CAUSES AND CONSEQUENCES OF AUTONOMIC DYSFUNCTION IN CHRONIC FATIGUE SYNDROME

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Abstract

Introduction

Chronic Fatigue Syndrome (CFS) is an incapacitating condition characterised by extreme fatigue. In the absence of an objective diagnostic test CFS remains a clinical diagnosis based on a broad spectrum of symptoms, including autonomic dysfunction and cognitive impairment. This has given rise to significant challenges, not least the development of multiple sets of diagnostic criteria that may represent different disease phenotypes. This thesis examines autonomic and cognitive features between subgroups that meet different diagnostic criteria to better understand this possibility. It also examines the overlap between symptoms of CFS and depression, a potential confounder.

Methods

A subset of data from a larger Medical Research Council funded observational study *Understanding the pathogenesis of autonomic dysfunction in CFS and its relationship with cognitive impairment* was examined. Patients were screened using the SCID-I assessment tool to exclude major depression prior to the main study. Depressive symptoms were compared to CFS Fukuda criteria. The DePaul Symptom Questionnaire (DSQ) was used to differentiate between diagnostic criteria. COMPASS and COGFAIL questionnaires were administered for self-reported autonomic and cognitive features respectively. The Task Force® Monitor was used for autonomic assessment and a battery of neuropsychological tests administered for objective cognitive assessment.

Results

Subjective autonomic and cognitive symptoms were significantly greater in CFS subjects compared to controls. There were no statistically significant differences in objective autonomic measures between CFS and controls. There were clinically significant differences between DSQ subgroups on objective autonomic testing. Psychomotor speed was significantly slower in CFS compared to controls. Visuospatial memory, verbal memory and psychomotor speed were significantly different between DSQ subgroups.

Conclusion

The findings indicate phenotypic differences between DSQ subsets and suggest that elucidating the symptoms seen in CFS, or its disease spectrum, will support research into its underlying pathophysiology and enable more tailored treatment. The absence of significant differences in objective autonomic function between CFS and controls in this cohort contrasts to findings of some other studies and may reflect study exclusion for depression. Together with the overlap between CFS and depressive symptoms, this reinforces the need to better understand the underpinning causality to allow appropriate identification and management.

For my Dad

I wish you could have seen what we've been up to for the last 21 years

And for his sister, my Beloved Aunt

Perhaps this research will form a piece of the picture one day

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Abbreviations

ACI	Acceleration change index
ACTH	Adrenocorticotrophic hormone
AD	Autonomic dysfunction
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AS	Active stand
AUC	Area under the curve
AVLT	Auditory verbal learning test
AVP	Arginine vasopressin
BEI	Baroreflex effectiveness index
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
BPV	Blood pressure variability
BRS	Baroreflex sensitivity
CDC	Centre for Disease Control
CFS	Chronic fatigue syndrome
CI	Cardiac index
CO	Cardiac output
COMPASS	Composite autonomic symptom score
CRA	Clinical research associate
CRF	Clinical research facility
CRH	Corticotrophin releasing hormone
CRN	Clinical research network
CVLT	California verbal learning test
dBP	Diastolic blood pressure
DSQ	DePaul symptom questionnaire
DSST	Digit symbol substitution test
EBV	Epstein Barr virus
ECG	Electrocardiogram

EDI	End diastolic index
FIS	Fatigue impact scale
GR	Glucocorticoid receptors
HF	High frequency
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRV	Heart rate variability
HUT	Head-up tilt
HVLT-R	Hopkins verbal learning test revised
IC	Index of contractility
IFN	Interferon
IL	Interleukin
IQ	Intelligence quotient
IQR	Inter-quartile range
LDN	Low dose naltrexone
LF	Low frequency
LVET	Left ventricular ejection time
LVWI	Left ventricular work index
IPAQ	International physical activity questionnaire
MDD	Major depressive disorder
MDE	Major depressive episode
ME	Myalgic encephalomyelitis
METS	Metabolic equivalents
mIBG	Meta-iodobenzylguanidine
mmHg	Millimetres of mercury
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
n	Number
NART	National adult reading test
NHS	National Health Service
NICE	National Institute for Health and Care Excellence

NK	Natural killer
NMH	Neurally mediated hypotension
OGS	Orthostatic grading scale
OH	Orthostatic hypotension
OI	Orthostatic intolerance
PBC	Primary biliary cirrhosis
PIS	Participant information sheet
PoTS	Postural tachycardia syndrome
PSD	Power spectral density
RCT	Randomised controlled trial
RRI	RR-interval
sBP	Systolic blood pressure
SCID-I	Structured clinical interview for DSM-IV
SD	Standard deviation
SEID	Systematic exertion intolerance disease
SI	Stroke index
SV	Stroke volume
TFC	Thoracic fluid content
TNF	Tumour necrosis factor
TPR	Total peripheral resistance
TPRI	Total peripheral resistance index
UK	United Kingdom
US	United States
VLF	Very low frequency
WAIS-R	Wechsler adult intelligence scale revised

Chapter 1. Introduction

1.1. Aims and objectives

The biological underpinning of Chronic Fatigue Syndrome (CFS) is unknown but dysregulation of the autonomic nervous system (ANS) is a strong candidate. This thesis examines the prevalence of autonomic dysfunction (AD) in CFS compared to controls and considers the origin and impact of this dysautonomia. It also considers its presence in the context of current diagnostic criteria and case definitions, which lack consistency and consensus.

It is widely acknowledged that there are limitations to current diagnostic criteria used for CFS and that there is disparity between them (1-4). Little is understood about the clinical implications of this or whether different criteria identify different phenotypes of CFS, although it has been suggested that criteria may select individuals with different levels of functional impairment (5, 6).

This thesis will examine both self-reported and objective autonomic and cognitive features between cohorts that meet different diagnostic criteria as per the DePaul Symptom Questionnaire (DSQ).

The aims and hypotheses of this thesis are given below:

1.1.1. Primary aims

- To identify differences in AD by subgroup of CFS patients, determined by diagnostic criteria;
- To identify differences in cognitive impairment by subgroup of CFS patients, determined by diagnostic criteria;
- To explore the prevalence and nature of depressive symptoms in this well-defined cohort of CFS patients.

1.1.2. Secondary aim

- To determine whether CFS patients can participate in a study that involves considerable personal burden.

1.1.3. Hypotheses

- AD does not differ by diagnostic criteria met;
- Cognitive impairment does not differ by diagnostic criteria met;
- Depressive symptoms are common in CFS patients;
- CFS patients are able to participate in and complete a complex and physically-demanding study.

1.2. History

CFS is a poorly-understood condition of unknown aetiology. It is characterised by a broad spectrum of symptoms, including myalgia, sore throat and cognitive impairment, and underpinned by an incapacitating fatigue that is exacerbated by exertion (7, 8). The name Chronic Fatigue Syndrome is now the most widely-used term for this condition, which has had a number of different labels since its recognition (8-10).

Its origins appear to be long-standing. As early as the 1800s, physicians began describing a condition called Neurasthenia to define a combination of symptoms of fatigue and neuralgia (9, 11). At this time, symptoms were thought to be behaviour-related and psychosomatic in origin. This perception of CFS as a psychological rather than physical condition is one which continues to divide clinicians and patients today and has fuelled debate and research into its aetiology throughout its history (12-15).

In the 1900s clusters of patients with similar symptoms steered clinicians and researchers to identify and shape the recognition and diagnosis of this condition. Three notable outbreaks have had a defining role in this. In 1930s Los Angeles an outbreak of symptoms, including muscle weakness and cramps largely in hospital clinical staff, led Alexander Gilliam to describe a polio-like illness, which he coined an atypical poliomyelitis (11, 16). A similar phenotype presented in an outbreak in Iceland in the 1940s, which was initially thought to be a new form of polio, however patients were not affected by paralysis but had significant cognitive impairment post-illness (16).

A later outbreak at the Royal Free Hospital in London in 1955, managed by Dr Melvin Ramsey, saw the start of better-documented research into the illness and revealed dominant symptoms of headache, sore throat, fatigue and low-grade fever (16). As a result, the condition was thought to be infectious with a neuroimmune origin and caused by inflammation of the brain and spinal cord. It was subsequently called benign Myalgic-Encephalomyelitis, or ME, which is a term that prevailed into the 21st century and is still commonly used today (8, 11). This paved the way in the 1960s for the World Health Organisation (WHO) classification of ME as a condition of the central nervous system (17).

The 1980s and 1990s saw a re-emergence of a trend towards viewing CFS as a behavioural condition and it was dubbed "*yuppie flu*" by many under the belief that it affected malingering professionals struggling to manage demanding jobs. This drove a stigmatisation that has since been difficult to remove and one which many sufferers still feel today (18).

Towards the end of the twentieth century there was a growing school of thought that CFS was secondary to a viral infection, such as Epstein-Barr Virus (EBV), and this gave rise to the term Post-Viral Fatigue Syndrome (8, 19). Diagnosis of ME or Post-Viral Fatigue Syndrome was complicated by the fact that the WHO classified Post-Viral Fatigue Syndrome as a separate condition. Since the 1980s there has been recognition, led by the Centre for Disease Control (CDC) and their 1988 research into a case definition, that one collective term was needed and consensus has determined that Chronic Fatigue Syndrome is the most representative (8, 16).

Since this time, the aetiology of CFS has continued to be researched and debated. The original 1988 CDC diagnostic criteria have been superseded by other consensus criteria, which aim to incorporate the condition's complex symptomatology (20-22). In the absence of a diagnostic test for the disease, there is continued debate about the suitability of existing criteria for its diagnosis and in 2015 the United States Institute of Medicine proposed that the condition be given a new name: Systemic Exertion Intolerance Disease (SEID) (23, 24).

There is acknowledgment that this new proposal offers more targeted diagnostic criteria to replace the existing lengthy criteria but there is continued concern that this proposed name and definition are, like their predecessors, still more descriptive than diagnostic and unlikely to appease the stigma associated with a condition that is often labelled as psychiatric (25). It has been suggested that priority should lie with classification of the number and severity of symptoms as a means of predicting prognosis rather than with the development of additional case definitions (1).

Anecdotally, the condition and its sufferers have never been able to escape the idea initially planted in the 1800s of behaviour and psychology playing a major role in the aetiology of CFS and studies of the disease continue to focus on psychological aetiological factors. This creates tension within and between clinicians, researchers and patients. Improved understanding of the condition's aetiology will enable us to better inform patients, their families and society and will ultimately lead to the development of targeted treatment strategies.

1.3. Symptoms and presentation

CFS is by definition a constellation of symptoms that cluster together. Not all patients with CFS experience all symptoms and, where present, symptoms appear to vary in presentation and in severity. A lack of understanding of the condition's pathophysiology may have resulted in multiple symptoms being required in its diagnosis. This has also given rise to the use of more than one set of criteria for clinical diagnosis, which not only has implications in clinical practice but also in terms of research. This is highlighted by the Institute of Medicine's recent commissioning of a panel to examine these criteria resulting in the proposal in February 2015 of a further new set of criteria (23).

The most common presenting symptoms (11, 26) are summarised below and in table 1 together with their reported sensitivity – the predictive ability of the symptom to diagnose CFS – where available. A summary of the different diagnostic criteria is outlined in table 2.

1.3.1. Fatigue

Fatigue is a pathological state defined as “extreme tiredness from mental or physical exertion or illness” (27) and is distinct from tiredness – the normal physiological “state of wishing for sleep or rest” (28), or malaise – “a general feeling of discomfort, illness, or unease whose exact cause is difficult to identify” (29, 30).

As the primary symptom that leads to a diagnosis of CFS (26), understanding the nature of what patients express as fatigue is central to clinical evaluation. Patients present with new-onset debilitating fatigue that often results in inactivity for prolonged periods, despite high levels of pre-morbid energy and fitness (31). Fatigue in CFS is incapacitating, not related to exertion, is often worsened by activity (post-exertional malaise) and is not alleviated by rest (8, 32).

1.3.2. Sore throat

Many patients describe having a viral-type infection prior to becoming unwell, often associated with a low-grade fever (26). One of the main features of this illness is a sore throat (8), which can be a presenting symptom, as well as a recurrent feature associated with relapse or chronic illness.

1.3.3. Muscle and joint pain

Persistent muscle ache is seen in 20-95% of patients and painful joints in the absence of swelling or inflammation are described in approximately half of patients (26).

1.3.4. Cognitive problems

Often described as a “brain fog” 85-95% of patients report problems with short-term memory and concentration (26, 33).

1.3.5. Other symptoms

These include dizziness, tachycardia, problems with bowel and bladder function, dry eyes and visual blurring (26, 32). Table 1 reports frequency of symptoms determined from a review of studies of CFS between 1991 and 2014.

Symptom	Frequency (%)	Sensitivity (%)
Fatigue	100	95.7
Low-grade fever	60-95	
Myalgia	20-95	75.1
Sleep disorder	15-90	85.4
Impaired cognition	50-85	72-82.9
Headache	35-85	
Muscle weakness	40-70	74.2
Visual blurring	50-60	
Nocturia	50-60	
Dizziness	30-50	
Tachycardia	40-50	
Dry eyes	30-40	
Dry mouth	30-40	
Diarrhoea	30-40	
Painful lymph nodes	30-40	

Table 1 Summary of symptoms in CFS and their frequency adapted from Komaroff *et al* and Watson *et al* (26, 34)

1.4. Diagnostic criteria

There is no definitive objective diagnostic test for CFS (35). Diagnosis is based on clinical presentation and the diagnostic process has supported four distinct sets of diagnostic criteria, outlined in table 2.

The development of a case definition and diagnostic criteria for CFS originated from the CDC in 1988 (8, 36). This case definition has been the foundation for subsequent criteria, which have attempted to encapsulate the broad symptomatology of CFS and improve the accuracy of diagnosis. These criteria are discussed below.

The 1991 Oxford criteria were developed by a United Kingdom (UK) based consensus group to address a lack of agreement about a case definition for CFS and concerns about the reliability of the 1988 definition (3, 37). The Oxford criteria proposed a

definition that required at least six months of mental and physical fatigue that inhibits function at least 50% of the time. It also proposed a subtype of CFS called Post-Infectious Fatigue Syndrome, associated with the presence of an infective agent (37). The Oxford criteria are recorded in table 2 and notably do not include problems with cognitive function.

In 1994 the CDC CFS study group further revised this definition to provide an integrated approach to CFS diagnosis and developed the Fukuda criteria (22). These require patients to have persistent or relapsing debilitating fatigue for at least six months with significant impairment in functioning and at least four minor symptoms, which include cognitive dysfunction, sore throat and myalgia. Criticisms of the Fukuda criteria include the lack of a rating scale for symptom severity and the challenges presented in their application (38).

The 2003 Canadian Consensus definition (39) was written to aid diagnosis on the basis of symptom clusters in an attempt to overcome the challenge of such a breadth of symptoms. As such, it includes more symptoms than the criteria above and does not describe fatigue as a defining feature of CFS, in recognition of the fact that this symptom is a feature of many diseases. Instead it describes post-exertional malaise as a central feature of the illness. It also requires at least one autonomic, neuroendocrine or immune symptom, as well as at least two other symptoms including arthralgia and poor sleep. The authors of the 2003 Canadian Consensus definition also criticised the Fukuda criteria for the overlap with depressive symptoms and differentiated between this by focussing on physical and cognitive functional impairment.

These 2003 criteria were further developed into the 2011 International Consensus Criteria (40). The most significant change was the removal of a temporal requirement, in that the six-month period of symptoms prior to diagnosis is no longer required before a diagnosis of CFS can be made. Once again, it also criticised the use of the word fatigue in the condition's name, arguing that focus on this as the primary symptom gives too great an emphasis on a symptom that is widespread through many chronic conditions (41).

Part of the challenge of having diverse sets of criteria is the possibility that they select a slightly different disease phenotype, which may not represent the same condition (5, 6, 42) and which complicates research into underlying aetiology. The 2003 Canadian criteria appear to select more functionally impaired patients, with physical and cognitive symptoms, compared to the Fukuda criteria and there is inconsistency about whether different criteria include or exclude patients with co-morbid psychiatric disease (3, 38).

Identifying diagnostic symptoms, rating their severity and standardising their measurement will improve the consistency of existing diagnostic criteria (3). Since the 1988 CDC case definition, revisions of CFS diagnostic criteria have attempted to arrive at a more focussed definition to improve reliability and to minimise overlap with other diseases, such as depression, and the hunt for an appropriate and accurate set of criteria continues.

The recently proposed case definition from the Institute of Medicine has been formed on the basis of a literature review and input from an expert committee. It places three common symptoms at the heart of the definition: impaired day-to-day function, post-exertional malaise and unrefreshing sleep (23). Yet, in the context of existing systematic, consistent definitions, some experts feel that emphasis on the identification of a definitive set of diagnostic criteria should be relegated in favour of focussing on number and severity of symptoms to help target treatment and improve prognosis (1).

Furthermore, the continued and varied development of polythetic diagnostic criteria for CFS, where some but not all symptoms must be present to reach a diagnosis, illustrates the challenge of knowing which symptoms are CFS-specific and may be a sign of the disorder's heterogeneity or which arise as co-morbidities secondary to the underlying pathophysiology. While there is no doubt that a definitive diagnostic test is key to recognition of this stigmatised disease and its accurate diagnosis, understanding these symptoms is central to better elucidating whether tests will identify different subsets of patients on a disease spectrum with different treatment requirements.

Although no set of diagnostic criteria is without limitations (43), the Fukuda case definition has widespread support (44-46). The lack of understanding of the true aetiological underpinning of CFS implies that these apparently comprehensive criteria may still not be valid. Nevertheless, they are the most widely used both in the United States (US) and in the UK, where they underpin the recommendations of the National Institute for Health and Care Excellence (NICE) (1, 3, 35). In the absence of an objective diagnostic test, the Fukuda criteria continue to present the most comprehensive and consistent set of criteria to date.

1.4.1. DePaul Symptom Questionnaire

The DSQ was devised by Leonard Jason *et al* to better assess the "core" symptoms of CFS and provide a consistent method of evaluating them (47-49). It is a self-reported measure of CFS symptoms, demographics and medical, occupational, and social history.

From these measures it is possible to give a "diagnosis" of CFS based on the Fukuda criteria (22), the 2003 Carruthers (Canadian) criteria (39) or the 2011 Carruthers (Canadian) consensus criteria (40). The 2003 Canadian criteria are further subdivided into clinical and research. The clinical criteria are the original 2003 Canadian criteria. The research criteria have been developed by a group led by Jason Leonard from the 2003 Canadian case definition with elements of the Fukuda criteria to limit symptoms and allow for better categorisation of patients (47). These research criteria are defined in table 2 and the principle differences between them are outlined in chapter 4, table 69.

A diagnosis is given according to four categories. The Fukuda criteria; the Fukuda+2003 Canadian Clinical criteria; the Fukuda+2003 Canadian Research criteria, or the Fukuda+2003+2011 Canadian criteria together. The Fukuda+2003 Canadian Clinical criteria build on the Fukuda alone criteria with the addition of neurological, neuroendocrine and autonomic symptoms. The Fukuda+2003 Canadian Research build on this by including these symptoms not as minor but major symptoms, core to a CFS diagnosis. Fukuda+2003+2011 make a further addition of more widespread, migratory pain and require wide-ranging and greater symptomatology (see table 2).

In broad terms, the difference in DSQ “diagnoses” represents increasing numbers of symptoms affecting more of the body’s systems. While this recognises the complex presenting symptoms, it may also complicate the picture with the inclusion of symptoms that are too broad and all-encompassing, and which may not all be core features of CFS.

Studies show that different diagnostic criteria identify patients with different functional impairment (50-52). To date no studies have assessed the difference in autonomic or cognitive phenotype using in-depth, objective dynamic tests according to DSQ criteria. This thesis addresses this knowledge gap, examining objective and subjective autonomic and cognitive performance by DSQ criteria to better determine whether they select different phenotypes.

Criteria	Major	Minor
1988 CDC criteria (36)	Fulfil both: • New onset of persistent or relapsing debilitating fatigue impairing daily activity to below 50% of premorbid for ≥ six months • Exclude other clinical conditions that may produce similar symptoms	≥ six of the symptom criteria and ≥ two of the physical criteria; or ≥ eight of the symptom criteria: Symptom criteria: • Mild fever • Sort throat • Lymphadenopathy • Generalised muscle weakness • Myalgia • Post-exertional fatigue • Headache • Arthralgia • Neuropsychologic difficulties • Sleep disturbance • Main symptoms developing over hours or days Physical criteria: • Low-grade fever • Non-exudative pharyngitis • Lymphadenopathy
1991 Oxford Criteria (37)	• Debilitating, unexplained fatigue of definite onset for ≥ six months at least 50% of the time	• Myalgia • Mood disturbance • Sleep disturbance

Criteria	Major	Minor
Fukuda criteria/ CDC 1994 criteria (22)	• ≥ six months of severe chronic fatigue not due to exertion or other illness and interfering with daily life	≥ four of the following eight symptoms: • Post-exertional malaise of >24 hours • Unrefreshing sleep • Impairment to short-term memory or concentration • Myalgia • Arthralgia without swelling/erythema • Headaches of new type • Lymphadenopathy • Frequent or recurring sore throat
Canadian consensus 2003 (39) **(DSQ 2003 clinical guidelines)**	≥ six months of: • Significant new-onset, unexplained, persistent fatigue that reduces activity level, and/or • Post-exertional malaise, and/or • Sleep dysfunction and/or • Pain in the form of widespread myalgia/arthralgia or headaches	≥ two neurological/cognitive symptoms: • Confusion • Poor concentration and short-term memory • Poor information processing, categorising and word retrieval • Perceptual and sensory disturbances • Ataxia • Muscle weakness • Fasciculations and ≥ one from two of the following categories: • Autonomic: orthostatic intolerance, PoTS, nausea, irritable bowel, urinary frequency, palpitations, exertional dyspnoea • Neuroendocrine: loss of thermostatic stability, weight change • Immune: lymphadenopathy, recurrent sore throat, general malaise, sensitivities to food/medications

Criteria	Major	Minor
Canadian (International) consensus 2011 (40)	• Postexertional neuroimmune exhaustion with marked, prolonged post-exertional physical or cognitive fatigue with extended recovery period	≥ one symptom from three of these categories: • Neurocognitive impairment • Pain • Sleep disturbance • Neurosensory, perceptual and motor disturbances ≥ one symptom from three of these categories: • Flu-like symptoms • Susceptibility to viral infections • Gastro-intestinal tract symptoms • Genitourinary symptoms • Sensitivities ≥ one symptom from these categories: • Cardiovascular • Respiratory • Loss of thermostatic stability • Intolerance of extremes of temperature
DSQ revised Canadian research criteria (47)	• New, persistent or recurring fatigue for at least six months which impacts usual activities • Post-exertional malaise and/or post-exertional fatigue • Unrefreshing or disturbed sleep • Widespread or migratory pain • ≥ two neurological or cognitive symptoms • ≥ one autonomic/neuroendocrine/immune manifestation	Absence of any active medical condition that may explain the presence of chronic fatigue

Criteria	Major	Minor
Institute of Medicine proposed criteria (23)	• Substantial reduction or impairment in pre-illness occupational, educational, social or personal activities for ≥ six months accompanied by fatigue • Post-exertional malaise • Unrefreshing sleep	≥ one of: • Cognitive impairment • Orthostatic intolerance

Table 2 Current and proposed diagnostic criteria

1.5. Aetiology

The biological underpinning of CFS is not known. Dysregulation of the ANS is a strong candidate, but infection, the immune system and genetics have also been implicated in its pathophysiology. These uncertain origins are highlighted by the fact that specialist CFS clinics in the UK can be based in different departments, including Immunology, Infectious Diseases and Psychiatry, and contributes to diagnostic uncertainty, difficulty in finding effective therapeutic interventions and patient frustration.

The WHO classifies CFS as a neurological condition (17) and, although opinion about its aetiology is divided, this is recognised by current NICE guidance in the absence of a better understanding of its pathogenesis (35). The focus of this thesis is on the ANS and its regulation and dysregulation, which is presented from section 1.10. Evidence for the roles of different underlying pathologies is discussed below.

1.5.1. Genetics and environment

An interaction of genetic and environmental risk factors is likely to underlie the development of CFS. Anecdotal stories of CFS affecting members of the same family have been evidenced in studies (53), suggesting there may be a genetic predisposition to developing the disease. Studies have also shown a higher concordance rate (55%) between monozygotic twins compared to dizygotic twins (19%) (54) suggesting that genetic make-up may be an important factor in developing CFS and may underlie its pathology in some individuals.

These studies are often small and few in number, however, and there is conflicting evidence for the role that genetic susceptibility versus environmental factors might play. Some studies have shown a strong genetic contribution to fatigue with negligible environmental influence (55, 56), while other research suggests that environment – with factors including infection and stress – plays a key role and that the interplay between both factors is central (57). The role of environmental factors is discussed below.

1.5.1.1. Infection

Patients frequently report symptoms of infection, including flu-like symptoms and sore throat (26, 42), which often precede the development of CFS – giving the name post-viral fatigue syndrome – and can reoccur or persist. In addition, several infectious organisms have been associated with CFS, including EBV, parvovirus, retrovirus, cytomegalovirus, coxsackie B virus and giardiasis (15, 58, 59). Research is inconsistent, however, and causality has not been demonstrated.

Furthermore, trials of treatment with antivirals have been ineffective (60), which may imply that, rather than one infectious agent causing CFS, a group of infections may trigger an individual susceptibility determined by a genetic predisposition.

1.5.1.2. Occupational stress

The role of stress in disease causality is uncertain (61). Conducting well-executed, high-validity studies is problematic because of significant ethical considerations. Despite this, there is some evidence of an association between psychological stress and certain conditions, including cardiovascular disease and depression, where long-term occupational stress and stressful life events appear to precede disease (61).

1.5.1.3. Early stress

Evidence for precipitating life events in the development of CFS is limited (62). A recent case control study has, however, shown that childhood trauma may be a predisposing factor for CFS and that greater levels of childhood trauma were associated with a higher risk of CFS (63). This may reflect a model of stress-vulnerability, in which underlying vulnerability is triggered by external events.

1.5.1.4. Current stress

Pre-morbid lifestyle is thought to have a role to play by some researchers and was implicated in causality during the 1980s when "*yuppie flu*" was thought to be a disease of individuals with high levels of occupational stress and long, demanding work hours. More recent research has shown an association between this *ergomania* – an excessive desire to work or exercise (64) – and development of CFS (65); however,

study limitations, including the role of confounders and subjective reporting, make it difficult to interpret and justify a causal role.

Despite gaps in research, there is a compelling argument that underlying stress, vulnerability and lifestyle may predispose to CFS, particularly when considered in the context of a possible underlying genetic predisposition, which affects only some family members.

1.5.2. Biological systems

These genetic and environmental factors may underlie the symptoms of CFS through an impact on the ANS and/or through the immune or endocrine systems.

1.5.2.1. The immune system

Some of the key symptoms of CFS, including sore throat, lymphadenopathy and arthralgia, can be linked to inflammatory processes. This has led researchers to believe that the immune system is implicated in the pathophysiology of CFS. A review of the physiology of the immune system is outwith the scope of this thesis but can be found in Jawetz, Melnick & Adelberg's Medical Microbiology (66). Relevant key findings related to CFS are discussed below.

A large systematic review found inconsistent evidence for the role of the immune system in CFS (67). Studies focussed on the role of natural killer (NK) cells, pro-inflammatory cytokines and T cells. The findings contribute to an overall picture that is inconclusive and mixed, as discussed below.

1.5.2.1.1. Innate immunity

Abnormalities of immune activation of the acute innate response have been demonstrated in CFS (68). Disrupted NK cell function (69, 70) has been shown, with reduced NK cell numbers and low cytotoxicity. Studies also suggest that a bigger response may be mounted by the complement system in CFS and may be implicated in changes in immune cells' gene expression and enhanced oxidative stress (71), an imbalance which is associated with pathophysiology and ageing (72).

1.5.2.1.2. Cytokines

Cytokines are proteins produced as a result of a mounted immunological response that act as 'messengers' in the immune system. Some – IL-1, IL-2, IL-4, IL-6, IL-12, tumour necrosis factor (TNF), interferon (IFN) g – are pro-inflammatory and others – IL-10, TGF-ß – are inhibitory (73). Measuring serum cytokines has challenges (74), nevertheless studies have successfully compared cytokine levels in CFS and control groups.

A recent study looking at the relationship between leptin – which stimulates the release of some pro-inflammatory cytokines – and fatigue found that self-reported daily fluctuations in fatigue positively correlated with leptin level (75), which in turn correlated to a number of cytokines, including IL-6, IFN-α and IFN-ß.

Nevertheless, evidence for the role of the immune system is conflicting. A number of studies, of varying quality, show no evidence of difference in cytokine levels between CFS and control subjects (67). Others have found opposing results with both increased and decreased levels of pro-inflammatory and inhibitory cytokines observed (71, 76).

1.5.2.1.3. Adaptive immunity

There is evidence indicating abnormal adaptive immunity in CFS. Some studies demonstrate no difference in T cell quality and function between CFS and control groups while others showed reduced T cell and CD4 cell number in CFS (58, 59). Other research has shown prolonged T cell survival (76) illustrating the challenges of interpreting study findings, which may be influenced by confounding factors including the presence of co-morbidities, such as depression.

Furthermore, there is also evidence of abnormal autoimmune reactions and some studies have shown elevated antibodies and B cells, as well as immunoglobulin-G deficiencies (42, 69).

Nevertheless, studies are often based on small sample sizes and causality, or the role of co-morbidities, cannot be determined. Together, these findings paint a picture of

the complexity of the immune system and the challenges faced in identifying consistent markers of the disease.

1.5.2.2. The nervous system

CFS is currently classified by the WHO as a chronic neurological condition (17). Symptoms including dizziness, palpitations, bowel and bladder disturbances and problems with temperature regulation, which are commonly seen in CFS patients (26), are regulated by the ANS (77) and strongly suggest that nervous system dysfunction might be implicated its pathophysiology. It has long been postulated that symptoms including orthostatic intolerance (OI) and postural hypotension, seen in both multiple sclerosis (MS) and CFS (78), imply a shared causal pathway with an underlying neuroimmune mechanism.

Disruption of the ANS is, however, observed in a number of chronic conditions (79), including depression (80), and studies have not yet demonstrated whether these symptoms underpin the pathogenesis of CFS or whether they result from a secondary mechanism.

One of the strongest evidence bases that CFS may be an abnormality of the nervous system comes from the overlap that has been demonstrated between CFS and Postural Tachycardia Syndrome (PoTS) – a dysautonomia (81). The symptoms of PoTS include OI and abnormal heart rate response and may encompass a group of dysautonomias (82) including CFS and is suggestive of a parallel underlying causal pathway.

The role of the ANS in CFS is discussed in more detail from section 1.10.

1.5.2.3. Endocrine dysregulation

The hypothalamic-pituitary-adrenal (HPA) axis is under homeostatic control and regulates many of the body's systems, including the cardiovascular, immune and central nervous systems (83). One of the hormones synthesised and secreted as a consequence of HPA axis function is cortisol, a glucocorticoid released in response to

stress. The HPA axis is sensitive to multiple influences, including diurnal rhythm and stress (84, 85). A summary of its regulation is depicted in figure 1.

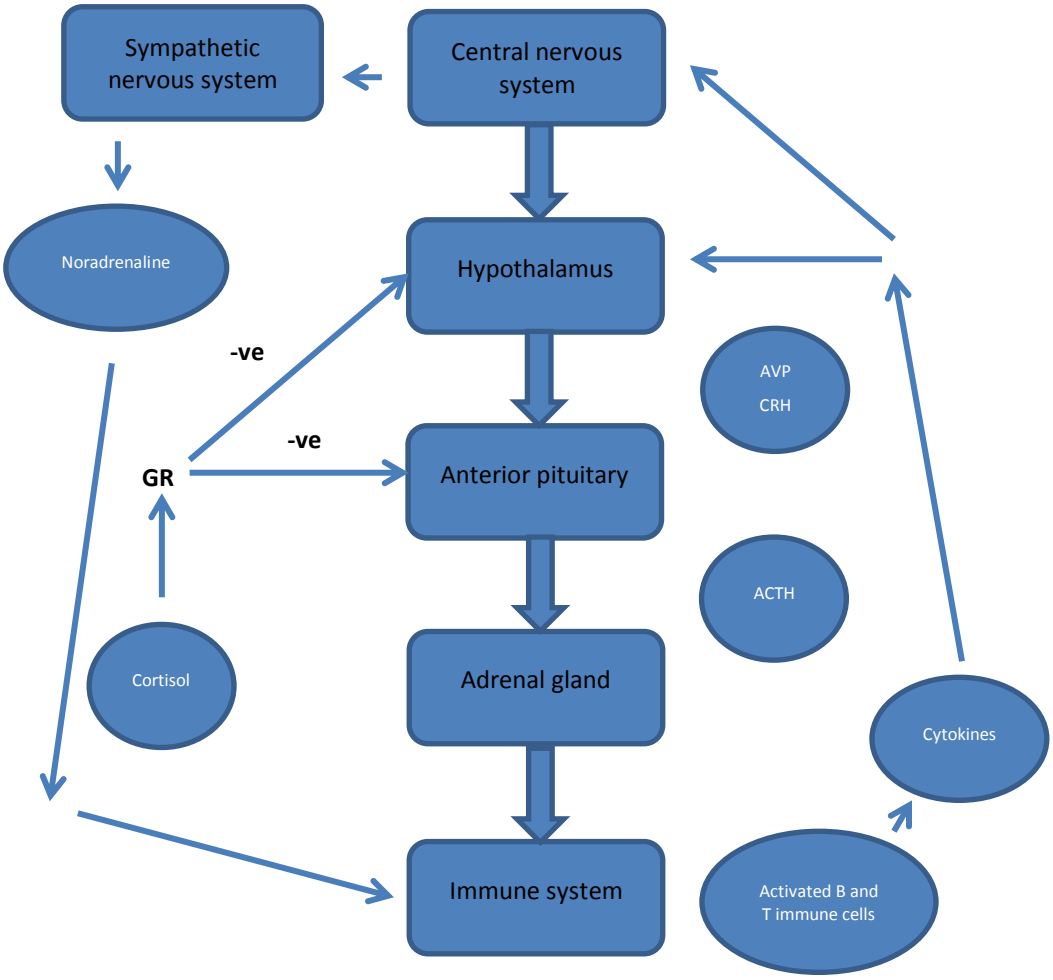

Figure 1 Schematic of the HPA axis showing regulation and negative feedback Adapted from Pocock and Richards, Human Physiology and Kohm and Sanders (86, 87)

Dysregulation of the HPA axis has been associated with CFS (15, 88). Down-regulation has been shown in CFS (89) by reduced cortisol levels (56, 90) and decreased diurnal variability of HPA axis hormones (91-93). A dose-response relationship has also been observed with increased HPA axis dysfunction associated with longer illness duration (94). Furthermore, low-dose hydrocortisone has been shown to be effective at reducing fatigue levels in the shorter term, strengthening support for an association between HPA axis dysregulation and CFS (95).

The ANS, HPA axis and the immune system are interconnected systems (96-99), as depicted in figure 1.

The immune system releases cytokines, including IL-1, IL-6, IL-10 and TNF-α, that can stimulate the HPA axis and the ANS (99). In itself it is regulated through hormonal and neural responses. HPA axis activation stimulates the release of glucocorticoids that inhibit the immune system. The ANS releases noradrenalin, which has a regulatory role on the release of pro-inflammatory cytokines (87). As such, changes in one system (hormonal/neural/immune) will affect the others and CFS may be the phenotypic expression of dysregulation of any aspect of these interconnected systems.

Existing research suggests that the complex interplay between the ANS and its immune and endocrine regulation may be implicated in the pathogenesis of CFS. Understanding the underlying pathophysiology will enable diagnosis to be made on pathophysiological grounds and may reveal that what we now think of as CFS is in fact a number of different disorders.

Further research is needed to explore this possibility and to better elucidate whether CFS, as currently diagnosed, represents one condition or a spectrum of disorders with a similar symptom base.

1.6. Prevalence

Estimates of CFS prevalence vary (100, 101). One of the principle challenges in obtaining accurate data stems from the lack of a pathophysiological diagnostic test. As a result, clinician-led diagnostic criteria are used. The variability in these criteria, as discussed, as well as inconsistency in use across clinics and the broad case definition for CFS give rise to a likelihood of both under- and over-diagnosis (102) and specifically the misdiagnosis of psychiatric conditions, such as depression (101).

Given the lack of epidemiological data, NICE bases prevalence estimates on extrapolations from other countries and gives the current UK prevalence of CFS as a minimum of 0.2-0.4% (103); however, recent studies suggest that such prevalence estimates may only account for 10% of those affected by the condition (104).

1.7. Cost and impact

The true economic burden of CFS is difficult to measure. Lack of adequate data regarding healthcare, welfare and productivity costs is exacerbated by probable underestimation of true disease prevalence. A US study estimated that household productivity in people with CFS reduced by one third and that labour productivity was over 50% lower, resulting in a total annual cost of $9.1 billion (105). A more recent paper from the UK looking solely at loss of productivity estimated the annual cost to the UK economy to be £75.5-£128.9 million (106). The cost of informal care is also thought to be substantial (107) and contribute to a high overall financial cost.

1.8. Prognosis

The natural history of CFS is variable and full recovery is rare (108). Studies point towards four patterns of recovery: 0-20% report full recovery; 8-63% report some improvement in symptoms; 5-20% have worsening of symptoms and 24-57% report no change (108).

Improved outcome appears to be associated with lower fatigue severity at baseline, shorter illness duration and absence of co-morbid psychiatric disorders (8, 109). Protective factors appear to be a younger age at diagnosis and absence of co-morbid depression or anxiety (110).

1.9. Management

Current NICE guidance centres on improving function and quality of life and focusses on improved sleep hygiene, adopting rest periods and maintaining a healthy diet (35). Pharmacological intervention is recommended only for symptom control, for example pain management or symptoms of hypotension.

More specialist care includes cognitive behavioural therapy and graded exercise therapy, which is aimed at a gradual increase in activity duration and intensity, as well as improving emotional and cognitive capacity and resilience (35).

1.10. The autonomic nervous system

The ANS is a complex neural network largely under involuntary control (77). It controls contraction and relaxation of organs, blood vessel dilation and constriction and the force and rate of heart contraction. There are two complementary divisions: the parasympathetic and the sympathetic systems. The sympathetic nervous system drives a flight-or-fight response in part by increasing heart rate and blood pressure. The parasympathetic system has competing effects and works to save energy and reduce heart rate and blood pressure.

1.10.1. The sympathetic nervous system

The sympathetic division of the ANS acts largely to prepare the body for action. Its origins are in the sympathetic preganglionic neurons, which originate in the thoracic and lumbar spines (T1-L2/L3) (77). The axons of these neurons travel to the sympathetic ganglia, most of which are located at the vertebrae, which then synapse forming the postganglionic sympathetic fibres. These postganglionic sympathetic fibres innervate multiple organs, including the heart, lungs, digestive tract, blood vessels and sweat glands (86).

1.10.2. The parasympathetic nervous system

The parasympathetic division is involved in more restorative functions than its sympathetic counterpart, for example digestion and slowing of heart rate. It origins are in the sacral spine (S3-S4) and brainstem (77). The parasympathetic ganglia are often close to or on the target organ and, like their sympathetic equivalents, innervate the heart, lungs, digestive tract and visceral organs (86).

Many organs are innervated by both arms of the ANS, often antagonistically, as outlined in table 3.

Affected organ	Sympathetic action	Parasympathetic action
Heart	Increased heart rate and force of contraction	Decreased heart rate
Blood vessels	Vasoconstriction (vasodilation in skeletal muscle)	Vasodilation in some exocrine glands
Lungs	Bronchial dilatation	Bronchial constriction
Eye	Pupillary dilatation	Pupillary constriction
Adrenal medullae	Secretion of epinephrine and norepinephrine	No innervation
Gastrointestinal tract	Decreased motility and secretion Sphincter constriction	Increased motility and secretion Sphincter relaxation
Urinary bladder	Inhibition of micturition	Initiation of micturition
Sweat glands	Sweat secretion	No innervation
Metabolism	Increase	No effect

Table 3 Principle antagonistic actions of the ANS

Adapted from Pocock and Richards (86)

The focus of this thesis is on the cardiac and blood pressure effects of the ANS. These are discussed below.

