulates corticosteroid sensitive CRF release from culture foetal hypothalamic cells. Role of protein kinases. *Brain Research* 1992; 574: 266-270.

128. Demitrack MA, Gold PW, Dale JK et al. Plasma and cerebrospinal fluid monoamine metabolism in patients with chronic fatigue syndrome: preliminary findings. *Biological Psychiatry* 1992; 32: 1065-1077.

129. Canadian MS research Group. A randomized controlled trial of amantadine in fatigue associated with multiple sclerosis. *Can J Neurol Sci* 1987; 14: 273-8.

130. O'Keane V, O'Flynn K, Lucey J, Dinan TG. Pyridostigmine induced growth hormone responses in healthy and depressed subjects: evidence for cholinergic supersensitivity in depression. *Psychol Med* 1992; 22: 55-60.

131.Bruno RL, Creange SJ, Fick NM. Parallels between post-polio fatigue and chronic fatigue syndrome: a common pathophysiology?. *Am J Med* 1998; 105 (3A): 66S-73S.

132. Buchwald D, Komaroff AF. Review of laboratory findings for patients with chronic fatigue syndrome. *Rev Inf Dis* 1991; 13 (S1): S12-S18.

133. Simpson LO. The role of nondiscocytic erythrocytes in the pathogenesis of myalgic encephalomyelitis/chronic fatigue syndrome. In: Hyde BM, Goldstein J, Levine P (eds). *The clinical and scientific basis of myalgic encephalomyelitis/ chronic fatigue syndrome.* Canada: The Nightingale Research Foundation 1992; pp 597-605

134.Tobi M, Morag A, Ravid Z et al. Prolonged atypical illness associated with serological evidence of persistent Epstein-Barr virus infection. *Lancet* 1982; 1: 61-64.

135.Strauss SE, Tosato G, Armstrong G et al. Persisting illness and fatigue in adults with Epstein-Barr virus infection. *Ann Intern Med* 1985; 102: 7-16.

136. Kuratsune H, Yamaguti K, Takahashi M et al. Acylcarnitine deficiency in chronic fatigue syndrome. *Clin. Inf. Dis.* 1994; 18 (suppl 1):S62-S67.

137.Majeed T, Simone CD, Famularo G, Marcellini S, Behan PO. abnormalities of carnitine metabolism in chronic fatigue syndrome. *Eur J Neurol* 1995; 2: 425-428.

138.Cox IM, Campbell MJ, Dowson D. Red blood cell magnesium and chronic fatigue syndrome. *Lancet* 1991; 337: 757-760.

139.Hinds G, Bell NP, McMaster D, McCluskey DR. Normal red cell magnesium concentrations and magnesium loading tests in patients with chronic fatigue syndrome. *Ann Clin Biochem* 1994; 31:93/162.1-3.

140.Read R, Spickett G, Harvey J, Edwards AJ, Larson HE. IgG1 subclass deficiency in patients with chronic fatigue syndrome. *Lancet* 1988; 1: 241-242.

141.Lloyd AR, Wakefield D, Boughton CR, Dwyer JM. Immunological abnormalities in chronic fatigue syndrome. *Med J Aust* 1989; 151:122-124.

142.Pearn J. Neurology of ciguatera. *J Neurol Neurosurg Psychiatry* 2001; 70: 4-8.

143. Chaudhuri A, Watson WS, Pearn J, Behan PO. Symptoms of chronic fatigue syndrome are due to abnormal ion channel function. *Medical Hypotheses* 2000; 54: 59-63.

144. Cannon SC. Ion channel defects and aberrant excitability in myotonia and periodic paralysis. *Trends in Neurosciences* 1996; 19: 3-10.

145. Boudoulas H, Tyson CC, Leighton RF, Wilt SM. Myocardial lactate production in patients with angina-like chest pain and angiographically normal coronary arteries and left ventricle. *Am J Cardiol* 1974; 34: 501-5.

146. Sylven C. Syndrome X. *J Intern Med* 1993; 234: 431-433.

147.Waldenstörm A, Ronquist G, Folhman J, Gerdin B, Ilback NG. Ionophoric interaction with the myocyte sarcolemma: a new insight in the pathophysiology of degenerative myocardial disease. *Scand Journal of Infectious Diseases* 1993; 88: 131-134.

148.Waldenstörm A, Ronquist G, Lagerqvist B. Angina pectoris patients with normal coronary angiograms but abnormal thallium perfusion scan exhibit low myocardial and skeletal muscle energy charge. *J Intern Med* 1992; 231: 327-331

149. Waldenstörm A, Fohlman J, Ilback NG, et al. Coxsackie B3 myocarditis induces a decrease in energy charge and accumulation of hyaluronan in the mouse heart. *Eur J Clin Invest* 1993; 23: 277-82.

150. Watson WS, McCreath GT, Chaudhuri A, Behan PO. Possible cell membrane transport defect in chronic fatigue syndrome. *J Chr Fatigue Synd* 1997; 3(3):1-13.

151. McClave SA, Snider HL. Use of indirect calorimetry in clinical nutrition. *Nutrition in Clinical Practice* 1992; 7: 207-221.

152. Watson WS, McMillan DC, Chaudhuri A, Behan PO. Increased resting energy expenditure in chronic fatigue syndrome. *J Chr Fatigue Synd* 1998; 4(4): 3-14.