1.10.3. Regulation: Baroreceptor reflex

The degree of pressure in circulation is monitored in the short-term by baroreceptors, located primarily in the carotid sinuses – informing the carotid sinus nerves – and aortic arch, which relay information to the vagus nerve by sensing the amount of stretch in the vessel wall. Arterial blood pressure is tightly regulated in the longer-term by the kidneys and more acutely by hormonal and neural control (86).

The baroreceptor reflex responds to subtle, prolonged changes in blood pressure over 15 minutes at which time the threshold for feedback increases, for example when exercising, to allow for a maintained heart rate and ensure adequate cardiac output.

It also plays an important role on standing to avoid postural hypotension. Starling's Law describes the phenomenon of a drop in venous return to the heart on standing, resulting in a drop in cardiac output and blood pressure. The baroreceptor reflex acts to increase heart rate via sympathetic drive, thereby increasing total peripheral

resistance and increasing blood pressure. Although this response is acute, delays in blood pressure restoration can lead to postural hypotension or dizziness.

1.10.3.1. At rest

At rest the heart is dominated by parasympathetic control via the vagus nerve (86). This exerts an inhibitory function, slowing an otherwise unfettered heart rate of approximately 100 bpm to a resting heart rate of 60-80 bpm.

1.10.3.2. On standing

After standing there is a significant fall in venous return to the heart, which results in a fall in blood pressure. This is also known as postural hypotension. In response, increased sympathetic discharge results in an increase in heart rate and total peripheral resistance, which acts to restore blood pressure (86).

1.10.3.3. The Valsalva manoeuvre

The Valsalva manoeuvre is the act of expiration against a closed glottis. This gives rise to an increase in intrathoracic pressure and an initial rise in blood pressure followed by a brief fall in heart rate. As venous return is restricted by continued raised intrathoracic pressure, cardiac output and mean arterial pressure fall resulting in an increase in heart rate to maintain blood pressure. The end of the manoeuvre signals a transient fall in blood pressure before increased venous return results in increased cardiac output and a rise in blood pressure (86).

There are four phases to the Valsalva manoeuvre. Their physiological changes and effect on blood pressure and heart rate are outlined in table 4.

Phase	Response	Systolic blood pressure	Heart rate
I			
Onset of strain: increased intrathoracic pressure	Baroreceptor activation	Increase	Stable
II			
Persistent strain: increased intrathoracic pressure	Low venous return and stroke volume	Decrease	Increase
III			
Release of breath-holding and glottic pressure: drop in intrathoracic pressure	Release	Decrease	Stable
IV			
Recovery: sudden increase in cardiac output	Baroreceptor activation	Increase	Decrease

Table 4 Phases of the Valsalva manoeuvre
Adapted from Yale and Zygmunt (111, 112)

1.10.4. Measuring autonomic nervous system function

ANS function (or dysfunction) can be assessed subjectively using self-reported questionnaires and objectively through the measurement of defined physiological parameters.

1.10.4.1. Subjective assessment

Validated self-reported questionnaires provide a measure of subjective symptoms of AD across affected organ systems.

The Orthostatic Grading Scale (OGS) is a validated tool (113) consisting of five items that assess the frequency and severity of orthostatic symptoms and the conditions under which they occur. Participants grade each item on a scale of 0–4, where 0 is the lowest and 4 the highest. These scores are then added to give a total score. Higher

scores indicate greater severity of OI. A limitation of this questionnaire is the narrow focus of symptoms covered, however it is an effective, easy-to-administer tool.

The Composite Autonomic Symptom Score (COMPASS) encompasses a broader set of symptoms of AD and is validated for use as a quantitative measure of autonomic symptoms (114). It consists of 73 questions grouped into eight domains corresponding to different aspects of the ANS. These domains – OI, vasomotor, secretomotor, gastrointestinal, bladder, pupillary responses, sleep disorder, and syncope – are weighted according to their clinical relevance and the individual scores are totalled. The highest possible sore is 179 and the higher the score the greater the symptom load.

The COMPASS 31 is an abbreviated version of the full COMPASS questionnaire. It has been shown to associate strongly with the full questionnaire (115, 116) and is suitable for use in a clinical environment. It consists of 31 questions across six domains. These domains are OI, vasomotor, secretomotor, gastrointestinal, bladder and pupillomotor. The highest possible score is 100 and is a marker of greatest symptom load.

1.10.4.2. Objective assessment

Responses of individual organs to the ANS can be measured. These include cardiac activity, respiration, pupillary responses, thermodynamics and the HPA axis (117). In relevance to this thesis, assessment of cardiovascular function is discussed below.

Clinical assessment of cardiac autonomic function can be conducted as a one-off measure or as a continuous beat-to-beat outcome. Limitations of these methods include the possible confounding effects of co-morbidities, such as anaemia or thyroid dysfunction, or medications.

1.10.4.2.1. One-off measures

Autonomic effects on the heart can be measured with a 12-lead electrocardiogram (ECG), which provides a transient measure of cardiac electrical activity. Usually performed supine when vagal tone is greatest (118), the ECG depicts waves of depolarisation and repolarisation and allows assessment of heart rate and

conductivity, as well as other pathology (86). Assessment of heart rate provides an indication of sympathetic and parasympathetic response, as depicted in table 4.

A limitation of this approach is that it measures heart rate at one time point, which may be affected by confounding factors including medication, co-morbidities and temperature. Furthermore, it is not a good distinguisher of sympathetic and parasympathetic activation (119).

1.10.4.2.2. Continuous monitoring

Continuous assessment over longer periods of time allows parameters such as heart rate, blood pressure variability and baroreceptor sensitivity to be measured. This can be achieved with an ambulatory ECG over a specified time period, for example 24 hours, or by using more specialist equipment, such as a Task Force® Monitor. Additional electrodes that allow measurement of impedance cardiography can also be applied to investigate parameters such as stroke volume (120).

Invasive measurement is also feasible and considered the Gold Standard, but rarely practical in circumstances other than in the context of therapeutic interventions in a clinical setting (121).

As with other methods, limitations of continuous measurement include the lack of controlled conditions, whereby medications, ambient temperature and co-morbidities can all affect the ANS.

1.10.4.2.3. Stimuli

Both one-off and continuous assessment can be performed in response to different stimuli. The cold pressor involves submerging a hand in cold water for one minute and provides a measure of sympathetic activity. Active stand and head-up tilt (HUT) give a measure of parasympathetic response and sympathetic activation. The Valsalva manoeuvre can be performed to measure both sympathetic and parasympathetic activity.

1.11. The prevalence of autonomic dysfunction in CFS

A picture is emerging of an association between AD and CFS on subjective and objective testing. Nevertheless, studies are not without their limitations and direction of causality cannot be proven. The evidence for AD in CFS is reviewed below.

A cross-sectional study of self-reported autonomic symptoms using COMPASS showed that almost 90% of CFS patients experience symptoms related to AD (122). These symptoms range from mild to severe and include palpitations, OI, urinary frequency and problems with temperature regulation (26). These subjective dysautonomic symptoms have been shown to be a better predictor of functional ability, or disability, than fatigue severity (122, 123). This strongly suggests that controlling autonomic symptoms could improve function in CFS and that effectiveness trials of medications that treat autonomic symptoms should be investigated.

Over the last two decades consecutive studies have found an association between objective signs of AD and CFS. Some of the challenges that arise from interpreting this research, however, include the observational nature of many of the studies, meaning that direction of causality cannot be established – that is to say that AD might result from a more sedentary lifestyle secondary to the distinguishing features of the disease, rather than being a primary feature in itself. Furthermore, studies often work with small sample sizes of "well" patients with milder disease who are able to attend for investigations, which suggests a significant potential for volunteer bias and implies that those with more severe disease are not assessed. This has important implications for a condition which may be a spectrum of disorders and may be limiting the phenotype that is investigated.

Despite the emerging picture of the role of the ANS, findings from existing research are not always reproducible and frequently describe small changes in autonomic function across a broad range of measures, which weakens the argument for its role. Nevertheless, evidence continues to suggest that ANS function differs in CFS compared to controls indicating that it may be a core feature of the disease and has utility as a potential clinical diagnostic biomarker.

1.12. Literature review: Autonomic dysfunction in CFS

A literature review was conducted to better understand existing evidence for the role of the ANS in CFS. A search was conducted via Ovid MEDLINE ® and Embase from 2000 to present day. English language articles were sought. The search terms *chronic fatigue syndrome* and *autonomic* were used, which produced 250 articles after deduplication. Abstracts were reviewed and non-relevant studies excluded. Further articles referenced by these articles were reviewed. Opinion was also sought from experts in the field regarding more recent and current research and these articles were identified. The procedure of the literature search is outlined in figure 2. In total 26 studies were reviewed (table 5).

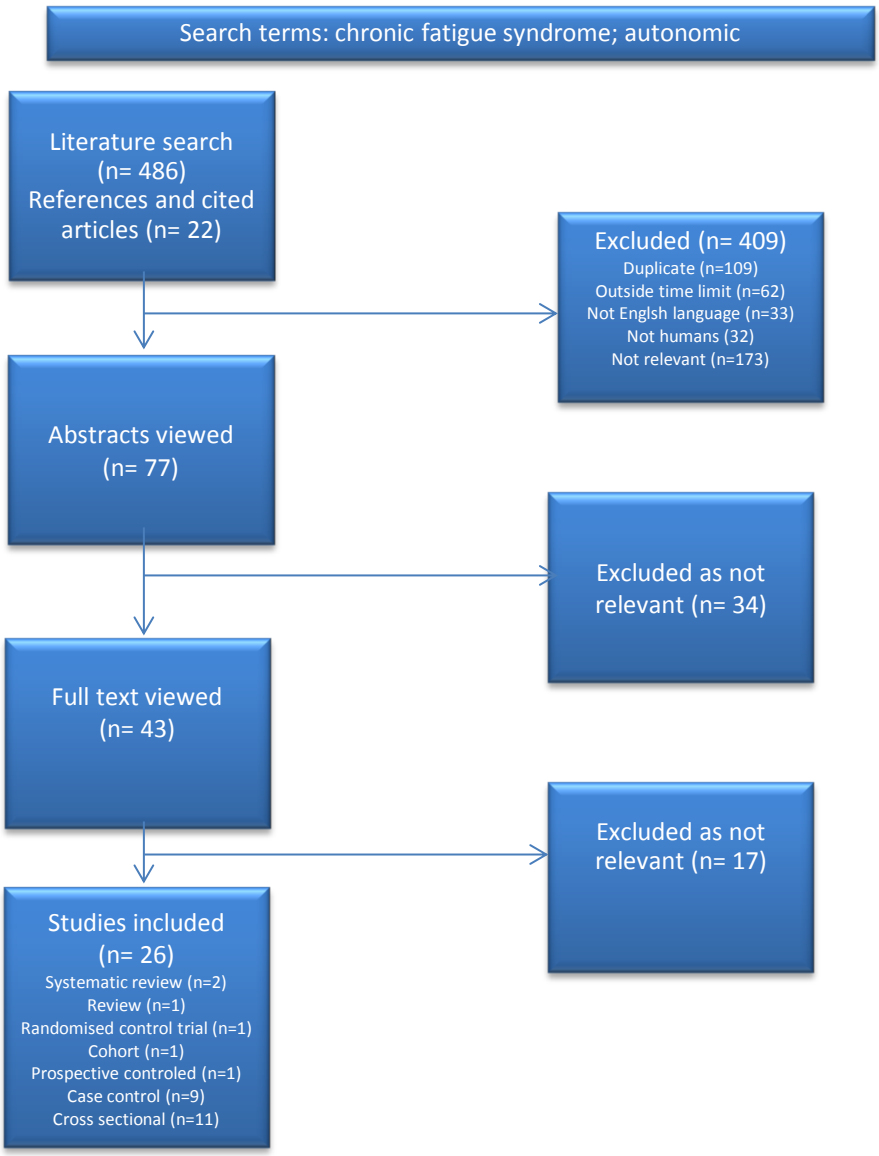

Figure 2 Procedure of literature search AD and CFS

Two recent systematic reviews support the finding of AD in CFS. In a recent review looking at 27 case-control studies van Cauwenbergh *et al* conclude that autonomic response to HUT appears to be an important diagnostic feature of CFS (124). A further systematic review by Meeus *et al* examines heart rate variability (HRV) in CFS and fibromyalgia (125) and concludes that CFS patients show increased sympathetic activity at night. Significantly, however, the quality of evidence reviewed in both articles is variable and focusses on case-control studies. This raises two principle issues: firstly of undetermined causality and secondly of the possibility of confounders, for which adjustment is not consistently made.

Concerns about the validity of Meeus *et al*'s review have been voiced by Tak *et al* (126) and centre on the complexity of measuring HRV and uncertainty about its validity as an outcome, as well as possible publication bias in existing studies which, when adjusted for, results in no demonstrable association between parasympathetic activity and conditions including CFS. In their response to Meeus *et al*, Tak *et al* discuss their own systematic review of the methodological quality of HRV studies in somatic disorders, in which they conclude that the quality of existing evidence is inadequate to determine the role of AD in somatic disorders such as CFS.

These reviews and their opposing conclusions highlight the challenges of both conducting research into a condition that is little understood and of interpreting findings. This is discussed further below.

1.12.1. Diagnostic criteria

One of the fundamental difficulties in identifying patients with CFS lies in the use of different diagnostic criteria, as discussed previously in section 1.4. Research has shown that the degree of subjective impairment differs between criteria (5). By implication this suggests that different pathologies may underpin the phenotypes; however, without an accurate and consistent identification of disease phenotypes research into underlying aetiology becomes complicated.

The studies reviewed considered patients who fulfilled established criteria for CFS (20, 22, 37, 39, 40). While these criteria are widely used in clinical practice and have similar

core features, each defines CFS in a slightly different manner (see table 2 section 1.4). The majority of studies used the Fukuda criteria (122, 127-133) and CDC definition (133-146), however a small number used the 1988 criteria, upon which the 1994 CDC definition was based (147, 148) and one used the Oxford criteria (149).

As discussed, there is disparity between criteria. The most noteworthy discrepancies include the limited symptom base in the Oxford criteria (37), as well as the absence of autonomic symptoms in the Fukuda criteria (22) and the CDC case definition (20). It is, therefore, plausible that different phenotypes across a spectrum of chronic fatigue *syndromes* are being investigated. If these phenotypes have different presenting features or underlying signs of dysautonomia, this will cloud research findings and make them difficult to interpret. Furthermore, it is accepted that fatigue can have different origins, as discussed by Newton *et al* (150), reinforcing the need for a consistent set of diagnostic criteria.

1.12.2. Average heart rate

Heart rate has been shown to be increased in CFS groups both at rest: at baseline (134, 148) and when asleep (134); and in response to stress: on standing (142, 148), after tilting (135) and after the cold pressor test (135).

The corrected QT interval (QTc) represents the time for ventricular depolarisation and repolarisation. It is influenced by autonomic – particularly vagal – tone. Naschitz *et al* (138) found that the average supine QTc in CFS was significantly shorter than the control group, indicating abnormality at rest. Scott *et al* (131) studied 220 patients with fatigue, 177 of whom fulfilled Fukuda criteria for CFS. They also observed that the QTc was significantly shorter in the CFS group compared to non-fatigued controls, indicating rapid repolarisation that has been associated with sudden cardiac death (151).

Changes in heart rate have been found in response to stress. The findings of van Cauwenbergh *et al*'s systematic review suggest that heart rate in response to the physiological stress of a HUT represents an important marker of AD in CFS, despite an overall moderate quality of case-control studies included (124).

Furthermore, mental arithmetic testing has been associated with an increased heart rate, suggestive of a decreased cardiac sympathetic response to mental stress (149). The same study by Soetekouw *et al* investigated response to a number of other physiological stimuli, including standing or Valsalva, and found no significant differences in heart rate between CFS patients and controls. This illustrates the lack of consistent findings across studies, perhaps as a consequence of small sample sizes, and implicates the possible role of confounders, such as deconditioning.

1.12.3. Heart rate variability

Individual studies have observed fluctuations in HRV between CFS and control groups. Like many studies of AD in CFS, however, findings are not always consistent and show variation in outcome measures.

This is illustrated by the findings of the following studies. Frith *et al* (152) found greater HRV in CFS – specifically representing low frequency (LF) variability – reflecting sympathetic function suggestive of an association between CFS and increased sympathetic activity. In response to the physiological stress of HUT, aperiodic spectral components of HRV have also been shown to be significantly lower in CFS compared to controls (144) and may relate to loss of HRV modulation.

Meeus *et al*'s recent systematic review looking at HRV found moderate evidence to support decreased HRV in sleep in CFS subjects. From their review they concluded that parasympathetic activity, as seen by reduced high frequency (HF) bands and low frequency/high frequency (LF/HF) ratio, and sympathetic activity on upright tilt were similar in CFS patients compared to controls (125). Limitations of this review have been discussed and include concerns over the methods used to measure HRV and lack of consistency in the outcomes assessed, such that an earlier meta-analysis concluded that the quality of existing evidence is insufficient to demonstrate that AD is associated with CFS (153).

Therefore, while there is evidence that supports abnormal HRV in CFS, lack of consistency and reproducibility suggest that potential confounders, such as medication

or co-morbid conditions, may have an important role to play and once again highlights the need for gold standard, reproducible research methods.

1.12.4. Blood pressure

Abnormal blood pressure has been associated with CFS. Newton *et al* (129) observed that, compared to a sedentary control group, a CFS group had statistically significantly (p=<0.0001) lower systolic blood pressure and lower mean arterial blood pressure (p=0.0002) assessed by 24-hour ambulatory blood pressure measurement.

This study also assessed a fatigue comparison group looking at patients with primary biliary cirrhosis (PBC). The CFS group had a lower systolic blood pressure compared to this fatigue comparison group; however, both 'fatigue' groups had comparable diastolic blood pressure. As with many studies of CFS, causality cannot be inferred from this cross-sectional study and it is important to note that the CFS group was younger at baseline, which may be a cause of the lower systolic blood pressure seen.

Nevertheless, this study did show an inverse relationship between subjective fatigue and diurnal variation of blood pressure in both CFS and PBC groups suggesting that a dysautonomia-associated fatigue may underpin CFS and PBC. This possibility is supported by a further study by the same team looking at a subjective measure of autonomic symptoms using the COMPASS questionnaire, which found that it has a positive predictive value for CFS of 0.96 95%CI 0.86–0.99) (122).

1.12.5. Orthostatic hypotension

Orthostatic intolerance is a common feature of many chronic diseases (154) with distinct aetiologies and may relate to secondary symptoms of these conditions rather than arise from a primary dysautonomia.

Jones *et al* (136) looked at the potential association between orthostatic instability and CFS. Their findings showed no orthostatic instability on stand-up test in either group and found that 30% of CFS patients had orthostatic instability on HUT compared to 48% of controls. They questioned the validity of primary dysautonomia in the

pathophysiology of CFS, as they found similar patterns of instability in both the CFS and control groups.

This is supported by Naschitz *et al*'s study conducting HUT in CFS subjects. The investigation had to be halted prematurely in 22.5% of CFS patients and 23.3% of non-CFS fatigued patients, as a result of orthostatic symptoms (146). Non-fatigued controls all tolerated the procedure, which does suggest a fatigue-associated dysautonomia (as Newton *et al* suggest (122)), however the similar termination rate in both fatigue groups raises the question of whether this dysautonomia is secondary to fatigue, or whether a similar underlying aetiopathogenesis is present.

Hollingsworth *et al* (128) examined the relationship between skeletal and cardiac function and symptoms on standing. They found a high prevalence of orthostatic problems in the CFS group, including increased cardiac contractility in response to the stress of standing. The CFS group also appeared to require greater cardiac activity on standing, reflected by a higher left ventricular work index (LVWI). Although a small study, its results have some clinical significance when considered in the context of evidence of an increased risk of cardiac mortality in fatigued PBC patients (155), which may imply an underlying causative mechanism.

1.12.6. Blood pressure variation

A number of studies have observed abnormalities in blood pressure variation in CFS patients in response to physiologic stress. Frith *et al* (152) found reduced systolic blood pressure variability on standing and greater LF HRV (a sympathetic marker) in CFS subjects compared to controls.

These findings are supported by Wyller *et al* (143) who examined blood pressure variability and closed-loop baroreflex function both at rest and during mild orthostatic stress. They found lower variability of HF systolic blood pressure and greater sympathetic baroreflex heart rate control during orthostatic stress in the CFS group.

Measures at rest do not appear to be significant (149), however greater diurnal variation in blood pressure has been observed (129) in CFS patients compared to

controls. Although Soetekouw's observation of greater responses in systolic and diastolic blood pressure during Valsalva in the CFS group was not statistically significant and therefore not considered to indicate a meaningful difference in cardiovascular autonomic function in CFS (149), evidence does appear to suggest that blood pressure variation is response to orthostatic stress may have potential for use as a biomarker of CFS.

1.12.7. Postural Tachycardia Syndrome

The hypothesis that PoTS patients may represent a clinically important subgroup of CFS is gaining momentum (156, 157).

PoTS is a disorder of OI characterised by an increase in heart rate on standing of at least 30 beats per minute (bpm) higher than the baseline value without concurrent orthostatic hypotension (OH), defined as a drop in systolic blood pressure of >25 mmHg or a drop in diastolic blood pressure of >10 mmHg (158). It is associated with symptoms of OI, such as dizziness, tingling, light-headedness or presyncope.

There is a growing body of evidence suggesting an association between postural tachycardia and CFS. Hollingsworth *et al* (128) observed that nearly one third of 64 participants in a CFS/ME cohort had diagnosable PoTS on haemodynamic testing compared to four in the control group. Although the prevalence of PoTs in the whole population is not known, it has been estimated to be 0.2-1% (159, 160).

A recent cross-sectional study from Australia of 306 CFS patients found co-morbid PoTS in 11% of the cohort (158) and suggests that associated haemodynamic between-group differences, including heart rate and blood pressure on standing, may be seen in a subset of CFS patients pointing towards the possibility of a disease spectrum.

This association is further seen when assessing fatigued PoTS patients according to CFS criteria. Okamoto *et al* (140) examined 47 patients with established PoTS. They found that 93% of this group had symptoms of severe fatigue and 30 fulfilled CDC criteria for CFS. They also observed greater orthostatic tachycardia in the CFS-PoTS group compared to the non-CFS-PoTS group and more variability in LF blood pressure,

suggesting greater sympathetic activity and the possibility of a similar underlying causal pathway.

Furthermore, a recent cross-sectional study examined subjective autonomic symptom burden, as assessed by the orthostatic grading scale. Findings of a significantly higher symptom burden in patients with PoTS alone and PoTS with co-morbid CFS compared to CFS alone suggest that these two conditions may share a similar aetiopathogenesis (132).

1.12.8. Sources of variation and potential confounders

The hierarchical level of evidence of these studies is predominantly low (125). Studies of AD and fatigue are largely observational (case control) and it is, therefore, not possible to determine the causal direction for abnormalities observed. In addition, many studies are limited by small cohort sizes, perhaps resulting from illness severity prohibiting participation, which weakens the statistical likelihood of findings being significant.

While most studies attempt to reduce confounding variables with specified exclusion criteria, including medications and co-morbid conditions, as well as matching baseline characteristic between cohorts, these are not uniformly adopted across all studies. The effect of this may be such that associated variables, rather than CFS, contribute to the observed dysautonomia.

1.12.9. Autonomic assessment

Most of the studies reviewed used continuous beat-to-beat monitoring; others assessed autonomic function as a series of one-off measurements. There is recognised difficulty in assessing and evaluating autonomic function (112, 161). Technology now allows assessment of continuous measurement of autonomic parameters over the short to medium term. This advancement is important for appropriate clinical assessment and future studies, and may represent a more comprehensive way of measuring autonomic function. The development of longer-term continuous measurement tools will further enhance future research and may be developed as a gold standard assessment tool.

1.12.10. Medication

Medications, such as antidepressants and analgesics, can affect the ANS. Some of the studies excluded medications for this reason, however it is not clear whether this was the case in all studies. Standardised exclusion of medication would strengthen results and reduce potential confounders.

1.12.11. Depression

Three studies excluded participants with a diagnosed psychiatric disorder. Of these, two found minimal change in autonomic function between the CFS and control groups. This raises a question of whether the demonstrated AD in CFS studies is accounted for by inclusion of patients with depression.

1.12.12. Co-morbidities

Conditions that affect cardiovascular or ANS function, such as existing cardiovascular disease, diabetes mellitus or anaemia, are potential confounders and are not consistently excluded.

1.12.13. Cardiovascular deconditioning

There are arguments that cardiovascular deconditioning related to less vigorous activity may be the cause of the ANS problems, including PoTS (162), in CFS (142, 148, 163). As with other studies investigating the underlying pathology of CFS, the direction of causality has not been proven and this remains, therefore, a controversial and much-debated subject.

Other studies (130) have examined BMI-matched CFS and control groups with similar patterns of sedentary behaviour and observed different autonomic findings in the CFS group, reinforcing the argument that it is an underlying pathology in this group that leads to a reduction in physical activity. Furthermore, a recent case control study identified a subgroup of fatigued CFS patients with no AD symptoms, suggesting that deconditioning cannot explain the principle symptoms of CFS (122) and supporting the finding in another study that physical deconditioning is not a perpetuating feature of CFS (164).

It may be that the autonomic profile represents an indicator of disease severity with sympathetic overactivity as an initial abnormality with subsequent associated parasympathetic withdrawal. There is evidence in favour of both decreased parasympathetic activity and increased sympathetic activity. Findings by Frith *et al* (152) and De Becker *et al* (135) are indicative of sympathetic overactivity. Similarly, Okamoto *et al* (140) found greater low-frequency blood pressure variability - a marker of sympathetic activation - in the CFS-PoTS group. Conversely, Naschitz *et al* (138) comment that if the shortened QTc intervals they observed were due to parasympathetic withdrawal they would have expected to see a shorter QTc on tilt testing compared to baseline.

While there are pointers that sympathetic activity may be increased and be the consistent feature of AD in CFS patients, future electrophysiological studies could delineate the relative contribution of parasympathetic and sympathetic change in CFS.

These are important considerations. Nevertheless, in the context of clinically-significant symptoms seen on subjective questionnaires such as COMPASS or during objective testing, there is a plausible basis for the hypothesis that dysautonomia is a central feature of CFS.

1.12.14. Treatment implications

Defining the specific autonomic abnormalities in CFS has implications not only for diagnosis but also for treatment. Sutcliffe *et al* examined the effectiveness of home orthostatic training in CFS and found that there was an improvement in blood pressure maintenance on standing in the group receiving training, which was maintained at six months (165). There was also a trend towards fatigue improvement at six months. This indicates that a larger-scale trial would be beneficial.

There is mixed evidence about pharmaceutical interventions. Fludrocortisone acetate has been shown to have some benefit in global wellness in CFS (141) but the study team concluded that there was insufficient difference compared to controls to determine that fludrocortisone acetate is an appropriate treatment option.

Newton *et al*'s (129) study looking at blood pressure circadian rhythm in CFS patients suggests an area for future research is the effectiveness of agents to increase blood pressure, such as midodrine.

Midodrine has not yet been subject to a randomised controlled trial but has been shown to be beneficial in case series. Naschitz *et al* (137) conducted a pilot study with midodrine in CFS patients with observed dysautonomia on HUT test. Six out of ten of the group had subjective and objective symptom improvement during ten months of treatment, which would indicate a place for further research into its efficacy and use in managing the symptoms of AD in CFS.

1.12.15. Conclusion

The findings of this literature review support the case for abnormalities in the autonomic nervous system in CFS. Subjective autonomic symptoms are well documented and appear to correlate to fatigue severity. There is an emerging picture across studies of subtle changes in objective autonomic function in CFS that include a raised heart rate – at rest and in response to stress – and lowered blood pressure, with possible sympathetic overactivity and corresponding decreased parasympathetic modulation. These changes may arise secondary to infection and a predisposing genetic susceptibility (166).

Nevertheless, this picture is blurred as a result of the use of different diagnostic criteria, which may be selecting CFS participants with different symptom burdens. Furthermore, inconsistent exclusion for comorbid disease – in particular depression where AD has also been demonstrated – means it is difficult to attribute abnormalities to one or other condition. The use of consistent criteria in future research would facilitate delineation of these autonomic symptoms.

1.12.16. Future research

Studies to date demonstrate AD in CFS. Future research is needed to explore AD with standardised measures and with more uniform diagnostic and exclusion criteria, including examining sedentary controls, in order to avoid the confounder of deconditioning, and ensuring consistency with psychiatric exclusion criteria.

Defining the specific abnormalities of autonomic function that occur in CFS, particularly whether they are central or peripheral, will lead to objective diagnostic criteria and identify potential treatment targets for this chronic debilitating condition.

Key to this is the exploration of potential functional and phenotypic differences by diagnostic criteria, which will enable better characterisation of a condition which may present across a spectrum of disease.

Study name	Study design	Cohort size	Diagnostic criteria	Exclusion criteria	Method for recording autonomics	Brief Results	Limitations
Boneva R *et al* **2007** Higher heart rate and reduced heart rate variability persist during sleep in chronic fatigue syndrome: A population-based study (134)	Case control	30 CFS 38 controls	CDC	Depression using Diagnostic Interview Schedule for DSM-IV	Continuous	CFS significantly higher mean HR with shorter mean RRI and reduced LF, VLF and total power of HRV CFS significantly lower plasma aldosterone and higher plasma norepinephrine HR correlated weakly with plasma norepinephrine	No measure of physical activity between controls and CFS therefore findings may be due to deconditioning
De Becker P *et al* **1998** Autonomic testing in patients with chronic fatigue syndrome (135)	Case control	21 CFS 13 control	CDC	Psychiatric disorder Cardioactive medications	Continuous	HR after tilting significantly higher is CFS (mean 88.9bpm v. 77.9bpm) LF power after tilting significantly higher in CFS. Trend towards increased HR during cold pressor test Sympathetic over-activity in response to stress. No parasympathetic differences between groups	Small sample sizes Healthy volunteer bias
Frith *et al* **2012** Impaired blood pressure variability in chronic fatigue syndrome-A potential biomarker (127)	Case control	68 CFS 68 controls	Fukuda	Vasoactive medication Diabetes mellitus Renal or hepatic disease	Continuous	LF-HRV (sympathetic) and significantly greater and reduced parasympathetic markers in CFS group Higher resting heart rate (p=0.006) and dBP (p=0.003) in the supine position in the CFS group DBP spectral power increased across all domains – shift towards sympathetic and away from parasympathetic. SBPV reduced on standing (parasympathetic and sympathetic)	Healthy volunteer bias Potential confounders not discussed
Freeman R *et al* **1997** Does the chronic fatigue syndrome involve the autonomic nervous system? (148)	Case control	20 CFS 20 controls	1988 case definition	Medications affecting ANS Screened for psychiatric conditions – not excluded but factored in	Beat-to-beat BP	Higher baseline and maximum heart rate on standing and tilting in CFS Test of parasympathetic nervous system function and measures of sympathetic nervous system less in CFS Physical activities index a predictor of autonomic test results	Small sample sizes Healthy volunteer bias and selection bias of patients with autonomic symptoms therefore not generalizable
Hollingsworth et al **2010** Impaired cardiovascular response to standing in chronic fatigue syndrome (128)	Cross-sectional	12 CFS 8 controls	Fukuda	Medication affecting haemodynamics Diabetes mellitus or renal/hepatic disease	Continuous	LVWI on standing in CFS significantly higher with symptoms. OGS scores higher in those with abnormal LVWI responses	Very small sample sizes Not possible to establish causality Volunteer bias
Jones J *et al* **2005** Orthostatic instability in a population-based study of chronic fatigue syndrome (136)	Cross-sectional	58 CFS 55 controls	CDC	Age > 55 years. Defined medical conditions. Cardioactive medications	Single measurements	No orthostatic instability on stand-up test in either group. Orthostatic instability in JUT test in 30% CFS and 48% controls	Small sample taking HUT

Study name	Study design	Cohort size	Diagnostic criteria	Exclusion criteria	Method for recording autonomics	Brief Results	Limitations
LaManca *et al* **1999** Cardiovascular response during head-up tilt in chronic fatigue syndrome (133)	Case control	39 CFS 31 controls	CDC Fukuda	Major psychiatric illness in five years prior to study Antihypertensives Cardioactive medication stopped before testing	Continuous	Haematocrit significantly raised in CFS Similar HUT cardiovascular adjustments between groups suggesting no difference in neurally-mediated blood pressure	Unclear what "major psychiatric co-morbidity excluded
McDonald *et al* **2014** Postural tachycardia syndrome is associated with significant symptoms and functional impairment predominantly affecting young women: A UK perspective (132)	Cross-sectional	136 PoTS patients	Fukuda		Subjective Orthostatic grading scale (OGS)	Statistically higher scores for OGS in PoTS group and in co-morbid PoTS and CFS	Systematic difference in participants (50% volunteer rate) Young, female PoTS volunteers – may not be representative
Meeus *et al* **2013** Heart rate variability in patients with fibromyalgia (FM) and patients with chronic fatigue syndrome: A systematic review (125)	Systematic review	6 case control studies on CFS		Included only studies using HRV for assessment		Moderate evidence for decreased parasympathetic activity: reduced HF and LF/HF Moderate evidence for increased sympathetic activity on upright tilt Decrease in HRV is sleep only	Limited number of studies Observing limited measured of autonomic function Case control studies reviewed
Naschitz J *et al* **2003** The head-up tilt test in the diagnosis and management of chronic fatigue syndrome (137)	Review of patient records	80 CFS	CDC		Continuous	Haemodynamic instability score calculated. HUT shows a dysautonomia specific to CFS and may be useful is supporting a diagnosis of CFS ≥0.98 Fractal and Recurrence Analysis-based Score ≥0.22 associated with CFS	Patients with mild to moderate CFS only tested Results may be due to deconditioning
Naschitz *et al* **2003** The head-up tilt test with haemodynamic instability score in diagnosing CFS (146)	Prospective controlled	40 CFS 73 fatigued 41 FM 58 syncope 28 anxiety 28 HTN 59 controls	CDC		Continuous	HUT stopped in 22% CFS patients with postural symptoms Median(IQR) HIS values CFS q2.14(4.67), fatigue 3.98(5.35), F 2.81(2.62), syncope 3.7(4.36), anxiety 0.21(6.05), controls 2.66(3.14), hypertensives 5.35(2.74) (p-0.0001 vs. CFS in all groups, except for anxiety disorder, p=NS). HIS cut-off at 0.98 has sensitivity 90.3% and specificity 84.5%	Not possible to score HIS on all CFS patients if HUT stopped early
Naschitz J *et al* **2006** Shortened QT interval: a distinctive feature of the dysautonomia of chronic fatigue syndrome (138)	Case control	30 CFS 22 controls	CDC	Caffeine and smoking restricted for 6 hours before tilt test	Continuous	Average supine QTc in CFS significantly shorter than control (supine 0.371 +/- 0.02 secs and on tilt 0.385 +/- 0.02 secs). Normal range 0.400 – 0.440 secs May be caused by atropine and reduced vagal tone	Small numbers One lead only to measure QT intervals not 12

Study name	Study design	Cohort size	Diagnostic criteria	Exclusion criteria	Method for recording autonomics	Brief Results	Limitations
Newton **2007** Symptoms of autonomic dysfunction in chronic fatigue syndrome (122)	Cross-sectional	Phase 1: derivation 40 CFS 40 controls Phase 2: validation 30 CFS 37 controls 60 PBC	Fukuda	Co-morbidities or medications with associated fatigue or AD	Subjective using COMPASS	Symptoms of AD measured using COMPASS associated with CFS and PBC and correlate with severity of fatigue Total COMPASS score >32.5 PPV 0.96 (95%CI 0.86-0.99) and NPV 0.84 (0.70-0.93) for the diagnosis of CFS Dysautonomia-associated fatigue underpins CFS and PBC	Cannot establish causality
Newton J *et al* **2009.** Lower ambulatory blood pressure in chronic fatigue syndrome (129)	Cross-sectional	38 CFS 120 controls 47 PBC	Fukuda	Vasoactive medication Hypertension Diabetes mellitus	Single ambulatory blood pressure at 30 minute intervals	Significantly (p=<0.0001) lower sBP and MABP (p=0.0002) and greater diurnal variation (p=0.009) in CFS group Inverse relationship between increasing fatigue and diurnal variation in CFS and PBC groups	CFS patients younger, which may act as confounder
Newton J *et al* **2011** Physical activity intensity but not sedentary activity is reduced in chronic fatigue syndrome and is associated with autonomic regulation (130)	Cohort	107 CFS 107 sedentary controls	Fukuda	Smoking and caffeine prior to test	Continuous	Active energy expenditure reduced in CFS group No association between activity and fatigue Physical activity was inversely associated with resting HR p=0.04; r^2=0.03 Reduced activity associated with reduced HRV in CFS	Volunteer bias: not representative of general population Role of deconditioning
Okamoto L *et al* **2012** Neurohumoral and haemodynamic profile in postural tachycardia and chronic fatigue syndromes (140)	Cross-sectional	47 PoTS 30 of these fulfilled criteria for CFS	CDC	Co-morbid disease affecting autonomic function: Renal/ liver/haematological/diabetes/arrhythmias Cardioactive medications	Continuous	CFS-PoTS had greater orthostatic tachycardia, greater low-frequency BPV, greater BP recovery from early to late phase II of Valsalva and higher supine and upright plasma renin activity	Deconditioning may be confounder Selection bias: PoTS cases more severe and CFS less severe
Reynolds *et al* **2014** Comorbidity of postural orthostatic tachycardia syndrome and chronic fatigue syndrome in an Australian cohort (158)	Cross-sectional	273 CFS 33 CFS PoTS	Canadian 2003	Not stated	Single measures at baseline, 2 minutes post stand and end	11% co-morbidity between CFS and PoTS groups Higher HR on standing in CFS-PoTS	Exclusion criteria not stated Possible measuring PoTS and associated fatigue or CFS
Rowe P *et al* **2001** Fludrocortisone Acetate to Treat Neurally Mediated Hypotension in Chronic Fatigue Syndrome (141)	RCT	83 CFS 45 placebo 38 treatment	CDC	Psychiatric disorder – unspecified Medications - unspecified	Continuous	Double blind trial examining efficacy of fludrocortisone acetate on CFS and NMH 15 point improvement in wellness score: 14% of treatment group v. 10% placebo group	Loss to follow-up uneven between groups Compliance not verified and harms not considered

Study name	Study design	Cohort size	Diagnostic criteria	Exclusion criteria	Method for recording autonomics	Brief Results	Limitations
Rowe P *et al* **1998** Neurally mediated hypotension and chronic fatigue syndrome (147)	Case control	23 CFS 14 control	1988 case definition	Not stated	Continuous HUT	Abnormal drop in BP in response to upright tilt seen in 22 of 23 and 4 of 14 controls In 45 minutes of upright tilt 16 CFS developed hypotension. Normal BP maintained in controls 23 CFS developed orthostatic symptoms during first stage of tilt testing – none of controls did 47% reported symptom improvement with fludrocortisone	Small cohort
Scott et al. **2012** Shortened QTc interval in chronic fatigue syndrome (131)	Cross-sectional	177 CFS 43 controls	Fukuda		12 lead ECG	Shortened QTc interval in CFS group. Higher symptom burden on OGS No significant difference in ECG or HR	Confounders not considered Small cohort of controls
Soetekouw P *et al* **1999** Autonomic function in patients with chronic fatigue syndrome (149)	Cross-sectional	37 CFS 38 controls	Sharpe *et al*	Medication for hypertension/COPD Antidepressant use. Cardiovascular/CNS disease/diabetes mellitus/COPD	Continuous	Lower inspiratory/expiratory difference HR in CFS group No major difference in HR or BP at rest Max increase in HR during standing similar. No significant differences in Valsalva ratio Greater responses in systolic and diastolic blood pressure during Valsalva in CFS group	Causality not proven
Van Cauwenbergh *et al* **2014** Malfunctioning of the autonomic nervous system in patients with chronic fatigue syndrome: A systematic literature review (124)	Systematic review	27 case control studies	Any	Children		Higher HR in CFS during HUT Overall appearance of different autonomous response in CFS v. controls particularly during HUT Fractal and Recurrence Analyses-based Scores and hemodynamic instability score valuable assessment tools	Varying methodological quality of studies Varying diagnostic criteria
Winkler A *et al* **2004** Autonomic function and serum erythropoietin levels in chronic fatigue syndrome (142)	Case control	22 CFS 18 controls 23 iron-deficient	CDC	Comorbid depression or anxiety All medication except prn paracetamol/ibuprofen	Continuous beat-to-beat	Statistically significant greater increase in HR and fall in SBP on standing with CFS Normal variation on standing and Valsalva	Volunteer bias and small sample Different gender balance between groups at baseline
Wyller et al **2011** Blood pressure variability and closed-loop baroreflex assessment in adolescent chronic fatigue syndrome during supine rest and orthostatic stress (143)	Cross-sectional	14 CFS 56 controls Aged 14-18	Modified CDC – 6 months fatigue by no accompanying symptoms	Chronic disease Regular medication	Continuous	Lower HF-SBPV in CFS group and rest and during LBNP LF:HF gain for baroreflex feedback increases significantly in CFS in response to stress due to increase LF gain and decreased HF gain suggesting increase in sympathetically-mediated baroreflex gain in CFS	Plasma volume not measured therefore hypovolaemia not ascertained Small sample size with potential for volunteer bias May not be representative

Study name	Study design	Cohort size	Diagnostic criteria	Exclusion criteria	Method for recording autonomics	Brief Results	Limitations
Yamamoto Y *et al* **2003** A measure of heart rate variability is sensitive to orthostatic challenge in women with chronic fatigue syndrome (144)	Case control	24 CFS 22 control (Female)	Modified CDC (< 6 years; Likert severity score ≥ 3 in month before	Depressive episode in five years before Antihypertensive and benzodiazepines Vasoactives		Supine: only mean RRI significantly lower in CFS. During HUT mean RRI, high frequency and fractal amplitudes were significantly lower in CFS. Looking at difference between baseline and HUT only A_{FR} significantly lower suggesting A_{FR} a disease specific response of HRV to HUT	Healthy controls paid Selection bias BPV and respiration not measured – can impact HRV
Yoshiuchi K *et al* **2004** Use of time-frequency analysis to investigate temporal patterns of cardiac autonomic response during head-up tilt in chronic fatigue syndrome (145)	Cross-sectional	18 CFS + PoTS 8 CFS 25 controls	Modified CDC – substantial symptoms 1 month before		Continuous	Significant postural change in both groups for all autonomic variables there were significant group x time interactions between CFS without PoTS and controls	Respiration not controlled Selection bias Very small sample size

Table 5 Summary of articles reviewed in ANS literature review

1.13. Depression: A potential confounder

There is a complex relationship between chronic diseases, particularly those that are difficult to manage and treat, and depression. This is further complicated in CFS by the absence of a biological marker and by the significant shared symptomatology. As such, it is an often-ignored confounder in studies. This section considers the implications of these common symptoms.

1.13.1. Shared symptoms

There is significant overlap between the symptoms of depression and the symptoms of CFS (table 8). Diagnostic criteria for CFS are detailed in table 2, section 1.4. Diagnostic criteria for depression are shown in tables 6 and 7. The WHO International Classification of Diseases version 10 (ICD-10) is the standard diagnostic tool for disease in clinical practice in the UK (167). The Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV) is the standard classification of mental disorders in research (worldwide and in clinical practice in the US (168)): it forms the basis of the diagnostic tool used in this study.

ICD-10 F32 Depressive episode		Core symptoms: • Depressed mood • Loss of interest and enjoyment • Increased fatigability Other symptoms: • Reduced concentration and attention • Reduced self-esteem and confidence • Guilt and unworthiness • Pessimistic views of the future • Ideas of self-harm/suicide • Disturbed sleep • Diminished appetite
	Mild	≥ two weeks of ≥ two core symptoms ≥ two other symptoms
	Moderate	≥ two weeks of ≥ two core symptoms ≥ three and preferably four other symptoms
	Severe	All three core symptoms ≥ four other symptoms of severe intensity

Table 6 ICD-10 diagnostic criteria for depressive episode

Taken from (169)

DSM-IV Major Depressive Disorder requires ≥ two episodes	≥ two weeks of • Depressed mood, and/or • Loss of interest or pleasure in life activities ≥ five of the following (causing clinically significant impairment in social, work, or other important areas of functioning almost every day): • Depressed mood most of the day • Diminished interest or pleasure in all or most activities • Significant unintentional weight loss or gain • Insomnia or sleeping too much • Agitation or psychomotor retardation noticed by others • Fatigue or loss of energy • Feelings of worthlessness or excessive guilt • Diminished ability to think or concentrate, or indecisiveness • Recurrent thoughts of death

Table 7 DSM-IV diagnostic criteria for depression

Taken from (168)

Proper exploration of the origin and nature of depressive symptoms is essential. Low mood is distinguishable from depressed mood and does not meet the diagnostic criteria. Furthermore, depressive symptoms may be better accounted for by the presence of a physical disorder, classified as F54 *Psychological and behavioural factors associated with disorders or diseases classified elsewhere*.

Within the WHO's ICD-10 criteria there is acknowledgement that differentiation between mild, moderate, and severe depressive episodes relies on a "complicated clinical judgement" of the number, nature, and severity of symptoms (169). Reaching a diagnosis of depression is a subjective judgement that is prone to inter and intra-individual variation.

Severity of depression is determined by the presence of increasing numbers of symptoms and increasing severity of functional impairment. This loss of function and its variable nature overlaps with features of CFS and complicates distinction between the two conditions.

Comparison of CFS and depression diagnostic criteria reveals the shared symptomatology. A recent study investigating this overlap found that 38% of those with Major Depressive Disorder (MDD) met the CDC criteria for CFS (38).

Symptoms	Forms part of ICD-10 criteria for depression	Forms part of Fukuda criteria of CFS	Prevalence (%)		
			Depressive disorder	CFS	Healthy controls
Depressed mood	✓		95	65	1
Loss of interest or pleasure	✓				
Fatigue or low energy	✓	✓	19	100	4
Disturbed sleep	✓	✓	95	98	9
Poor memory or concentration	✓	✓	79	83	1
Low self-confidence	✓				
Change in appetite	✓				
Suicidal ideation	✓				
Agitation or slowing of movement	✓				
Guilt	✓				
Sore throat		✓	11	64	8
Tender lymph nodes		✓	11	65	4
Myalgia		✓	68	89	31
Arthralgia		✓	50	73	17
Headache		✓	22	59	7

Table 8 ICD-10 Symptoms shared between depression and CFS and their prevalence Taken from Komaroff (26, 46)

This shared symptomatology may characterise an underlying common causal pathway (170) whose phenotype represents a spectrum of disease. This spectrum may include patients who are currently classified as having either CFS or depression, some with more fatigue-type symptoms and some with more depressive-type symptoms (171).

Evidence examining the potential for a shared aetiopathogenesis is discussed in section 1.13.2. Alternatively, CFS and depression may indeed be distinct disorders, which will one day be biologically separable.

It may be that clinicians intuitively apply more sophisticated assessment to differentiate CFS from MDD than that available in ICD or DSM. Anecdotally, patients with CFS report frustration and inability to perform tasks as limiting factors rather than a lack of motivation, this suggests a definite lack of physical capacity to participate in activities rather than a lack of impetus or motivation.

Moreover, low mood experienced by CFS subjects is described as secondary to frustration about physical symptoms and a sense of profound helplessness, which may be distinguishable from the low mood seen in depression that is often accompanied by pessimistic feelings about the future with or without suicidal ideation (172). Furthermore there is evidence that low self-esteem is not a feature of CFS, whereas it is common in depression (172, 173), and indicates that a detailed exploration of each symptom is central to reaching the correct diagnosis.

Studies show that the criteria for depression that are met by CFS patients relate to changes and disturbances in mood, weight, appetite and sleep, as well as somatic symptoms. If this mood change reflects and arises from a change in physical functioning there is an argument that the underlying origin is different from that in depression.

Criteria that are currently recognised as the Gold Standard diagnostic tool for these conditions may, in fact, be confounding a distinction between depression and CFS. If they are two distinct disorders, diagnosed clinically on the basis of many shared symptoms, patients with one condition may meet the criteria for the other. This has important implications, firstly in terms of clinical management and the wider perception of CFS, and secondly in terms of research into its pathogenesis.

Studies have attempted to differentiate between these overlapping symptoms and elucidate whether discrimination between their presence in these two conditions is

possible. A randomised controlled trial of the efficacy of fluoxetine on symptoms in CFS patients found no beneficial effect on any CFS characteristics (174). Although it is difficult to draw conclusions about the underlying pathophysiology from this (not all MDD patients respond to antidepressants (175)) it may be an indication that CFS is distinct from major depression (where Fluoxetine can improve symptoms (176)).

Deeper understanding of the nature of symptoms and associated features, for example mood and esteem, will help differentiate between the two. In the context of the challenge of differentiating between these two conditions, proposals that specific symptoms are suited to enable distinction offer promise. A better understanding of these symptoms – post-exertional malaise, unrefreshing sleep, and impaired memory or concentration (177) – as well as a deeper exploration of individual patients' clinical presentation will allow more accurate diagnosis.

1.13.2. Biological mechanisms

Some researchers hypothesise that the same biological mechanisms underpin these common characteristics, forming part of a disease spectrum. Similar underlying inflammation and cell-mediated immune activation has been seen in both CFS and depression, suggesting a common underlying causal pathway may be responsible for the overlapping phenotype (178).

1.13.2.1. Autonomic dysfunction

Depression has been associated with markers of AD, including elevated levels of catecholamines (80) and abnormalities of heart rate, such as lower HRV. A number of studies have shown elevated resting heart rate in patients with depression, as well as higher responses to physical stressors, such as standing (179), and suggest that parasympathetic activity may be diminished with an associated increase in sympathetic activity (180). This differs from some studies exploring CFS, which have found decreased LF and very LF in CFS subjects (134), suggesting an impaired sympathetic drive.

Altered HRV with lower HF components and higher LF have also been associated with depression, again suggesting an enhanced sympathetic drive, with a positive dose-

response shown by greater severity of AD correlating to greater depression severity (181). Other studies have found no significant association between depression and LF or HF after adjustment for confounders (182).

Although AD may be important, lack of consistent research makes it difficult to determine the direction of causality or to fully support the hypothesis that AD is a common mechanism for CFS and depression. Furthermore, AD is found in a number of other conditions and may therefore not be disease specific or may arise from common symptoms such as fatigue.

1.13.2.2. Immune system

While some research has shown higher inflammatory markers in CFS compared to depression (183), other studies suggest that the presence of somatic symptoms may be the determining factor in immune dysfunction in both conditions. Patients with depression who also exhibit somatic symptoms have been shown to have significantly increased IL1 and TNF-α compared to those without somatic symptoms (178), which mirrors the findings of some studies that have found these to be elevated in CFS (76). This points towards the possibility of a spectrum of related disorders.

The role that co-morbid depression has in this is unclear. The neurotransmitter neuropeptide Y, a stress mediator produced by neurons of the sympathetic nervous system, has been shown to be elevated in CFS and also correlates with both stress and depression (184), complicating interpretation of the underlying reasons for this elevation.

1.13.2.3. Hypothalamic-pituitary-adrenal axis

Both depression and CFS are linked to disruption of the HPA axis. Findings suggest opposing directions of dysfunction with down-regulation of the HPA axis seen in CFS and up-regulation in depression (185-187). Research findings are not consistent, however, and it is likely that findings are confounded by the presence of both CFS and depression in some subjects.

1.13.3. Cognitive impairment

Cognitive impairment has been shown in CFS and MDD. Similar motor impairment on cognitive testing has been seen in both conditions with MDD subjects demonstrating impaired performance compared to CFS (188).

Cognitive impairments in MDD subjects have been shown to be strongly associated with depression severity and subjective fatigue; in patients with CFS, one study has shown a weaker correlation between cognition and depression (and no correlation with fatigue) (189). This study found that CFS subjects were less depressed than MDD subjects and raises the question of whether the observed cognitive impairment was secondary to CFS or arose from co-morbid depressive symptoms.

1.13.4. Current challenges

There are important questions to be considered. Depression and CFS may lie on the same disease spectrum, sharing symptoms but manifesting with different phenotypes (170, 171). Another important consideration is whether depression predisposes to or causes CFS. Early adversity may also be important, as a trigger for subsequent major depressive disorder which may, as a series of stages or as a result of complex interplay, cause CFS.

If depression and CFS are different disease processes, co-morbid depression may impact the effect of CFS and resilience to symptoms. Furthermore, it may alter the recollection or impact of adversity. Future research should, therefore, control for co-morbid depression to enable better understand of the relationship between the conditions.

The available evidence relating to commonalities in the symptom profiles and biological associations of these two disorders mean that it is not possible to confirm or exclude the supposition that they are distinct and separable disorders, neither that they have a unitary shared pathogenesis nor that the disorders represent the phenotypic expression of complex interplay between the body's regulatory homeostatic systems. In research and in clinical practice differentiation between the

two disorders and the determination of comorbidity is imprecise and subject to judgement without clear anchor points.

1.13.5. Future direction

A better understanding of the qualitative nature of the distinguishing symptoms that can be taken into field trials for revised diagnostic criteria, utilisation of more sophisticated clinical precision in pathophysiological studies, a better understanding of the impact of potentially shared aetiological factors, such as early adversity, and the use of a systems-based approach to examine the relationships between symptoms and biological concomitants will allow the field to progress and will support stratified or shared treatment decisions.

1.14. Impact of autonomic dysfunction in CFS: Cognitive function

Autonomic dysfunction in CFS affects many of the body's systems. This thesis focusses on its potential role in cognitive impairment.

Cognitive impairment is a recognised symptom of CFS and is one of the diagnostic criteria for Fukuda (22). Up to 95% of people with CFS have reported problems with cognitive function at some point during the course of their illness (26, 33). Studies examining cognitive problems in CFS are limited and interpretation complicated by the use of different test batteries and by a lack of consistency, both in terms of results and control conditions. Despite this, findings suggest that abnormalities of information processing speed, impaired working memory and information learning are features of cognitive impairment in CFS and appear to occur independently of fatigue and co-morbid depression (190).

The possible underlying aetiology of cognitive impairment encompasses degenerative, vascular, metabolic, psychiatric and iatrogenic causes (191). Some of these are implicated in the presence of autonomic dysfunction, which has been associated with cognitive impairment and is discussed in section 1.14.2.

The heterogeneous clinical presentation and aetiology of cognitive impairment is illustrated though its association with many chronic diseases, including

neuroendocrine disorders and organic brain disease. Sleep disorders have been shown to be associated with an increased risk of developing OH and cognitive impairment (192). Similarly, type 2 Diabetes Mellitus has been shown to negatively impact cognitive function, which may represent a link between metabolic syndrome and vascular dementia (193). Elevated cortisol has also been associated with cognitive impairment – and with dementia (194, 195) – suggesting that HPA axis dysregulation may be a risk factor for poor cognitive function.

Both hypertension and hypotension have been associated with impaired cognitive function (196-200). This has been evidenced particularly among elderly populations but research has also shown an association between hypotension and impaired cognitive function, specifically visuospatial function, in younger individuals (201), possibly secondary to cerebral hypoperfusion.

1.14.1. Cognitive function

Table 9 outlines cognitive function and its corresponding neuroanatomical control.

In broad terms, the frontal regions of the brain – the limbic system, thalamus, hypothalamus, basal ganglia, and cerebral cortex – are used for planning and thinking and the posterior – medulla, cerebellum, and pons – for vision, memory, movement and sleep. Exact functioning is not fully understood and results from a complex interplay of processes and regions (202).

Function		Control
Memory	Verbal	Prefrontal cortex; left temporal lobe; hippocampus; limbic system
	Visuospatial	Prefrontal cortex; hippocampus; occipital lobe; posterior parietal lobe
Executive		
Composed of several higher cognitive skills, including working memory, reasoning, problem solving, execution		Pre-frontal cortex
Psychomotor speed		
Relationship between cognition and physical movement		Frontoparietal lobe
Attention		Parietal lobe

Table 9 Cognitive function and corresponding brain centre

Taken from (202-209)

1.14.2. Autonomic dysfunction and cognitive function in non-CFS

Studies of the pathophysiology of organic psychiatric disorders, including dementia, have demonstrated a 'U' or 'J'-shaped association with abnormalities in blood pressure – that is they are more prevalent at both extremes and the upper end of blood pressure. A systematic review examining the relationship between blood pressure and Alzheimer's disease found an inverse association between hypertension later in life and Alzheimer's. Studies included are, however, limited by selection bias, lack of participant homogeneity and an inability to determine causality (210).

Hypotension has been found to be protective of memory, as tested using the Mini Mental Status Examination in an elderly population over 80 years of age (211). Conversely, OH appears to be negatively associated with memory and overall global functioning in both younger and older populations (196, 212, 213) and has been

associated with poorer cognitive performance, both in terms of a drop in blood pressure on standing and longer time to recovery to baseline blood pressure (214).

This may reflect the 'U'-shaped association and point towards the existence of a protective threshold, below which risk of developing cognitive problems increases. Furthermore, blood pressure variability has also been linked to cognitive impairment with increased variability being associated with greater dysfunction in elderly patients (215, 216).

Evidence currently provides a mixed picture for the role that AD has to play in cognitive impairment and studies tend to focus on effects in older patients. This means that comparison with CFS patients, where the patient demographic is younger (mean age of onset age 30 years (217)) is difficult. This mixed picture is mirrored in research into cognitive impairment in CFS, as described in the following review.

1.15. Literature review: Cognitive function in CFS

A literature review was conducted to examine existing research assessing subjective and objective cognitive problems in CFS and to explore the pathophysiological processes that might be underpinning these problems.

The search was conducted via Ovid MEDLINE ® and Embase from 2000 to present day. English language articles were sought. The search terms *chronic fatigue syndrome* and *cognitive dysfunction* were used, which produced 44 articles after deduplication. Abstracts were reviewed and non-relevant studies excluded. Further articles referenced by these articles were reviewed. Opinion was also sought from experts in the field regarding more recent and current research and these articles were identified. The procedure of the literature search is outlined in figure 3. In total 10 studies were reviewed (table 11).

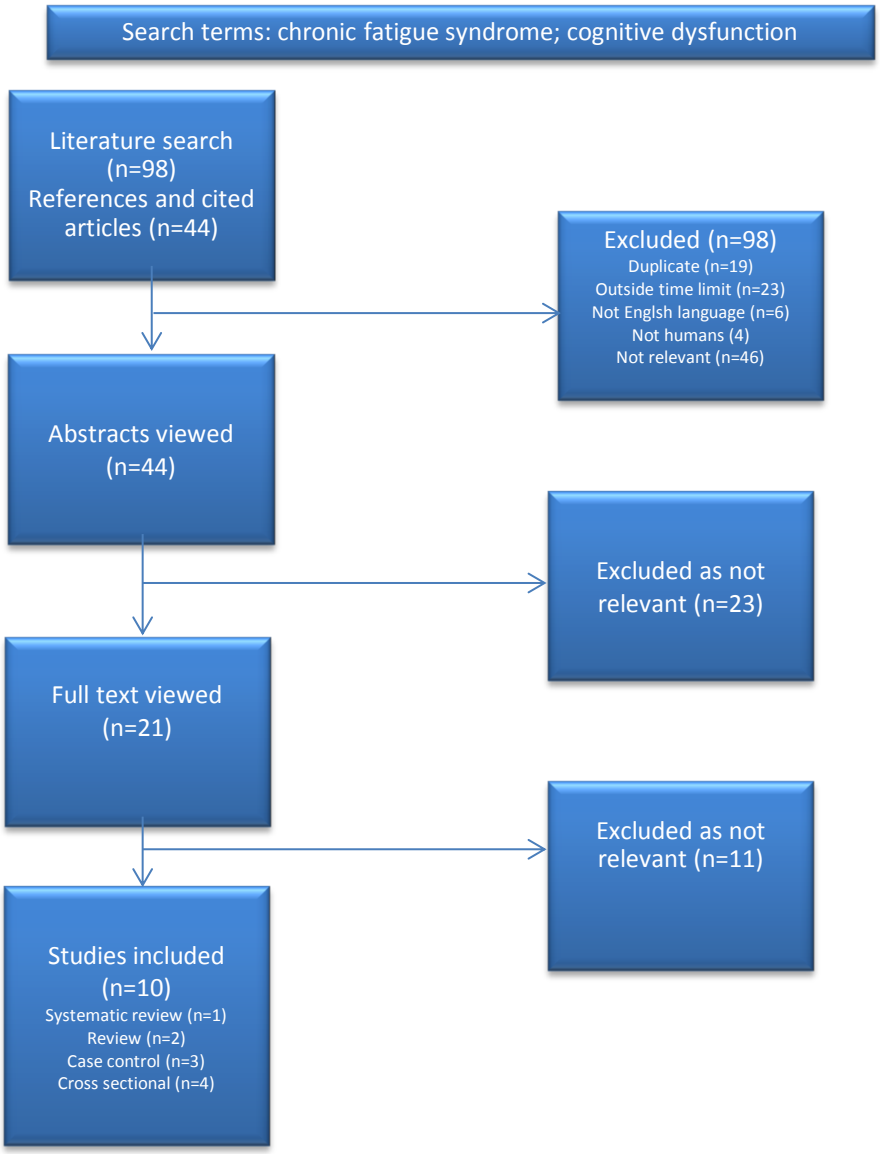

Figure 3 Procedure of literature search cognitive function and CFS

1.15.1. Challenges of testing cognitive function in CFS

The limitations of investigating cognitive dysfunction in CFS are well recognised (218). As with studies examining the role of the ANS in CFS, research investigating cognitive function is limited by lack of heterogeneous cohorts and comparison groups and small sample sizes leading to insufficient statistical power.

One of the primary challenges lies with a lack of consistent test battery for neuropsychological testing. Table 10 shows some of the tests used in research, as well as the domain assessed, and gives a picture of the breadth of tools available. While they examine the same overarching area of functioning, many of the deficits observed

in studies are subtle and as such they are sensitive to the use of varying tests, which can result in variation in findings (219).

Another significant challenge of assessing cognitive function stems from the fact that individual participants have a unique ability, with strengths and weaknesses in different cognitive domains. This makes assessment at group level difficult, as variation between individual participants may be significant. In addition, there is an absence of literature examining individual ability pre- and post-morbidity and therefore assessing the direct impact that CFS might have on individual cognitive function presents huge challenges.

1.15.1.1. Subjective measures of cognitive function

Subjective measures of cognitive function are an important tool in determining self-perception of cognitive problems. Tools used include the Cognitive Failures Questionnaire (COGFAIL) (220), Everyday Attention Questionnaire (221), and questionnaires of memory and attention symptom severity. Some studies have also used the Mental Fatigue Scale and rating of energy (222, 223).

Metacognition – self-perception and understanding about one's own thought processes (224) – is an important concept to consider when evaluating self-reported assessment. Self-recognition of individual strengths and weaknesses in learning and information processing has been shown to be positively associated with symptom severity (225-228). Furthermore, subjective rating of cognitive function is not a simple proxy measure for objective assessment. There is evidence that the two measures do not correlate (229). Metacognition helps to explain this.

CFS patients report greater subjective cognitive impairment (230), particularly in terms of problems with memory and attention (231), which impacts functional ability (123). Studies show conflicting evidence about whether these self-reported measures correlate to objective measures or to performance. Perceived fatigue has been shown both to have no impact on objective performance (230), as well as correlate to significant impairment of spatial working memory and sustained attention (232). The studies reviewed examined small cohorts and each used different test batteries. While

these limitations may explain the different findings, they also highlight the challenges of investigating cognitive function in CFS.

A recent study by Cockshell and Mathias supports the finding of a mismatch between subjective and objective assessment. They examined the relationship between subjective perception of cognitive problems and objective assessment (231). The findings show that CFS patients rated themselves more highly in terms of cognitive deficit compared to controls, but objectively both cohorts had similar results.

The reasons for this disparity might be explained by metacognitive function and relate to a perception of pre-morbid cognitive function, which is rarely measured, or to a persistence to push towards "expected" levels when CFS subjects undergo objective testing.

1.15.1.2. Objective measures of cognitive function

The challenges of objective measurement are discussed in section 1.15.1. In this context, a pooled analysis of research may provide an overall picture of cognitive dysfunction in CFS. A recent meta-analysis of 50 studies over a 20-year period suggests patients with CFS have cognitive deficits in attention, memory and reaction time but no significant deficit in fine motor speed, vocabulary, reasoning and global functioning (219).

Claypoole *et al* examined a number of domains in 22 pairs of monozygotic twins and found statistically significant differences in motor functioning, speed of information processing, verbal memory functioning and executive functioning but similar intellectual and visual memory functioning. These results are suggestive of poorer neuropsychological performance in CFS (233).

Domain-specific deficits are discussed below.

1.15.1.2.1. Psychomotor speed

Delineating between psychomotor speed (coordinating thinking and doing) and processing speed (the ability to understand and retrieve information) can be

challenging (234). Collated evidence suggests that motor speed is not impaired in CFS subjects (219, 232) pointing towards a greater role of impaired processing speed rather than motor function.

There is consistency in evidence of impaired cognitive and processing speed among CFS subjects compared to controls (190, 230, 232, 235-237). This finding is supported by evidence that orthostatic stress impairs neurocognitive abilities of working memory, accuracy and information processing in CFS/PoTS patients (238).

Beaumont *et al*'s case-control study of the relationship between HRV and cognitive function found a mismatch between low perceived fatigue and effort in CFS subjects and actual performance (230). Although testing relatively small numbers of participants and a limited range of cognitive functions, they found that while ability was comparable between groups, CFS participants were statistically significantly slower at completing the tasks. They also observed a higher baseline heart rate and a sustained increase with long recovery period in this group compared to controls, suggesting that cognitive and autonomic function may be related.

1.15.1.2.2. Attention

Attention has been shown to be similar across CFS and control groups (231, 232, 236). This picture is complicated by the fact that some tests encompass both attention and working memory and assessment with specific tests (for example STROOP) has shown attention deficits while others (for example Digit Span) have not.

Some researchers suggest that this discrepancy may be explained by deficits in verbal rather than working memory (231), on the basis that attention and working memory (the ability to store and manipulate information) may interact closely with one another (239) and that immediate attention span may be considered a form of working memory (207). This differs from verbal memory – the ability to store and retrieve verbal information.

1.15.1.2.3. Memory

Memory (verbal and visuospatial) has been seen to be impaired in CFS patients suffering with self-reported mental fatigue, in particular working memory, which has

been shown to positively correlate with fatigue and immediate and delayed recall (219, 232). Increasing fatigue levels have also been shown to be associated with decreasing attention (240), highlighting the potential interplay between the two domains and the challenges of identifying specific deficits on testing.

Studies have demonstrated impaired visual and verbal episodic memory compared to controls (189, 235). Nevertheless, others have shown no deficit in memory functions between CFS and control groups (231), illustrating the disparity in results and challenges of testing and interpreting findings as discussed above.

1.15.2. Role of psychiatric co-morbidities

Depression and psychiatric co-morbidities have been associated with poor cognitive performance (241, 242), once again raising the question of a relationship between depression and CFS. This is further complicated by the possibility that depression may present as a result of existing cognitive impairment (243).

Nevertheless, studies investigating this relationship point towards distinct features and no association between depression and CFS (190). Claypoole *et al*'s study of monozygotic twins suggests that cognitive performance in CFS subjects was independent of MDD, implying a separate causal pathway (233).

This is supported by Cockshell and Mathias' study of cognitive impairment in CFS and its relationship with function and psychological status. On testing for information processing speed, attention, memory, motor functioning and verbal and visuospatial performance they found information processing speed alone was different in the CFS group compared to controls. On further examining its association with CFS symptoms, psychological co-morbidities and functional ability they found no correlation and suggest that the cognitive impairment seen in CFS is not secondary to other variables, including depression and anxiety (236).

1.15.3. Impact

The impact of cognitive impairment can be significant with a strong association between functional disability and poor cognitive ability (244, 245). Many patients

remain functionally impaired over time with consequent unemployment (246) and long-term detriment to confidence and quality of life.

1.15.4. Underpinning

Many hypotheses regarding the possible biological underpinning of cognitive dysfunction in CFS centre on a neuroimmune origin. Similarities in symptomatology between CFS and MS have added weight to this argument. Impaired (low) levels of NK cells have been demonstrated in both conditions (78) and have been associated with poorer performance on objective measures of cognitive functioning in CFS (247).

Furthermore, the nature of the impairment in MS – of domain-specific deficits rather than global cognitive decline, subtle in nature and characterized by great inter-patient variability – appears to be mirrored by the type of impairment seen in CFS (248). The cause of this impairment in MS is thought to be related to its autoimmune or inflammatory origins (249), an aetiology that may explain the same symptoms in CFS.

Studies also suggest that cognitive impairment may result from AD, which results in impaired cerebral blood flow and low-level hypoxia leading to reduced cognitive ability over time. Cognitive impairment has been associated with reduced cardiac vagal tone and reduced vagal activity (230). Increasing orthostatic stress with a cognitive challenge has also been shown to impair neurocognitive abilities of working memory, accuracy and information processing in CFS/PoTS (238, 250).

Co-morbid depression – or perhaps the common pathogenesis underlying both it and CFS – has been proposed as the cause of cognitive impairment (251), however greater deficits have been found in CFS patients without depression (189, 219, 252) and other studies show no association between psychological status and cognitive function (236).

An important consideration is the fact that it may be the fatigue itself that is central to understanding cognitive impairment. Fatigue, a feature of many diseases (41, 253), is associated with significant objective impairment of cognitive functioning and motor performance (254).

Lack of understanding of the pathogenesis of CFS and the ubiquitous nature of cognitive deficits across a broad range of diseases makes it is difficult to postulate the mechanism that underlies cognitive impairment in CFS. Nevertheless, evidence does suggest that there may be a neuroimmune role.

1.15.5. Longitudinal impact

Few studies have assessed cognitive performance over time. A recent cross-sectional study suggests that cognitive impairment does not deteriorate with disease duration (237); however, this study is significantly limited by a lack of longitudinal data and the use of different participants over time and is therefore difficult to interpret.

There is also little evidence to support the presence of mental fatigability with performance over time (219, 255). Studies assessing this impact are limited as they do not examine performance over consecutive days or weeks but instead focus on performance over the course of one-off "sessions". Future research to understand the impact of repeated testing over consecutive days would better discern whether fatigability is a feature of cognitive impairment seen in CFS.

1.15.6. Limitations

There are limitations to the tests used to assess cognitive function, not least the fact that different tests are used in different research studies. While each battery of tests broadly assesses similar gross areas of functioning, the use of different tests and methods of application does present problems in drawing comparisons between studies. Sensitivity and specificity of tests used differs, which leads to varying results across studies (256). Despite the fact that there are merits and problems with each, there is scope for multiple studies using the same tests to strengthen comparison between groups.

There are limitations with individual tests and with their interpretation. In the digit span test forward and backward measures reflect different areas of functioning – immediate recall and working memory respectively – and these scores should be assessed separately. Some studies have, however, assessed the aggregate score of

forward and backward measures, which therefore poses a problem in terms of what this actually measures.

Mode of delivery of the tests can impact results, for example the time of day that the tests are completed, their duration and the way in which they are administered - by computer or manually. Comparison between CFS and control cohorts also creates challenges with different age groups and different education levels.

These challenges are exacerbated by the fact that any research project investigating CFS faces the difficulty of maintaining heterogeneity in and between cohorts, not only in terms of cohort demographics but also in terms of the diagnostic criteria used, as described in Chapter 1.

Area of functioning	Test	Description
Attention	Digit Span from Wechsler batteries	Asked to recall a series of numbers: forwards then backwards Forward: Immediate verbal recall involving auditory attention and short-term retention capacity Backward: Working memory
	Phasic Alertness Task	Asked to respond quickly when stimulus appears
	Trail making	Part A: draws lines to connect numbered circles consecutively Part B: connects numbered and lettered circles consecutively, alternating between the two
	Paced Auditory Serial Addition Test	Adds pairs of random digits by adding each digit to the digit immediately preceding it Considered by participants do be very stressful
	Rapid Visual Information Processing Test	Asked to detect target sequences of digits Measure of sustained attention
	Digit Symbol (Wechsler)	Symbol substitution test: part 1 copies symbol; part 2 matches symbol to a number in a key; part 3 identifies errors from another completed key
	Corsi Block-Tapping/Spatial Span	Nine cubes in random order on a board; examiner taps blocks in an order; participant asked to repeat the order; increasing length Immediate visual recall
Concentration	Continuous Performance Test II	Brief presentation of stimuli to which participant responds e.g. indicates each time a letter other than “X” appears Measure of focussed attention
	Stroop	Part 1: participant reads from list of printed word names comprised of three colours Part 2: participant names the colour of the ink from a list of XXXXs printed in one of three different coloured inks Part 3: participant names the colour of the ink from a list of three colours printed in different coloured inks e.g. the word RED printed in BLUE ink answer BLUE Timed and incorrect responses recorded

Area of functioning	Test	Description
Verbal Memory	Auditory-Verbal Learning Test (AVLT)	Asked to remember and recall a 15 word list (A) read out five consecutive times; followed by one single interference list (B); asked to recall list A post interference – one immediate, one delayed
	Hopkins Verbal Learning Test-Revised (HVLT-R)	Asked to learn and recall 12 nouns: assesses verbal short-term learning
	California Verbal Learning Test (CVLT)	Asked to recall items in any order; not told about categories, but expected to recognise this and use it to aid recall: assesses semantic associations e.g. animals, vegetables
Visual Memory	REY	Asked to reproduce a complicated drawing, first by copying then from memory
	Benton Visual Retention	Recall of three figures
	Continuous Visual Memory	Task to discriminate new from repeated stimuli
	Wechsler WMS-III Faces	Facial recognition memory of new and repeated faces
Psychomotor Coordination	Reaction Time Test	Tests of response speed
	California Computerized Assessment Package	Series of ten reaction time measures to test reaction time and speed of information processing
Processing Speed	Reaction Time Test	As above
Motor Speed	Reaction Time Test	As above
Executive Function	Trail making	As above
	Stroop	As above
	Wisconsin Card Sorting	Asked to sort cards by rules with different colour, number and formed shapes
	Attentional shift: intra/ extra dimensional shift (IED) task	Must correctly identify stimuli of white lines overlying colour-filled shapes
	Stockings of Cambridge	Shown two displays containing three coloured balls and must recreate a specified pattern Time taken to complete and accuracy recorded

Area of functioning	Test	Description
Working Memory	Digit Span backward	Working memory
	Alpha Span	Alphabetically orders increasingly long list of unrelated words: working memory
	Digit Sequencing	Listens to strings of random numbers and immediately recalls in ascending order
	Paced Auditory Serial Addition Test-Revised	Adds pairs of random digits; adds each digit to preceding digit Considered by participants do be very stressful – often feel they are doing badly when doing well
	N-Back Task	Reports when an item is the same as an item seen a specified number (n) of steps back, for example in 2-back the participant says yes after the 3 in this sequence 1-4-5-3-8-3

Table 10 Summary of cognitive tests

Article	Study type	Aim	Diagnostic criteria and Methods	Cognitive performance measures – objective	Cognitive performance measures - subjective	Cognitive performance with time	Findings - Objective	Findings - Subjective	Overall finding	Limitations
Beaumont *et al* **2012** Reduced Cardiac Vagal Modulation Impacts on Cognitive Performance in Chronic Fatigue Syndrome (230)	Case-control	Examine the relationship between HRV and cognitive performance	Fukuda Sydney: 30 CFS recruited from clinic; 40 controls recruited by advert in local paper No stimulants for 12 hours before HR: 3-lead ECG HRV calculated	Digit Symbol Test; Spatial Working Memory Task; Stroop. Sustained attention, working memory, response flexibility	Rated fatigue; physical and mental SPHERE and Kessler 10 validated for psychological symptoms and distress Brief disability Q and Pittsburgh Sleep Quality Index McGill Pain Q		Baseline characteristics comparable CFS – no less accurate but significantly slower Higher baseline HR in CFS (72 v. 67bpm p= 0.02) HR increase in both groups on starting testing but continued in CFS and longer to recover; stabilised in controls Lower HRV in CFS	More perceived fatigue and effort but no impact on performance	CFS: reduced cognitive speed; higher resting HR	Small numbers in cohort Small number of tests Self-paced
Becker *et al* **1991** Methodologic Considerations in Assessment of Cognitive Function in Chronic Fatigue Syndrome (218)	Review	Workshop to consider design of cognitive testing in CFS		Tests may have to be non-specific as symptoms often non-specific	Difficulty comparing individuals, i.e. have different strengths and pre-morbid function				Include verbal and non-verbal as well as recall, recognition and learning Should be challenging and have time pressure and consider impact of co-morbidities	

Article	Study type	Aim	Diagnostic criteria and Methods	Cognitive performance measures – objective	Cognitive performance measures - subjective	Cognitive performance with time	Findings - Objective	Findings - Subjective	Overall finding	Limitations
Capuron *et al* **2006** Cognitive Dysfunction Relates to Subjective Report of Mental Fatigue in Patients with Chronic Fatigue Syndrome (232)	Cross-section	Examine subjective mental fatigue and cognitive function	Fukuda Wichita 43 CFS 53 non-fatigued adult controls Excluded psych and co-morbidity SCID-I MDD excluded SF-36 for functional impairment	CANTAB computerised battery: Psychomotor RTT; Reasoning SOC; Memory SWM, PRM, SRM; Attention IED, RVIP	Fatigue: Mental fatigue subscale		No difference in psychomotor co-ordination or motor speed, attention or reasoning skills. CFS with mental fatigue had impaired memory		Mental fatigue correlates to impaired short-term memory	Selection bias
Claypoole *et al* **2001** Cognitive Compromise Following Exercise in Monozygotic Twins Discordant for Chronic Fatigue Syndrome: Fact or Artifact? (235)	Cross-section	Examine neuropsychological after ex response in CFS twins compared to healthy co-twins	Fukuda 22 pairs of monozygotic twins	Intellectual functioning WAIS; motor functioning RTT; speed of information processing STROOP, PASAT; verbal memory Wechsler WMS-R; visual memory R-AVLT; executive Functioning trail making			Adjusted for age, sex, education CFS lower motor functioning, speed of information processing, verbal memory functioning, and executive functioning Similar intellectual and visual memory functioning			Volunteer and reporting bias

Article	Study type	Aim	Diagnostic criteria and Methods	Cognitive performance measures – objective	Cognitive performance measures - subjective	Cognitive performance with time	Findings - Objective	Findings - Subjective	Overall finding	Limitations
Cockshell & Mathias **2010** Cognitive functioning in chronic fatigue syndrome: a meta-analysis (219)	System-atic review	Examine pattern and size of cognitive deficits in CFS	50 studies from 1998-2008			Impaired information processing speed and working memory over time			Deficits in attention, memory, reaction time No deficits in fine motor speed, reasoning, global functioning	Variation in findings due to methods
Cockshell & Mathias **2013** Cognitive Deficits in Chronic Fatigue Syndrome and Their Relationship to Psychological Status, Symptomatology , and Everyday Functioning (236)	Cross-section	Assess cognitive function in CFS and relationship to psychological status, CFS symptoms and functioning	Fukuda 50 CFS 50 controls Exclusion for other disorders affecting cognitive performance				Impaired information processing speed in CFS: not related to co-morbidity, symptoms or function Comparable attention, memory, motor function, verbal and visuo-spatial ability		Slow information processing speed in CFS	Healthy volunteer bias
Cockshell & Mathias **2014** Cognitive functioning in people with CFS: A comparison between subjective and objective measures (231)	Case-control	Examine relationship between subjective and objective assessments of memory and attention	Fukuda 50 CFS 50 controls Exclusion for other disorders affecting cognitive performance	California Verbal Learning Test II; Rey-Osterreith Complex Figure Test; PASAT; STROOP; NART	CDC CFS Symptom Inventory (memory and concentration) COGFAIL Everyday Attention Questionnaire		No significant difference in memory or attention between groups	CFS statistically significantly worse self-reported memory and attention than controls	Objective and subjective tests do not correlate	Volunteer bias – CFS from database and may not represent wider population

Article	Study type	Aim	Diagnostic criteria and Methods	Cognitive performance measures – objective	Cognitive performance measures - subjective	Cognitive performance with time	Findings - Objective	Findings - Subjective	Overall finding	Limitations
Michiels & Cluydts R **2001** Neuropsychological functioning in chronic fatigue syndrome: a review (190)	Litera-ture review	Review neurocognitive studies in CFS patients	Medline and psychinfo Memory and attention						Slow processing speed, impaired working memory, poor learning Fatigue and depression do not correlate to cognitive impairment	Unclear search strategy Articles reviewed lack heterogeneity of samples Small sample sizes and not uniform tests
Ocon *et al* **2012** Increasing orthostatic stress impairs neurocognitive functioning in chronic fatigue syndrome with postural tachycardia syndrome (238)	Case-control	To examine whether orthostatic stress is associated with neurocognitive impairment in CFS/PoTS	1994 CDC criteria 16 CFS/PoTS 20 controls	N-back task during tilt table test			No RT differences between groups CFS/PoTS subjects responded less correctly during the n-back task test Progressively worse with more tilt		Increasing orthostatic stress with a cognitive challenge impairs neurocognitive abilities of working memory, accuracy and information processing in CFS/PoTS	Small sample size
Santamarina-Perez & Eiroa-Orosa **2011** Length of Illness Does Not Predict Cognitive Dysfunction in Chronic Fatigue Syndrome (237)	Cross-section	To examine the evolution of cognitive impairment in CFS	Fukuda 56 CFS Excluded for psychiatric conditions of organic causes of cognitive impairment	WAIS-III; PASAT; California Computerized Assessment Package; Rey; STROOP; Trail-making, verbal fluency		Different illness durations evaluated (<12 months; 12-44 months; >48 months)	>50% had impaired processing speed Length of illness did not correlate		Length of illness did not correlate to cognitive impairment	No longitudinal data Not same participants over time

Table 11 Summary of articles reviewed in cognitive literature review

Chapter 2. Methodology

I was appointed as Clinical Research Associate (CRA) with Medical Research Council (MRC) funds obtained by my supervisor Professor Julia Newton to conduct a study investigating the pathogenesis of AD in CFS. I was involved in its setup and delivery. The data that I present here are from the MRC study.