153. Ferrannini E. The theoretical basis of indirect calorimetry: a review. *Metabolism* 1988; 37: 287-301.

154. Gilbert A, Lereboullet P, Herschen M. *Bull. et mem Soc med d hop de Paris* 1907; 24: 1203.

155. Zhang C, Baumer A, Mackay IR, Linnane AW, Nagley P. Unusual pattern of mitochondrial DNA deletions in skeletal muscle of an adult human with chronic fatigue syndrome. *Hum Mol Genet* 1995; 4: 751-4.

156. Buchwald D, Herrell R, Ashton S, et al. A twin study of chronic fatigue. *Psychosom Med* 2001; 63: 936-43.

157. Petrou S, Ordway RW, Kirber MT, et al. Direct effects of fatty acids and other charged lipids on ion channel activity in smooth muscle cells. *Prostaglandins Leukotrienes and Essential Fatty Acids* 1995; 52: 173-78.

158.Martin DW, Mayes PA, Rodwell VW. *Harper's Review of Biochemistry* (18th edition). Singapore: Lange Medical Publications, 1981.

159. Horrobin DF, Bennett CN. Phospholipid Metabolism and the pathophysiology of psychiatric and neurological disorders. In: Peet M, Glen I, Horrobin DF (eds). *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (2nd edition). Marius Press 2003, Canforth: pp 3-48.

160. Foster DW, Rubenstein AH. Hypoglycemia, insulinoma and other hormone secreting tumour of the pancreas. In: Petersdorf RG, Adams RD, Braunwald E, Isselbacher KJ, Martin JB, Wilson JD (eds). *Harrison's Principles of Internal Medicine* (10th edition). New York: McGraw Hill 1987; pp 682-89.

161. Matsui S, Fu ML, Shimizu M, et al. Dilated cardiomyopathy defines serum autoantibodies against G-protein-coupled cardiovascular receptors. *Autoimmunity* 1995; 21: 85-88.

162. Hinson JP, Dawnay AB, Raven PW. Why should we give a qualified welcome to ouabain: a whole new family of adrenal steroid hormones? *J Endocrinol* 1995; 146: 369-72.

163.Hilton PJ, White RW, Lord GA, et al. An inhibitor of the sodium pump obtained from human placenta. *Lancet* 1996; 348: 303-5.

164.Sakurai M, Mannue T, Kanazawa I, Tanabe H. Lidocaine unmasks silent demyelinative lesions in multiple sclerosis. *Neurology* 1992; 42:2088-93.

165.Brinkmeier H, Aulkemeyer P, Wollinsky KH, Rudel R. An endogenous pentapeptide acting as a sodium channel blocker in inflammatory autoimmune disorders of the central nervous system. *Nat Med* 2000; 6:808-11.

166. Muir WA, McLaren GD, Braun WE, Askari AK. Evidence for heterogeneity in hereditary haemochromatosis. Evaluation of 174 persons in nine families. *Am J Med* 1984; 76: 806-14.

167. Simon M, Bourel M, Genetet B, Fauchet R. Idiopathic haemochromatosis: demonstration of recessive transmission and early detection by family HLA typing. *N Engl J Med* 1977; 297: 1017-21.

168. http://www.ncbi.nlm.nih.gov/entrez/dispomim/cgi?id=235200(Online Mendelian Inheritance in Man; accessed on 7th December, 2003).

169. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormality in people heterozygous for hereditary haemochromatosis. *N Eng J Med* 1996; 335: 1799-1805.

170. Dfrup I, Skajaa K, Clausen T, Kjeldsen K. Reduced concentrations of potassium, magnesium and sodium potassium pumps in human skeletal muscle during treatment with diuretics. *BMJ* 1988; 296: 455-58.

171. Stacpoole PW. Lactic acidosis. *Endocrin Metab Clin North Am* 1993; 22: 221-45.

172. De Stefano N, Matthews PM, Ford B, et al. Short-term dichloroacetate treatment improves indices of cerebral metabolism in patients with mitochondrial disorders. *Neurology* 1995; 45: 1193-98.

173. Burnet RB, Yeap BB, Chatterton BE, Gaffney RD. Chronic fatigue syndrome: is total body potassium important? *Med J Aust* 1996; 164: 384.

174.Nashef L, Lane RJM. Screening for mitochondrial cytopathies: the sub-anaerobic threshold exercise test (SATET). *J Neurol Neurosurg Psychiatry* 1989; 52: 1090-4.

175.Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14: 377-81.

176.Harris JA, Benedict FG. *A Biometric Study of Basal Metabolism in Man.* Washington: Carnegie Institute, 1919.

177.Watson WS, Chaudhuri A, Lyon DT, Gallacher SJ, Behan PO. DXA body composition studies are not affected by extracellular water measurements using stable sodium bromide dilution. *Physiol Meas* 2001; 21:541-7.

178.Watson WS, Sammon AM. Body composition in cachexia resulting from malignant and non-malignant diseases. *Cancer* 1980; 46:2041-6.

179.Watson WS. Total body potassium measurement--the effect of fallout from Chernobyl. *Clin Phys Physiol Meas* 1987; 8:337-41.

180. Skrabal F, Arnot RN, Joplin GF. Equations for the prediction of normal values for exchangeable sodium, exchangeable potassium, extracellular fluid volume, and total body water. *BMJ* 1973; ii:37-8.

181.Boddy K, King PC, Hume R, Weyers E. The relation of total body potassium to height, weight, and age in normal adults. *Journal of Clinical Pathology* 1972; 25:512-7.

182.Pols MA, Peeters PH, Bueno-De-Mesquita HB, Ocke MC, Wentink CA, Kemper HC et al. Validity and repeatability of a modified Baecke questionnaire on physical activity. *Int J Epidemiol* 1995; 24:381-8.

183.Brooks JCW, Roberts N, Whitehouse G, Majeed T. Proton magnetic resonance spectroscopy and morphometry of hippocampus in chronic fatigue syndrome. *Br J Radiol* 2000; 73: 1206-8.

184. Haley RW, Marshall WW, McDonald GG, et al. Brain abnormalities in Gulf War syndrome: evaluation with 1H MR spectroscopy. *Radiology* 2000; 215: 807-17.

185. Chaudhuri A, Condon BR, Gow JW, Brennan D, Hadley DM. Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *NeuroReport* 2003; 14: 225-8.

186. Kawagoe R, Takikawa Y, Hikosaka O. Expectations of reward modulates cognitive signals in the basal ganglia. *Nature Neurosci* 1998; 411-6.

187. Verhoeff NP, Bobeldijk M, Feenstra MG, et al. In vitro and in vivo D2-dopamine receptor binding with [^{123}I] S(-)iodobenzamide [^{123}I] IBZM in rat and human brain. *International Journal of Radiation Application and Instruments* 1991; 18: 837-46.

188. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of $5HT_2$ and D_2 receptor occupancy of clozapine, risperidone and olanzapine in schizophrenia. *Am J Psychiat* 1999; 156: 286-93.

189. Verhoeff NP, Brucke T, Podreka L, et al. Dynamic SPECT in two healthy volunteers to determine the optimal time for an in vivo D2 dopamine receptor imaging with [^{123}I] IBZM using the rotating gamma camera. *Nuclear Medicine Communications* 1991; 12: 687-697.

190. Kandel ER, Schwartz JH, Jessell TM. The nervous system. In: *Essentials of neural science and behavior.* Connecticut: Appleton and Lange 1995; pp 71-88.

191. Owens D, Evans J. Population studies on Gilbert's syndrome. *J Med Genetics* 1975; 12: 152-26.

192. Foster DW, Rubenstein AH. Hypoglycemia. In: Fauci AS, Martin JB, Braunwald E, et al (eds). *Harrison's Principles of Internal Medicine* (14th edition). New York: McGraw-Hill 1998; pp 2081-7.

193. Yin H, Berg AK, Westman J, Hellenstorm C, Fisk G. Complete nucleotide sequence of a Coxsackievirus B-4 strain capable of establishing persistent infection in human pancreatic islet cells: effects on insulin release, proinsulin synthesis, and cell morphology. *J Med Virol* 2002; 68 : 544-57.

194. Cunningham L, Bowles NE, Lane RJ, Dubowitz V, Archard LC. Persistence of enteroviral RNA in chronic fatigue syndrome is associated with the abnormal production of equal amounts of positive and negative strands of enteroviral RNA. *J Gen Virol* 1990 ; 71 :1399-402.

195.Jacobson W, Saich T, Borysiewicz LK, et al. Serum folate and chronic fatigue syndrome. *Neurology* 1993; 43: 2645-7.

196. Hille B. *Ionic channels of excitable membranes.* Boston: Sinaeur Association 1992.

197. Zuberi S, Eunson L, Spauschus R, et al. A novel mutation in the human voltage-gated potassium channel gene (kv1.1) associated with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain* 1999; 122: 817-25.

198. Hart I, Waters C, Vincent A, et al. Autoantibodies detected to expressed K+ channels are implicated in neuromyotonia. *Ann Neurology* 1997; 41: 238-46.

199. Rhodes K, Monaghan M, Barrezutea et al. Voltage-gated K+ channel B subunits: expression and distribution of KVβ1 and KVβ2 in adult rat brain. *J Neurosci* 1996; 16: 4846-60.

200. Morvan A. de la choree fibrillaire. *Gaz. Hebdomadaire Med Chir* 1870; 27: 173-200.

201.Barber P, Anderson N, Vincent A. Morvan's syndrome associated with voltage gated K+ channel antibodies. *Neurology* 2000; 54: 771-2.

202. Gow JW, McGarry F, Behan WMH, Simpson K, Behan PO. Molecular analysis of cell membrane ion channel function in chronic fatigue syndrome. (Abstract). International Meeting on Chronic Fatigue Syndrome. Dublin, 1994.

203.Spence VA, Khan F, Belch JJF. Enhanced sensitivity of the peripheral vascular response in patients with chronic fatigue syndrome. *Am J Med* 2000; 108: 736-9.

204.Martin SD, Martin E, Rai SS, et al. Brain blood flow changes in depressed patients treated with interpersonal psychotherapy or venlafaxine hydrochloride. Preliminary findings. *Arch Gen Psychiatry* 2001; 58: 641-8.

205. Lane RJM, Barnett MC, Taylor DJ, Kemp GJ, Lodi R. Heterogeneity in chronic fatigue syndrome: evidence from magnetic resonance spectroscopy of muscle. *Neuromuscular Disorders* 1998; 8:204-9.