Participants were recruited as part of the larger MRC-funded project *Understanding the pathogenesis of autonomic dysfunction in chronic fatigue syndrome and its relationship with cognitive impairment*. MRC grant number: MR/J002712/1; Trust R&D number: 6132.

This thesis looks at a subset of data from the MRC project to examine the prevalence of AD and cognitive impairment in CFS and to better understand whether prevalence differs depending on which diagnostic criteria are used. It also examines the prevalence and nature of depressive symptoms in this cohort of CFS patients.

This chapter will introduce the MRC study and will describe the aspects of the methodology of the MRC project that are relevant to this thesis.

2.1. Introduction to the MRC study

The MRC project was designed to better understand the role that AD has to play in the pathophysiology of CFS and was a case-control study intended to address two areas:

1. The pathogenesis of AD in CFS

To determine whether AD in CFS is

- a primary abnormality in brain autonomic centres;
- due to abnormalities in the HPA axis;
- a problem of downstream ANS control;
- secondary to hypovolaemia which has resulted in compensatory autonomic abnormalities.

2. The relationship between AD and cognitive impairment in CFS

To explore whether AD and cognitive impairment in CFS are caused by similar underlying central processes, causing damage to both autonomic and cognitive brain centres, or whether cognitive impairment is secondary to peripheral hypotension arising from AD and resulting in reduced cerebral perfusion and cerebral damage.

In order to investigate the above, participants were required to undergo a series of investigations for the MRC project. These were as follows:

- autonomic assessment with the Task Force® Monitor for continuous beat-to-beat blood pressure and heart rate measurement comprising of a ten minute rest, two minute active stand and Valsalva manoeuvre;
- cognitive assessment using a battery of established neurocognitive tests lasting approximately 90 minutes;
- HPA axis testing including blood tests for cytokines and a dexamethasone suppression test;
- cardiac, liver and brain Magnetic Resonance Imaging (MRI);
- plasma volume and red cell mass;
- cardiac meta-iodobenzylguanidine (mIBG).

The approximate timeline of these investigations is outlined in figure 4 below. The aim was to carry out all investigations within an eight-week period. The interval between each investigation was predominately determined by participant choice and in part by resource availability.

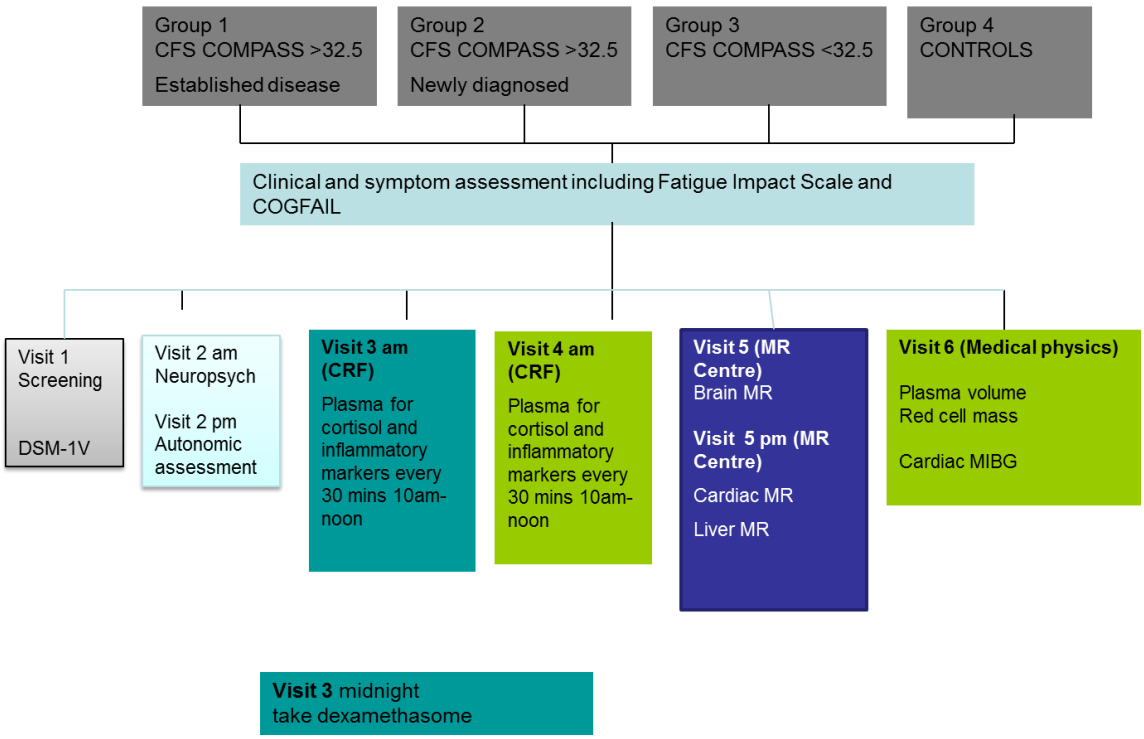

Figure 4 Schematic depicting timeline of investigations for the MRC project

Recruitment commenced in October 2012 and ended in April 2014. Assessments were conducted at the Clinical Research Facility (CRF), Royal Victoria Infirmary, Newcastle upon Tyne; the Magnetic Resonance Centre at the Campus for Ageing and Vitality, Newcastle University, and the Nuclear Medicine Department at the Freeman Hospital, Newcastle upon Tyne.

The MRC project was delivered by a team of clinicians and non-clinicians, whose involvement is depicted in figure 5 below.

The project was designed by Professor Julia Newton, Professor Andrew Blamire, Dr Stuart Watson and Dr Peter Gallagher. The Research Associate was involved in the protocol development, analysis and interpretation of the MRI scans. The CRA had responsibility for the day-to-day management of the project, including recruitment, screening and autonomic and cognitive assessment.

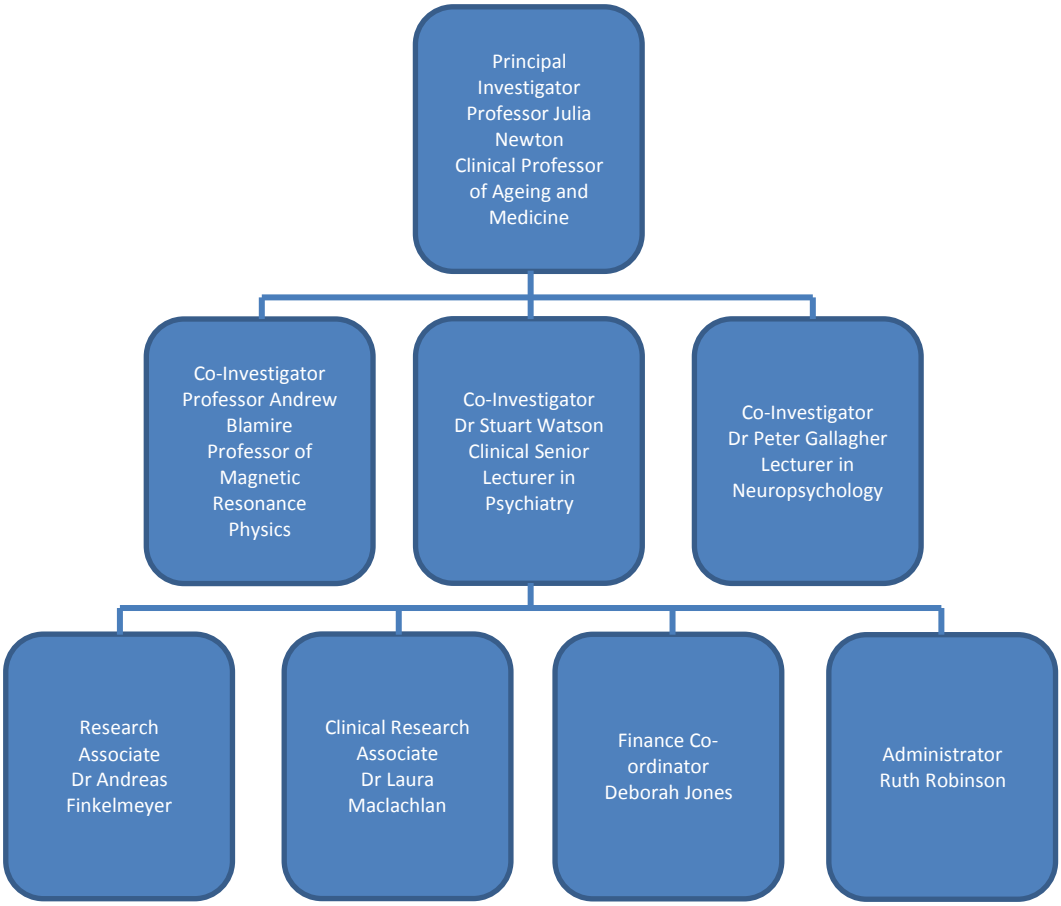

Figure 5 Schematic depicting the MRC project team and their roles

2.2. Ethics

The MRC study was conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions (257).

Favourable ethical opinion was obtained from the NRES Committee North East - Newcastle & North Tyneside 2 prior to commencement of the study (see Appendix D). Local research and development approval was obtained from Newcastle upon Tyne Hospitals NHS Foundation Trust's Research and Development office.

2.3. Confidentiality

Personal data were regarded as strictly confidential. All data leaving the site identified participants by a unique study identification number only. The study complied with the Data Protection Act 1998 (258) and Caldicott principles (259). The study records

and Investigator Site Files were kept on site in a locked filing cabinet with restricted access.

2.4. Participants

Participants were patients with CFS and age-matched sedentary, community controls.

2.5. Recruitment

Potential participants with CFS were initially identified through routine clinic outpatient appointments in the Newcastle upon Tyne specialist CFS clinics and regional specialist CFS services. All CFS subjects met the Fukuda criteria for CFS (see table 2 for diagnostic criteria).

Eligible participants were invited to take part by the consultant and the study was explained to them. A study participant information sheet (PIS) was provided at this time.

Other CFS participants contacted the team after learning about the study via support groups and the Clinical Research Network (CRN) website.

Controls were recruited by established methods including via University-held volunteer databases, local advertisements and by "word of mouth", for example via friends, colleagues and family members of CFS participants.

The contact details of the research team were made available to potential controls to allow them to contact the team. A controls version of the PIS was then provided.

Following receipt of information about the study, participants were given a minimum of 24 hours to decide whether or not they would like to participate. Those interested in taking part in the study returned a form expressing their interest and were subsequently contacted by telephone by a member of the study team to discuss the project and undergo an initial telephone screening. Telephone screening was conducted to avoid the need for potentially unwell CFS subjects to attend in person, resulting in unnecessary exertion, if there was a clear exclusion criterion. If no obvious

exclusion criteria were identified, potential participants invited to a more detailed screening visit.

A screening log was kept to document details of subjects were invited to participate in the study. For subjects who declined to participate or who were not eligible to take part reasons for non-participation were documented where available.

2.6. Consent procedures

Informed consent was taken by the CRA, trained in Good Clinical Practice, as per the consent standard operating procedure (SOP) (Appendix B). Participants had an opportunity to ask any questions. Those wishing to take part provided written informed consent by signing and dating the study consent form, which was witnessed and dated by a member of the research team with documented, delegated responsibility to do so. Written informed consent was obtained prior to study specific investigations.

The original signed consent form was retained in the investigator site file, with a copy in the clinical notes and a copy provided to the participant. The participant specifically consented to their GP being informed of their participation in the study.

2.7. Withdrawal

Participants had the right to withdraw from the study at any time for any reason, and without giving a reason. The investigator was also able to withdraw participants from the study if this was felt to be in the in the participant's best interests.

2.8. Inclusion criteria

- Participant able to provide written informed consent for participation in the study prior to any study specific procedures;
- Aged 18 years or older;
- Not pregnant;
- Available for the duration of the study;
- Willing and able to comply with the procedures required as described in the information leaflet and as directed by the study doctor.

Controls were required to live a sedentary lifestyle. There is no agreed definition of sedentary in terms of activity, however it has been described as a group of behaviours that occur whilst sitting or lying down while awake typically requiring very low energy expenditure (260). The National Health Service (NHS) describes it as activities such as sitting or lying down for long periods (261).

Control subjects were eligible for inclusion in the study if they participated in moderate exercise for fewer than 30 minutes three times a week.

2.9. Exclusion criteria

- Previous participation in this study;
- Co-morbid depression as assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) for research.
  Participants were excluded if, on assessment, they had any current or past diagnosis according to SCID-I with the following exceptions:
    - Simple bereavement
    - Pain Disorder
    - Anxiety Disorders
    - Substance Abuse if not in the last six months and for substance dependence if not in the last year;
- Currently being treated for hypertension.

2.10. Medications

Cardioactive medications were stopped 72 hours prior to autonomic and cognitive testing. These included, but were not limited to:

- Amitriptyline;
- Antidepressants;
- Antihistamines;
- LDN (low dose naltrexone);
- Opioid analgesics;
- Fludrocortisone for PoTS;
- Bendroflumethiazide for idiopathic oedema;
- Midodrine for management of PoTS was stopped 12 hours prior to testing.

2.11. Data Handling & Record Keeping

The original autonomic data were summarised in the case report form. Cognitive results were written into the cognitive assessment booklet with the exception of the Attentional Network Task, which was kept on the laptop used for cognitive testing.

Results were databased using Excel. The database was set up by the CRA and the data were entered by the MRC project administrator. Ten percent of the data were checked by the CRA for accuracy.

This thesis focusses on the following data from the MRC study:

- phenotype data comprising clinical, symptom and demographic assessment;
- autonomic assessment;
- cognitive assessment;
- depression symptoms.

The methods of collection for these data are detailed below.

2.12. Characteristics of the study cohort

To better define the phenotype of the study population a number of parameters were examined using validated tools, as discussed below. Questionnaires (detailed below and included in Appendix A) were given to participants after screening and inclusion, completed independently and returned at the time of the initial visit to the CRF.

2.12.1. Demographics

Details regarding medication use, past and current medical history, length of history, mobility, education and employment status were gathered by the CRA at the time of screening.

2.12.2. Fatigue severity

Fatigue status was determined using the generic fatigue measure the Fatigue Impact Scale (FIS). This tool helps to quantify individual perception of the impact that fatigue has on daily functioning (262). There are 40 items, each scored on a 5-point Likert scale

(no problem=0, small problem=1, moderate problem=2, big problem=3, extreme problem=4) with an option to indicate not applicable, thus providing a continuous scale of 0-160. It comprises three subscales looking at the impact that fatigue has on physical (10 items: motivation, effort, stamina and coordination), psychosocial (20 items: isolation, emotions, coping and workload) and cognitive (10 items: concentration, memory, thinking and though organisation) functioning (263).

2.12.3. Autonomic symptoms

Subjects completed the validated autonomic symptom assessment tool Composite Autonomic Symptom Scale (COMPASS). The COMPASS forms a comprehensive and highly sensitive assessment of the prevalence, degree, and association between symptoms of AD (115).

It consists of 73 questions grouped into eight domains corresponding to different aspects of the ANS. These domains are OI, vasomotor, secretomotor, gastrointestinal, bladder, pupil responses, sleep disorder, and syncope. The domains are weighted according to their clinical relevance and individual scores are then totalled, which provides an indicator of overall symptom burden. The highest possible score is 179 and the higher the score, the greater the symptom load.

The COMPASS 31 is an abbreviated version with 31 questions addressing six domains. These domains are OI, vasomotor, secretomotor, gastrointestinal, bladder and pupillomotor. It provides a more refined measure of autonomic symptoms and can be calculated from the full COMPASS questionnaire. Both are included in this analysis.

2.12.4. Cognitive function

Cognitive symptoms were quantified by COGFAIL (220), which is a measure of self-reported failures in perception, memory and motor function. The questionnaire consists of 25 questions measuring the frequency of cognitive failures in the previous six months. The questions are answered on a 5-point Likert scale from (0=never, 1=very rarely, 2=occasionally, 3=quite often, 4=very often) giving a possible total score of 100. The higher the score, the greater the cognitive impairment.

2.12.5. DePaul Symptom Questionnaire

The DSQ (47) is a self-reported measure of CFS symptoms, demographics, and medical, occupational, and social history. From these measures, it is possible to give a "diagnosis" of CFS based on the Fukuda criteria (22), the 2003 Carruthers (Canadian) criteria (39) or the 2011 Carruthers (Canadian) consensus criteria (40) (see table 2 and section 1.4.1).

The development of the DSQ was based upon the CFS Questionnaire (264), which has been shown to have good inter-rater and test-retest reliability and to differentiate between individuals with CFS, MDD, and healthy controls (265, 266). The DSQ has been demonstrated as a valid tool for assessing CFS symptoms and discriminating between diagnostic criteria (49).

Participants rate each symptom's frequency over the past six months on a 5-point Likert scale (0=none of the time, 1=a little of the time, 2=about half the time, 3=most of the time, and 4=all of the time), as well as each symptom's severity over the past six months (0=symptom not present, 1=mild, 2=moderate, 3=severe, 4=very severe).

2.12.6. International Physical Activity Questionnaire

There is currently no standardised way to measure self-reported activity levels. The International Physical Activity Questionnaire (IPAQ) was established in 1998 to provide a measure of physical activity and has been shown to be reliable.

The short version of the IPAQ questionnaire was used. It is an instrument used to assess activity levels in adults aged 15-69. This questionnaire asks about three types of activity: walking, moderate-intensity activities and vigorous-intensity activities, undertaken in four domains: leisure time physical activity, domestic and gardening (yard) activities, work-related physical activity and transport-related physical activity. It applies to the preceding seven days and as such is not necessarily a good indicator of longer-term lifestyle.

The duration in minutes and frequency in days of the three types of activity is calculated and scored. The score is a measure of energy required defined in metabolic equivalents (METs). METs are multiples of resting metabolic rate.
Scores are then equated to levels of activity, described as low, moderate or high. This is depicted in table 12.

Low	• No activity is reported, or • Some activity is reported but not enough to meet moderate or high
Moderate	Any of the following three criteria • ≥ three days of vigorous activity of at least 20 minutes per day, or • ≥ five days of moderate-intensity activity and/or walking of at least 30 minutes • ≥ five days of any combination of walking, moderate-intensity, or vigorous-intensity activities achieving a minimum of at least 600 MET-minutes/week
High	Any one of the following two criteria per day, or • vigorous-intensity activity on at least three days and accumulating at least 1500 MET-minutes/week, or • ≥ seven days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 MET-minutes/week

Table 12 IPAQ activity level

2.12.7. Reproducibility and reliability of subjective questionnaires

The questionnaires and assessment tools used in this study have been tested for consistency and reproducibility. The Cronbach alpha coefficient provides a measure of reliability and internal consistency (267). The coefficient is expressed as a number between 0 and 1, where a value above 0.70 indicates acceptable internal consistency. The Spearman's correlation coefficient is used for non-parametric data and shows the strength of relationship between data. It derives a value between 0 and 1 where 0 shows a very weak association and 1 a very strong one.

The fatigue impact scale has been shown to be a valid and reliable measure of fatigue in a number of chronic conditions, including MS, with a very high Cronbach's alpha coefficient of 0.88-0.98 (267, 268). Both the COMPASS and COMPASS-31 have been shown to have a Cronbach's alpha of >0.7 indicating good internal validity (269, 270). The COGFAIL questionnaire has been shown to have excellent internal reliability with a Cronbach's alpha of 0.92 (271). The IPAQ has been shown to be reliable and have good

test re-test reliability with a Spearman coefficient clustered around 0.8 indicating strong to very strong reproducibility (272).

2.13. Depression

All potential participants were screened prior to inclusion in the study. Part of the screening process involved assessment for psychiatric co-morbidities using the SCID-I (273).

2.13.1. Structured Clinical Interview for DSM-IV Axis I Disorders

The SCID-I for research is a standardised tool used to screen for mood episodes and disorders, psychotic symptoms and disorders, anxiety and substance use disorders as per DSM-IV (274). This is a validated tool often used as the "gold standard" as a screening tool (275) and is considered to be a reliable reflection of DSM-IV diagnoses (276). Conducting the SCID-I in its entirety takes approximately 45-90 minutes.

The following sections were not included during the screening interview:

- A38 Dysthymic Disorder;
- G1-5 Somatoform Disorders.

This is due to shared features with CFS symptoms, with the potential to inaccurately exclude potential participants.

A diagnosis of past or current depressive episode requires criteria to be met for five specified symptoms, which must include depressed mood or loss of interest, be present for a two-week period and be associated with functional impairment. The structure of the SCID-I determines that subjects who do not meet criteria for depressed mood or lack of interest are not asked about other depressive symptoms.

A database of results from the SCID-I was kept and provided the starting point to better understand the overlap between symptoms of depression and CFS. All SCID-I assessments, with the exception of one, were completed by the CRA.

2.14. Autonomic nervous system

Participants underwent autonomic testing using the Task Force® Monitor. This was conducted at the CRF by the CRA. Prior to commencement of the study the Task Force® Monitor was calibrated by the Newcastle-upon-Tyne Hospital Medical Physics Department.

2.14.1. Autonomic Testing

A ten minute rest, two minute active stand and Valsalva manoeuvre were performed. The procedures for performing these tests are outlined below and their SOPs can be viewed in Appendix B.

The Task Force® Monitor programme version 2.2 was used to record and analyse ECG and blood pressure (BP) recordings. An individual report is provided for each participant, detailing mean heart rate (HR bpm), mean systolic BP (sBP, mmHg), mean diastolic BP (dBP, mmHg), mean low frequency normalised units (LFnu, %), mean very low frequency (VLF, ms^2), mean LF (ms^2), mean HF (ms^2), mean power spectral density [PSD, ms^2), LFnu:HFnu, LF:HF, baroreceptor slope mean (a measure of baroreceptor sensitivity, ms/mmHg) and baroreflex effectiveness index (BEI, %). These are explained in tables 13 and 14.

2.14.2. Task Force® Monitor Set up

The CRA, trained in the use of the Task Force® Monitor, performed all autonomic assessments. A pack of eight electrodes (CNSystems Medizintechnik GmbH, accessory 01616) were attached to each individual. These consisted of four ECG electrodes; one on the anterior surface of the left shoulder, one on the anterior surface of the right shoulder, one over the lower left thorax in the anterior axillary line and one over the lower right thorax in the anterior axillary line, providing a four channel ECG, and three impedance electrodes; one on the posterior neck, one superior to the ECG electrode over the lower left thorax crossing the anterior axillary line and the mid-clavicular line and one superior to the ECG electrode over the lower right thorax crossing the anterior axillary line and the mid-clavicular line. One electrode was attached to the left lateral ankle. A pictorial representation is shown in the autonomic assessment SOP in Appendix B.

2.14.3. Blood pressure

For continuous beat-to-beat BP recording the appropriately-sized “Flying-V” finger cuff and oscillometric BP cuff were selected. The finger cuff was then connected to the Task Force® Vascular Unloading Monitor and placed proximally over two fingers on the left hand. The Task Force® Vascular Unloading Monitor was attached to the forearm using a Velcro fixing cuff. The appropriately-sized oscillometric BP cuff was placed on the opposite upper arm.

2.14.4. ECG

ECG recordings were based on the bipolar principles of Einthoven. Oscillometric and continuous beat-to-beat BP recording has an accuracy of ± 5 mmHg between 50 and 250 mmHg (277).

2.14.5. Impedance cardiography

Impedance cardiography provides measures of AD by examining haemodynamic activity. It provides parameters of continuous beat-to-beat stroke volume and maximum ejection speed (278). These are outlined in table 13.

Parameter	Measure	Normal range
Acceleration index (ACI)	Measure of myocardial contractility: maximum acceleration of the impedance signal (2nd deviation) between opening of aortic valve and dZ/dtmax	1-400 /100s^2
Baroreflex effectiveness index (BEI)	A measure of baroreflex response to changes in blood pressure	%
Baroreflex sensitivity (BRS)	Measure of baroreflex control on the heart	ms/mmHg
Cardiac index (CI)	Cardiac output normalised for body surface area	2.5 - 4.0 l/min/m2
Cardiac output (CO)	Volume of blood pumped from left ventricle over one minute	4.0 - 8.0 l/min
Ejection fraction	Proportion of blood ejected during systole	55-70 %
End diastolic index (EDI)	Ratio of stroke index to ejection fraction	60-110 ml/mm^2 at rest
Index of contractility (IC)	Maximum blood flow during left ventricular ejection	1000/s
Left ventricular ejection time (LVET)	Time interval from opening to closing of the aortic valve	0-1500 msec
Left ventricular work index (LVWI)	Work of left ventricle for each heartbeat	0-200 gm-m/m2
Stroke index (SI)	Stroke volume normalised for body surface area	33 - 47 ml/m2/beat
Stroke volume (SV)	Volume of blood ejected from left ventricular contraction	60 - 100 ml/beat
Thoracic fluid content (TFC)	Total fluid content of the thorax	10-150 1/kOhm
Total peripheral resistance (TPR)	Total resistance of all peripheral vessels	800-1200 dyn*sec/cm^5
Total peripheral resistance index (TPRI)	Total peripheral resistance normalised for body surface area	1970-2390 dyn*sec/cm^5

Table 13 Parameters to examine autonomic dysfunction

Taken from (86, 278-283)

2.14.6. Heart rate variability

Heart rate variability – beat-to-beat variations in heart rate measured at the RRI – is a reliable, objective marker of autonomic activity and the balance between sympathetic and parasympathetic activation (279, 284). ECGs of healthy individuals at rest show regular variations in RRI described as respiratory sinus arrhythmia where the RRI is shortened during inspiration and prolonged during expiration (86).

Studies have shown that spectral analysis of HRV can provide a quantitative measure of sympathovagal function in a number of conditions including OH (285), post myocardial infarction prognosis (286) and diabetic neuropathy (287). Figure 6 shows a spectral analysis of HRV.

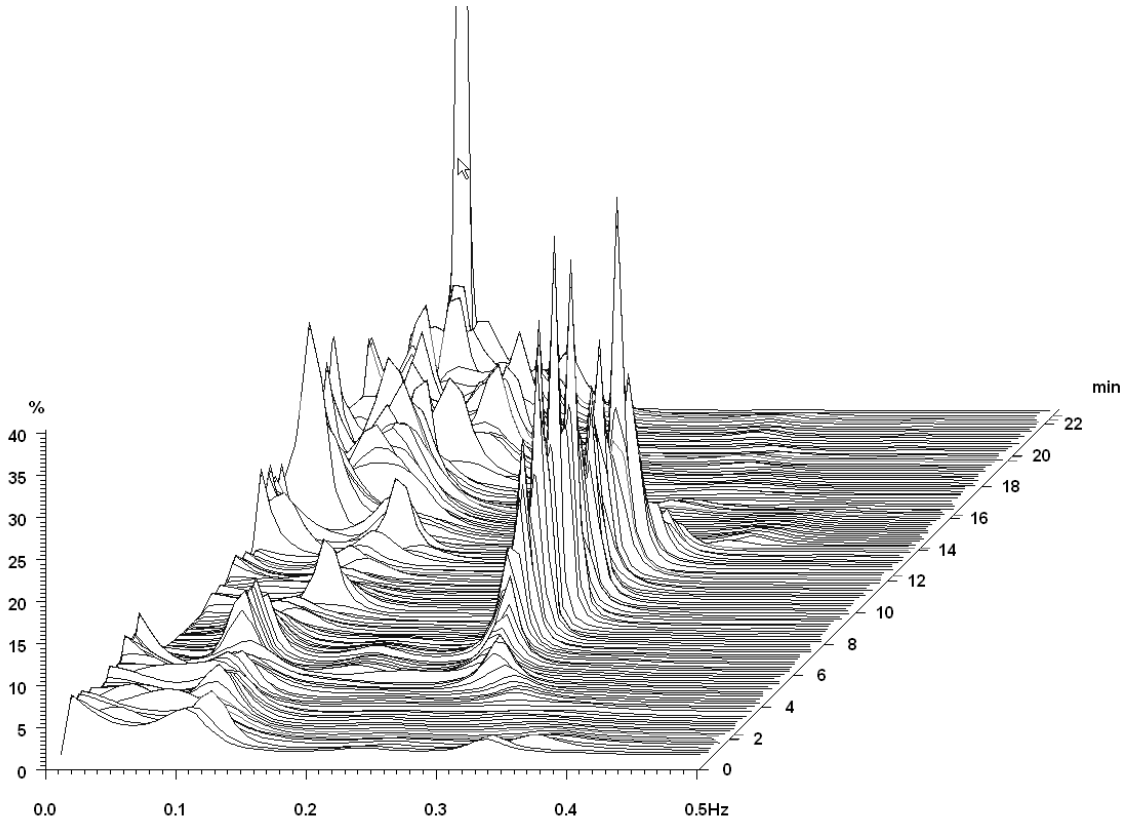

Figure 6 Spectral analysis of heart rate variability

Taken from (288)

In figure 6, ten minute supine rest is taken between four and 14 minutes (z axis). Parasympathetic drive is seen as the large peaks (y axis) between 0.3 and 0.4 Hz (x axis). At 15 minutes upright stand is performed. The change in peaks from high to low frequency represents reduced parasympathetic drive and increased sympathetic stimulation.

The Task Force® Monitor programme version 2.2 is an effective tool to assess HRV over periods of more than five minutes, producing a measure of how variance distributes as a function of frequency using the Fast Fourier Transformation (279).

Power spectral density (PSD) is divided into HR fluctuations and subsequently into LF, VLF and HF. The Task Force® Monitor programme also provides normalised units of the LF and HF spectral components. These are outlined in figure 6 and table 14. The LF/HF ratio is used as a measure of sympathovagal balance where increases in LF/HF ratio are thought to reflect sympathetic dominance. Accuracy of calculating the LF/HF ratio in this way has, however, been criticised, as it relies on several assumptions about the changes in parasympathetic and sympathetic activity, which may not be present (289). It should therefore be interpreted in this context.

Parameter		Measure
High frequency (ms^2)	HF	Reflection of parasympathetic modulation
High frequency normalised units (%)	HFnu	HF/(LF+HF) Index of parasympathetic modulation
	HFnu-dBP	Parasympathetic modulation of dBP
	HFnu-RRI	Parasympathetic modulation of cardiovascular activity
	HFnu-sBP	Parasympathetic modulation of sBP
Low frequency (ms^2)	LF	Reflection of sympathetic modulation
Ratio of low to high frequency	LF/HF	Sympathovagal balance index Increases reflect a shift to sympathetic dominance
	LF/HF-dBP	Sympathovagal balance index dBP
	LF/HF-RRI	Sympathovagal balance index of cardiovascular function
	LF/HF-sBP	Sympathovagal balance index sBP
Low frequency normalised units (%)	LFnu	LF/(LF+HF) Index of sympathetic modulation
	LFnu-dBP	Sympathetic modulation of dBP
	LFnu-RRI	Sympathetic modulation of cardiovascular activity
	LFnu-sBP	Sympathetic modulation of sBP
Normalised units	Nu	A measure of sympathetic and parasympathetic balance: proportion each domain contributes towards total PSD minus VLF contribution
Power spectral density (ms^2)	PSD	Measure of power distribution across frequencies
Slope mean (ms/mmHg)		Baroreceptor sensitivity

Table 14 Task Force® Monitor measures

Sources (127, 290, 291)

2.14.7. Active standing

A dynamic measure of sympathetic and parasympathetic function can be achieved on orthostatic challenge, where the drop in sBP or dBP on active stand is measured. An abnormal response is defined as a drop of ≥ 20 mmHg in sBP or ≥ 10 mmHg in dBP (292).

One of the limitations of this definition is the fact that it does not recognise the significance of smaller, prolonged, symptomatic blood pressure drops and long recovery times. To address this, the area under the curve (AUC) can be calculated as a measure of the "whole picture", using software developed by the medical physics department of Newcastle upon Tyne Hospitals NHS Trust. This is depicted in figure 7.

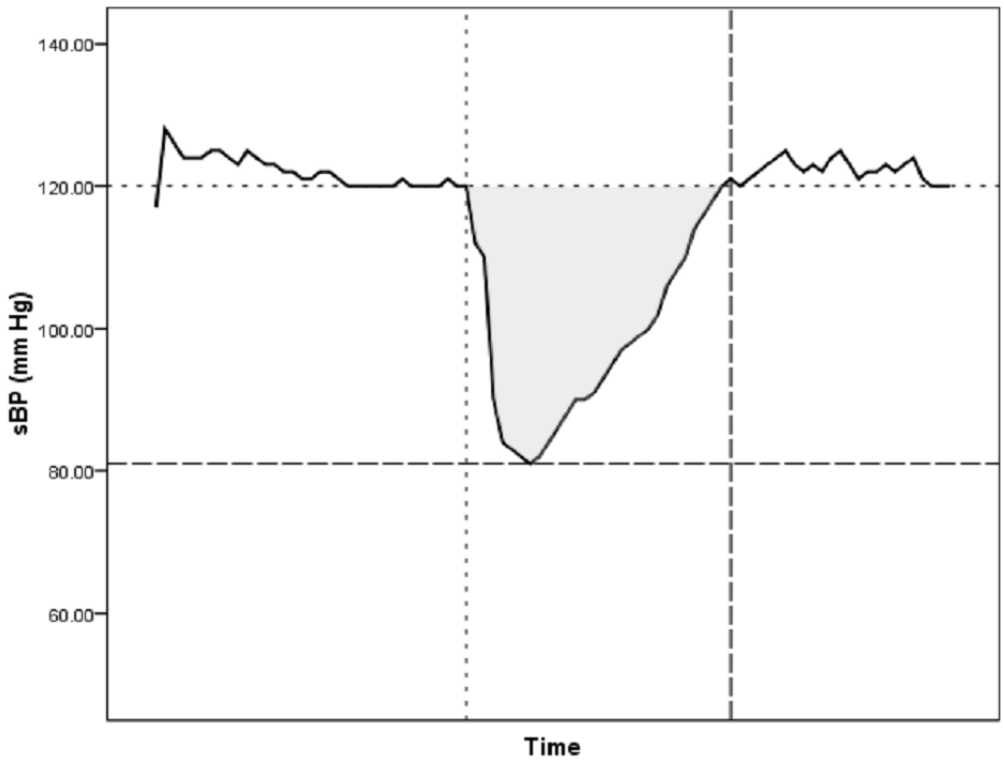

Figure 7 AUC of sBP upon standing

Taken from (288)

Figure 7 shows the AUC of sBP on orthostatic challenge. Baseline sBP (at the end of the supine rest period) is shown as the dotted horizontal line. Active stand is signified by the dotted vertical line. The solid line represents actual sBP - it drops on active stand

and its nadir is seen as the horizontal dashed line. Its return to baseline is seen as the vertical dashed line. The AUC is the shaded area.

2.14.8. Baroreflex Sensitivity

Changes in peripheral vascular tone and the force and rate of heart contraction in response to changes in baroreceptor activation are known as the baroreflex. Baroreflex sensitivity (BRS) has been found to be reduced in subjects with vasovagal syncope (293).

BRS can be calculated by the Task Force® Monitor programme version 2.2 by identifying sBP changes occurring in association with either prolonged or shortened RRI over three or more beats. Gradients of these associations are then calculated to give a mean value for the ten minute supine rest. This is the slope mean.

The baroreflex effectiveness index (BEI) can also be calculated. This provides a measure of baroreflex response to changes in blood pressure by calculating the ratio of spontaneous changes in sBP to corresponding changes in RRI (294).

2.14.9. Valsalva manoeuvre

The Valsalva manoeuvre is a measure of parasympathetic activity. It is calculated as RR min/RR rebound or HR Phase IV/HR Phase II. A normal response results in increased HR in phase II, as BP drops, and transient bradycardia secondary to baroreflex response in phase IV (295) (see table 4, section 1.10.3.3). A normal Valsalva ratio is >1.21 (normal range has also been stated as 1.29-1.46 depending on age and gender). A ratio of <1.21 indicates dysautonomia (296, 297).

2.14.10. Standard operating procedure

The SOP for autonomic assessment is included in Appendix B. Participants attended the CRF at the Royal Victoria Infirmary in Newcastle upon Tyne. They were tested between 9am and 10am. A light breakfast was permitted. Participants were asked to refrain for the following:

- caffeine the morning prior to testing;
- alcohol the morning prior to testing;

- nicotine for two hours prior to testing.

2.14.11. Assessment during ten minute supine rest

The initial part of the assessment involved a ten minute rest to measure parameters while supine. This was conducted as follows:

- electrodes were attached as described in section 2.14.2;
- the participant was asked to lie supine on a clinic bed and made as comfortable as possible;
- the participant rested quietly, remaining awake;
- recording was started when resting HR and BP were seen on the Task Force® Monitor screen;
- the start point of the recording indicated the beginning of the ten minute rest.

2.14.12. Assessment in response to an active stand

At the end of the ten minute rest participants underwent a two minute active stand as follows:

- two assistants were required;
- participants were asked to bend at the waist to come to a sitting position with legs in front of them. This was achieved with the help of two assistants who used a back strap to lever the participant;
- participants were asked to swing their legs off the bed;
- participants were asked to stand immediately and remain standing for a duration of two minutes;
- the beginning of the active stand was marked by “Active stand start” on the monitor and the end of the stand by “Active stand end”;
- after completion of the active stand participants were asked to sit down on the bed;
- participants were rested for two minutes to return to their cardiovascular baseline before performing the Valsalva manoeuvre.

2.14.13. Assessment in response to the Valsalva manoeuvre

Prior to commencing the ten minute rest, each participant was given the opportunity to practice the technique used to perform the Valsalva manoeuvre in order to maximize good technique. This was limited to two or three seconds so participants did not tire.

The Valsalva manoeuvre was performed as follows:

- participants sat on the clinic bed;
- a 10ml syringe was attached to a baumanometer;
- participants were asked to take a deep breath in and blow into the 10ml syringe performing the Valsalva manoeuvre (exhaling against a closed glottis) to achieve a reading of 40mmHg on the baumanometer;
- this was sustained for 15 seconds if possible;
- the beginning and end of the manoeuvre was recorded by "Valsalva 1 start" and "Valsalva 1 end" respectively;
- mmHg and time in seconds achieved were recorded if they differed from the target.

2.14.14. Recovery

Participants were asked to sit for two minutes to allow for recovery to baseline autonomic measures.

2.15. Cognitive function

A battery of neuropsychological tests were administered by the CRA at the CRF to assess cognitive function.

2.15.1. Rationale for choice of test

The battery of tests used focus on memory and concentration, as current literature indicates that these domains are most affected in CFS patients (219). Reviews also indicate that in order to optimise cognitive testing in CFS patients verbal and non-verbal, as well as recall, recognition and learning skills should be assessed. These tests should be challenging and carried out under time pressure (218). The tests used and their order of administration is shown in table 15.

The battery of neurocognitive tests was designed to assess a number of broad cognitive domains, as outlined below. The National Adult Reading Test (NART) was used before cognitive testing began, as a measure of pre-morbid Intelligence Quotient (IQ).