206.Jakobi JM, Killinger DW, Wolfe BM, Mahon JL, Rice CL. Quadriceps muscle function and fatigue in women with Addison's disease. *Muscle Nerve* 2001; 24: 1040-9.

207.McCully KK, Natelson BH. Impaired oxygen delivery to muscle in chronic fatigue syndrome. *Clinical Science* 1999; 97: 603-8.

208. Petty RKH, Harding AE, Morgan-Hughes JA. The clinical features of mitochondrial myopathy. *Brain* 1986; 109: 915-38.

209. Jackson MJ, Schaefer JA, Johnson MA, et al. Presentation and clinical investigation of mitochondrial respiratory chain disease- a study of 51 patients. *Brain* 1995; 118: 339-57.

210. Wagenmakers AJM. Muscle function in critically ill patients. *Clinical Nutrition* 2001

211.Low PA, Opfer Gehring TL, McPhee BR, et al. Prospective evaluation of clinical characteristics of orthostatic hypotension. *Mayo Clin Proc* 1995; 70: 617-22.

212.Kurpad AV, Khan K, Calder AG, Elia M. Muscle and whole body metabolism after norepinephrine. *Am J Physiol* 1994; 266: E877-E884.

213. Mense S, Simons DG, Russell IJ. *Muscle pain: understanding its nature, diagnosis and treatment.* Philadelphia: Lippincott Williams & Wilkins 2001.

214. Kress M, Fickenscher H. Infection by human varicella-zoster virus confers norepinephrine sensitivity to sensory neurons from rat dorsal ganglia. *FASEB Journal* 2001; 15: 1037-43.

215. Drummond RD. The effect of sympathetic activity on thermal hyperalgesia in capsaicin-treated skin during body cooling and warming. *Eur J Pain* 2001; 5: 59-67.

216. Llau ME, Durrieu G, Tran MA, et al. A study of dopaminergic sensitivity in Parkinson's disease: comparison in "de novo" and levodopa-treated patients. *Clin Neuropharmacol.* 1996;19: 420-7.

217. Queiroz CM, Frussa-Filho R. Effects of buspirone on dopaminergic supersensitivity. *Life Sci* 1997; 61:371-82.

218. Association of British Pharmaceutical Industry. *ABPI Compendium of data sheets and summary of product characteristics* (1999-2000). London: Datapharm Publications 1999; pp 238-9.

219.Fallini A, Calabrese G, Origgi D, et al. Proton magnetic resonance spectroscopy and intracranial tumours. Clinical Perspectives. *J Neurol* 1996; 243: 706-14.

220. Davie CA, Hawkins CP, Barker GJ, et al. Serial proton resonance spectroscopy in acute multiple sclerosis lesions. *Brain* 1994; 117: 49-58.

221. Brand A, Reichter-Landberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci* 1993;15: 289-98.

222. Forton DM, Allsop JM, Main J, et al. Evidence for a cerebral effect of the hepatitis C virus. *Lancet* 2001; 358: 38-9.

223. Tracey I, Carr CA, Guimaraes AR, et al. Brain choline-containing compounds are elevated in HIV-positive patients before the onset of AIDS dementia complex: a proton magnetic resonance spectroscopic study. *Neurology* 1996; 46: 783-8.

224. Tomoda A, Miike T, Yamada E, et al. Chronic fatigue syndrome in childhood. *Brain Dev* 2000; 22: 60-4.

225. Puri BK, Counsell SJ, Zaman R, et al. Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Arch Psychiatr Scand* 2002; 106: 224-6.

226. Carrasco L. Modification of membrane permeability by animal viruses. In: *Advances in virus research* (Vol 45). New York: Academic Press 1995; pp 61-112.

227. Shin JS, Abraham SN. Co-option of endocytic functions of cellular caveolae by pathogens. *Immunology* 2001; 102: 2-7.

228. McCully KK, Smith S, Rajae S, Leigh JS Jr, Natelson BH. Blood flow and muscle metabolism in chronic fatigue syndrome. *Clinical Science* 2003; 104: 641-7.

229.von Economo C. *Encephalitis Lethargica. Its sequelae and management.* (Translated by K.O. Newman). London: Oxford University Press 1931.

230. Denny-Brown D. *The basal ganglia and their relation to disorders of movement.* Oxford: Oxford University Press, 1952.

231.DeLong M, Georgopoulos AP. Motor functions of the basal ganglia. In: Brookhart JM, Mountcastle VB, Brooks VB (eds). *Handbook of Physiology* (Vol 2). Bethesda: American Physiological Society 1981; pp 1017-62.

232. Stahl SM. Basal ganglia neuropharmacology and obsessive-compulsive disorder: the obsessive-compulsive disorder hypothesis of basal ganglia dysfunction. *Psychopharm Bull* 1988; 24: 370-4.

233. Fuster JM. *The prefrontal cortex: anatomy, physiology and neuropsychology of the frontal lobe.* New York: Raven Press, 1989.

234. American Psychiatric Association. *Diagnosis and Statistical Manual of Mental Disorders* (4th ed). Washington DC: APA 1994.

235. Horrobin D. *The Madness of Adam and Eve. How Schizophrenia shaped Humanity*. London: Corgi Books 2002, p 296.

9. APPENDIX : Clinical Proforma and the Glasgow Fatigue Scale

One difficulty that exists in the assessment of CFS patients and their response to a therapeutic intervention is the lack of an appropriate rating scale. Most fatigue rating scale used in the assessment of CFS are not specifically designed for CFS patients and do not take into account their fatigue symptoms and the overlapping features that often co-exist, such as syndrome X or irritable bowel syndrome. Since it is impossible to ascertain the efficacy of any therapeutic claim in CFS in the absence of a specific and sensitive disease marker, we have recently developed a *CFS Rating Scale* based on symptom severity. This has a total score in excess of 200 points and takes into account not only the symptoms of fatigue and myalgia and the activities of daily living but also other symptoms related to cardiac, neuropsychiatric and gastrointestinal changes frequently experienced by these patients. This scale has been used in the clinical assessment of the CDC-defined CFS patients.

The final score in the CFS rating scale was calculated by adding all individual subscores and then dividing this figure by 10 and approximating the value to the nearest full number. Accordingly, the maximum possible score in this scale is 25. The scale was used in 90 CFS patients with a duration of symptoms of 1-15 years (mean 4.5 years). The normative data was derived from 30 healthy subjects.

In the CFS patients, the final score ranged from 3-19 (mean score: 9) out of a possible 25. The final score in the normal subjects was always <1 (mean score: 0). Thus, the rating scale may reliably distinguish CFS patients from normal healthy subjects. However, it needs to be validated against other fatiguing disorders and depression to determine its specificity and sensitivity in the CDC-defined CFS population.

CHRONIC FATIGUE SYNDROME

PATIENT'S PROFORMA

A: Patient Characteristics

Name: _____ Sex: M/F D.O.B._____

Study No: _____ Hospital (Unit No.)
_____

Address :_____ Contact Phone No. _____

GP details: _____

B: Personal History

Main Complaints:	**Duration:**
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

C: Duration of CFS and related symptoms:

When were you first diagnosed as CFS/ME?

Duration of fatigue: more than 5 yrs, 3-5 yrs, 2-3 yrs, 1-2 yrs, 6 months to 1 year?

Onset of fatigue:

acute onset (can recall the precipitating event with the date),
subacute
gradual or insidious onset.

*How did you get it** - after a 'flu-like illness?

- after a chest infection?
- after diarrhoea?
- after a stressful event?
- after immunisation?
- after trauma?

* (In each of these, please specify the nature of the event, i.e. type of stress, such as bereavement, type of trauma such as surgical intervention, and the nature of the vaccine given.)

Is its severity influenced by
a) menstruation
b) mental activity
c) drugs
d) injection
e) smoking
f) alcohol
g) heat/cold

Did you test positive for any of the following viruses or bacteria after you had the symptoms of CFS?

Coxsackie A/B	Infectious mononucleosis (EBV)
Chlamydia	Rubella
Influenza	Herpes simplex
Brucella	Campylobacter jejuni

Have you had any change in weight since being diagnosed with CFS? YES/NO.

Do you have any of the following:

Gilbert's syndrome

Hepatitis B vaccination

Jaundice

Coeliac disease

Irritable bowel syndrome

Asthma/ other atopic diseases

Allergies to food or drugs (specify)

Craving for salt, bread, sweets, or chocolate

Chest pain

Prostatism (in males)

Endometriosis / Polycystic ovarian disease (in females)

Loss of sexual drive

Old poliomyelitis

Alcohol intolerance

Depressive symptoms

Anxiety disorder

Is there any history of asthma or eczema in any members of your family?

Is there anyone else in your family who suffers from CFS?

Did you participate in any of the studies of CFS? If yes please indicate when, where (which hospital) and nature of the study.

D: Exposure to Toxins/Chemicals: YES/NO

If YES:

time of exposure __/__/__
dd mm yy

name of chemical/suspected toxin _____

route of entry: ingestion/inhalation/skin contamination_____

approximate duration of exposure _____ (days, weeks, months)

symptoms:

_____

_____

_____

_____

Laboratory tests: POSITIVE/NEGATIVE

diagnosis: CERTAIN/UNCERTAIN

treatment taken for presumed toxic exposure _____

E: Treatment History

Have you taken any of the following medications for treatment of CFS?

Antidepressants
(specify)

Sertraline

Clomipramine

Amitriptyline

MAOI

Newer antidepressants (e.g. RMAOI, Effexor, Edronex)

Drug treatment for fatigue (duration, dose and treatment effect)

Amantadine

Primoline

Methylphenidate

Lamotrigine or Carbamazepine

Gabapentin

Complementary therapy: Fish oil, Primrose oil, Chinese herbs, St. John's Wort, aromatherapy, reflexotherapy, acupuncture, nutritional therapy
Others (please specify):

Have your been under any kind of anaesthesia before or after the development of CFS? YES/NO.
If YES, please specify the nature of the anaesthetic and whether it had precipitated or changed the course of the fatigue symptoms.

Have you taken any of the following antibiotics in the course of your current illness due to CFS or for any other reason?

Penicillins or cephalosporins

Quinolones

Tetracycline

Macrolide antibiotics

(Erythromycin, Clarithromycin, Roxithromycin) - (please record if any of the antibiotics had any influence in the course of the fatigue symptoms)

Have you taken any other medications or therapy that might have influenced the course of your fatigue?