Order of administration	**Test**	**Domain**
1	National Adult Reading Test (NART)	Screening for pre-morbid IQ
2	Rey Auditory-verbal learning test (AVLT)	Verbal learning and memory
3	STROOP Colour Word Test	Executive function and attention
4	Digit Symbol Substitution Test (DSST)	Psychomotor speed
5	Digit Span	Working memory and short-term memory
6	Trail Making	Executive function and attention
7	Spatial Span	Working memory and short-term dynamic visuospatial memory
8	Visual Patterns Test (VPT)	Short-term fixed visuospatial memory
9	FAS Verbal Fluency	Executive function and attention
10	Attention Network Task (ANT)	Executive function and attention

Table 15 Tests performed depicted by order of administration

2.15.2. Standard Operating Procedure

The SOP for cognitive testing and the participant booklet are included in Appendices B and C.

Cognitive testing was conducted immediately following autonomic assessment. It was commenced between 9.30am and 10.30am and lasted for approximately 90 minutes. Subjects refrained from caffeine and alcohol on the morning of the tests and from

nicotine for at least two hours before testing. Cardioactive medication was stopped for 72 hours prior to testing as per the SOP for medication. The cognitive battery comprised a mixture of computerised, verbal and written tests, which were administered as per standardised instructions (see participant booklet Appendix C).

2.15.3. Tests administered

2.15.3.1. National Adult Reading Test

The NART was used as a screening tool to measure pre-morbid IQ as an estimate of level of intellectual functioning before onset of illness. It is the most widely-used tool to estimate pre-morbid IQ and has been validated for this use (298). A list of 50 phonetically irregular words is given to the subject who is asked to read them aloud. The number of errors is converted to give a verbal IQ score.

2.15.3.2. Rey Adult Verbal Learning Test

The Rey AVLT looks at immediate memory span, learning curve and short-term and long-term retention. It can also be used to assess retroactive and proactive interference tendencies, as well as tendencies to confusion and confabulation on memory tasks (207, 299). The Rey AVLT has been shown to be sensitive for verbal memory, outwith the associative context achieved with prose (294).

To assess immediate recall the examiner reads 15 words (list A) at a rate of one per second. The subject is asked to repeat back as many as they can remember, in any order. This procedure is conducted a total of five times (trials I-V).

The examiner immediately reads another list of 15 words (list B) once and the subject is asked to repeat back as many as possible. The subject is then asked to recall as many words as possible from list A without further hearing the list (trial VI). After approximately 30 minutes delay the subject is asked to recall as many words as possible from list A (trial VII).

Finally, the subject is given a list of 50 words which contains all the items on lists A and B as well as semantically associated or phonetically similar words. They are asked to identify whether the words were on list A or B or not on either list.

This thesis examines total score, calculated by adding the first five recalls (trials I-V), to assess immediate verbal declarative memory, and percentage retained from trial VI on trial VII as a measure of delayed recall – the ability to recall information after interference.

2.15.3.3. STROOP Colour Word Test

The Golden version of the STROOP test was performed in three parts. Parts 1 and 2 were used to look at cognitive processing and motor speed. Part 3 was also for focussed attention and ability to inhibit autonomic response tendencies.

In part 1 the subject is given a list of 100 words (the colours *red*, *green* or *blue*) divided into five columns and printed in black ink. They are asked to read out as many as possible, as accurately as possible, in 45 seconds. If they reach the end of the 100 words before the end of 45 seconds they start again at the beginning of the list (as is the case in parts 2 and 3).

In part 2 the subject is given a list comprising 100 sets of four crosses printed in different coloured ink (red, green, blue). They are asked to read out as many as possible, as accurately as possible, in 45 seconds.

In part 3 the subject is given a list of 100 words (*red*, *green*, *blue*) printed in a colour ink (red, green, blue) that does not match the word. They are asked to read out as many as possible, as accurately as possible, in 45 seconds.

For each part, the number of words read out correctly and the number of errors made is recorded. This thesis examines "colour word" minus average of word reading plus colour naming. This provides a measure of interference. A negative score indicates a poorer ability to suppress word reading (300).

2.15.3.4. Digit Symbol Substitution Test

The DSST examines psychomotor speed, set shifting and selective sustained attention. It forms part of the Wechsler Adult Intelligence Scale Revised (WAIS-R) (301).

Three parts were used in this study. In part 1 the subject is presented with four rows of 25 squares. Each square contains a symbol. The subject is asked to copy the symbol exactly into a square immediately underneath. Seven samples are used as an untimed practice. Subjects are asked to work as “quickly and accurately” as possible. They are timed for 90 seconds. The number correct or the time taken to complete all 93 symbols is recorded.

In part 2 the subject is presented with a key where the symbol is matched to a corresponding number. They are asked to put the number that corresponds to the symbol into the bottom box as “quickly and accurately as possible” in 90 seconds. The number correct or the time taken to complete all 93 symbols is recorded.

In part 3 the subject is presented with a new key (symbols and numbers) and a sheet where the symbol has already been matched to a number. They are asked to identify errors, working from left to right, as “quickly and accurately as possible”. The number correct or the time taken to complete all 93 symbols is recorded. The results are largely unaffected by education (207).

This thesis examines symbols per second from part 2, which provides a measure of psychomotor speed and attention.

2.15.3.5. Digit Span

Digit span looks at retention (forward version) and effort using working memory, short term memory and attention (backward version) (207).

In the first part of the test the examiner reads out a series of numbers at a rate of one per second. The first sequence contains three numbers and after two sequences for each number of digits (i.e. two sequences of three digits) an additional digit is added up to a total of nine. When a sequence is repeated correctly the examiner moves on to the next number sequence. This continues until the participant fails to recall a pair of sequences with the same number of digits or until they reach the end of the task.

In the second part the examiner reads out a series of numbers and asks the participant to recall them in reverse order. The first sequence contains two digits and continues to eight digits.

A total score summing the number of sequences recalled correctly (maximum 14) and a clinical measure (the highest number of digits recalled in one sequence) are recorded.

This thesis examines the clinical measure. This provides a measure of verbal memory and mental manipulation.

2.15.3.6. Trail Making Test

The trail making test looks at divided attention and executive function. It also has a motor component (207, 302). There are two parts to the test. In part A the subject is asked to connect a series of numbered circles consecutively by drawing a line between numbers. In part B the subject is asked to connect numbered and lettered circles, alternating between the two and working consecutively. They are asked to work as quickly and accurately as possible. The time taken to complete in seconds and any errors made are recorded.

This thesis examines shift (trial B minus trial A). This is a reliable measure of executive set shifting function - the ability to alternate between tasks – and correlates to Stroop colour word score (303).

2.15.3.7. Spatial Span

Spatial Span is a computerised version of Corsi's block tapping. It assesses visuospatial short-term memory (304).

Subjects watch a sequence of blue squares turn yellow one at a time. They are then asked to repeat this sequence via a touch screen. After a correct response the length of sequence increases by one. After maximum recall has been achieved subjects are then asked to repeat sequences in reverse order.

Subjects are given four scores: a total forward score and a score for the longest forward sequence recalled; and a total backward score and a score for the longest backward sequence recalled.

This thesis examines longest forward and backward scores. These provide a measure of immediate working memory.

2.15.3.8. Visual Patterns Test

The VPT is a computerised version of the matrices test. It assesses short-term visual memory (305) and is designed to assess visual recall.

The subject is presented with a chessboard-like pattern where half the squares are black and half are white. The pattern begins with a 2x2 matrix and continues to a 5x6 matrix (where 15 cells are filled) (305).They are asked to recreate the pattern on a blank matrix by identifying the squares that were black. A touch screen format is used.

The subject is given a total score and a score for the maximum number of targets recalled. This thesis examines the maximum number of targets to provide a measure of short-term memory.

2.15.3.9. FAS Verbal Fluency

The FAS Verbal Fluency test is a controlled oral word association test. It examines verbal fluency, an executive function (207).

There are three trials. Subjects are asked to list as many words as possible beginning with the letters F, A and S over a duration of 60 seconds per letter. Proper nouns or derivatives are not allowed. Accuracy is recorded as the overall number of correct words stated.

2.15.3.10. Attention Network Task

The ANT is used to assess attention. It is a computerised task.

Subjects are asked to press the left or right mouse button in response to visual stimuli in the form of five arrows. They are asked to focus on the middle arrow, which is flanked on either side by two either congruent or incongruent arrows. A cross appears in the centre of the screen and the arrows appear either above or below this cross. On some occasions before the arrows appear on the screen an asterix flashes above or below the cross as an alert that the arrows will appear in this position.

The ANT assesses arousal (alerting), attention shifting (orienting/inhibition) and executive control (central and spatial difference).

2.16. Data analysis

Data were analysed using GraphPad Prism version 6.

The data were tested for normality by plotting a histogram and by performing a Kolmogorov-Smirnov Test for Normality. Normally-distributed data are summarised using the mean and standard deviation (SD). Skewed data are summarised using the median and interquartile range (IQR). Proportions are summarised using number and percentage.

The relationship between categorical nominal and binary data was analysed using the Chi-squared test. The relationship between normally-distributed continuous data was analysed using the unpaired t-test, at a 5% significance level. The relationship between multiple means was analysed using ANOVA and a 5% significance level is given.

Unpaired continuous skewed data were analysed using a non-parametric test, Mann Whitney U test. The Kruskal-Wallis test was performed to compare multiple non-parametric datasets. A 5% significance level is given.

2.17. Methodological limitations

Limitations of the study methodology are discussed below.

2.17.1. Recruitment

It is difficult to rule out the possibility of selection bias. The participants recruited necessarily had to be sufficiently mobile to attend appointments and participate in investigations. More unwell sufferers of CFS who are not ambulatory would not be able to participate, which is likely to mean that a less severe CFS phenotype is investigated in this study as those with more severe illness, necessitating confinement in bed, could not participate.

There is a chance that the study is subject to "healthy volunteer bias". Participants were initially contacted through the CFS clinic at the Royal Victoria Infirmary and ongoing recruitment was through local support groups. There may be a systematic difference in those who volunteered to take part compared to those who did not, for example they may be more well and therefore more motivated. This also applies to control subjects, who were recruited via family members or from a university database.

2.17.2. Implementation

There is a strong possibility of recall bias with questionnaires asking about historically significant issues. It is often difficult to recall details of past events but this may be particularly the case in subjects with CFS who subjectively report problems with cognitive function.

Similarly, there may be response bias when completing questionnaires. The possibility that participants answered the questionnaires in a particular way or exaggerated symptoms, in particular in view of the stigma that often surrounds this condition, cannot be ruled out as the questionnaires were self-reported. Some of the controls recruited were family members of subjects with CFS, which may bias them to respond to questions in a particular way to emphasise a difference. Conversely, it may also have encouraged more honest responses.

Response bias was minimised through the use of validated questionnaires. The DSQ, used to define participants by diagnostic criteria met, was analysed in the US by an independent analyst blinded to the study cohorts.

There is a possibility of interviewer bias, as the CRA was not blinded to whether the participant was in the CFS or the control group.

2.17.3. Confounders

The possible confounding effect of medications was minimised by withholding medications prior to investigation as in section 2.10. Furthermore, participants meeting criteria for MDD were excluded. Controls were screened only if sedentary, to minimise the possibility of deconditioning accounting for any results.

Baseline characteristics were recorded and comparison between two groups performed to ensure matched controls.

2.17.4. Statistics

The aim of the MRC study was to recruit a total of 81 participants (71 CFS; ten controls) to achieve the correct statistical power. The original application to the MRC set out to recruit three groups of CFS and a comparator control group. Budget limitations applied by the MRC reduced the available funding and therefore the number of groups reduced. As such, the study involves relatively small numbers of participants and it is possible that any observations could have been observed by chance and that the study is underpowered.

ANOVA and non-parametric tests were used to compare outcome measures between DSQ groups. There are limitations to these methods. ANOVA allows multiple comparison, but it does not give information about which outcomes differ from one another. It is therefore difficult to draw conclusions about the nature and location of statistical differences. The use of non-parametric tests to compare skewed data is also limited in that they compare ranked data (the median not the mean).

Chapter 3. Results

3.1. Study cohort

In total 170 information sheets were sent to CFS and control participants. One hundred subjects responded with interest in taking part. Figure 8 depicts the study consort diagram, recorded over the course of the study.

Twenty four potential participants were excluded after the initial telephone screening. The reasons for their exclusion are shown in the consort diagram below. Three were taking antihypertensives; ten had a history of depression; two (CFS) had not been diagnosed at a specialist service, three (controls) were not sedentary and one could not be reached by telephone for the initial screening.

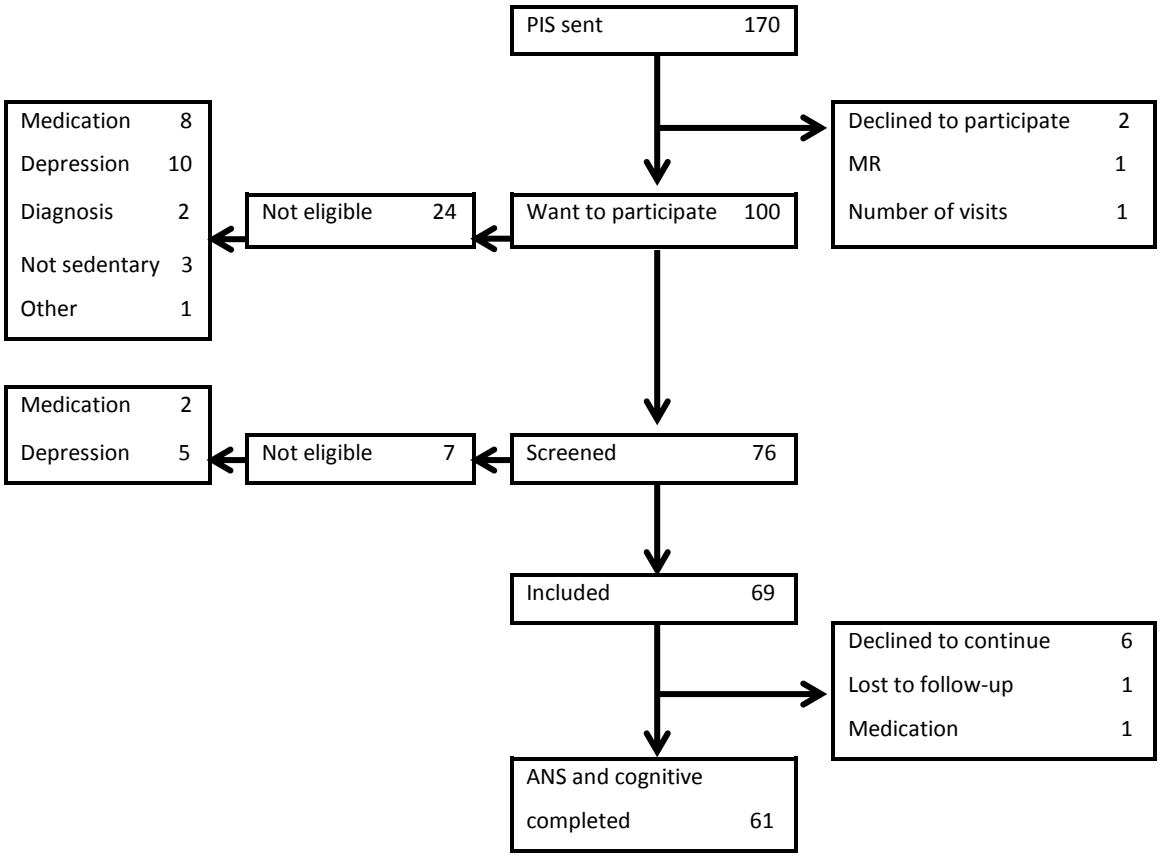

Figure 8 Consort diagram of recruitment

Seventy six potential participants were invited for screening. Of these, two were excluded for taking antihypertensives and five were excluded for meeting criteria for a current or past major depressive episode.

Sixty nine participants were eligible for entry into the study. Eight participants did not continue past the screening stage. Six withdrew from the study; one was lost to follow-up and one commenced a new medication resulting in loss of eligibility.

Sixty one participants underwent autonomic and cognitive testing. These were comprised of 51 CFS participants and ten controls.

3.1.1. Baseline characteristics in CFS and controls

Baseline characteristics of the CFS and control groups are depicted in table 16. All eligible CFS (n=59) and control (n=10) participants are included, as they were enrolled in the study at baseline. Co-morbidities were self-reported by affected system.

The groups were well-matched at baseline in that there were no significant differences in age, gender, employment or education status, or pre-morbid IQ. The control group was slightly older than the CFS group (45 versus 49) but with a higher standard deviation is unlikely to be clinically significant.

Two characteristics that merit further mention are the presence of co-morbidities, which is higher in the CFS group (92% versus 70%) and use of cardioactive medication (41% versus 0%). One of the initial aims of the study was to exclude participants on cardioactive medication or with co-morbidities associated with AD. In order to include sufficient numbers of CFS subjects it was necessary to allow participation of subjects on cardioactive medication. It was easier to identify healthy controls without co-morbid disease and not taking medication. Cardioactive medication was stopped temporarily for a specified period before assessments (see section 2.10) to reduce the possibility of confounding.

	CFS (n=59)	**Controls** (n=10)
Age (years) mean (SD)	45 (12.4)	49 (15.3)
Male n (%)	15 (25%)	3 (30%)
Female n (%)	44 (75%)	7 (70%)
Employed n (%)		
No	39 (66%)	7 (70%)
Yes	20 (34%)	3 (30%)
Full time	*7 (35%)*	*1 (33%)*
Part time	*13 (65%)*	*2 (67%)*
Paid	*19 (95%)*	*3 (100%)*
Voluntary	*1 (5%)*	*0 (0%)*
Student n (%)	2 (3%)	2 (20%)
Years in Education n (%)		
≤11	11 (19%)	1 (10%)
>11	48 (81%)	9 (90%)
Highest qualification n (%)		
None	3 (5%)	0 (0%)
GCSE	14 (24%)	2 (20%)
A level	14 (24%)	2 (20%)
Degree	28 (47%)	6 (60%)
Premorbid IQ mean (SD)	117 (7.9)	122 (4.3)
Co- morbidities		
Yes	54 (92%)	7 (70%)
No	5 (8%)	3 (30%)
Cardioactive medication		
Yes	24 (41%)	0 (0%)
No	35 (59%)	10 (100%)

Table 16 Baseline characteristics CFS and controls

3.1.2. Physical activity in CFS and controls

All eligible participants included in the study were given the *IPAQ Short Last 7 Days Self-administered* version of the IPAQ questionnaire to complete in order to establish a baseline measure of activity and to try and ensure similar levels of activity between control and CFS groups. Mean scores and outcomes are reported in table 17.

	CFS (n=50)*	**Controls** (n=10)	**p value** (Mann Whitney)
IPAQ score median (IQR)	198 (941)	1413 (1345)	0.0013
Low n (%)	32 (64%)	2 (20%)	
Moderate n (%)	17 (34%)	6 (60%)	
High n (%)	1 (2%)	2 (20%)	

* one questionnaire not completed

Table 17 IPAQ scoring and outcome measures CFS and controls

Scores are lower than those seen in active, healthy volunteers in other studies, who typically show median scores of 4500-6000 (306, 307), and reflect the sedentary lifestyle of participants in this study. Nevertheless, controls had significantly higher IPAQ scores compared to CFS subjects. A greater proportion of controls met criteria for moderate activity compared to CFS subjects (60% versus 34%). More CFS subjects were classified as having low activity levels compared to controls (64% versus 20%). Two controls and one CFS subject had high activity levels.

Despite participating in fewer than 30 minutes of exercise a week and living a sedentary lifestyle, controls still show higher levels of activity than CFS subjects. This may represent an aggregation of movement – which may not be vigorous – but which has an additive effect on IPAQ scoring to show higher activity levels. This finding may be an indicator of greater loss of functional ability in CFS subjects.

3.1.3. Participant questionnaires in CFS and controls

Participants completed the FIS, COGFAIL and COMPASS questionnaires. The results comparing outcomes between CFS and controls are shown in table 18.

	CFS (n=49*) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (unpaired t-test)
FIS	91.7 (32.5)	0.2 (0.6)	<0.0001**
COGFAIL	53.9 (19.1)	30.4 (10.2)	0.0004**
COMPASS	37.3 (15.1)	9.9 (4.8)	<0.0001**
COMPASS 31	26.4 (10.0)	8.1 (4.9)	<0.0001**

* two questionnaires not completed

** significant at 5% level

Table 18 Participant questionnaires CFS and controls

CFS subjects have statistically significantly higher self-reported fatigue, as measured by the FIS (91.7 versus 0.2 (p=<0.0001)). There is greater variability in score in the CFS group compared to controls.

CFS subjects have statistically significantly higher subjective cognitive impairment on COGFAIL (53.9 versus 30.4 (p=0.0004)). Both groups had some variability in scores, but the spread of scores was greater in the CFS group.

CFS subjects have statistically significantly higher subjective autonomic symptoms. COMPASS scores were 37.3 versus 9.9 (p=<0.0001). There is greater variability in score in the CFS group compared to controls. CFS subjects also had significantly higher scores on COMPASS 31 (26.37 versus 8.1). This is statistically significant at the 5% level (p=<0.0001). There is greater variability in score in the CFS group compared to controls.

Overall these results show that on subjective measurement CFS subjects reported greater impairment in terms of fatigue, cognitive function and autonomic symptoms

compared to controls. Furthermore, there was greater spread in scores in the CFS group, which suggests variability in symptoms and loss of function between CFS subjects, which may indicate different levels of disease severity.

3.1.4. Baseline characteristics by DSQ

All participants had been diagnosed with CFS and met the Fukuda criteria. Participants who were included in the study were given the DSQ to complete. The results from the CFS cohort are depicted in table 19 and show the number of CFS subjects that met each DSQ diagnostic subgroup.

Fukuda alone	**Fukuda + 2003 Research**	**Fukuda + 2003 Clinical**	**Fukuda + 2003 + 2011**
6	8	9	26

Table 19 Number of participants by DSQ

One participant completed the DSQ but did not go on to complete further questions or other investigations. For this reason they are not included in subsequent analyses.

Baseline characteristics by DSQ diagnosis are shown in table 20.

The groups are moderately well-matched. The mean age of participants meeting the Fukuda+2003 Clinical criteria and those meeting the Fukuda+2003+2011 criteria is slightly higher than the other two diagnostic groups, however this is not clinically significant. More men appear to meet the Fukuda+2003 Clinical criteria compared to the other three subgroups, however the actual numbers here are small and unlikely to represent a significant increase.

Participants meeting the Fukuda+2003 Clinical and Fukuda+2003+2011 criteria are less likely to be in employment and also have a greater prevalence of co-morbidities. This may indicate greater functional disability in participants meeting these criteria.

	Fukuda alone (n=6)	Fukuda + 2003 Research (n=8)	Fukuda + 2003 Clinical (n=9)	Fukuda + 2003 + 2011 (n=26)
Age (years) mean (SD)	43 (14.5)	41 (10.9)	49 (13.5)	47 (11.1)
Male	1 (17%)	1 (12.5%)	4 (44%)	6 (30%)
Female	5 (83%)	7 (87.5%)	5 (66%)	20 (70%)
Employed				
No	3 (50%)	5 (38%)	5 (66%)	19 (73%)
Yes	3 (50%)	3 (62%)	4 (44%)	7 (27%)
Full time	0 (0%)	0 (0%)	4 (100%)	3 (43%)
Part time	3 (100%)	3 (100%)	0 (0%)	4 (57%)
Paid	3 (100%)	3 (100%)	4 (100%)	7 (100%)
Voluntary	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Student	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Years in education				
≤11	1 (17%)	0 (0%)	1 (11%)	8 (31%)
>11	5 (83%)	8 (100%)	8 (89%)	18 (69%)
Highest qualification				
None	0 (0%)	1 (12.5%)	0 (0%)	2 (8%)
GCSE	1 (17%)	0 (0%)	1 (11%)	11 (42%)
A level	0 (0%)	3 (37.5%)	1 (11%)	6 (23%)
Degree	5 (83%)	4 (50%)	7 (78%)	7 (27%)
Premorbid IQ mean (SD)	119 (6.6)	123 (3.1)	117 (6.6)	116 (9.2)
Co- morbidities				
Yes	5 (83%)	7 (87.5%)	8 (89%)	25 (96%)
No	1 (17%)	1 (12.5%)	1 (1%)	1 (4%)
Cardioactive medication				
Yes	1 (17%)	2 (25%)	5 (66%)	10 (38%)
No	5 (83%)	6 (75%)	4 (44%)	16 (62%)

Table 20 Baseline characteristics by DSQ

3.1.5. Physical activity by DSQ

The results of the IPAQ by DSQ are shown in table 21.

	Fukuda alone (n=6)	**Fukuda + 2003 Research** (n=8)	**Fukuda + 2003 Clinical** (n=8)*	**Fukuda + 2003 + 2011** (n=26)	**p value** (Mann Whitney)
IPAQ score median (IQR)	819 (1418)	30 (938)	908 (983)	33 (480)	0.0414**
Low	2 (33%)	5 (62.5)	4 (50%)	20 (77%)	
Moderate	4 (66%)	3 (37.5)	4 (50%)	5 (19%)	
High	0 (0%)	0 (0%)	0 (0%)	1 (4%)	

* Questionnaire not completed by one participant

** significant at 5% level

Table 21 IPAQ scoring and outcome measures by DSQ

There is a statistically significant difference in IPAQ score between DSQ groups. CFS participants who meet the Fukuda alone criteria and those who meet the Fukuda+2003 Clinical criteria appear to have higher levels of activity compared to those meeting the Fukuda+2003 Research criteria and those who meet the Fukuda+2003+2011 criteria. Participants who meet Fukuda+2003+2011 have lower levels of activity compared to the other three groups, with 77% of participants reporting 'low' activity. This may represent greater functional impairment. This group also has the greatest spread of activity, with one participant achieving high levels.

3.1.6. Participant questionnaires by DSQ

The results of the participant questionnaires by DSQ diagnosis are shown in table 22.

There are statistically significant differences in FIS and COMPASS scores. Participants meeting the Fukuda+2003+2011 criteria have higher subjective levels of fatigue, cognitive impairment and symptoms of dysautonomia. The lowest scores are seen in the group that meet Fukuda alone.

	Fukuda alone (n=6) mean (SD)	**Fukuda + 2003 Research** (n=8) mean (SD)	**Fukuda + 2003 Clinical** (n=9) mean (SD)	**Fukuda + 2003 + 2011** (n=26) mean (SD)	**p value** (ANOVA)
FIS	58.2 (34.1)	93.1 (22.4)	81.7 (19.3)	102.5(33.3)	0.0131*
COGFAIL	42.0 (17.4)	54.1 (20.9)	49.6 (15.8)	58.2 (19.3)	0.25
COMPASS	27.5 (13.7)	37.3 (6.6)	28.3 (9.6)	42.6 (16.7)	0.0253**
COMPASS 31	17.3 (9.6)	24.0 (5.4)	21.0 (8.7)	31.0 (9.4)	0.0017**

** significant at 5% level

Table 22 Participant questionnaires by DSQ

These findings point towards an additive effect as more diagnostic criteria are met and, in combination with the findings on IPAQ, suggest greater functional impairment in the subgroup meeting Fukuda+2003+2011.

3.1.7. Completion of study protocol

The number of participants who completed all visits of the full MRC study within eight weeks is shown in table 23. Fifty nine CFS subjects were enrolled on the MRC study. Eight did not continue. In total 86% of the CFS group completed the study. Forty eight of 61 participants (79%) completed the full MRC project within eight weeks (56 days). All those who completed the study did so within 12 weeks.

	Within 8 weeks	**Within 8-12 weeks**
All participants (%)	44 (72)	17 (28)
CFS only (%)	37 (73)	14 (27)

Table 23 Time to completion

Difficulties in completing the study within eight weeks arose from participant-determined and other factors. Delivery of the nuclear medicine equipment delayed some visits, as did limited MRI appointment availability. Participants had difficulty

attending appointments each week because of other commitments. CFS participants also preferred to space out appointments in order to pace themselves to enable completion of the study (in contrast to control subjects who preferred to compact investigations into a short timeframe).

These results suggest that this cohort was comprised of highly-motivated subjects who were able and prepared to undergo considerable physical burden to complete all investigations.

3.2. Depression

Initial telephone screening excluded ten potential participants because of psychiatrist-diagnosed MDD. A total of 65 participants were screened. Sixty three participants have been included in the analysis. It was not possible to include two assessments for the following reasons. One CFS participant's SCID-I assessment was performed by a third party and contained inaccuracies. This participant was noted to have depression but was included in the study. They subsequently withdrew from the study before it was possible to verify the results. One SCID-I assessment was misplaced. The misplaced SCID-I related to a CFS subject who was excluded because of past major depressive episodes, however more detailed information about this could not be recorded and is therefore not included in the analysis.

The prevalence of depressive symptoms in the CFS cohort is reported in table 24. The UK prevalence of depression has been reported to be 4-10% for major depression and between 2.5-5% for low-grade chronic depressive symptoms (308, 309). In this study cohort 15% of the 100 people who expressed interest in participating and underwent at least a telephone screening had a diagnosis of previous or current MDD.

Thirty two percent of CFS participants reported feeling depressed or down for at least two weeks. Sixteen percent reported loss of interest or pleasure and 13% reported both. The most prevalent of the "secondary" symptoms was a change in appetite, as well as change in sleep, loss of energy and concentration and feelings of worthlessness. Suicidal ideation and psychomotor change were the least prevalent.

Symptom		n (%)
At least two weeks of:		
	Feeling depressed or down	20 (32)
	Loss of interest or pleasure in things usually enjoyed	10 (16)
	Both of the above	8 (13)
Of those with the above symptoms		
	Change in appetite	11 (47)
	Change in sleep	6 (26)
	Psychomotor change	4 (17)
	Loss of energy	6 (26)
	Feelings of worthlessness	9 (39)
	Loss of concentration	5 (22)
	Suicidal ideation	3 (13)

Table 24 Depressive symptoms on SCID-I assessment tool at screening

The number of depressive symptoms and percentage of participants in each category, as well as associated antidepressant use is reported in table 25.

The majority (63.5%) of CFS participants in this study had no symptoms of depression. Eight percent had one or two symptoms and 22.2% had three or four symptoms. Four participants (6.3%) had five or more symptoms and were excluded from the study.

Seventy five percent of participants who met a diagnosis of MDD were prescribed antidepressant medication. Of the participants with no recorded symptoms of depression 15% were being prescribed antidepressants. One participant with two symptoms of depression was prescribed antidepressant medication.

Number of symptoms	n (%)	Participants prescribed antidepressant medication n (% of those in symptom category)
0	40 (63.5)	6 (15)
1	2 (3.2)	0 (0)
2	3 (4.8)	1 (33.3)
3	12 (19.0)	0 (0)
4	2 (3.2)	0 (0)
5+	4 (6.3)	3 (75)

Table 25 Number of symptoms of depression and concomitant antidepressant use

These findings highlight the challenges of diagnosing and managing two conditions (CFS and depression) that share symptoms. This is discussed in section 4.1.3.

3.3. Autonomic nervous system

3.3.1. CFS and controls: At rest

The results below compare autonomic testing between the CFS and control groups at rest.

Table 26 shows a comparison of mean values for beat statistics between CFS and control groups. Overall there is no statistically significant difference between CFS and controls. Mean and standard deviations of all measures are broadly comparable.

CFS and control groups both had a resting mean heart rate of 73-74 bpm, which lies in the normal range (86). Mean sBP is slightly lower in the control group compared to the CFS group, but CFS subjects show a wider standard deviation. Both groups have a mean sBP, dBP and mBP that lie towards the lower end of normal (310, 311). The reasons for this may include the fact that participants abstained from caffeinated drinks prior to testing, which may imply low fluid intake and hypovolaemia, and because blood pressure was measured supine (312).

Beat statistics	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
HR (bpm)	74.34 (10.26)	73.62 (7.11)	0.84
sBP (mmHg)	108.8 (18.78)	104.9 (11.19)	0.52
dBP (mmHg)	69.25 (11.04)	67.86 (6.55)	0.70
mBP (mmHg)	79.54 (12.32)	78.23 (7.64)	0.75

Table 26 Beat statistics CFS and controls

Table 27 depicts a comparison of cardiac statistics. There are no statistically significant differences between CFS and control groups.

Cardiac statistics	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
SI (ml/m^2)	41.03 (11.79)	42.74 (10.16)	0.67
CI ($l/min*m^2$)	3.00 (0.84)	3.2 (0.93)	0.61
TPRI	2255 (810.9)	2052 (549.4)	0.45
EDI	67.99 (19.51)	68.58 (16.19)	0.93
IC	44.91 (18.15)	45.74 (15.51)	0.89
ACI	64.81 (31.31)	56.99 (24.10)	0.46
LVWI	3.145 (0.94)	3.285 (1.16)	0.68
LVET	303.8 (17.80)	312.1 (11.08)	0.16
TFC	27.50 (5.94)	28.41 (6.51)	0.67

Table 27 Cardiac statistics CFS and controls

Myocardial contractility has been measured using IC and ACI and LVWI. Values are on the lower side of the normal range. Afterload has been measured using TPRI. EDI has been used to measure preload. Both are within normal limits. Stroke index and cardiac index are within the normal range (280).

TFC is a measure of intra and extra-vascular fluid volume in the chest cavity. As fluid content increases, TFC decreases. TFC in CFS and control subjects was comparable to healthy volunteers in other studies and within normal limits (313).

This shows that in this cohort CFS subjects and control subjects were within normal parameters when testing cardiac function using the above measures.

Table 28 shows HRV statistics representing modulation of cardiovascular activity. There are no statistically significant differences between groups. Both cohorts appear to have a shift in balance towards increased sympathetic modulation of cardiovascular activity, as seen in the higher LFnu-RRI values and the LF/HF-RRI.

Heart rate variability statistics	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
LFnu-RRI	59.16 (16.87)	62.42 (12.89)	0.57
HFnu-RRI	40.39 (16.94)	37.58 (12.89)	0.62
LF/HF-RRI	3.06 (4.85)	2.25 (1.16)	0.72

Table 28 Heart rate variability statistics CFS and controls

Table 29 shows a comparison of dBP variability statistics between CFS and control groups. There are no statistically significant differences between groups. LFnu-dBP, a marker of sympathetic modulation of dBP, is higher than HFnu-dBP (a marker of parasympathetic modulation) and shows a balance towards greater sympathetic modulation in both groups. This is supported by the high LF/HF-dBP sympathovagal balance index.

Blood pressure variability statistics (dBP)	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
LFnu-dBP	54.83 (13.78)	46.81 (19.01)	0.12
HFnu-dBP	13.82 (10.64)	12.48 (10.75)	0.72
LF/HF-dBP	7.05 (5.32)	6.14 (4.20)	0.61

Table 29 Blood pressure variability statistics (dBP) CFS and controls

There are no statistically significant differences in sBP BPV statistics between groups, as seen in table 30. Mean measures and variation are comparable in both CFS and control cohorts. Like dBP, higher LFnu-sBP indices in both groups suggest greater sympathetic activation than parasympathetic. This is also supported by the LF/HF-sBP, which is towards the higher side.

Blood pressure variability statistics (sBP)	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
LFnu-sBP	46.73 (14.33)	38.48 (13.86)	0.1
HFnu-sBP	17.37 (11.75)	15.43 (10.68)	0.63
LF/HF-sBP	4.70 (4.37)	3.80 (2.43)	0.53

Table 30 Blood pressure variability statistics (sBP) CFS and controls

Table 31 shows comparison of baroreflex sensitivity statistics at LAG1 (at rest). There are no statistically significant differences between CFS and control cohorts. Baroreflex sensitivity provides a measure of baroreflex control of the heart. The baroreflex effectiveness index provides a measure of the baroreflex response to changes in blood pressure. Both are within normal limits (314). A study comparing subjects with vasovagal syncope to controls did find comparable values to those seen in this study (281), and may reflect the fact that BP in these groups was at the lower end of normal.

Baroreflex sensitivity statistics LAG1	**CFS** (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
Baroreflex sensitivity	13.32 (8.14)	11.66 (9.58)	0.57
Baroreflex effectiveness index	59.98 (20.58)	58.08 (15.49)	0.78

Table 31 Baroreflex sensitivity statistics LAG1 CFS and controls

3.3.2. CFS and controls: Standing

Table 32 shows a comparison of mean sBP on standing. Mean sBP and nadir on standing are not statistically significantly different between CFS and control groups. The overall drop of sBP on standing is statistically significantly different between groups, with less drop in the CFS cohort (11.43 versus 19.8 (p=0.0411)).

	CFS (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
Mean sBP	110.2 (25.04)	109.5 (10.94)	0.93
Nadir	98.75 (26.12)	89.7 (16.67)	0.3
Drop	11.43 (11.98)	19.8 (9.18)	0.0411*

* significant at 5% level

Table 32 Systolic blood pressure on standing CFS and controls

The nadir represents the lowest sBP value during the two minute active stand. OH is diagnosed with a fall in sBP of 20mmHg or greater (315). Both CFS and control groups lie within normal limits, although control subjects have a higher mean drop in sBP on standing.

Table 33 shows comparison of mean dBP on standing. There is no statistically significant difference in mean dBP, nadir or drop between groups. Although not

statistically significant, the control group has a bigger mean drop on standing compared to the CFS cohort (8.85 versus 14.01).

Both groups are within normal limits for mean dBP, although at the lower end of normal. A drop of 10mmHg in dBP on standing meets the definition of OH (315). The control group meets this definition. This may be explained by hypovolaemia.

	CFS (n=51) mean (SD)	**Controls** (n=10) mean (SD)	**p value** (two sample t-test)
Mean dBP	77.44 (17.73)	73.51 (6.49)	0.99
Nadir	64.59 (16.33)	59.5 (7.68)	0.34
Drop	8.85 (9.69)	14.01 (7.56)	0.12

Table 33 Diastolic blood pressure on standing CFS and controls

Table 34 depicts the 30:15 ratio during the active stand. This is a dynamic measure of parasympathetic activity. It represents the longest RRI at approximately the 30th beat to the shortest RRI at the 15th beat and is taken directly after standing. Small ratios are abnormal.

	CFS (n=51) median (IQR)	**Controls** (n=10) median (IQR)	**p value** (Mann-Whitney test)
30:15 ratio	1.12 (1.07,1.17)	1.07 (1.04, 1.16)	0.24

Table 34 30:15 ratio CFS and controls

There is no significant difference in median 30:15 ratio between CFS and control groups. Ratios in both groups are lower than findings from control subjects in other studies (314), however both groups lie within the normal range of > 1.04 (316).

Table 35 shows comparison of AUC on standing. Median AUC at baseline is statistically significantly lower in the CFS group compared to the controls group, however this is not clinically significant.

	CFS (n=51) median (IQR)	**Controls** (n=10) median (IQR)	**p value** (Mann-Whitney test)
AUC (Baseline)	24.18 (0.0, 118.2)	129.2 (57.02,407.8)	0.0249*
AUC – (20mmHg)	0.0 (0.0,0.0)	0.0 (0.0,0.0)	>0.99

* significant at 5% level

Table 35 Area under the curve CFS and controls

3.3.3. CFS and controls: Valsalva

Table 36 shows comparison of Valsalva measurements between the CFS and control groups. The CFS group has a statistically significantly longer Valsalva ratio compared to the control group (0.70 versus 0.53 (p=0.0245)). There were no statistically significant differences in mean measures of Phase I, IIi or IV between groups. Ratios <1.21 imply dysautonomia.

The Valsalva ratio for both groups is abnormal (<1.21 (296, 297)), which indicates dysautonomia in both CFS and control subjects. Age, gender and baseline blood pressure can affect the Valsalva ratio, with lower ratios seen in women, with increasing age (296). The low baseline blood pressure seen in both groups may also help to explain these results in these cohorts.

	CFS (n=49*) mean (SD)	**Controls** (n=9*) mean (SD)	**p value** (two sample t-test)
Valsalva ratio	0.70 (0.21)	0.53 (0.20)	0.0245**
Phase I	12.08 (9.81)	17.64 (6.23)	0.11
Phase IIi	8.61 (14.62)	4.53 (17.64)	0.46
Phase IV	17.49 (15.70)	25.98 (27.47)	0.1955

* unable to analyse due to missing BP readings

** significant at 5% level

Table 36 Valsalva ratio CFS and controls

Overall there are no significant differences in objective autonomic measures between CFS and control groups on standing. This is discussed in section 4.1.1.

3.3.4. DSQ: At rest

The results reported below show comparison of objective measures of autonomic function in CFS subgroups as defined by the DSQ.

One-way ANOVA has been used to compare means between groups. There are limitations to this method, which are discussed in section 2.17.

Table 37 shows beat statistics between DSQ groups. There are no statistically significant differences between groups. All DSQ subgroups had HR in the normal range (86). Fukuda alone subjects had the lowest HR and Fukuda+2003+2011 the highest.

Beat statistics	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
HR (bpm)	69.57 (6.25)	75.69 (6.81)	73.07 (9.24)	76.59 (11.50)	0.43
sBP (mmHg)	108.9 (17.24)	101.9 (13.11)	122.3 (24.99)	107.2 (17.36)	0.12
dBP (mmHg)	71.61 (17.37)	64.91 (6.08)	71.90 (14.75)	69.62 (9.47)	0.59
mBP (mmHg)	82.21 (16.71)	75.14 (7.75)	83.65 (15.55)	79.56 (11.48)	0.54

Table 37 Beat statistics by DSQ

Table 38 shows a comparison of cardiac statistics between DSQ groups.

There are no statistically significant differences in any measures of cardiac function. Mean measures in the Fukuda+2003+2011 group are lower for all measures except TPRI. Measures in the Fukuda alone group are higher compared to the other subgroups, also with the exception of TPRI. Although not statistically significant, this is suggestive of different phenotypes between subgroups.

Cardiac statistics	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
SI (ml/m^2)	47.30 (12.59)	45.04 (12.52)	41.10 (12.32)	37.46 (10.33)	0.16
CI ($l/min*m^2$)	3.28 (0.90)	3.36 (0.82)	2.98 (0.99)	2.83 (0.80)	0.39
TPRI	2026 (506.9)	1849 (609.3)	2464 (1105)	2394 (802.0)	0.3
EDI	78.78 (22.03)	73.17 (19.82)	68.15 (21.03)	62.38 (17.11)	0.20
IC	56.14 (21.94)	51.46 (19.19)	43.66 (17.42)	39.68 (16.19)	0.14
ACI	81.76 (38.24)	74.57 (32.03)	64.97 (34.75)	56.08 (27.31)	0.21
LVWI	3.68 (1.574)	3.28 (0.8251)	3.26 (1.065)	2.97 (0.7564)	0.38
LVET	312.1 (18.44)	304.2 (17.06)	296.4 (13.55)	303.4 (18.56)	0.41
TFC	28.59 (3.894)	28.09 (6.559)	28.75 (6.877)	26.68 (6.218)	0.78

Table 38 Cardiac statistics by DSQ

Myocardial contractility has been measured using IC and ACI and LVWI. These are within the normal range in all groups, however on the lower side of normal. The Fukuda+2003+2011 subgroup shows the lowest values across these parameters and may indicate decreased myocardial contractility compared to the other subgroups, in particular the Fukuda alone group, which has the highest value.

Afterload has been measured using TPRI and EDI has been used to measure preload. TPRI is within normal limits for all subgroups except Fukuda+2003 Research, where it is low. EDI is lowest in the Fukuda+2003+2011 group, and reflects a lower cardiac index in this group (317). Stroke index and cardiac index are within the normal range (252) and are lowest in the Fukuda+2003+2011 subgroup.

TFC is a measure of decreased intra and extra-vascular fluid volume in the chest cavity. It is comparable across all subgroups.

Table 39 depicts HRV statistics by DSQ sub-group. One measure has statistically significant differences between groups. LF/HF-RRI differs across groups (2.26;7.72;3.14;1.84 (p=0.0262)). This reflects a shift in balance between sympathetic and parasympathetic activity between groups with Fukuda+2003 Research showing greater sympathetic activity and Fukuda+2003+2011 lower sympathetic modulation of cardiovascular activity.

Heart rate variability statistics	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
LFnu-RRI	57.10 (11.11)	62.37 (20.12)	68.60 (13.42)	54.74 (17.64)	0.19
HFnu-RRI	42.90 (11.11)	34.77 (19.84)	31.40 (13.42)	45.26 (17.64)	0.14
LF/HF-RRI	2.26 (1.92)	7.72 (11.01)	3.14 (1.87)	1.84 (1.62)	0.0262*
LF/HF	2.03 (1.50)	3.34 (3.63)	2.36 (1.18)	1.55 (1.26)	0.13

* significant at 5% level

Table 39 Heart rate variability statistics by DSQ

Table 40 shows dBP BPV statistics between DSQ subgroups. There are no statistically significant differences between groups, however the Fukuda+2003 Research group has the highest LF/HF-dBP ratio, which may indicate clinically significantly greater sympathetic modulation of dBP.

The Fukuda+2003+2011 subgroup has the lowest LFnu-dBP, indicating lower sympathetic modulation of dBP. This is supported by a low LF/HF-dBP ratio. Overall, however, there is a mixed picture.

Blood pressure variability statistics (dBP)	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
LFnu-dBP	57.39 (12.11)	63.52 (11.29)	55.66 (10.97)	50.17 (14.50)	0.09
HFnu-dBP	11.52 (4.90)	9.66 (7.49)	14.59 (8.75)	15.65 (13.01)	0.54
LF/HF-dBP	6.07 (2.68)	11.30 (8.16)	5.91 (3.87)	6.36 (4.97)	0.11

Table 40 Blood pressure variability statistics (dBP) by DSQ

Table 41 shows BPV of sBP between groups. LF/HF-sBP is statistically significantly different between groups.

LF/HF-sBP is highest in the Fukuda+2003 Research group suggesting a balance towards greater sympathetic activity in this group. It is lowest in the Fukuda+2003+2011 group and suggests a balance to lower sympathetic activity in this subgroup. This is supported by the finding of a low LFnu-sBP in this subgroup, suggesting low sympathetic modulation of sBP, and of a higher HFnu-sBP, pointing towards greater parasympathetic modulation of sBP.

Blood pressure variability statistics (sBP)	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
LFnu-sBP	45.06 (17.60)	57.48 (12.15)	46.85 (11.71)	43.14 (14.15)	0.10
HFnu-sBP	14.17 (6.105)	9.53 (8.29)	16.77 (10.22)	21.02 (13.23)	0.09
LF/HF-sBP	3.51 (1.29)	10.49 (7.29)	4.14 (2.80)	3.30 (2.48)	0.0002*

* significant at 5% level

Table 41 Blood pressure variability statistics (sBP) by DSQ

Table 42 shows BRS statistics at LAG1 compared between DSQ subgroups.

Baroreflex sensitivity statistics LAG1	**Fukuda** n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
Baroreflex sensitivity	12.26 (4.74)	19.10 (7.50)	13.17 (9.35)	10.47 (4.50)	0.0135*
Baroreflex effectiveness index	55.97 (32.27)	73.53 (18.61)	56.26 (16.39)	57.51 (18.69)	0.23

* significant at 5% level

Table 42 Baroreflex sensitivity statistics LAG1 by DSQ

At LAG1 baroreflex sensitivity is statistically significantly different across groups and is lower in the Fukuda+2003+2011 group suggesting decreased baroreflex sensitivity to

controlling heart rate in this group, in particular compared to the Fukuda+2003 Research group.

The baroreflex effectiveness index is comparable across all groups, although slightly higher in the Fukuda+2003 Research group, which may indicate greater control.

3.3.5. DSQ: Standing

Tables 43 and 44 show mean drop, baseline and nadir in sBP and dBP between DSQ groups. There are no statistically or clinically significant differences between groups.

	Fukuda n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
Mean sBP	108.3 (18.68)	99.54 (22.39)	125.3 (27.82)	108.9 (25.94)	0.2
Nadir	101.3 (17.64)	85.75 (28.50)	111.8 (29.45)	97.88 (25.96)	0.25
Drop	7.00 (6.51)	13.79 (8.00)	13.57 (17.13)	11.01 (12.54)	0.72

Table 43 Systolic blood pressure on standing by DSQ

Mean sBP is comparable across all groups and lowest in the Fukuda+2003 Research subgroup. This is reflected by a lower nadir. No group meets criteria for OH on sBP.

This is also reflected in dBP on active stand, as shown in table 44. Mean dBP is comparable across groups and not statistically significantly different.

	Fukuda n=6 mean (SD)	**Fukuda + 2003 Research** n=8 mean (SD)	**Fukuda + 2003 Clinical** n=9 mean (SD)	**Fukuda + 2003 + 2011** n=26 mean (SD)	**p value** (ANOVA)
Mean dBP	74.14 (19.23)	69.26 (13.61)	77.07 (18.53)	73.08 (17.67)	0.83
Nadir	67.00 (21.86)	59.13 (18.07)	65.44 (18.16)	65.12 (15.10)	0.80
Drop	7.14 (5.10)	10.14 (5.63)	11.63 (11.90)	7.96 (11.12)	0.76

Table 44 Diastolic blood pressure on standing by DSQ

Table 45 shows the 30:15 ratio on standing – a dynamic measure of parasympathetic activity.

	Fukuda n=6 median (IQR)	**Fukuda + 2003 Research** n=8 median (IQR)	**Fukuda + 2003 Clinical** n=9 median (IQR)	**Fukuda + 2003 + 2011** n=26 median (IQR)	**p value (Kruskal-Wallis)**
30:15 ratio	1.12 (1.06,1.55)	1.10 (1.02,1.33)	1.16 (1.07,1.36)	1.11 (1.06,1.17)	0.73

Table 45 30:15 ratio by DSQ

Median measures and IQR are comparable across groups and not statistically significantly different. Ratios across all subgroups are normal (>1.04).

Table 46 shows median AUC between DSQ subgroups. There is no statistically or clinically significant difference between groups.

	Fukuda n=6 median (IQR)	**Fukuda + 2003 Research** n=8 median (IQR)	**Fukuda + 2003 Clinical** n=9 median (IQR)	**Fukuda + 2003 + 2011** n=26 median (IQR)	**p value** (Kruskal-Wallis test)
AUC (Baseline)	16.91 (0.0,121.0)	51.74 (14.20,299.1)	0.0 (0.0,128.4)	20.04 (0.0,112.4)	0.68
AUC – (20mmHg)	0.0 (0.0,0.0)	0.0 (0.0,0.0)	0.0 (0.0,69.48)	0.0 (0.0,0.0)	0.50

Table 46 Area under the curve by DSQ

3.3.6. DSQ: Valsalva

Table 47 shows median measures of Valsalva ratio. There are statistically significant between-group differences in Phases I and IIi.

The Valsalva ratio is abnormal (<1.21) in all subgroups, which indicates dysautonomia. The statistically significant differences in Phases I and IIi may reflect parasympathetic activation by the baroreceptors (318). However, blood pressure in phases I and IIi has not been shown to decrease, which would be expected, and may therefore reflect poor Valsalva technique.

	Fukuda n=6 median (IQR)	**Fukuda + 2003 Research** n=8 median (IQR)	**Fukuda + 2003 Clinical** n=9 median (IQR)	**Fukuda + 2003 + 2011** n=24* median (IQR)	**p value** (Kruskal-Wallis test)
Valsalva ratio	0.56 (0.40,0.82)	0.68 (0.47,0.96)	0.81 (0.68,0.97)	0.74 (0.52,0.87)	0.22
Phase I	13.30 (8.14,20.55)	14.44 (7.25,26.69)	3.54 (-0.96,5.98)	13.29 (2.98,18.15)	0.0282**
Phase IIi	134.5 (114.5,146.5)	3.16 (-1.12,17.82)	10.03 (-7.89,25.50)	8.96 (0.35,22.81)	0.0013**
Phase IV	21.16 (7.01,37.89)	17.77 (7.43,37.59)	15.77 (4.11,22.93)	16.36 (1.89,30.95)	0.70

* unable to analyse two data sets due to missing BP readings

** significant at 5% level

Table 47 Valsalva ratio by DSQ

3.4. Cognitive function

Results of the cognitive function assessments are shown below. Comparison to previous studies is made where no normal reference range is available.

3.4.1. CFS and controls

The number of participants is as per table 48 (Premorbid IQ CFS and controls) unless otherwise indicated.

3.4.1.1. Premorbid IQ

Table 48 shows premorbid IQ score as per NART. Premorbid IQ is neither statistically nor clinically different between the CFS and control groups. Although evidence for its effectiveness at predicting premorbid IQ is debated (298, 319), both groups have an IQ above the average score for the UK (a score of 108.5) (320).

	CFS (n=51) mean (SD)	**Controls (n=10)** mean (SD)	**p value** (unpaired t-test)
NART	117.9 (7.9)	122.3 (4.4)	0.09

Table 48 Premorbid IQ CFS and controls

3.4.1.2. Memory: Verbal

Table 49 shows the results of measures of verbal memory. There are no statistically significant differences between CFS and controls.

	CFS (n=51) mean (SD)	**Controls (n=10)** mean (SD)	**p value** (unpaired t-test)
Rey AVLT			
Total recall trials 1-5	46.9 (10.4)	50.5 (6.2)	0.30
Forgetting (% retained from A6 on trial A7)	76.3 (21.1)	82.1 (18.4)	0.42
Forward Digit Span			
Clinical measure	6.6 (1.4)	6.9 (1.3)	0.52

Table 49 Verbal memory CFS and Control

Rey AVLT total recall is comparable to control subject in other studies and other normative data (207, 321, 322) and shows no statistically significant difference in immediate memory in CFS subjects. Delayed recall, as shown by percentage retained, is not statistically significantly different between groups and is comparable to that seen in healthy controls in other studies (322). Control participants recalled a higher total number of words over five trials of Rey AVLT and retained a higher percentage, although this was not clinically significant.

Forward digit span is comparable to controls in other studies (321, 322).

3.4.1.3. Memory: Visuospatial

Table 50 shows the results of measures of visuospatial memory. There are no statistically significant differences between CFS and control groups.

Both groups recalled a comparable sequence number on spatial span and VPT, indicating no deficits in immediate visuospatial memory. All are comparable to control subjects in other studies (321, 322).

	CFS (n=50)* mean (SD)	**Controls (n=10)** mean (SD)	**p value** (unpaired t-test)
Spatial span			
Forward longest sequence	5.1 (1.3)	5.4 (1.4)	0.52
Backward longest sequence	4.9 (1.2)	4.4 (1.6)	0.25
VPT			
Maximum number of targets	9.9 (2.2)	9.9 (2.6)	> 0.99

* One participant was unable to complete the task

Table 50 Visuospatial memory CFS and controls

3.4.1.4. Executive: Verbal fluency

Tables 51-54 show measures of executive function.

Table 51 shows the results of measures of verbal fluency as per FAS. The number of correct words achieved on FAS is not statistically significantly different between groups, however controls achieved more correct words compared to CFS participants (46.0 versus 39.3).

The mean score by CFS subjects is consistent with the results of a meta-analysis finding of a mean FAS score of 40.5 (SD 6.1) (323). Controls in this study performed slightly better, however this is likely to be explained by the wide standard deviation.

FAS	CFS (n=50)* mean (SD)	Controls (n=10) mean (SD)	p value (unpaired t-test)
Total correct	39.3 (10.6)	46.0 (14.2)	0.09

* One participant was unable to complete the task

Table 51 Verbal fluency CFS and controls

3.4.1.5. Executive: Inhibition

Table 52 shows the results of measures of inhibition whereby the subject's ability to supress a usual response with a less habitual one is tested (324). There are no statistically significant differences between CFS and control subjects.

STROOP	CFS (n=50)* mean (SD)	Controls (n=10) mean (SD)	p value (unpaired t-test)
"Colour word" minus "average of word reading plus colour naming"	-35.1 (14.3)	-41.4 (9.2)	0.18

* One participant was unable to undertake the task due to colour blindness

Table 52 Inhibition CFS and controls

3.4.1.6. Executive: Mental manipulation

Table 53 shows the results of measures of mental manipulation. Mean clinical measures (highest number of digits recalled) on reverse digit span are not statistically significantly different between CFS and controls. Controls had a higher mean clinical measure on reverse digit span compared to CFS participants, although this was not statistically significant (5.8 versus 5.0 (p=0.06)).

Scores in both CFS and control groups are similar to normative measures (325).

Reverse digit span	**CFS** mean (SD)	**Controls** mean (SD)	**p value** (unpaired t-test)
Clinical measure	5.0 (1.3)	5.8 (1.1)	0.06

Table 53 Mental manipulation CFS and controls

3.4.1.7. Executive: Set shifting

Set shifting represents the ability to alternate attention between simultaneous tasks and requires the use of cognitive, perception and motor skills. There are no statistically significant differences in set shifting between CFS and controls, as seen in table 54.

Trail making test	**CFS (n=50)*** mean (SD)	**Controls (n-10)** mean (SD)	**p value** (unpaired t-test)
Shift (B-A) (seconds)	32.9 (23.1)	27.0 (11.2)	0.69

* One participant was unable to complete the task

Table 54 Set shifting CFS and controls

3.4.1.8. Psychomotor speed

Table 55 shows comparison of psychomotor speed between CFS and controls, as measured with the DSST.

There is a statistically significant difference in mean number of symbols per second on DSST. Control participants completed more symbols per second compared to CFS (0.6 versus 0.70 (p=0.0351)). This shows that this cohort of CFS subjects had statistically significantly reduced psychomotor speed (and attention) compared to control subjects.

DSST	CFS mean (SD)	Controls mean (SD)	p value (unpaired t-test)
Symbols per second	0.6 (0.2)	0.7 (0.1)	0.0351*

* significant at 5% level

Table 55 Psychomotor speed CFS and controls

3.4.1.9. Attention

Table 56 shows comparison of attention between CFS and controls on ANT. There are no statistically significant differences between groups.

ANT	CFS (n=51) mean (SD)	Controls (n=10) mean (SD)	p value (unpaired t-test)
Alerting	13.6 (29.8)	20.7 (24.7)	0.48
Orienting	57.9 (46.2)	79.0 (42.8)	0.19
Inhibition	119.2 (48.4)	104.4 (46.0)	0.38
Central difference	135.1 (67.1)	126.9 (72.0)	0.73
No stimulus difference	104.5 (63.4)	99.1 (58.0)	0.81
Spatial difference	114.8 (58.3)	87.1 (41.1)	0.16

Table 56 Attention CFS and controls

3.4.2. DSQ

The following results show comparison of cognitive measures by DSQ subgroup. Participant numbers are as per premorbid IQ table 57, unless otherwise indicated.

3.4.2.1. Premorbid IQ

Premorbid IQ, as measured using NART, is comparable across all DSQ subgroups and not statistically significantly different, as seen in table 57.

All groups have a mean verbal IQ above average (320). Reading single words, as is done with NART, requires little sustained concentration. Significantly, however, NART has been shown to discriminate between healthy controls and cognitively impaired dementia subjects (326). The findings below, of a lower NART score in the Fukuda+2003+2011 subgroup, may reflect impaired cognitive ability in comparison to the Fukuda+2003 Research group or a loss of function (as reflected in findings of FIS and COMPASS scores), which may have contributed to an overall lower level of educational attainment.

	Fukuda (n=6)	**Fukuda + 2003 Research** (n=8)	**Fukuda + 2003 Clinical** (n=9)	**Fukuda + 2003 + 2011** (n=26)	**p value** (ANOVA)
NART	119.2 (6.6)	123.1 (3.1)	117.1 (6.6)	116.0 (9.2)	0.16

Table 57 Premorbid IQ by DSQ

3.4.2.2. Memory: Verbal

Tables 58 and 59 show comparisons of measures of verbal memory.

Table 58 compares verbal memory. There is a statistically significant inter-group difference in total recall over trials 1-5 on AVLT. Participants in the Fukuda+2003+2011 group performed worse (42.2) than the other three groups and those meeting a Fukuda+2003 Research 'diagnosis' performed best (53.0). This suggests that Fukuda+2003+2011 subjects have a less efficient verbal declarative memory compared to the other groups.

The Fukuda+2003+2011 group also had the lowest percentage retention on trial A7 (70.5%) and the Fukuda+2003 Research group the highest (84.2%). Although this was not statistically significant, it does suggest that delayed recall may be less efficient in the Fukuda+2003+2011 group.

Forward digit span measures are not statistically or clinically different across groups and are comparable to scores observed in control subjects in other studies (321).

	Fukuda (n=6) mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=26)** mean (SD)	**p value** (ANOVA)
Rey AVLT					
Total recall trials 1-5	51.3 (6.3)	53.0 (4.47)	49.4 (11.7)	42.15 (10.1)	0.014*
Forgetting (% retained from A6 on trial A7)	80.1 (23.2)	84.2 (14.8)	80.3 (12.0)	70.5 (24.2)	0.32
Forward Digit Span					
Clinical measure	6.7 (1.2)	6.4 (1.2)	6.9 (1.5)	6.6 (1.5)	0.91

* significant at 5% level

Table 58 Verbal memory by DSQ

3.4.2.3. Memory: Visuospatial

Table 59 shows the results of comparisons of visuospatial memory across DSQ subgroups. There are statistically significant differences across all measures on spatial span and VPT. Participants in the Fukuda+2003+2011 group underperformed in all measures compared to the other subgroups. This suggests that the Fukuda+2003+2011 group has a deficit in immediate working memory compared to the other groups. The scores in this group are lower than scores seen in control subjects in other studies (321, 322).

There is a statistically significant difference in VPT scores between groups. The Fukuda+2003+2011 group underperforms compared to the other subgroups.

	Fukuda (n=6) mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=26)** mean (SD) (n=25)**	**p value** (ANOVA)
Spatial span					
Forward longest sequence	6.3 (0.8)	5.6 (0.9)	5.4 (1.5)	4.6 (1.2)	0.0054*
Backward longest sequence	5.0 (0.9)	5.9 (0.6)	4.8 (1.6)	4.6 (1.2)	0.0655*
VPT					
Maximum number of targets	10.5 (1.2)	11.4 (1.9)	11.0 (2.0)	8.8 (2.2)	0.0045*

* significant at 5% level

** One participant was unable to complete the task

Table 59 Visuospatial memory by DSQ

These findings suggest that visuospatial working memory, which allows temporary retention and manipulation of information (327) – as assessed by spatial span – and short-term visuospatial memory – as assessed by VPT (328), differ by diagnostic criteria and may be impaired in participants meeting Fukuda+2003+2011.

3.4.2.4. Executive: Verbal fluency

Tables 60-63 show comparisons of measures of executive function.

There are no statistically significant differences in verbal fluency between subgroups, as seen in table 60. Nevertheless, participants in the Fukuda+2003+2011 group performed worse than other groups, with fewer correct words on FAS. The Fukuda+2003 Research group achieved the most number of words.

Although mean score in the Fukuda+2003+2011 group is lower than the other groups, it is in the lower range of normal when compared with mean normative scores on meta-analysis (323). These findings suggest that verbal fluency in this cohort of CFS subjects is within normal limits.

FAS	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=25)*** mean (SD)	**p value** (ANOVA)
Total correct	41.7 (10.9)	43.4 (4.9)	41.4 (11.2)	36.8 (10.9)	0.34

* One participant was unable to complete the task

Table 60 Verbal fluency by DSQ

3.4.2.5. Executive: Inhibition

Table 61 shows the results of measures of inhibition.

STROOP	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=25)*** mean (SD)	**p value** (ANOVA)
"Colour word" minus "average of word reading plus colour naming"	-41.5 (13.0)	-34.7 (15.7)	-34.7 (12.0)	-33.9 (15.7)	0.73

* One participant was unable to undertake the task due to colour blindness

Table 61 Inhibition by DSQ

There are no statistically significant differences between groups, however the Fukuda+2003+2011 subjects were the worst performers. Nevertheless, inhibition appears to be comparable across diagnostic criteria.

3.4.2.6. Executive: Mental manipulation

Table 62 shows a comparison of mental manipulation between DSQ subgroups assessed by clinical measure of reverse digit span. There are no statistically significant differences between groups.

Reverse digit span	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=26)** mean (SD)	**p value** (ANOVA)
Clinical measure	4.8 (1.9)	5.4 (1.3)	5.2 (0.8)	4.9 (1.3)	0.75

Table 62 Mental manipulation by DSQ

3.4.2.7. Executive: Set shifting

Table 63 shows a comparison of set shifting between DSQ subgroups as assessed with the trail making test. There are no statistically significant differences between groups, however the Fukuda+2003+2011 group took longer to complete the task on average – almost twice as long as the Fukuda alone group.

Trail making test	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=25)*** mean (SD)	**p value** (ANOVA)
Shift (B-A)	22.0 (9.5)	33.5 (20.9)	26.0 (10.1)	39.4 (27.8)	0.26

* One participant was unable to complete the task

Table 63 Set shifting by DSQ

3.4.2.8. Psychomotor speed

Table 64 shows that there are statistically significant differences in psychomotor speed between groups, measured using the DSST. The Fukuda+2003+2011 group under-performed compared to the other subgroups. This group also had a significantly reduced psychomotor speed and attention compared to other groups.

DSST	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=26)** mean (SD)	**p value** (ANOVA)
Symbols per second	0.7 (0.1)	0.7 (0.1)	0.6 (0.1)	0.5 (0.2)	0.0162*

* significant at 5% level

Table 64 Psychomotor speed by DSQ

3.4.2.9. Attention

Table 65 shows comparison of measures of attention using ANT. There are no statistically significant differences between groups. Nevertheless, the Fukuda+2003 Research and Fukuda+2003+2011 groups performed worse on the alerting compared to the other two groups.

ANT	**Fukuda (n=6)** mean (SD)	**Fukuda + 2003 Research (n=8)** mean (SD)	**Fukuda + 2003 Clinical (n=9)** mean (SD)	**Fukuda + 2003 + 2011 (n=24)*** mean (SD)	**p value** (ANOVA)
Alerting	23.0 (21.9)	5.4 (23.1)	18.6 (33.6)	5.6 (39.7)	0.59
Orienting	53.9 (52.5)	73.7 (37.8)	47.7 (18.1)	60.8 (53.8)	0.70
Inhibition	110.3 (57.3)	105.0 (30.7)	125.6 (28.6)	124.1 (57.6)	0.74
Central difference	136.2 (86.1)	127.3 (29.8)	131.6 (28.0)	140.3 (83.4)	0.97
No stimulus difference	94.8 (74.1)	89.8 (50.7)	117.4 (52.1)	109.0 (72.4)	0.81
Spatial difference	99.5 (56.9)	98.13 (59.2)	127.7 (35.8)	123.0 (64.6)	0.59

* Two participants were unable to complete the task

Table 65 Attention by DSQ

There were some statistically significant differences on cognitive assessment between DSQ subgroups. These results are discussed in section 4.1.2.

3.5. Summary

A summary of the main findings and the principle differences between DSQ subgroups is shown below, for ease of reference (tables 66-69).

	Fukuda alone	Fukuda + 2003 Research	Fukuda + 2003 Clinical	Fukuda + 2003 + 2011
IPAQ * (median)	819	30	908	33
FIS * (mean)	58.2	93.1	81.7	102.5
COGFAIL (mean)	42.0	54.1	49.6	58.2
COMPASS 31 * (mean)	17.3	24.0	21.0	31.0

Table 66 Summary of subjective questionnaire results by DSQ

	Fukuda alone	Fukuda + 2003 Research	Fukuda + 2003 Clinical	Fukuda + 2003 + 2011
Memory				
Rey AVLT total recall *	51.3	53.0	49.4	42.2
Spatial span forward *	6.3	5.6	5.4	4.6
Spatial span backward *	5.0	5.9	4.8	4.6
VPT *	10.5	11.4	11.0	8.8
Executive function				
FAS	41.7	43.4	41.4	36.8
Trail making	22.0	33.5	26.0	39.4
Psychomotor speed DSST *	0.7	0.7	0.6	0.5
Attention Alerting	23.0	5.4	18.6	5.6

Table 67 Summary of cognitive results by DSQ

* indicates result statistically significant at 5% level with ANOVA

	Fukuda alone	Fukuda + 2003 Research	Fukuda + 2003 Clinical	Fukuda + 2003 + 2011
Beat statistics (mean)				
HR (bpm)	69.57	75.69	73.07	76.59
mBP (mmHg)	82.21	75.14	83.65	79.56
Cardiac statistics (mean)				
SI (ml/m^2)	47.30	45.04	41.10	37.46
EDI	78.78	73.17	68.15	62.38
LVWI	3.68	3.28	3.26	2.97
LF/HF-RRI * (mean)	2.26	7.72	3.14	1.84
LF/HF-dBP (mean)	6.07	11.30	5.91	6.36
LF/HF-sBP (mean)	3.51	10.49	4.14	3.30
Parasympathetic modulators (mean)				
HFnu-RRI	42.90	34.77	31.40	45.26
HFnu-dBP	11.52	9.66	14.59	15.65
HFnu-sBP	14.17	9.53	16.77	21.02
Sympathetic modulators (mean) LFnu-RRI	57.10	62.37	68.60	54.74
LFnu-dBP	57.39	63.52	55.66	50.17
LFnu-sBP	45.06	57.48	46.85	43.14

Table 68 Summary of autonomic results at rest by DSQ

* indicates result statistically significant at 5% level with ANOVA

Criteria	Differentiating symptom requirements (condensed)
Fukuda criteria	Baseline criterion: ≥ six months of severe fatigue Plus ≥ four of the following: Post-exertional malaise Unrefreshing sleep Memory/concentration impairment Myalgia Arthralgia Headaches Lymphadenopathy Sore throat
Canadian consensus/DSQ clinical 2003	Addition of autonomic and neuroendocrine symptoms Perceptual and sensory disturbances Ataxia Muscle weakness Fasciculations Autonomic: orthostatic intolerance, PoTS, nausea, irritable bowel, urinary frequency, palpitations, exertional dyspnoea Neuroendocrine: loss of thermostatic stability, weight change Joint/muscle pain: can be widespread/migratory but specifically not involving abdomen or chest and no hyperalgesia
Canadian consensus/DSQ research 2003	All symptoms considered major Pain described as migratory and widespread: myofascial/joint/abdominal/head/chest
Canadian (International) consensus 2011	As Canadian consensus clinical 2003 but symptoms further categorised and greater number required to meet diagnosis Nature of pain can be migratory and widespread and can include chest and abdominal pain/generalised hyperalgesia

Table 69 Summary of differentiating symptoms by DSQ criteria

Chapter 4. Discussion

4.1. Main findings and comparison with existing studies

The main findings of this study will be discussed in the context of the original study aims and hypotheses and current literature. Differences between CFS and control groups are discussed first to provide context for the DSQ results.

4.1.1. Autonomic dysfunction

One of the aims of this study was to identify differences in AD by subgroup of CFS subjects, characterised by DSQ diagnostic criteria. It was hypothesised that AD does not differ by DSQ subgroup. A summary of the study findings is given below.

Subjective measures of autonomic function were statistically significantly different between CFS and control groups, with CFS subjects reporting greater impairment on COMPASS, as well as greater fatigue.

Objective testing did not reveal any statistically significant differences between CFS and control subjects. Resting HR and BP were at the lower end of the normal clinical reference range in both groups, as was cardiac function measured using SI, CI, EDI and LVWI. Examination of heart rate and blood pressure variability appeared to show a shift to increased sympathetic modulation in both groups. Baroreflex control was within normal limits in both CFS and control subjects.

Neither mean BP nor nadir on standing was statistically or clinically significantly different and 30:15 ratio – an indicator of parasympathetic activity – was within normal parameters. Valsalva manoeuvre findings suggest a dysautonomia in both groups with a reduced Valsalva ratio.

The findings summarised above show that there was no statistically significant objective difference in autonomic function in this cohort of CFS and control subjects. This differs from previous studies that have typically demonstrated a difference in autonomic function between CFS subjects and controls (124, 125) and may reflect a number of factors.

Firstly, sedentary controls were selected for participation. The intention in doing so was to match activity levels between CFS subjects and controls. The inclusion of non-sedentary (that is, potentially active) control subjects in previous studies may have resulted in autonomic dysfunction being demonstrated in CFS subjects compared to controls, arising secondary to inactivity and consequent deconditioning.

Both controls and CFS subjects appeared to exhibit some degree of dysautonomia and it is possible that this results from a sedentary lifestyle. Nevertheless, there is inconsistency in this study, in that controls – with higher IPAQ scores (and by implication therefore subjectively more activity) than CFS subjects – exhibit more features suggestive of dysautonomia compared to CFS subjects. Furthermore, this appears to be inconsistent with findings across DSQ subgroups, where the Fukuda+2003+2011 group has both lower activity levels on IPAQ and more features of clinical impairment on objective autonomic testing. This strongly suggests that there are other explanatory variables rather than deconditioning. This is further discussed in section 4.1.7.

A second possible explanation for the findings may stem from that fact that this study excluded potential participants with a history of co-morbid depression. Autonomic dysfunction has been found in depression. Subjects in this study did not meet criteria for MDD according to the SCID-I assessment tool. It is possible that autonomic dysfunction seen in other studies results from co-morbid depression and is not a primary feature of CFS. Consistent exclusion criteria across studies and research centres would enable better delineation of these two conditions and their presenting symptoms.

Finally, it is feasible that autonomic dysfunction, such as abnormalities in heart rate and blood pressure, experienced by CFS subjects is intermittent. Although assessment using the Task Force® Monitor provides a continuous measure, a limitation of the study methodology was that assessment was conducted during one time frame of approximately 20 minutes. If symptoms are experienced intermittently it is possible they are not being captured at the time of autonomic assessment, which may be exacerbated by testing under research – that is non-real life – conditions. This is a

possible explanation for the mismatch between subjective and objective autonomic features.

Subjective autonomic symptoms between DSQ subgroups were statistically significantly different, with the lowest mean score in the Fukuda alone group and the highest in the Fukuda+2003+2011 group (and Fukuda+2003 Research group). This pattern was reflected in findings from the IPAQ and FIS questionnaires, which revealed statistically significantly greater subjective fatigue and lower activity in the Fukuda+2003+2011 and Fukuda+2003 Research groups compared to Fukuda alone.

There were no statistically significant differences between groups on objective measures – with the exception of LF/HF-RRI. At rest, HR and BP were similar across DSQ subgroups groups. Cardiac statistics indicated decreased myocardial contractility in the Fukuda+2003+2011 group and decreased EDI and low CI and SI compared to the other subgroups. This group also showed decreased sympathetic modulation on HRV (LF/HF-RRI) and on BPV and decreased baroreflex sensitivity.

Measures on standing were comparable between diagnostic subgroups. Valsalva ratio was abnormal in each group, consistent with findings comparing CFS and control subjects. This may reflect an underlying dysautonomia or the low baseline blood pressure. It may also reflect a poor Valsalva technique where the manoeuvre was not maintained for an adequate duration or the optimum pressure was not reached.

Although not statistically significant, there were differences in objective autonomic parameters between DSQ subgroups. When considered at a clinical level these results show emerging between-group differences with a picture of greater objective 'impairment' in the Fukuda+2003+2011 subgroup where mean measures are overall lower for all parameters except TPRI. Measures in the Fukuda alone group are higher compared to other subgroups, also with the exception of TPRI.

It is significant that the Fukuda criteria do not require the presence of autonomic symptoms and this in itself may explain the findings. Equally, it is possible that there is an additive or cumulative effect across DSQ subgroups where more symptoms and the

potential for more migratory, widespread and potentially hyperalgesic pain with the Canadian 2011 criteria compared to Fukuda and Canadian 2003 Clinical results in greater symptom burden and disease severity.

This picture may represent different disease phenotypes across different diagnostic groups, suggesting that the preliminary hypothesis – AD does not differ by diagnostic criteria met – may not be true when considered at a clinical level. Subjects meeting the Fukuda+2003+2011 criteria appear to exhibit potentially clinically significant greater impairment compared to the other subgroups, in particular those meeting the Fukuda alone criteria. Results suggesting lower cardiac muscle elasticity, lower stroke volume and reduced cardiac output in this subgroup add weight to this argument.

The challenge is determining whether these additional symptoms are core to CFS and point towards a subgroup of patients with greater functional impairment and the possibility that CFS is a condition that lies across a disease spectrum, or whether they represent co-morbid disease or other non-specific, prevalent somatic symptoms and are confounding research into this condition. In either instance improved and consistent study criteria and delineation of CFS "diagnoses" through the use of a tool such as the DSQ in future research will provide a strong basis from which to answer these questions.

4.1.2. Comparison with existing autonomic studies

Study findings on self-reported symptoms of autonomic dysfunction – showing statistically significantly greater impairment among the CFS cohort compared to controls – are consistent with those of other studies (26, 122, 123). This strengthens existing evidence that subjective autonomic symptoms are common in CFS patients.

This study found no statistically significant differences on objective autonomic testing between CFS and control subjects, which is consistent with some previous studies and conflicts with findings from others. This highlights the challenges of determining underlying pathophysiology where study findings are not always reproducible. Furthermore, it weakens the argument for a causal relationship (between dysautonomia and CFS) according to the Bradford-Hill criteria (329).

Meeus *et al*'s recent systematic review of six case control studies concluded that there is moderate evidence of decreased parasympathetic activity in CFS subjects with reduced heart rate and LF/HF and evidence of increased sympathetic activity on HUT (125). As discussed previously, concerns have been raised regarding the methodological quality of these studies and the validity of objective assessment. Tak *et al* argue that there is inadequate evidence to determine the role that AD has in CFS (153), with some studies showing no evidence of AD in CFS (135, 144).

The findings reported in this thesis may result from a lack of statistical power, such that statistical significance at the 5% level was not detected. Furthermore, the inclusion and exclusion criteria may be significant. If these findings are considered in the context of the use of sedentary controls, this could indicate that deconditioning has a role in the presence of the dysautonomia in CFS found in other studies where comparison is made to physically active controls. Another important factor may be the exclusion of MDD. AD has been found in MDD and raises the question of whether autonomic symptoms seen in other studies result from co-morbid depression, or even whether MDD and CFS are conditions which lie on the same disease spectrum.

COMPASS scores analysed by DSQ criteria revealed a consistently high score across all subgroups with statistically significant variation in score by subgroup. Fukuda alone scored the lowest and Fukuda+2003+2011 the highest.

This may be explained by the fact that Fukuda criteria do not require the presence of autonomic symptoms. It also points towards the Fukuda+2003+2011 criteria representing a more severe disease phenotype with greater functional impairment. This is similar to findings in other studies, where comparison of Fukuda and Canadian clinical criteria has shown that those meeting the Canadian criteria have more physical symptoms and greater functional impairment (5, 330, 331).

No other studies to date have examined differences in objective autonomic assessment between DSQ subgroups.

Although difficult to determine the role that deconditioning and MDD have in CFS and associated AD, the mixed findings indicate that there is a need to use matched controls in future studies, where MDD is excluded and activity levels are determined to enable recruitment of controls with sedentary lifestyles.

4.1.3. Cognitive function

Another aim of this thesis was to identify differences in cognitive impairment by subgroup of CFS subjects, determined by DSQ diagnostic criteria, to investigate the hypothesis that cognitive impairment does not differ by diagnostic criteria met.

Subjective measurement of cognitive function with COGFAIL showed statistically significant differences between CFS and control subjects with CFS participants reporting impaired function.

Objective measurement with a validated battery of tests showed statistically significant differences in psychomotor speed on DSST with CFS participants performing slower than controls. Other measures were comparable between groups and gave normative results. These included verbal and visuospatial memory, all assessed measures of executive function (verbal fluency, inhibition, mental manipulation and set shifting) and attention.

Performance on DSST is dependant particularly on psychomotor speed and attention (207). The dissociation with impaired DSST performance and normal performance on tests of attention in CFS suggests that the DSST impairment in CFS may be caused by reduced psychomotor speed.

Studies show that physical activity is associated with a protective effect on psychomotor processing speed (208). In this context, the finding that CFS subjects reported lower IPAQ scores compared to controls may offer an explanation. It is also possible that this represents an important feature of CFS where loss of psychomotor ability is a defining characteristic. Delineating the roles that processing and motor speed have in this is challenging, however, and has important implications in terms of the nature of the impairment, the domain affected and possible treatment.

Memory and executive function were not statistically different between controls and CFS groups but the different levels of functioning seen may have clinical significance. CFS participants recalled a lower total number of words over five trials of AVLT and retained a lower percentage, a measure of verbal memory. Similarly, controls appeared to demonstrate better executive function in verbal fluency and mental manipulation domains, achieving more correct words on FAS and recalling a greater number of digits on reverse digit span.

This may indicate a loss of function in CFS that is subjectively significant and represents or exacerbates symptom burden and loss of functioning, in particular in terms of ability to continue in employment or studies.