F: Past Medical History

Pre-morbid psychiatric disorders (e.g. depression, anxiety neurosis, hyperventilation, hypochondriasis or major affective disorder)

1._____
2._____
3._____
4._____

Endocrine disorder (e.g. diabetes, thyroid diseases including thyroiditis, hypo-pituitarism)

1._____
2._____
3._____
4._____

Recurrent infectious diseases (e.g. gastroenteritis, influenza)

1._____
2._____
3._____
4._____

Immunologic/hypersensitivity disorders (e.g. asthma, drug-sensitivity, rhinitis, thyroiditis, SLE, rheumatologic disorder)

1._____
2._____
3._____
4._____

GI disorders (e.g. Dyspepsia, reflux, IBS)

1._____
2._____
3._____
4._____

Neurological disorders
(e.g. migraine, multiple sclerosis, polio
Parkinson's disease, epilepsy)

1._____
2._____
3._____
4._____

Cardiovascular disorder
(e.g. coronary artery disease,
syndrome X, hypertension,
peripheral vascular disease)

1._____
2._____
3._____
4._____

G: Previous laboratory investigations

Full blood count (please record if there was any neutropaenia)

ESR

Urea and Electrolytes

Liver function tests

Plasma bilirubin

Random glucose

Serum CK

Thyroid function

Urine (dipstix)

Any other tests:

H: Family History

Do you have any member of your family suffering from asthma, eczema, irritable bowel syndrome, Gilbert's syndrome, haemochromatosis, cirrhosis of liver, Parkinson's disease, polio, Alzheimer's disease, suicidal depression, epilepsy, migraine or cardiovascular disease.?

Is there anyone in your family or in close neighbourhood to you who could be suffering from chronic fatigue syndrome or CFS?

Relationship	Nature of illness	Survived/Died
spouse/partner		
sibling		
parents		
children		
first degree relatives (e.g. cousins)		

I: History of concomitant medications

Name of medication	Condition for which prescribed	Duration of Treatment	Adverse effects if any

J: Personal History

Place of residence in the recent past (beginning with current one)

1._____

2._____

Occupation:_____ Employed/Unemployed _____

Employment status in past five years (beginning with current one)

1_____

2_____

Marital status (single/married/living with partner) _____

(for females between 15-45 years): contraception practiced YES/NO

If YES, mode of contraception _____

Handedness: right/left/ambidextrous _____

Smoker? YES/NO

If YES, number of cigarettes smoked per day _____

		AMOUNT
Alcohol consumption (per week)	Whisky	_____
	Beer	_____
	Wine	_____

K: Social History

Is the patient living at home/at an institution? If the patient is at home, please record: other family members/carers looking after the patient.

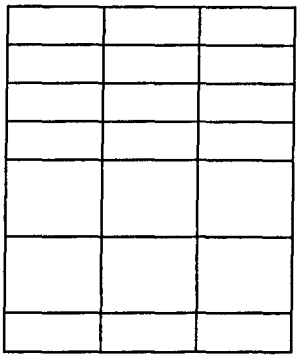
Are the patient's home circumstances adequate for the level of his/her disability?

Has the patient applied for any kind of disability benefit on account of CFS?

CFS Rating Scale

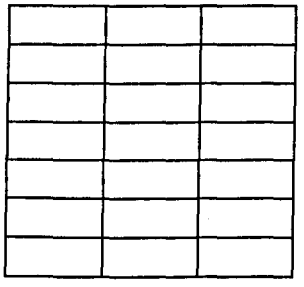
I.1 Fatigue
(maximum score = 20)

20 = at rest
15 = after getting up from restful night's sleep
12 = after any of: bathing, dressing self, eating a meal
9 = after daily routine household activities
6 = after less than half (50%) of previous level of outdoor activity
3 = after less than usual (but more than half of) the previous level of outdoor activity
0 = after strenuous activity



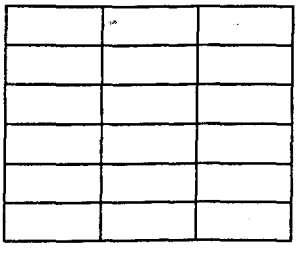
1.2 Activities of daily living
(maximum score = 20)

20 = bed or chairbound for most of the waking period
15 = requires support for some of the ADL
12 = requires no support for ADL, but housebound
9 = left job due to CFS
6 = working at less than half (50%) of the normal capacity
3 = working at less than previous capacity
0 = working at normal capacity



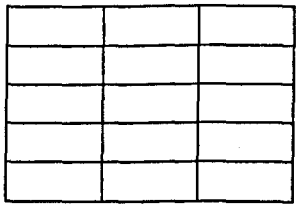
1.3 Walking at a normal pace (unsupported)
(maximum score = 12)

12 = cannot walk
9 = can walk up to 100 metres
6 = can walk more than 100 but less than 300 metres
3 = can walk more than 300 but less than 600 metres
1 = can walk more than 600 metres but less than a km
0 = can walk more than a km



I.4 Course of fatigue
(maximum score = 6)

6 = fluctuating fatigue, but without any clear remission
3 = relapsing and remitting fatigue
2 = progressive fatigue
1 = stationary fatigue state, or fluctuation AM more than PM
0= improving fatigue



I.5 Post-exertion malaise
(maximum score = 6)

6 = severe worsening of fatigue
3 = moderate worsening of fatigue
1 = mild worsening of fatigue
0= no difference from fatigue at rest

SECTION 1 TOTAL MAXIMUM SCORE = 64

II Physical symptoms (other than GI or Psychological)

II.1 Myalgia in more than one limb
(maximum score = 6)