There were no statistically significant differences between DSQ subgroups on self-reported cognitive impairment with COGFAIL. Nevertheless, Fukuda+2003+2011 subjects reported higher scores compared to Fukuda alone, pointing towards greater self-perceived cognitive impairment in this group. This may be significant given the findings that this subgroup also appears to exhibit clinically greater impairment on autonomic assessment.

Comparison of objective measures between DSQ diagnostic subgroups revealed statistically significant between-group differences.

Verbal memory – as assessed using the AVLT total score – was more impaired in the Fukuda+2003+2011 subgroup than the Fukuda alone group. Digit span was comparable across groups.

Assessment using AVLT is thought to provide a reflection of arousal, motivation, attention and concentration, as well as immediate verbal memory. Age has been shown to affect total recall score, with those above aged 60 scoring lower, as has gender, with women tending to score higher (207); however, this is unlikely to offer explanation for the study findings as age and gender spread were balanced between groups.

These differences in verbal memory may result from a stimulus overload, as seen when differences in AVLT and digit span favour AVLT (207). This arises from confusion where there is otherwise intact immediate memory and concentration. This overload may reflect greater fatigue or loss of function, which is consistent with the findings reported on subjective questionnaires. Stimulus overload is a common feature of CFS and patients can experience problems processing large amounts of information. This may be due to impaired inhibition, although this is not shown in the results here (with STROOP), or to abnormalities in the neurotransmitter functioning, such as serotonin.

Statistically significant differences were observed between groups on visuospatial memory assessment. Working memory and short-term visuospatial memory appeared to be more impaired in the Fukuda+2003+2011 group. This group also had greater impairment compared to findings in controls in other studies (321, 325). The Fukuda alone group showed the least impairment.

Assessment of executive function revealed no statistically significant differences between DSQ subgroups. Although executive verbal function on FAS was lower in the Fukuda+2003+2011 group, it was comparable to normative scores seen on meta-analysis (323). Set shifting measured on TMT was worse in the Fukuda+2003+2011 group compared to the other subgroups and may reflect impaired psychomotor function (332), particularly when considered in the context of findings on DSST.

There were statistically significant between-group differences on DSST assessment. The Fukuda+2003+2011 group achieved fewer symbols per second compared to other groups. This suggests that there may be some psychomotor impairment in this group, which may reflect the increased symptom burden, the presence of more widespread, severe or hyperalgesic pain – as seen in the 2011 diagnostic criteria, or impaired processing speed.

There were no statistically significant between-group differences in attention, as measured on ANT. Nevertheless, alerting (a measure of arousal) appeared to be clinically worse in the Fukuda+2003 Research and the Fukuda+2003+2011 groups. The

executive element was similar between subgroups, which is consistent with other findings.

In summary, there were statistically significant differences in psychomotor speed, visuospatial memory and some measures of verbal memory across DSQ subgroups, with Fukuda+2003+2011 consistently underperforming compared to other cohorts. This mirrors subjectively higher scores on COGFAIL, indicating more cognitive impairment, greater fatigue on FIS and more AD symptoms on COMPASS. Fukuda alone and Fukuda+2003 Clinical appeared to perform better.

It is not possible to draw conclusions regarding statistically significant between-group differences when using ANOVA, however there does appear to be a subset of CFS subjects in this study that is more severely cognitively impaired - that is those meeting the Fukuda+2003+2011 criteria (who also appear to have clinically greater autonomic dysfunction).

This differs from the original hypothesis that cognitive impairment does not differ by diagnostic criteria met and points towards greater functional impairment in subjects meeting the Fukuda+2003+2011 criteria, suggesting that this may represent a more severe disease phenotype compared to Fukuda alone.

The interplay between fatigue and cognitive function is important to consider, particularly in the context of possible stimulus overload and different apparent levels of impairment between DSQ subgroups.

Cognitive impairment has been seen in a number of conditions associated with impaired sleep, including sleep apnoea. A study comparing cognitive performance between CFS and sleep apnoea subjects assessed verbal and visuospatial memory using AVLT and digit span and found impairment in both groups compared to normative data. They found no between-group differences on AVLT recall but more severe impairment in sleep apnoea subjects on digit span, as well as on measures of psychomotor performance, concluding that overall impairment was worse in sleep apnoea subjects compared to CFS subjects (254).

This suggests that cognitive impairment may arise secondary to symptoms of fatigue, rather than be a primary feature of CFS. This is supported by the fact that the most severe cognitive impairment in this study was found in the DSQ subgroup Fukuda+2003+2011, which also reported greater fatigue on FIS.

The role that pain may have to play in this also deserves further investigation, in light of evidence that pain can impair cognitive function (333) and in view of the potentially different characteristics of the pain included in different criteria.

The nature of the pain described in the Canadian 2011 criteria – which can be widespread, hyperalgesic in nature and include abdominal and chest pain - may be more severe or widespread than in subjects in other subgroups and may exacerbate poorer cognitive function.

Finally, the consistent clinical underperformance in the Fukuda+2003+2011 group across autonomic and cognitive assessment also points towards a possible relationship between these two processes. The analysis and research conducted here do not allow for more than supposition, however in light of the link between autonomic measures such as blood pressure and cognitive function (see section 1.14) it is possible that there is an association between the two. This merits further investigation.

4.1.4. Comparison with existing cognitive studies

The findings of greater subjective symptoms of cognitive impairment in this study cohort are consistent with findings in other studies (230, 231) and strengthen the evidence that CFS patients self-report cognitive problems.

The challenges of objective assessment of cognitive impairment include the use of different tests and lack of knowledge of baseline ability. Current literature of cognitive impairment in CFS shows a mixed picture, with meta-analysis indicating possible overall deficits in attention, memory and reaction time (219). Few studies have reported impaired psychomotor skills in CFS.

This contrasts to findings in this study, which showed no statistically significant differences in memory or attention between CFS and control subjects and statistically significant differences in psychomotor speed on DSST. This may reflect a loss of physical rather than cognitive function or impaired processing speed resulting from stimulus overload.

The lack of consistency with other studies does contribute to an overall picture of the challenges associated with the use of different test batteries and gives weight to the argument that adopting a uniform set of tests (and inclusion/exclusion criteria) across all studies may aid comparison of results.

No other studies to date have assessed objective cognitive parameters by DSQ subgroup. A small number of studies examining phenotypes by different diagnostic criteria on self-reported symptoms show that there is greater subjective functional impairment and fatigue in patients meeting the Canadian Clinical criteria (2003) compared to the Fukuda alone criteria (5, 51). This is consistent with the findings presented here, which further build on this and show that subjects meeting Fukuda+2003+2011 have the greatest functional impairment compared to other DSQ subgroups.

4.1.5. Depressive symptoms

This thesis aimed to explore the prevalence and nature of depressive symptoms in this well-defined cohort of CFS subjects, with the hypothesis that depressive symptoms are common in CFS.

The nature of conducting this research study meant that there was time at each screening visit to explore depressive-type symptoms in depth to understand their nature and origins and help differentiate whether they were better accounted for by depression or CFS. It is possible that patients seen in a primary care setting may have been found to meet criteria for depression, where time restraints necessitate a more "tick box" approach to diagnosis. Equally, while mental health professionals also explore the nature of these symptoms in greater depth, is it possible that CFS is not a

diagnosis at the forefront of their minds and is therefore missed, in favour of a diagnosis of depression.

The prevalence of MDD in the study cohort compared to the UK as a whole was higher. This may be due to the considerable disease burden associated with CFS, which predisposes to co-morbid depression: the prevalence of depression in chronic disease is estimated to be 20% (334). It may also be explained by the fact that there are overlapping symptoms, the natures of which are difficult to distinguish and characterise and this may result in misdiagnosis. Equally, these overlapping symptoms may be the manifestation of a common underlying aetiopathogenesis encompassing two conditions that lie across a disease spectrum.

Nearly 40% of this CFS cohort reported symptoms of depression, which included secondary symptoms such as change in appetite, sleep disturbance and loss of energy and concentration. Some of these occur in many diseases and are therefore not defining features of depression. They are significant as they may be presenting symptoms of patients later diagnosed with CFS but may initially be thought to be associated with depression, a more prevalent diagnosis.

Subsequent exploration of these depressive-type symptoms may go on to reveal low mood or cessation of activities, contributing to a diagnosis of depression, but which arise as a consequence of CFS and resultant functional impairment, frustration and guilt about the burden on family members.

Differentiating between the “depressed mood” of depression and the “frustration and low mood” in CFS is key to correct diagnosis. One study participant with co-morbid CFS and MDD stated that feeling down in MDD improved with exercise and did not with CFS, thereby enabling them to differentiate between the two. CFS participants also highlighted a difference between a lack of motivation and loss of pleasure to perform activities (with depression) compared to a lack of ability but with a strong motivation (with CFS). These differentiations are significant but may be under-explored by clinicians.

In total, 63% of the CFS subjects in this study reported no symptoms of depression on SCID-I assessment. A significant limitation of screening for depression using DSM criteria was that if patients reported no "primary" symptoms – feeling depressed or down, or loss of interest – no enquiry was made regarding other "secondary" symptoms. It is therefore likely that secondary symptoms that overlap with CFS symptoms, such as sleep disturbance, psychomotor change and loss of concentration, were under-reported and not included in the analysis.

Anecdotally, CFS patients report trials of antidepressant medication. In this study, 15% of CFS participants with no recorded depressive symptoms were prescribed antidepressants. It is important to note that actual numbers of participants are small; however, the finding may have important implications.

Estimation of antidepressant use in a non-depressed population without CFS is difficult. A Canadian study examined data from the Canadian Community Health Survey from 2002 and showed that 5.8% of Canadians were prescribed antidepressants, compared to a 4.8% prevalence of a major depressive episode. Of those taking antidepressants 33.1% had a past-year episode of major depression. Of those without a past-year episode of depression, over 60% had had migraine, fibromyalgia, anxiety disorder, or past depression (335).

This suggests that antidepressant medication is used for reasons other than depression, perhaps as a form of pain management or as a trial for hard-to-treat symptoms. The lack of improvement in CFS symptoms seen in studies exploring their efficacy (174) suggests that their use may be unsuitable and that in the absence of co-morbid depression their prescription may be inappropriate and perhaps erroneously intensify the perception of CFS as a psychiatric disorder.

These findings illustrate the challenges of understanding which symptoms are better attributed to CFS and which to depression. They also suggest that the original hypothesis that depressive symptoms are common in CFS may be correct but that there are significant challenges in determining whether these symptoms are better

accounted for by depression or by CFS and that their proper exploration is central to correct diagnosis.

4.1.6. Completion of a complex and physically-demanding study

The final aim of this thesis was to determine whether CFS patients can participate in a study that involves considerable personal burden.

Of the CFS subjects enrolled, 86% (51/58) completed the full study and were able to undergo combinations of six half-day or three full-day visits, plus a screening visit, within a three-month period. Furthermore, 100 CFS patients (59% of the total sent information sheets) expressed interest in participating, requesting that they be contacted for initial screening by the study team.

Not only does this indicate that this cohort of CFS subjects was highly motivated to complete the study, it also implies that there is a widespread motivation from patients to investigate the aetiology of CFS. This motivation may stem from a wish to determine the physiological basis for symptoms and enable treatment. It may also be to counter the stigma associated with the condition and "prove" to disbelievers that CFS has been and continues to be wrongly attributed to malingering.

This determination and resilience – particularly in the face of stigma – also strongly suggests that this cohort of CFS subjects was highly motivated and optimistic about the outcome of research, which may signal an important differentiating feature from depressive symptoms, where feelings of hopelessness tend to dominate.

These findings indicate that the opening hypothesis is correct: CFS patients are able to participate in and complete a complex and physically-demanding study. Nevertheless, the longer-term impact of the physical, mental and emotional exertion of participation was not measured in this study and the consequences may be considerable.

Of particular note, and in relation to this, is the fact that participants necessarily had to be ambulatory in order to meet the inclusion criteria and actively participate in investigations. As a result, while there was a spectrum of disease severity amongst the

CFS subjects included in this study, it is likely that at the time of investigation those enrolled largely represented a “milder” phenotype and more severely affected patients were not included. This has implications for the study findings, as including participants with more severe disease may have shown a larger effect size – with more autonomic and cognitive impairment – in comparison to controls or between DSQ groups.

4.1.7. The role of deconditioning

All participants were considered sedentary at the start of the study, undergoing fewer than 30 minutes of exercise three times a week. Despite this, the CFS group reported significantly lower levels of activity on IPAQ compared to controls. Even with the possibility of bias when using subjective methods to assess activity levels, this indicates that there is considerable variation in levels of activity within populations that are classified as sedentary. In the context of this study, it suggests that the CFS cohort has a significantly less active lifestyle than sedentary controls, which may result from the impact of greater functional impairment.

Furthermore, there is a significant difference in IPAQ score between DSQ groups, with Fukuda alone and Fukuda+2003 Clinical scoring higher than Fukuda+2003 Research and Fukuda+2003+2011.

An important question concerns direction of causality: whether lower activity results from symptom burden, for example fatigue or autonomic symptoms, or whether these symptoms arise from a less active lifestyle and deconditioning.

It is not possible to draw conclusions regarding this. Nevertheless, there is inconsistency between IPAQ results and objective markers of dysautonomia – reduced IPAQ score does not correspond to reduced cardiac output or increased heart rate (see tables 64 and 66). Although this should be interpreted cautiously, given the limitations of the IPAQ questionnaire and the absence of statistical analysis to corroborate the observation, this implies that the dysautonomia seen in the Fukuda+2003+2011 group may be part of the disease symptomatology that in itself contributes to increased fatigue and loss of function rather than due to deconditioning.

Furthermore, there is a statistically different level of subjective functional impairment between DSQ subgroups on FIS and COMPASS, with Fukuda+2003+2011 showing greater impairment across all measures including COGFAIL. This group of subjects was also less likely to have continued education longer than 11 years and had fewer further education qualifications (27% had obtained degree level education compared to 83% of Fukuda alone subjects). Therefore, it may be that greater impairment impedes conventional achievement and exacerbates a loss of social functioning, including activity levels.

Overall, these findings suggest that the Fukuda+2003+2011 subgroup has a higher degree of functional impairment, which is reflected in a lower IPAQ score. This may result from the greater number of symptoms required and the addition of potentially more widespread, migratory pain where hyperalgesia is a feature (stemming from the 2011 criteria).

4.2. Conclusion

The findings of this study show significant differences in subjective cognitive and autonomic measures between DSQ subgroups. They also show clinical between-group differences in objective assessment of autonomic and cognitive function. This suggests that CFS – as classified using current diagnostic criteria – may constitute a disease spectrum, with different phenotypes and severities.

The differences observed between DSQ subgroups may be a reflection of the *additive* effect of the diagnostic criteria. The absence of autonomic symptoms in the Fukuda criteria implies a different, less severe, disease phenotype with fewer features of AD. In contrast, symptoms of AD are present in the 2003 criteria and include ataxia, muscle weakness and OI. Further still, the 2011 criteria have a requirement for a greater symptom burden, which can include widespread migratory pain and hyperalgesia.

The Fukuda+2003+2011 subjects appear to have greater autonomic and cognitive impairment. The presence of autonomic symptoms, such as muscle weakness, and the possibility of more widespread, severe pain may explain poorer psychomotor performance in the Fukuda+2003+2011 subgroup. However, given that the

Fukuda+2003 (Research and Clinical) criteria subjects appeared to perform better, this suggests that, rather than the presence of autonomic symptoms, it may be increased symptom burden or type of pain that reflects a more severe disease phenotype.

The possibility that current criteria include symptoms that are not primary features of CFS, that represent distinct, undiagnosed co-morbid disease and that may be confounding clinical presentation and research, must also be considered. The number of symptoms included in the Canadian 2011 criteria and the number of physiological systems that can be implicated may mean that, rather than diagnosing a more severe CFS phenotype, these criteria in fact capture CFS and other co-morbidities with non-specific symptoms. This is important not only because it is likely to affect management and subsequent prognosis, but also as it may give rise to an inaccurate and confused picture of which condition (or conditions) is being researched.

Furthermore, it is possible that the inclusion of more widespread pain with or without hyperalgesia in the 2011 criteria may be a confounding symptom – particularly in view of the association between pain and both autonomic dysfunction and cognitive impairment (attention, psychomotor speed, verbal and working memory) (333, 336, 337).

There were significant differences in subjective measures between CFS and control subjects, with CFS subjects reporting greater impairment. Objective autonomic and cognitive assessment provided a mixed picture and may reflect the small number of control subjects and signify that the study was underpowered. Another potential explanation comes from the fact that this study both excluded depression and recruited sedentary controls.

The role that co-morbid psychiatric disease and deconditioning may play in results from other studies must be considered. While some of those excluding co-morbid depression have found autonomic features in CFS subjects and not in controls, others have not (134, 142, 144). It is therefore important to consider whether AD arises from co-morbid depression or whether both depression and CFS – with their overlapping symptomatology – represent a spectrum of disease where AD is a feature in some but

not all patients. Future research should explore this possibility and adopt a consistent approach to establish the presence of depression in CFS subjects.

Similarly, the impact of deconditioning in CFS, its potentially confounding role and the implications for findings of AD is significant and one which should be recognised in the design of future studies.

Finally, this study shows that CFS patients are highly motivated to participate in research to explore the pathology underlying this condition. This presents a strong argument for larger-scale studies which include more severely affected patients that will enable broader and more in-depth research into what may be a spectrum of disease.

4.3. Strengths and limitations

4.3.1. Strengths

The MRC study recruited a highly-motivated cohort of CFS subjects who were able to undergo a comprehensive series of investigations to give a dataset which is almost 100% complete. The number of questionnaires and objective tests conducted means that for the first time it has been possible to look in-depth at potential phenotypic differences between DSQ subgroups.

The data have also illustrated that CFS patients are able – and prepared – to undergo and complete such a comprehensive study, which may allow for future comprehensive research.

Screening participants for depression has made it possible to begin to understand the overlap between MDD and CFS, as well as helping to define some of the differentiating features. Furthermore, it sets a president for future research that controls for co-morbid depression that will allow greater understanding of whether they are distinct pathologies or whether they lie on the same disease spectrum.

4.3.2. Limitations

There are limitations to these data. The study may lack statistical power. The original intention was to recruit 71 CFS participants and ten controls, which was not possible within the specified time period. The sample sizes in the DSQ groups are small, which means it is difficult to conclude that the differences seen are significant, however these differences are consistent across all questionnaires, which points towards variation in phenotypes. Finding healthy volunteers who were prepared to act as controls and participate in all investigations of the original MRC study was difficult and this is in part reflected by the small number of controls, which does limit comparison between groups.

The smaller cohort may have resulted in a small effect size and subsequent statistical significance remaining undetected. Findings in this study of no statistically significant differences may therefore have resulted from a type II error – wrongly concluding there was no difference between cohorts. The need for a realistic and pragmatic approach to recruitment meant that further recruitment was not possible within the study timeframe and therefore analysis was conducted using existing data but this is noted as a significant limitation.

The challenge of recruiting control subjects who were both sedentary and prepared to commit to the number of investigations required for the wider MRC study meant it was necessary to permit inclusion of family members of CFS participants. This aided the recruitment of a minimum number of controls and concerns a very small number of subjects. Nevertheless, research suggests that there is an underlying genetic component to the pathophysiology of CFS and this implies that family members may exhibit similar phenotypic features to CFS subjects and therefore not allow for adequate differentiation on objective assessment.

The Task Force® Monitor was calibrated prior to the start of the study. This calibration was not repeated at the study close. It is therefore difficult to determine that after 18 months of autonomic assessment the measures were still being accurately recorded. This is a limitation that is acknowledged here and that should be addressed in

subsequent studies. This lack of calibration may also have affected the equipment used to conduct the Valsalva, giving erroneous readings.

For the purposes of the study the CFS and control groups were matched in terms of living a sedentary lifestyle. The IPAQ was used to give a more in-depth understanding of activity levels between groups. There are a number of limitations with using the IPAQ, which may have affected results. Firstly, caution has been advised when using the IPAQ score as an outcome measure in studies (338) and its validity as an objective measure of activity and its test-retest reliability have been questioned (339, 340). Secondly, a number of participants reported problems completing the questionnaire, as they found it difficult to quantify how much time they spend doing particular activities.

All the participant questionnaires examined self-reported measures and were completed independently by each participant. There is a possibility of both reporting and recall bias with this approach. CFS participants may be more likely to report severe symptoms in order to raise awareness of their condition, or may be more acutely aware of such symptoms than a healthy participant who may never have considered their presence previously. Recall bias is also likely to be a problem, in particular in consideration of CFS participants who consider themselves to have reduced cognitive function and poor memory.

Participants in this CFS cohort necessarily had to be well enough to attend hospital for appointments and therefore more severely affected CFS patients are significantly under-represented in this study. Subjectively reported fatigue severity has been found to positively associate with higher COMPASS score (122). It is possible that more severe disease may be associated with greater AD and would therefore differ from controls.

There is a high degree of inter-rater and test-retest variation when implementing the SCID-I (341). All except one assessment – which was subsequently excluded – were conducted by one assessor, however, there is a possibility that outcomes would vary depending on variables such as day of assessment and does imply that participants

with co-morbid or past depression may have been wrongly included. Participants may also have under-reported symptoms to enter into the study or be subject to recall bias.

In order to better clarify the potential for misdiagnosis between the two conditions future screening using the SCID-I would be improved by asking all participants about all symptoms, not limiting questions about "secondary" symptoms dependent on the presence of depressed mood or loss of pleasure.

Finally, this was an observational study. As a consequence, it is not possible to determine causality. Such a design is, however, more practical given the relatively low incidence of CFS, which would mean that a cohort study – although providing stronger evidence – would be extremely costly and would need to be conducted over a considerable time period.

4.4. Implications

This is the first study investigating objective differences in autonomic and cognitive features across DSQ subgroups. Its findings begin to paint a picture that CFS represents a disease spectrum, with different disease severity, or even different diseases with distinct underpinning aetiologies.

Subjects meeting the Fukuda+2003+2011 criteria appear to perform less well on objective cognitive and autonomic assessment and report greater functional impairment on subjective questionnaires compared to subjects meeting Fukuda alone. Significant differences between criteria include the addition of autonomic symptoms, greater number of symptoms and the nature and extent of pain.

On the basis of the study findings, the current use of different diagnostic criteria to define CFS in different studies appears at best to be assessing different severity phenotypes and may even be assessing different diseases. This has potentially very significant repercussions for furthering understanding of the underlying aetiopathogenesis of this debilitating condition and is likely to be contributing to the sometimes conflicting and mixed results seen across studies.

This is exacerbated by the overlap between CFS symptoms and symptoms of depression. Lack of consistent exclusion for this co-morbidity and lack of in-depth knowledge of the complex interplay between the two conditions imply that existing research is exploring subsets of patients with CFS, depression or both conditions. Better understanding these symptoms is key to distinguishing between the two and examining whether they lie across one disease spectrum or represent distinct pathologies. Furthermore, it will aid identification of appropriate treatment and management options with the aim of improving quality of life for patients.

There is evidence from the findings of this study of clinical differences in objective autonomic parameters across groups of CFS subjects. Damage to the autonomic nervous system appears to take the form of an initial sympathetic over-modulation followed, in more severe disease, by sympathetic underactivity and increased parasympathetic modulation, as seen with subjects meeting the Fukuda+2003 Research and Fukuda+2003+2011 criteria respectively. This supports the theory that abnormalities in the autonomic nervous system are a potentially important feature of CFS and hold promise for better understanding the underlying pathophysiology of this condition.

When considered in the context of wider research findings and current theories there is a strong argument for a trigger in the form of an infectious agent, such as EBV or human herpes virus 6, with subsequent damage to the autonomic nervous system resulting from vagal nerve damage. This may be particularly so in individuals who have an underlying genetic susceptibility.

Adopting a consistent approach to the inclusion of controls with matched activity levels and exclusion or in-depth classification and understanding of co-morbid depression will allow for better differentiation between what may be a CFS spectrum or different diseases.

Furthermore, a greater understanding of the implications of adopting different diagnostic criteria needs to be prioritised, and is central to better elucidating the core characteristics of CFS.

These approaches will aid researchers in their understanding of whether AD is a central feature of the disease and whether it represents a neurological underpinning aetiopathogenesis or a confounder that arises secondary to underlying pathology.

4.5. Future research

This research did not assess the longer-term impact on CFS subjects of completing a study of this magnitude. Capturing this information, in the form of repeat questionnaires or qualitative interview, would enable understanding of the way in which such a physically, mentally and emotionally demanding study affects CFS participants.

Anecdotally, CFS patients report fatigability with exertion over time. Conducting repeated cognitive assessment over consecutive days may aid understanding of whether this self-reported fatigability is also seen on objective testing, and this may represent an important disease marker which has not hitherto been investigated.

Better defining the phenotype of DSQ subgroups is central to improving understanding of the pathophysiology that underpins CFS and what may be a spectrum of disease. Current research – and the use of different diagnostic criteria – may be investigating different disease phenotypes or even different diseases and co-morbidities.

Furthermore, the shared symptomatology between MDD and CFS raises the possibility that some or all of these shared symptoms are complicating research by incorrectly selecting individuals with either one or both conditions, or that these diseases may be part of a similar disease spectrum.

Investigating patients with more severe disease is key to understanding whether it is a disease spectrum or distinct conditions that are seen in what is currently termed CFS. This might be achieved through the use of portable equipment that can be taken to patients' bedsides at home.

Finally, a more consistent approach to conducting future research would enable better comparison between studies and reduce the role that confounders have to play in

findings. The use of uniform assessment methods and tools, as well as consistent exclusion for co-morbid depression and well-matched sedentary controls, should allow for improved within and between study comparisons.

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Appendices

A Presentations resulting from the study

Maclachlan, L., Watson, S., Blamire, A., Gallagher, P., He, J., Finkelmeyer, A., Newton, J.L.. *Understanding the pathogenesis of autonomic dysfunction in chronic fatigue syndrome (CFS) and its relationship with cognitive impairment*. Poster presentation at ME/CFS Research collaborative launch, London, UK. April 2013.

Maclachlan, L., Watson, S., Gallagher, P., Finkelmeyer, A., Blamire, A., Newton, J.L.. *Prevalence of depressive symptoms assessed using the structured clinical interview for DSM-IV in chronic fatigue syndrome*. Poster presentation at IACFS/ME biennial conference, San Francisco, USA. March 2014.

B Questionnaires

Autonomic Symptom Profile

Answer every question by darkening the appropriate oval. If you are unsure about how to answer a question, please give the best answer you can. Please darken the corresponding oval completely. Fill in the number in the box if provided. This is an American questionnaire – so some of the spellings are strange and the numbers erratic, please just ignore this and answer the questions as they appear. Many thanks.

18. In the past year, have you ever felt faint, dizzy or 'goofy' or had difficulty thinking soon after standing up from a sitting or lying position ?
    - O 1 Yes *If you marked Yes go to question 19.*
    - O 2 No *If you marked No go to question 37.*

19. When standing up, how frequently do you get these feelings or symptoms ?
    - O 1 Rarely
    - O 2 Occasionally
    - O 3 Frequently
    - O 4 Almost always

20. How would you rate the severity of these feelings or symptoms ?
    - O 1 Mild
    - O 2 Moderate
    - O 3 Severe

21. For how long have you been experiencing these feelings or symptoms ?
    - O 1 Less than 3 months
    - O 2 3-6 months
    - O 3 7 to 12 months
    - O 4 13 months to 5 years
    - O 5 more than 5 years
    - O 6 as long as I can remember.

22. In the past year, how often have you ended up fainting soon after standing up from a sitting or lying position ?
    - O 0 Never
    - O 1 Once
    - O 2 Twice
    - O 3 Three times
    - O 4 Four times
    - O 5 Five or more times

23. How cautious are you about standing up from a sitting or lying down position ?
O 1 Not cautious at all
O 2 Somewhat cautious
O 3 Extremely cautious

24. What part of the day are these feelings worst ? (check one only)
O 1 Early morning
O 2 Rest of the morning
O 3 Afternoon
O 4 Evening
O 5 At night, when I get up after I've been sleeping
O 6 No particular time is worst
O 7 Other time, please specify ……

25. In the past year, have these feelings or symptoms that you have experienced:
O 1 Got much worse
O 2 Got somewhat worse
O 3 Stayed about the same.
O 4 Got somewhat better
O 5 Got much better
O 6 Completely gone.

Please rate the average severity you have experienced in the past year for each of the following symptoms.

	never had	Mild	Moderate	Severe
26. Rapid or increased heart rate (palpitations)	O 1	O 2	O 3	O 4
27. Sickness to your stomach (nausea) or vomiting ?	O 1	O 2	O 3	O 4
28. A spinning or swimming sensation ?	O 1	O 2	O 3	O 4
29. Dizziness ?	O 1	O 2	O 3	O 4

30. Blurred vision ? O 1 O 2 O 3 O 4

31. Feeling of weakness ? O 1 O 2 O 3 O 4

32. Feeling shaky or shaking sensation? O 1 O 2 O 3 O 4

33. Feeling anxious or nervous ? O 1 O 2 O 3 O 4

34. Turning pale ? O 1 O 2 O 3 O 4

35. Clammy feeling to your skin ? O 1 O 2 O 3 O 4

36. Do you have any biological (blood, natural) relatives among your patients, grand parents, brothers, sisters, or children who have frequent dizziness after standing from a sitting or lying position ?

O 1 Yes O 2 No If Yes, please list their names and relationships to you.

Name	Relationship
…….	…….
…….	…….
…….	…….

In the past year, have you ever felt faint, dizzy, or 'goofy' or had difficulty thinking:

37. soon after a meal ? O 1 Yes
O 2 No

38. after standing for a long time ? O 1 Yes
O 2 No

39. during or soon after physical activity or exercise ? O 1 Yes
O 2 No

40. during or soon after being in a hot bath, shower, tub or sauna ? O 1 Yes
O 2 No

41. Have you ever felt dizzy or faint or actually fainted
when you saw blood or had blood samples taken ? O 1 Yes
O 2 No

In the past year, have you fainted:

42. while passing urine ? O 1 Yes
O 2 No

43. while coughing ? O 1 Yes
O 2 No

44. while pressing on your neck ? O 1 Yes
O 2 No

45. before a public speech ? O 1 Yes
O 2 No

46. any other time ? O 1 Yes
O 2 No

If you checked Yes to any of these questions on fainting please describe circumstances.
…….
…….

47. In the past year, have you ever completely lost consciousness after a spell of dizziness ?

O 1 Yes O 2 No

48. In the past year, have you had any seizures or convulsions ? O 1 Yes
O 2 No
please describe circumstances ……..

In the past 5 years how would you rate the amount of trouble, if any you have had:

None Some A lot
Constant

49. with paralysis in parts of your face ? O 1 O 2 O 3 O 4

50. with feelings of complete weakness
all over your body ? O 1 O 2 O 3 O 4

51. with attacks of uncontrollable
movements of your arms and legs ? O 1 O 2 O 3 O 4

52. with attacks in which you couldn't
control your speech ? O 1 O 2 O 3 O 4

53. Have you ever in your adult life had a spell of dizziness ? O 1 Yes O 2 No

54. In the past year, have you ever noticed colour changes in your skin, such as red, white or purple ?

O 1 Yes *If yes, continue with question 55.* O 2 No *If no, go to question 65.*

What colour skin changes have occurred (check all that apply)

55. O My skin turns red.

56. O My skin turns white.

57. O My skin turns purple.

58. O Other, please specify ……

What parts of your body are affected by these colour changes ? (check all that apply)

59. O My hands.

60. O My feet.

61. O Other parts, please specify ……

62. O Entire body.

63. For how long have you been experiencing these changes in skin colour ?
O 1 Less than 2 months
O 2 3-6 months

O 3 7-12 months

O 4 13 months to 5 years

O 5 More than 5 years

O 6 As long as I can remember

64. Are these changes in skin colour:

O 1 Getting much worse

O 2 Getting somewhat worse

O 3 Staying about the same

O 4 Getting somewhat better

O 5 Getting much better

O 6 Completely gone

65. In the past year, after a long hot bath or shower, have you ever noticed the pads on the ends of your fingers wrinkle up ?

O 1 Yes O 2 No

66. In the past 5 years, what changes, if any, have occurred in your general body sweating?

O 1 I sweat much more than I used to.

O 2 I sweat somewhat more than I used to.

O 3 I haven't noticed any changes in my sweating.

O 4 I sweat somewhat less than I used to.

O 5 I sweat much less than I used to.

67. In the past 5 years, what changes, if any, have occurred in the amount your feet sweat ?

O 1 They sweat much more than they used to.

O 2 They sweat somewhat more than they used to.

O 3 I haven't noticed any changes.

O 4 They sweat somewhat less than they used to.

O 5 They sweat much less than they used to.

68. In the past 5 years, what changes, if any, have occurred in facial sweating after eating spicy foods ?

O 1 I sweat much more than I used to.

O 2 I sweat somewhat more than I used to.

O 3 I haven't noticed any changes in my sweating.

O 4 I sweat somewhat less than I used to.

O 5 I sweat much less than I used to.

O 6 I avoid eating spicy foods because I sweat so much.

O 7 I avoid eating spicy foods for other reasons.

In the past 5 years, what changes, if any, have occurred in your ability to tolerate heat during a hot day, strenuous work or exercise, hot bath or shower, hot tub or sauna ? (check all that apply).

69. O I now get more overheated.

70. O I now get dizzy.

71. O I now get short of breath.

72. O Other changes, please specify ……..

73. O No change.

74. Do your eyes feel excessively dry ? O 1 Yes O 2 No

75. Does your mouth feel excessively dry ? O 1 Yes O 2 No

76. Do you have excessive amounts of saliva formation ? O 1 Yes O 2 No

77. What is the longest period of time that you have had any one of these symptoms: dry eyes, dry mouth, or increased saliva production ?

O 0 I have not had any of these symptoms.
O 1 Less than 3 months.
O 2 3 to 6 months.
O 3 7 to 12 months.
O 4 13 months to 5 years.
O 5 More than 5 years.
O 6 As long as I can remember.

78. For the symptom of dry eyes, dry mouth, or increased saliva production that you have had for the longest period of time, is this symptom:

O 0 I have not had any of these symptoms.
O 1 Getting worse.
O 2 Getting somewhat worse.
O 3 Staying about the same.
O 4 Getting somewhat better.
O 5 Getting much better.
O 6 Completely gone.

79. What weight changes, if any, have you had over the past year ?

O 1 I have lost about …… pounds.

O 2 My weight has not changed.

O 3 I have gained about ……..pounds.

80. In the past year, have you noticed any changes in how quickly you get full when eating a meal ?

O 1 I get full a lot more quickly now than I used to.

O 2 I get full more quickly now than I used to.

O 3 I haven't noticed any change.

O 4 I get full less quickly now than I used to.

O 5 I get full a lot less quickly now than I used to.

81. In the past year, have you felt excessively full or persistently full (bloated feeling) after a meal ?

O 1 Never O 2 Sometimes O 3 A lot of the time

82. In the past year, have you felt like you had a persistent upset stomach (nausea) ?

O 1 Never O 2 Sometimes O 3 A lot of the time

83. In the past year, have you vomited after a meal ?

O 1 Never O 2 Sometimes O 3 A lot of the time

84. In the past year, have you had a cramping or colicky abdominal pain ?

O 1 Never O 2 Sometimes O 3 A lot of the time

85. Are these pains usually after a meal ? O 1 Yes O 2 No

86. How long have you had these cramping or colicky abdominal pains ?

O 1 Less than 3 months

O 2 3 to 6 months

O 3 7 to 12 months

O 4 13 months to 5 years

O 5 More than 5 years

O 6 As long as I can remember

87. In the past year, have you had any bouts of diarrhea ?

O 1 Yes *If yes continue with question 88* O 2 No *If no go to question 94*

88. How frequently does this occur ?

O 1 Rarely O 2 Occasionally

O 3 Frequently ……..times per month O 4 Constantly

89. How severe are these bouts of diarrhoea ?

O 1 Mild O 2 Moderate O 3 Severe

90. What part of the day do they seem to be worse ?

O 1 First thing in the morning

O 2 Rest of the morning

O 3 Afternoon

O 4 Evening

O 5 During the night

O 6 No particular time

91. Do these bouts of diarrhoea usually occur after meals O 1 Yes O 2 No

92. Are these bouts of diarrhoea accompanied with lots of rectal gas (flatus)

O 1 Never O 2 Occasionally O 3 Frequently O 4 Always

93. Are your bouts of diarrhea getting:

O 1 Much worse

O 2 Somewhat worse

O 3 Staying the same

O 4 Somewhat better

O 5 Much better

O 6 Completely gone

94. In the past year, have you been constipated ?

O 1 Yes *If Yes continue below with question 95* O 2 No *If No go to question 98.*

95. How frequently are you constipated ?

O 1 Rarely O 2 Occasionally

O 3 Frequently ……..times per month O 4 Constantly

96. How severe are these bouts of constipation ?

O 1 Mild O 2 Moderate O 3 Severe

97. Is your constipation getting:

O 1 Much worse

O 2 Somewhat worse

O 3 Staying the same

O 4 Somewhat better

O 5 Much better

O 6 Completely gone

98. Overall, are your abdominal symptoms of vomiting, diarrhoea, constipation, or weight loss getting:

O 0 I have not had these symptoms.

O 1 Much worse

O 2 Somewhat worse

O 3 Staying the same

O 4 Somewhat better

O 5 Much better

O 6 Completely gone

99. Which one of the following symptoms have been most troublesome for you (check only one).

O 0 None

O 1 Vomiting

O 2 Diarrhoea

O 3 Constipation

O 4 Weight loss

100.How long have you had this most troublesome symptom.

O 0 I do not have any of these symptoms

O 1 less than 3 months

O 2 3 to 6 months

O 3 7 to 12 months

O 4 13 months to 5 years

O 5 more than 5 years

O 6 As long as I can remember

101 Is this most troublesome symptom getting:

O 0 I do not have any of these symptoms

O 1 Much worse

O 2 Somewhat worse

O 3 Staying the same

O 4 Somewhat better

O 5 Much better

O 6 Completely gone

102 In the past 5 years, how would you rate the amount of trouble, if any, you have had with difficulty swallowing.

O 1 No trouble

O 2 Some trouble

O 3 A lot of trouble

O 4 Constant trouble

103. In the past 5 years, how would you rate the amount of trouble, if any, you have had with everything you eat tasting the same.

O 1 No trouble

O 2 Some trouble

O 3 A lot of trouble

O 4 Constant trouble

Have you ever in your life:

104 Been nauseated or vomited O 1 Yes O 2 No

105 had a bout of diarrhea O 1 Yes O 2 No

106. lost your appetite for at least part of the day O 1 Yes O 2 No

107. Felt discomfort or pain in the pit of the stomach O 1 Yes O 2 No

108. In the past year, have you ever leaked urine or lost control of your bladder function ?

O 1 Never O 2 Occasionally

O 3 Frequently ……..times per month O 4 Constantly

109. In the past, have you had difficulty passing urine ?

O 1 Never O 2 Occasionally

O 3 Frequently ……..times per month O 4 Constantly

110. In the past year, have you had trouble completely emptying your bladder ?

O 1 Never O 2 Occasionally

O 3 Frequently ……..times per month O 4 Constantly

111. How would you describe your current sexual desire ?

O 1 Completely absent O 2 Greatly reduced

O 3 Somewhat reduced O 4 About the same or more than in the past

IF MALE COMPLETE QUESTIONS 112 -123 . FEMALES GO TO QUESTION 124

112. Are you able to have a full erection ?

O 1 Never, under any circumstances

O 2 Much less frequently than in the past

O 3 Somewhat less frequently than in the past

O 4 The same, or more frequently, than in the past

Which of the following statements apply to your situation ? (Fill in all that apply)

113. O 1 My ability to have intercourse has not changed.

114. O 1 I have erections but am unable to have intercourse.

115. O 1 I can have intercourse only some of the time.

116. O 1 My erections are definitely impaired.

117. O 1 I am able to have intercourse, but am unable to ejaculate

118. O 1 I have 'dry' orgasms and afterward my urine looks milky.

119. O 1 I have been unable to have erections or they have been impaired since I started taking a medication called …….

120. O 1 Other situation, please describe ……..

121. O 1 None of the above apply.

122. How long have you had difficulty with erectile function ?

O 0 I do not have this difficulty

O 1 Less than 3 months

O 2 3 to 6 months

O 3 7 to 12 months

O 4 13 months to 5 years

O 5 More than 5 years

O 6 As long as I can remember

123. Is this difficulty getting:

O 0 I do not have difficulty

O 1 Much worse

O 2 Somewhat worse

O 3 Staying the same

O 4 Somewhat better

O 5 Much better

O 6 Completely gone

124. In the past year, without sunglasses or tinted glasses, has bright light bothered your eyes ?

O 1 Never O 2 Occasionally

O 3 Frequently O 4 Constantly

125. How severe is the sensitivity to light ?

O 1 Mild O 2 Moderate O 3 Severe

126. In the past year, have you had trouble focussing your eyes ?

O 1 Never O 2 Occasionally

O 3 Frequently O 4 Constantly

127. How severe is this focusing problem ?
O 1 Mild O 2 Moderate O 3 Severe

128. In the past year have you had blurred vision ?
O 1 Never O 2 Occasionally
O 3 Frequently O 4 Constantly

129. How severe is the focusing problem
O 1 Mild O 2 Moderate O 3 Severe

130 In the past year, have you had difficulty seeing at night ?
O 1 Never O 2 Occasionally
O 3 Frequently O 4 Constantly

131. How severe is the focusing problem
O 1 Mild O 2 Moderate O 3 Severe

132 In the past year, has the same degree of light seemed:
O 1 Excessively dimmer O 2 Much dimmer
O 3 About the same O 4 Much brighter
O 5 Excessively brighter

133. Which one of the following eye symptoms is the most troublesome for you ?
O 0 None O 1 Trouble focusing O 2 Blurred vision
O 3 Difficulty seeing at night.