For any of the upper or lower extremities
3 = severe
2 = moderate
1 = mild

II.2 Neck & shoulder pain (must be present for some time daily)
(maximum score = 3)

3 = severe, requires rest and analgesic
2 = moderate, requires analgesic only
1 = mild and tolerable, usually analgesics not required

II.3 Arthralgia (without joint swelling or redness) in more than one limb.
(maximum score = 6)
(for both upper and lower extremities, add score for individual extremity)
For any limb
3 = severe
2 = moderate
1 = mild

II.4 Unexplained muscle weakness (Grade 4 or less in MRC Scale)
(maximum score = 6)
For any of the upper or lower extremities.
3 = severe
2 = moderate
1 = mild

II.5 Headache
(maximum score = 6)

6 = severe, throbbing headache preventing work, requiring complete rest.
3 = moderately severe headache with visual symptoms or nausea.
2 = daily headache requiring intermittent use of analgesics.
1 = mild and occasional headache.
0 = mild and tolerable headache, usually analgesics not required.

II.6 Dysequilibrium (symptoms of dizziness)
(maximum score = 6)

6 = symptoms of dizziness accompanied by nausea and vertigo present almost daily, severe, requiring rest and medication.
3 = symptoms of nausea and vertigo present frequently (3 days a week), requiring rest or medication
2 = symptoms of nausea or vertigo or dizziness present sometime during the week
1 = any of the symptoms of nausea or vertogo, present occasionally (less than once a week.

II.7 General physical symptoms
(maximum score = 6)

2 = documented fever
2 = documented non-exudative pharyngitis
2 = documented lymphadenopathy
0 = undocumented symptoms

II.8 Visual symptoms
(maximum score = 6)

2 = photosensitivity or meningism
2 = temporary visual obscuration or difficulty in focussing
2 = dark spots in front of eyes or scintillating scotomas

II.9 Sweating
(maximum score = 6)

6 = profuse, unexplained drenching sweats, frequent, at night time and daytime, necessitating change of clothing.
3 = frequent unexplained night time and daytime sweating - no change of dress required
2 = occasional unexplained night or daytime sweats
1 = sweating due to environmental reasons.
0 = no sweating

II.10 Chest Pain (maximum score = 9)

9 = syndrome X
6 = frequent attacks of chest pain, diagnosed as atypical angina. ECGs normal or showing only mild changes
3 = occasional attacks of precordial chest pain occurring at rest or on effort
2 = attacks of precordial chest pain only on severe effort
1 = any other chest pain (eg left inframammary chest pain)
0 = no chest pain

II.11 Frequent 'flu' like illness/sore throat or other viral illnesses (maximum = 3)

3 = > 6 attacks a year
2 = 3 - 5 attacks a year
1 = 1 - 2 attacks a year

II.12 Craving for sweets/chocolates/breads. (maximum score = 6)

6= insatiable craving - must take out of meal chocolates, sweets or bread every day and night.
3= strong craving - frequent consumption of chocolates and sweets almost daily.
2= modest craving - feels happy to take sweets or chocolates but never every day.
1= occasional craving.
0= no craving.

II.13 Change in body weight since the onset of fatigue. (maximum score =9)

9= persistent (static or progressive) loss of weight
6= cyclic weight changes throughout the month, with or without oedema (idiopathic cyclic oedema).
3= initial loss of weight, followed by gain but not above the baseline weight.
2= Initial loss of weight, followed by steady gain with substantial increase in body weight.
1= no loss, but steady weight gain.
0= no change in body weight (any loss or gain).

II.14 Neurological signs (documented)
(Maximum score = 15)

3 = tremors
3 = fasciculation and myokymia
3 = hemisensory syndrome
3 = alternate feelings of hot and cold.
3 = unsteadiness of gait, Rombergism or difficulty in walking in tandem

.

SECTION II TOTAL MAXIMUM SCORE = 93

III GI Symptoms (Irritable Bowel Syndrome)

III.1 Chronic abdominal pain
(maximum score = 6)

6 = chronic severe generalised abdominal pain interfering with work and social function
3 = intermittent generalised moderately severe pain but associated with constipation - diarrhoea
2 = chronic intermittent pain confined to lower abdomen, relieved by passage of flatus or stool
1 = occasional pains with bloating or heartburn or waterbrash
0 = no pain

III.2 Vomiting
(maximum score = 6)

6 = frequent vomiting for no apparent cause
4 = vomiting induced by food
3 = occasional nausea associated with abdominal pain
2 = constant nausea
1 = occasional nausea
0 = no vomiting or nausea

III.3 Borborygmi
(maximum score = 3)

3 = continuous borborygmi with social embarrassment
2 = occasional borborygmi with abdominal pain
1 = borborygmi with abdominal distension
0 = no borborygmi

III.4 Abdominal distention
(maximum score = 6)

6 = marked distension causing discomfort in sitting, lying or wearing clothes
4= distension occurring at times, i.e. before menstrual periods
3= distension with synptoms of indigestion and heartburn
1 = mild occasional distension alone
0 = no distension

1II.5 Change in bowel movement
(maximum score = 6)

6 = episodes of diarrhoea alternating with prolonged constipation requiring laxative or suppository
3 = variable patterns, alternating with hard and soft stools or intermittent diarrhoea or constipation.
2 = constipation alone or increased frequency of motions
1 = transient changes in bowel habit lasting no more than one to two days
0 = regular bowel movement

SECTION III TOTAL MAXIMUM SCORE = 27

IV. Neuropsychological symptoms

IV.1 Change in sleep pattern
(maximum score = 9)

9 = difficulty in falling asleep, frequently broken sleep & increased daytime sleepiness; frequent nightmares.
6 = broken sleep, increased daytime sleepiness but without nightmares.
4 = sleeping excessively (more than 12 hours a day) or too little sleep (less than 5 hours a day).
3 = frequently falling off to sleep without any change in night time sleep.
1 = slight increase or change in sleep pattern
0 = normal sleeping patters.

IV.2 Concentration difficulties
(maximum score = 6)

6 = difficulty in concentrating in all kinds of activities including visual and recreational with impaired attention span on formal testing.
3 = inability to concentrate in visual and conversational tasks more than recreational activities.
2 = some impairment in the ability to read or hold a conversation but usually not affecting social life; normal concentration in recreational activities.
1 = intermittent difficulties in collecting one's thoughts but no persistent symptoms.
0 = no difficulty in concentrating

IV.3 Apathy
(maximum score = 3)

3 = complete apathy: unable to do anything without help
2 = difficulties in starting simple routine activities which are carried out with effort
1 = difficulty in starting routine activities, but these can be carried out without much effort
0 = no difficulty in getting started with routine activities

IV.4 Self-assessment of symptoms
(maximum score = 3)

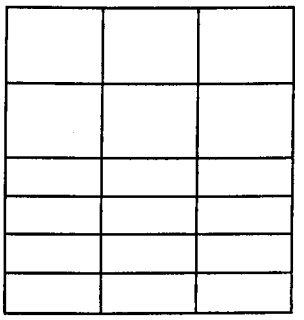
3 = loss of interest in life
2 = loss of interest in surroundings; loss of feeling for friends and family
1 = reduced ability to enjoy usual interests
0 = normal interest in surroundings and in other people

IV.5 Sadness
(maximum score = 6)

6 = looks sad and generates comments from family and friends that patient appears depressed.
3 = looks sad, rarely smiles and work and duties affected
2 = looks sad but can brighten up with appropriate social contact
1 = feels sad but not all of the time.
0 = normal

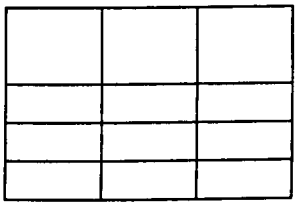
IV.6 Excessive irritability
(maximum score = 6)

6 = highly irritable, cannot participate in usual conversation without frequent outbursts of temper
4 = irritable; can converse but cannot hide anger or irritation; makes frequent gestures
3 = spouse and family complain of irritability
2 = excessive reaction at times to normal stimuli
1 = occasional irritability and outbursts of temper
0 = no or transient irritability



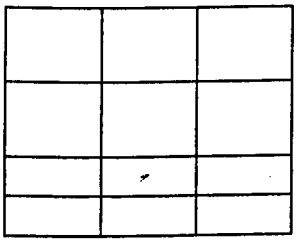
IV.7 Rapid changes in mood
(maximum score = 3)

3 = totally unpredictable mood, with changes from low to high several times during a day.
2 = changes in mood at least on one occasion every day.
1 = occasional changes in mood.
0 = no major mood changes.



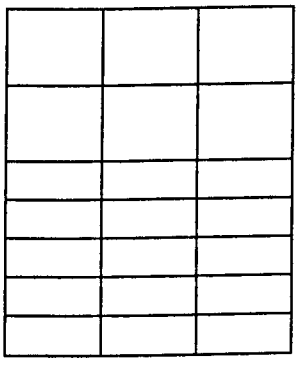
IV.8 Hallucinatory symptoms
(maximum score =3)

3 = frequent hallucinatory symptoms (visual or auditory) interfering with daily life.
2 = intermittent hallucinatory symptoms without any impact on daily life
1 = occasional or transient hallucinations
0 = no hallucinations



IV.9 Anomia
(maximum score = 6)

6 = excessive delay and pause in most sentences due to inability to name objects and persons
5 = conspicuous difficulty in use of words and naming with substitution
4 = errors in naming in routine conversation
3 = occasional errors in naming
2 = difficulty with names of people
1 = minor difficulties in naming with substitution
0 = normal speech



IV.10 Memory impairment
(maximum score = 6)

6 = progressive impairment affecting short-term memory and interfering with daily life.
4 = impairment of short term memory, non-progressive, but causing social embarrassment.
3 = memory deficits connected by prompting
2 = frequent difficulty in remembering recent events but without any interference with social or daily life.
1 = occsasional or transient difficulty in short term memory.
0 = no impairment.

IV.11 Intolerance to alcohol or antidepressants or antibiotics.
(Maximum score = 6)

2 = intolerance to alcohol
2 = intolerance to tricyclic antidepressants
2 = intolerance to antibiotics

SECTION IV TOTAL MAXIMUM SCORE = 57

Maximum Score: (Sections I -IV) = 241

Subscores:

Fatigue and neuropsychiatric symptoms: (I+IV)

Musculoskeletal and gastrointestinal symptoms: (II+III)

FATIGUE SCORE: Total score divided by 10 (range: 0-24)

Definitions of improvement or deterioration:

Change of **Final Score** by 6 or 25% of the baseline.