134. How long have you had this troublesome eye symptom ?
O 0 I don't have any of these symptoms
O 1 Less than 3 months
O 2 3 to 6 months
O 3 7 to 12 months
O 4 13 months to 5 years
O 5 More than 5 years
O 6 As long as I can remember

135. Is this most troublesome symptom with your eyes getting:
O 0 I don't have any of these symptoms
O 1 Much worse
O 2 Somewhat worse
O 3 Staying the same
O 4 Somewhat better
O 5 Much better

O 6 Completely gone

136. In the past year, have you ever noticed or been told that while sleeping you stop breathing for several seconds ?
O 1 Yes O 2 No

137. In the past year, have you ever noticed or been told that while sleeping you snore loudly ?
O 1 Yes O 2 No

Have you ever been told you have or been diagnosed as having :

138. Narcolepsy O 1 Yes O 2 No O 3 Don't know

139. Obstructive sleep apnoea O 1 Yes O 2 No O 3 Don't know

140. Abnormal or disordered sleep Patterns O 1 Yes O 2 No O 3 Don't know

141. Currently, how refreshing and restorative is your sleep
O 1 Not at all restorative – derive no benefit
O 2 Some slight restorative value
O 3 Restorative, but not adequate
O 4 Relatively satisfactory
O 5 Very satisfactory – feel completely refreshed

142. Compared with a year ago, how would you rate your own sleep over the last month ?
O 1 Last month was much worse than a year ago
O 2 Last month was slightly worse than a year ago
O 3 Last month was about the same as a year ago
O 4 Last month was slightly better than a year ago
O 5 Last month was much better than a year ago

143. Have you ever in your adult life had difficulty getting to sleep or staying asleep once you were asleep?
O 1 Yes O 2 No

144. In the past year, have you ever noticed or been told that during the day you sometimes breathe very loudly (e.g. croup) ?
O 1 Yes O 2 No

How would you describe your alcohol use of the past year (check all that apply)

145. O 1 I have not drunk any alcohol over the last year

146. O 1 I drink socially only.

147. O 1 I have used alcohol excessively in the past year.

148. O 1 I have been intoxicated one or more times in the past year.

149. O 1 I have passed out from drinking too much alcohol one or more times in the past year.

How would you describe your drug use over the past year ? (check all that apply)

150. O 1 I have not used any drugs in the last year

151. O 1 I have used drugs excessively in the last year

152. O 1 I have been intoxicated from drugs one or more times in the last year.

153. O 1 I have passed out from taking drugs one or more times in the last year.

154. Have you ever felt that you have used alcohol or drugs excessively ? O 1 Yes
O 1 No

155. Have you ever been told or have you been diagnosed as having alcohol of drug dependency ?
O 1 Yes O 2 No

156. Have you received treatment for alcohol or other drug dependency
O 1 Yes O 2 No Please list the drugs involved including alcohol

……..
Which of the following describe your cigarette smoking ? (check all that apply)
157. O 1 I have never smoked cigarettes

158. O 1 I have smoked cigarettes in the past but stopped: Date stopped :

163. O 1 I am currently smoking about …… cigarettes per day.

166. In the past 5 years, how would you rate the amount of trouble, if any you have had with over sensitive hearing ?
O 1 None O 2 Some O 3 A lot O 4 Constant

167. Have you ever in your adult life had difficulty keeping your mind on your job or task ?

O 1 Yes O 2 No

What medications have you taken in the past month ?

Name of medication	How often do you take it	How much do you take each time
……..	……	
…….	……	
……	……	
…….	……	
……..	……	
…….	……	
……..	……	
…….	……	

We welcome below (or on a separate sheet) any comments you might have about what might have caused or been associated with your current illness or anything that might be helpful to us in understanding your current condition.

The Cognitive Failures Questionnaire (Broadbent, Cooper, FitzGerald & Parkes, 1982)

The following questions are about minor mistakes which everyone makes from time to time, but some of which happen more often than others. We want to know how often these things have happened to your in the past 6 months. Please circle the appropriate number.

		Very often	Quite often	Occasion-ally	Very rarely	Never
1.	Do you read something and find you haven't been thinking about it and must read it again?	4	3	2	1	0
2.	Do you find you forget why you went from one part of the house to the other?	4	3	2	1	0
3.	Do you fail to notice signposts on the road?	4	3	2	1	0
4.	Do you find you confuse right and left when giving directions?	4	3	2	1	0
5.	Do you bump into people?	4	3	2	1	0
6.	Do you find you forget whether you've turned off a light or a fire or locked the door?	4	3	2	1	0
7.	Do you fail to listen to people's names when you are meeting them?	4	3	2	1	0
8.	Do you say something and realize afterwards that it might be taken as insulting?	4	3	2	1	0
9.	Do you fail to hear people speaking to you when you are doing something else?	4	3	2	1	0
10.	Do you lose your temper and regret it?	4	3	2	1	0
11.	Do you leave important letters unanswered for days?	4	3	2	1	0
12.	Do you find you forget which way to turn on a road you know well but rarely use?	4	3	2	1	0
13.	Do you fail to see what you want in a supermarket (although it's there)?	4	3	2	1	0

		Very often	Quite often	Occasion-ally	Very rarely	Never
14.	Do you find yourself suddenly wondering whether you've used a word correctly?	4	3	2	1	0
15.	Do you have trouble making up your mind?	4	3	2	1	0
16.	Do you find you forget appointments?	4	3	2	1	0
17.	Do you forget where you put something like a newspaper or a book?	4	3	2	1	0
18.	Do you find you accidentally throw away the thing you want and keep what you meant to throw away – as in the example of throwing away the matchbox and putting the used match in your pocket?	4	3	2	1	0
19.	Do you daydream when you ought to be listening to something?	4	3	2	1	0
20.	Do you find you forget people's names?	4	3	2	1	0
21.	Do you start doing one thing at home and get distracted into doing something else (unintentionally)?	4	3	2	1	0
22.	Do you find you can't quite remember something although it's "on the tip of your tongue"?	4	3	2	1	0
23.	Do you find you forget what you came to the shops to buy?	4	3	2	1	0
24.	Do you drop things?	4	3	2	1	0
25.	Do you find you can't think of anything to say?	4	3	2	1	0

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Broadbent, D.E., Cooper, P.F., FitzGerald, P., & Parkes, K.R. (1982). The Cognitive Failures Questionnaire (CFQ) and its correlates. *British Journal of Clinical Psychology*, *21*, 1-1

DePaul Symptom Questionnaire

Please answer the following questions.

1. What is your height?_____

2. What is your weight?_____

3. What is your date of birth?_____

4. What is your gender?_____

5. To which of the following race(s) do you belong?

- ☐ Black, African-American
- ☐ White
- ☐ American Indian or Alaska Native
- ☐ Asian or Pacific Islander
- ☐ Other race (*Please specify*)_____

6. Are you of Latino or Hispanic origin?

☐ Yes ☐ No

7. What is your current marital status?

- ☐ Married or living with partner
- ☐ Separated
- ☐ Widowed
- ☐ Divorced
- ☐ Never married

8. Do you have any children?

☐ Yes ☐ No (*Skip to Question 9)*

8a. How many children do you have?_____

8b. How many of your children are under 18 years old?_____

9. How many people live in your home?_____

10. What grade or degree have you completed in school?

- ☐ Less than high school
- ☐ Some high school
- ☐ High school degree or GED
- ☐ Partial college (at least one year) or specialized training
- ☐ Standard college degree
- ☐ Graduate professional degree including masters and doctorate

11. What is your current work status? **(Check all that apply)**

- ☐ On disability
- ☐ Student
- ☐ Homemaker
- ☐ Retired
- ☐ Unemployed
- ☐ Working part-time
- ☐ Working full-time

11a. If you are on disability, for what condition do you receive disability compensation?

Please Specify_____

12. What is your current occupation?

Current_____

12a. If you are currently not working, what was your most recent occupation?

Most Recent_____

*For the following questions (13-66), we would like to know **how often you have had each symptom** and **how much each symptom has bothered you over the last 6 months**. For each symptom please circle **one number for frequency and one number for severity**. Please fill the chart out from left to right.*

Symptoms	Frequency: *Throughout the **past 6 months**, **how often** have you had this symptom?* *For each symptom listed below, circle a number from:* ***0 = none of the time*** ***1 = a little of the time*** ***2 = about half the time*** ***3 = most of the time*** ***4 = all of the time***	Severity: *Throughout the **past 6 months**, **how much** has this symptom bothered you? For each symptom listed below, circle a number from:* ***0 = symptom not present*** ***1 = mild*** ***2 = moderate*** ***3 = severe*** ***4 = very severe***
13) Fatigue/extreme tiredness	0 1 2 3 4	0 1 2 3 4
14) Dead, heavy feeling after starting to exercise	0 1 2 3 4	0 1 2 3 4
15) Next day soreness or fatigue after non-strenuous, everyday activities	0 1 2 3 4	0 1 2 3 4
16) Mentally tired after the slightest effort	0 1 2 3 4	0 1 2 3 4
17) Minimum exercise makes you physically tired	0 1 2 3 4	0 1 2 3 4
18) Physically drained or sick after mild activity	0 1 2 3 4	0 1 2 3 4
19) Feeling unrefreshed after you wake up in the morning	0 1 2 3 4	0 1 2 3 4
20) Need to nap daily	0 1 2 3 4	0 1 2 3 4
21) Problems falling asleep	0 1 2 3 4	0 1 2 3 4
22) Problems staying asleep	0 1 2 3 4	0 1 2 3 4
23) Waking up early in the morning (e.g. 3am)	0 1 2 3 4	0 1 2 3 4
24) Sleep all day and stay awake all night	0 1 2 3 4	0 1 2 3 4
25) Pain or aching in your muscles	0 1 2 3 4	0 1 2 3 4
26) Pain/stiffness/tenderness in more than one joint without swelling or redness	0 1 2 3 4	0 1 2 3 4
27) Eye pain	0 1 2 3 4	0 1 2 3 4
	Frequency:	Severity:

Symptoms	*Throughout the **past 6 months**, **how often** have you had this symptom?* *For each symptom listed below, circle a number from:* ***0 = none of the time*** ***1 = a little of the time*** ***2 = about half the time*** ***3 = most of the time*** ***4 = all of the time***	*Throughout the **past 6 months**, **how much** has this symptom bothered you?* *For each symptom listed below, circle a number from:* ***0 = symptom not present*** ***1 = mild*** ***2 = moderate*** ***3= severe*** ***4 = very severe***
28) Chest pain	0 1 2 3 4	0 1 2 3 4
29) Bloating	0 1 2 3 4	0 1 2 3 4
30) Abdomen/stomach pain	0 1 2 3 4	0 1 2 3 4
31) Headaches	0 1 2 3 4	0 1 2 3 4
32) Muscle twitches	0 1 2 3 4	0 1 2 3 4
33) Muscle weakness	0 1 2 3 4	0 1 2 3 4
34) Sensitivity to noise	0 1 2 3 4	0 1 2 3 4
35) Sensitivity to bright lights	0 1 2 3 4	0 1 2 3 4
36) Problems remembering things	0 1 2 3 4	0 1 2 3 4
37) Difficulty paying attention for a long period of time	0 1 2 3 4	0 1 2 3 4
38) Difficulty finding the right word to say or expressing thoughts	0 1 2 3 4	0 1 2 3 4
39) Difficulty understanding things	0 1 2 3 4	0 1 2 3 4
40) Only able to focus on one thing at a time	0 1 2 3 4	0 1 2 3 4
41) Unable to focus vision and/or attention	0 1 2 3 4	0 1 2 3 4
42) Loss of depth perception	0 1 2 3 4	0 1 2 3 4
43) Slowness of thought	0 1 2 3 4	0 1 2 3 4
44) Absent-mindedness or forgetfulness	0 1 2 3 4	0 1 2 3 4

45) Bladder problems	0 1 2 3 4	0 1 2 3 4
46) Irritable bowel problems	0 1 2 3 4	0 1 2 3 4
Symptoms	Frequency: *Throughout the **past 6 months**, **how often** have you had this symptom?* *For each symptom listed below, circle a number from:* ***0 = none of the time*** ***1 = a little of the time*** ***2 = about half the time*** ***3 = most of the time*** ***4 = all of the time***	Severity: *Throughout the **past 6 months**, **how much** has this symptom bothered you?* *For each symptom listed below, circle a number from:* ***0 = symptom not present*** ***1 = mild*** ***2 = moderate*** ***3= severe*** ***4 = very severe***
47) Nausea	0 1 2 3 4	0 1 2 3 4
48) Feeling unsteady on your feet, like you might fall	0 1 2 3 4	0 1 2 3 4
49) Shortness of breath or trouble catching your breath	0 1 2 3 4	0 1 2 3 4
50) Dizziness or fainting	0 1 2 3 4	0 1 2 3 4
51) Irregular heart beats	0 1 2 3 4	0 1 2 3 4
52) Losing or gaining weight without trying	0 1 2 3 4	0 1 2 3 4
53) No appetite	0 1 2 3 4	0 1 2 3 4
54) Sweating hands	0 1 2 3 4	0 1 2 3 4
55) Night sweats	0 1 2 3 4	0 1 2 3 4
56) Cold limbs (e.g. arms, legs, hands)	0 1 2 3 4	0 1 2 3 4
57) Feeling chills or shivers	0 1 2 3 4	0 1 2 3 4
58) Feeling hot or cold for no reason	0 1 2 3 4	0 1 2 3 4
59) Feeling like you have a high temperature	0 1 2 3 4	0 1 2 3 4
60) Feeling like you have a	0 1 2 3	0 1 2 3

low temperature				4				4
61) Alcohol intolerance	0	1	2	3 4	0	1	2	3 4
62) Sore throat	0	1	2	3 4	0	1	2	3 4
63) Tender/sore lymph nodes	0	1	2	3 4	0	1	2	3 4
64) Fever	0	1	2	3 4	0	1	2	3 4
65) Flu-like symptoms	0	1	2	3 4	0	1	2	3 4
66) Some smells, foods, medications, or chemicals make you feel sick	0	1	2	3 4	0	1	2	3 4

67. Have you **always** had persistent or recurring **fatigue/energy problems**, even back to your earliest memories as a child? (By persistent or recurring, we mean that the fatigue/energy problems are usually ongoing and constant, but sometimes there are good periods and bad periods.)

□ Yes □ No □ Not having a problem with fatigue/energy

68. Since your **fatigue/energy related illness** began, do your headaches either happen more often, feel worse or more severe, or are they in a different place or spot?

□ Yes □ No □ Not having a problem with fatigue/energy

69. How long ago did your problem with **fatigue/energy** begin?

□ Less than 6 months

□ 6-12 months

□ 1-2 years

□ Longer than 2 years

□ Had problem with fatigue/energy since childhood or adolescence

□ Not having a problem with fatigue/energy

70. Have you been diagnosed with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

□ *Yes* □ *No*

*70a. If yes, what year were you diagnosed?*_____

70b. Do you currently have a diagnosis of Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

□ *Yes* □ *No*

70c. Who diagnosed you with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

□ *Medical Doctor* □ *Alternative Practitioner* □ *Self-Diagnosed*

70d. Have any of your family members been diagnosed with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

□ *Yes* □ *No*

If yes, please list their relation to you and current age_____

_____ _____

_____

71. Did you experience any of the following symptoms regularly and repeatedly in the months and years before your fatigue/energy problems began?

□ Sore throat

□ Tender/sore lymph nodes

□ Unrefreshing sleep

□ Impaired memory and concentration

□ Prolonged fatigue following physical or mental exertion

□ Muscle pain

□ Headaches

□ Joint Pain

□ Not having a problem with fatigue/energy

72. If you rest, does your problem with **fatigue/energy** go away? **(Check one)**

□ Entirely

□ Partially

□ My fatigue/energy problem is not improved by rest *(Skip to Question 73)*

□ I am not having a problem with fatigue/energy *(Skip to Question 73)*

72a. How long do you have to rest for your problem with **fatigue/energy** to entirely or partially go away?

□ less than 30 minutes □ 30 to 59 minutes □ 1-2 hours □ more than 2 hours

73. If you were to become exhausted after actively participating in extracurricular activities, sports, or outings with friends, would you recover within an hour or two after the activity ended?

□ *Yes* □ *No*

*74. Do you reduce your activity level to avoid experiencing problems with **fatigue/energy**?*

□ *Yes* □ *No* □ *Not having a problem with fatigue/energy*

*75. Do you experience a worsening of your **fatigue/energy related illness** after engaging in minimal physical effort?*

□ *Yes* □ *No* □ *Not having a problem with fatigue/energy*

*75a. Do you experience a worsening of your **fatigue/energy related illness** after engaging in mental effort?*

□ *Yes* □ *No*

*75b. If you feel worse after activities, how long does this last? **(Check one)***

□ *1 hour or less* □ *2-3 Hrs* □ *4-10 Hrs* □ *11-13 Hrs*

□ *14-23 Hrs* □ *More than 24 Hrs (Please specify_____)*

76. Are you currently engaging in any form of exercise?

□ *Yes* (Skip to Question 77) □ *No*

*76a. If you do not exercise, why aren't you exercising? **(Check all boxes that you agree with)***

□ *Not interested*
□ *No time*
□ *Would like to but cannot because of problems with fatigue/energy*
□ *Cannot because exercise makes symptoms worse*

77. Over what period of time did your **fatigue/energy related illness,** develop? **(Check one)**

□ Within 24 hours
□ Over 1 week

- Over 1 month
- Over 2-6 months
- Over 7-12 months
- Over 1-2 years
- Over 3 or more years
- I am not ill

78. How would you describe the course of your **fatigue/energy related illness? (Check one)**

- *Constantly getting worse*
- *Constantly improving*
- *Persisting (no change)*
- *Relapsing & remitting (having "good" periods with no symptoms & "bad" periods)*
- *Fluctuating (symptoms periodically get better and get worse, but never disappear completely)*
- *No Symptoms/I am not ill*

79. Which statement best describes your **fatigue/energy related illness** during the **last 6 months**? **(Check one)**

- I am not able to work or do anything, and I am bedridden.
- I can walk around the house, but I cannot do light housework.
- I can do light housework, but I cannot work part-time.
- I can only work part-time at work or on some family responsibilities.
- I can work full time, but I have no energy left for anything else.
- I can work full time and finish some family responsibilities but I have no energy left for anything else.
- I can do all work or family responsibilities without any problems with my energy.

80. Did your **fatigue/energy related illness** start after you experienced any of the following? **(Check one or more and please specify)**

- An infectious illness_____
- An accident_____
- A trip or vacation_____
- An immunization (shot at doctor's office)_____
- Surgery_____
- Severe stress (bad or unhappy event(s))_____
- Other_____
- I am not ill

81. Have you ever consulted a medical doctor or health professional about your **fatigue/energy** problem?

□ *Yes* □ *No* (Skip to Question 83)

82. Do you currently have a medical doctor overseeing your **fatigue/energy** problem?

□ Yes □ No

83. Do you have any medical illness (es) that might be causing your symptoms?

□ Yes□ No *(Skip to Question 84)*

83a. What medical illnesses do you have?

Illness name(s) and year it began:_____

*83b. For which of these conditions are you currently receiving treatment?*_____

84. Are you currently taking any medications (over the counter or prescription)?

□ *Yes* □ *No* (Skip to Question 86)

84a. What medications are you taking? _____

*85. Do you think any medication(s) is (are) causing your problem with **fatigue/energy**?*

□ *Yes* □ *No* (Skip to Question 86)

□ *I do not have a problem with fatigue/energy* (Skip to Question 86)

*85a. Please specify which medications:*_____

86. Have you ever been diagnosed and/or treated for any of the following: ***(Check all that apply and write year (s) experienced, years treated, and medication (if applicable) in the blank)***

□ *Major depression*_____

□ *Major depression with melancholic or psychotic features*_____

□ *Bipolar disorder (Manic-depression)*_____

□ *Anxiety*_____

□ *Schizophrenia*_____

□ *Eating disorder*_____

- ☐ *Substance abuse*_____
- ☐ *Multiple chemical sensitivities*_____
- ☐ *Fibromyalgia*_____
- ☐ *Allergies*_____
- ☐ *Other (*Please specify*)*_____
- ☐ *No diagnosis/treatment*

87. What do you think is the cause of your problem with ***fatigue/energy****?* ***(Check one)***

- ☐ *Definitely physical*
- ☐ *Mainly physical*
- ☐ *Equally physical and psychological*
- ☐ *Mainly psychological*
- ☐ *Definitely psychological*
- ☐ *No problem with fatigue/energy*

88. Do you think anything specific in your personal life or environment accounts for your problem with ***fatigue/energy****?*

☐ *Yes* ☐ *No* (Skip to Question 89)

☐ *I do not have a problem with fatigue/energy (*Skip to Question 89)

*88a. Please specify:*_____

89. In the **past 4 weeks**, approximately how many hours per week have you spent doing:

Household related activities?_____hours per week

Social/Recreational related activities?__hours per week

Family related activities?_____hours per week

Work related activities?_____hours per week

90. In the ***past 4 weeks****, have you had to reduce the number of hours you previously spent (prior to your illness) on occupational, social or family activities because of your health or problems with* ***fatigue/energy****?*

☐ *Yes* ☐ *No* (Skip to Question 91) ☐ *Not having a problem with fatigue/energy*

90a. ***Before your fatigue/energy related illness****, approximately how many hours did you used to spend on:*

Household related activities? _____*hours per week*

Social/Recreational related activities? _hours per week

Family related activities? _____hours per week

Work related activities?_____hours per week

91. Please rate the amount of **energy** you had available **yesterday**, using a scale from 1 to 100 where 1= no energy and 100 = your pre-illness energy level. **(If you don't have a fatigue/energy related illness, a score of 100 = having abundant energy such that you could work full time and complete your family responsibilities)**_____

92. Please rate the amount of **energy** you expended (used) **yesterday**, using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy expended_____

93. Please rate the amount of **fatigue** you had **yesterday**, using a scale from 1 to 100 where 1 = no fatigue and 100 = severe fatigue_____

94. For the **past week**, please rate the amount of **energy** you had available using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy level_____

95. For the **past week**, please rate the amount of **energy** you have expended (used) using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy expended___

96. For the **past week**, please rate the amount of **fatigue** you have had using a scale from 1 to 100 where 1 = no fatigue and 100 = severe fatigue_____

97. Since the onset of your problems with fatigue/energy, have your symptoms caused a 50% or greater reduction in your activity level?

□ Yes □ No □ Not having a problem with fatigue/energy

98. Do you experience frequent viral infections with prolonged recovery periods?

□ Yes □ No

99. Are you intolerant of extremes of temperatures (when it is extremely hot or cold)?

□ Yes □ No

MOS SURVEY

This survey asks for your views about your health. This information will help keep track of how you feel and
how well you are able to do your usual activities. Answer every question by marking the answer as
indicated. If you are unsure about how to answer a question, please give the best answer you can.

1.In general, would you say your health is: ***(Please circle one)***

.....Excellent 1
.....Very good 2
.....Good 3
.....Fair 4
.....Poor5

2. **Compared to one year ago,** how would you rate your health in general now? ***(Please circle one)***

.....Much better than one year ago 1
.....Somewhat better now than one year ago 2
.....About the same as one year ago 3
.....Somewhat worse now than one year ago 4
.....Much worse now than one year ago 5

3. The following items are about activities you might do during a typical day. Does your health now
limit you in these activities? If so, how much?

Activities	**Yes, Limited A Lot**	**Yes, Limited A Little**	**No, Not Limited At All**
Vigorous activities: running, lifting heavy objects, participating in strenuous sports	1	2	3
Moderate activities: moving a table, pushing a vacuum cleaner, bowling, playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing **several** flights of stairs	1	2	3
Climbing **one** flight of stairs	1	2	3
Bending, kneeling, or stooping	1	2	3
Walking **more than a mile**	1	2	3

Walking **several blocks**	1	2	3
Walking **one** block	1	2	3
Bathing or dressing yourself	1	2	3

4. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities as a result of your **physical health**?

Problems	**Yes**	**No**
Cut down on the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the **kind** of work or other activities	1	2
Had **difficulty** performing the work or other activities (For example, it took extra effort)	1	2

5. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

Problems	**Yes**	**No**
Cut down the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Didn't do work or other activities as **carefully** as usual	1	2

6. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, neighbors, or groups? ***(Please circle one)***

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

7. How much bodily pain have you had during the **past 4 weeks**?

None 1
Very mild 2
Mild 3
Moderate 4
Severe 5
Very Severe 6

8. During the **past 4 weeks**, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all 1
Slightly 2

Moderately.....3

Quite a bit 4

Extremely.....5

9. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time **during the past 4 weeks**-

Questions	**All of the Time**	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
Did you feel full of pep?	1	2	3	4	5	6
Have you been a nervous person?	1	2	3	4	5	6
Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
Have you felt calm and peaceful?	1	2	3	4	5	6
Did you have a lot of energy?	1	2	3	4	5	6
Have you felt down-hearted and blue?	1	2	3	4	5	6
Did you feel worn out?	1	2	3	4	5	6
Have you been a happy person?	1	2	3	4	5	6
Did you feel tired?	1	2	3	4	5	6

10. During the **past 4 weeks**, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time.....1

Most of the time 2

Some of the time.....3

A little of the time 4

None of the time 5

11. How **TRUE** or **FALSE** is each of following statements for you?

Statements	**Definitely True**	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
I seem to get sick a little easier than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

Fatigue Impact Scale (FIS)

Please put a tick or cross in the box that best addresses your fatigue
[If the question is not relevant to you, please tick “not applicable”]

		no problem	small problem	moderate problem	big problem	extreme problem	Not applicable
1.	I feel less alert						
2.	I feel that I am more isolated from social contact						
3.	I have to reduce my workload or responsibilities						
4.	I am more moody.						
5.	I have difficulty paying attention for a long period						
6.	I feel I cannot think clearly.						
7.	I work less effectively (this applies to work inside or outside the home)						
8.	I have to rely more on others to help me or do things for me.						
9.	I have difficulty planning activities ahead of time						
10.	I am more clumsy and uncoordinated						
11.	I find that I am more forgetful						
12.	I am more irritable and more easily angered						
13.	I have to be careful about pacing my physical activities						
14.	I am less motivated to do anything that requires physical effort						
15.	I am less motivated to engage in social activities						
16.	My ability to travel outside my home is limited						
17.	I have trouble maintaining physical effort for long periods						
18.	I find it difficult to make decisions						
19.	I have few social contacts outside my own home						
20.	Normal day to day events are stressful for me						

		no problem	small problem	moderate problem	big problem	extreme problem	Not applicable
21.	I am less motivated to do anything which requires thinking						
22.	I avoid situations that are stressful for me						
23.	My muscles feel much weaker than they should do						
24.	My physical discomfort is increased						
25.	I have difficulty dealing with anything new						
26.	I am less able to finish tasks that require thinking						
27.	I feel unable to meet the demands that people place on me						
28.	I am less able to provide financial support for myself and my family						
29.	I engage in less sexual activity						
30.	I find it difficult to organise my thoughts when I am doing things at home or at work						
31.	I am less able to complete tasks that require physical effort						
32.	I worry about how I look to other people						
33.	I am less able to deal with emotional issues						
34.	I feel slowed down in my thinking						
35.	I feel it hard to concentrate						
36.	I have difficulty participating fully in family activities						
37.	I have to limit my physical activities						
38.	I require more frequent or longer periods of rest						
39.	I am not able to provide as much emotional support to my family as I should.						
40.	Minor difficulties seem like major difficulties						

IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

C Standard Operating Procedures

SOP for Pre-screening telephone call

SOP Number: 1	
Version Number & Date: V1.0. dated 9/11/12	**Effective Date:**
Superseded Version Number & Date (if applicable):	**Review Date:**

Author signature

Approval signature

Background

This SOP is to provide clear instructions on the pre-screening telephone call.

Scope

The SOP covers what to discuss in the pre-screening telephone call.

Roles and Responsibilities

All personnel involved in the task must be fully trained and deemed competent by their supervisor.

Procedure

1. Clarify what the participant understands about the project.
2. Answer any questions or offer further explanation as appropriate.
3. Explain that there are some criteria that mean people cannot participate and that you would like to check a few things now before calling people out to the hospital.
4. Clarify that it is ok to ask these questions.
5. Run through exclusion criteria:

a. Have you previously participated in this study before?

b. Are you involved in any other trials?

Are you able to attend Newcastle Hospitals?

c. What medications do you take? (any affecting autonomic nervous system should be excluded)

d. Do you have other medical problems? (clarify which. If diabetes should be excluded)

e. Have you ever been diagnosed with any problems with your mood? (if yes cannot participate)

f. Could you be pregnant? (if yes advise taking pregnancy test before participation)

g. Do you have a pacemaker? (if yes cannot participate)

h. Have you ever had an operation where metal has been inserted into your body? (if yes seek clarification from MR centre about contra-indications)

6. If there are any questions about patient responses speak to another member of the team and arrange to phone the patient back.

SOP for Taking consent

SOP Number: 1	
Version Number & Date: V1.0. dated 9/11/12	**Effective Date:**
Superseded Version Number & Date (if applicable):	**Review Date:**

Author signature
Approval signature

Background

This SOP is to provide clear instructions on taking consent.

Scope

The SOP covers what to discuss when taking consent and what to record in the notes.

Roles and Responsibilities

All personnel involved in the task must be fully trained and deemed competent by their supervisor.

Things to discuss

Ask the patient what they understand about the project.
Are there any questions they would like to ask?
Explain that need to mention some things. Discuss:

1. The aim of the study in to see how blood pressure regulation and brain function interrelate. To explore how this may help us to develop treatments and improve quality of life. Participant may not get any direct benefit from the research.
2. Number of visits. Talk about plan to complete all visits within four weeks if possible. Understand that the number and nature of visits is quite demanding so can be spread out.
3. Nature of the visits:
    a. Screening. This includes a DSMIV assessment.
    b. Assess for mood disorders
    c. If found will not be able to participate
    d. Will inform GP of diagnosis for management
    e. Autonomic assessment and cognitive assessments.
    f. Blood tests.
        i. Looking at cortisol, cytokines.
        ii. Further sample will be stored for future tests.
    g. MRI scans.
        i. Cannot participate if any contraindications as per safety questionnaire.
    h. Medical physics.
    i. Involves blood tests as per SOP.
4. Draw attention to possible side effects including:
    a. bruising form blood taking, vein blockage, small nerve injury causing numbness and pain. Usually resolve with time.
    b. MR scan noisy/claustrophobic
    c. Small dose of radiation in medical physics tests. Equivalent to 13 months of background radiation in Newcastle area or CT head.
    d. Consider the number of visits.
5. All data is recorded anonymously. Data collected by Nuclear Medicine will be stored under patient's details on NHS system and transferred anonymously.
6. Participation is voluntary. Free to withdraw at any time without giving a reason and this will have no effect on future care.
7. The sponsor of the research study in Newcastle upon Tyne Hospitals NHS Foundation Trust.

8. The funding has come from Medical Research Council.
9. Explain that participant's medical notes and data collected in the study may be looked at by people working for Newcastle upon Tyne Hospitals NHS Foundation Trust and regulatory authorities where necessary. Data will be entered in these notes by researchers working on the project.
10. GP will be informed of participation in the study.
11. When having the tests, ie MRI scans, there is a possibility that an abnormality will be observed. The scans will be looked at by radiographers and by members of the research team. Tests not being done for diagnostic purposes so there is no guarantee that abnormalities would be detected. If anything abnormal was seen the scan would be sent to a radiologist for an expert opinion about whether further investigation or treatment were necessary. In this instance your GP would be contacted and informed of the results.
12. Draw attention to PALS in the instance of wanting advice.
13. Contact for the study:
    a. Laura Maclachlan, Tel: 0191 208 1357
    b. Professor Julia Newton, Tel: 0191 222 6000, email: julia.newton@ncl.ac.uk

What to include in the notes

1. Patient has read version 5 1 5 12 of the patient information sheet. Understand what the research involves.
2. They have had at least 24 hours to consider the information. Have had the opportunity to ask questions.
3. They understand their participation is voluntary and they are free to withdraw at any time.
4. Patient understands that parts of their medical notes and data collected during the study may be looked at by individuals from the Newcastle upon Tyne Hospitals NHS Foundation Trust, their representatives/agents or regulatory authorities where it is relevant to my taking part in this research.
5. Agree to the use of blood samples
6. Agree to GP being informed of participation and any important findings
7. Side effects discussed including radiation, MR scans and risks of blood taking.
8. Discussed potential benefits of taking part.
9. Given information about contact person.

Original consent form into site file.
Copy of consent form and PIS into patient notes
Copy of consent form to the CRF

NB use the latest version of consent form and PIS (currently v5 1 5 12)

SOP for SCID assessment

SOP Number: 1	
Version Number & Date: V3.0. dated 08/03/13	**Effective Date:**
Superseded Version Number & Date (if applicable):	**Review Date:**

Author signature
Approval signature

Background

This SOP is to provide clear instructions on the SCID assessment.

Scope

The SOP covers what to discuss in the SCID assessment.

Roles and Responsibilities

All personnel involved in the task must be fully trained and deemed competent by their supervisor.

Procedure

Explain that these questions are to ensure there are no confounding factors in the study.
Ask patient about how they are feeling.
Explore mood over the course of the illness and diagnosis.
Use wording as per SCID.
Do not include A38 dysthymic disorder.
Do not include module G1-5 Somatoform Disorders.

Exclusion

Any current or past diagnosis according to SCID with the following exceptions:
Simple bereavement
Module G6 Pain Disorder but do not use this as an exclusion criteria.
Module F Anxiety Disorders but do not use this as an exclusion criteria.
Module E Substance Abuse if **not** in the last six months and for substance dependence if **not** in the last year.

SOP for Medications

SOP Number: 1	
Version Number & Date: V3.0. dated 28/11/13	**Effective Date:**
Superseded Version Number & Date (if applicable):	**Review Date:**

Author signature
Approval signature

Background

This SOP is to provide clear instructions on what medications are exclusion criteria and what to do with other medication.

Scope

The SOP covers information about what medications are exclusion criteria and what to do with other medication.

Exclusion

Antihypertensive mediation, including but not limited to:

- ACE-inhibitors;
- Diuretics *;
- Calcium channel blockers;
- Beta-blockers.
- Naltrexone.

Inclusion

Simple analgesia taken prn, including

- Paracetamol;
- Ibuprofen.

Other medications

The following medications should be stopped for 72 hours prior to autonomic testing and blood tests. Can be continued for MR scans and nuclear medicine. This is a participant decision, if they wish to take part in the research:

- SSRIs
- Amitriptyline
- Antihistamines
- LDN (low dose naltrexone)
- Vesicare
- Opioid analgesics
- PPIs
- Fludrocortisone
- Nortriptyline
- Duloxetine
- * Bendroflumethiazide for idiopathic oedema

The following medication should be stopped for 24 hours prior to autonomic testing and blood tests. Can be continued for MR scans and nuclear medicine. This is a participant decision, if they wish to take part in the research:

- Midodrine

SOP for Autonomic testing

SOP Number: 1	
Version Number & Date: V1.1. dated 24/04/13	**Effective Date:**
Superseded Version Number & Date (if applicable):	**Review Date:**

Author signature

Approval signature

Background

This SOP is to provide clear instructions on using the Taskforce monitor for autonomic testing.

Scope

The SOP covers how to use the Taskforce Monitor.

Roles and Responsibilities

All personnel involved in the task must be fully trained and deemed competent by their supervisor.

Procedure

The autonomic testing should be carried out between 9am and 10am.
Participant refrains from caffeine and alcohol the morning prior to testing and for nicotine for two hours before testing. A light breakfast can be eaten. Refer to SOP for medications version 2 for instructions about medications.
Explain mechanism and procedure for Valsalva technique before attaching electrodes.

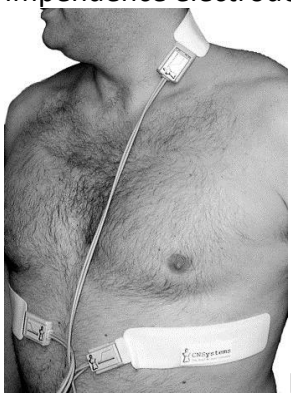
10 minute Rest

Equipment needed

Task Force Monitor
Electrodes, including impedance electrodes

1. Ask patient to gel their hands
2. Rest patient on bed
3. Attach patient to Task Force Monitor Equipment as per below:

Impendence electrodes:



In addition: Small electrode and clip attaches to left ankle.
Image taken from
http://www.sciencedirect.com/science/article/pii/S0010482505000685 Accessed 13/11/12.
Place remaining electrodes on anterior thorax in approximately the 2nd intercostal space mid-axillary on the left and right side and below the impedance electrodes on the left and right side. Start from right 2nd intercostal space and place in the following order: red, yellow, green, blue.

4. Set intervention time: 10 min Rest: Start
5. Patient rests quietly for 10 minutes while heart rate and blood pressure are monitored
6. Set intervention time: 10 min Rest: End

Active Stand

Equipment needed

Task Force Monitor

2 people are needed to do this procedure

After patient has rested for 10 minutes

1. Assist patient to stand
2. Insert intervention: Active Stand: Start as patient comes to the standing position
3. Stand patient for 2 minutes
4. Ask and observe patient for any symptoms
5. Insert intervention: Active Stand: End
6. Sit patient down

Valsalva

Equipment needed

Task Force Monitor
10 ml Syringe
baumanometer

1. Set intervention: Valsalva1: Start
2. Allow patient to recover to baseline for two minutes.
3. Patient takes a deep breath in
4. Place syringe in mouth
5. Blow until baumanometer reads 40mmHg for 15 seconds
6. Set intervention: Valsalva1: End while patient is blowing
7. Patient stops blowing, sits quietly, breathing normally, and not talking until recordings have returned to normal

D Cognitive testing booklet

NATIONAL ADULT READING TEST (NART)

Participant Code…… Date ……. Time ……

Instruction: Give NART sheet to participant and ask them to read out each word.

CHORD	HEIR	PLACEBO
ACHE	RADIX	ABSTEMIOUS
DEPOT	ASSIGNATE	DETENTE
AISLE	HIATUS	IDYLL
BOUQUET	SUBTLE	PUERPERAL
PSALM	PROCREATE	AVER
CAPON	GIST	GAUCHE
DENY	GOUGE	TOPIARY
NAUSEA	SUPERFLUOUS	LEVIATHAN
DEBT	SIMILE	BEATIFY
COURTEOUS	BANAL	PRELATE
RAREFY	QUADRUPED	SIDEREAL
EQUIVOCAL	CELLIST	DEMESNE
NAIVE	FACADE	SYNCOPE
CATACOMB	ZEALOT	LABILE
GAOLED	DRACHM	CAMPANILE
THYME	AEON	

Number of errors: ……

Verbal IQ: ……

Rey AVLT word recall

Participant Code…… Date ……. Time ……

*Please write down **all** words that the subject recalls (including any that have already been said, and ones that may not actually be on the list)*

List A	after reading List A each time					List B	after reading list B	without reading List A
	A1	**A2**	**A3**	**A4**	**A5**		**B**	**A6**
Drum						Desk		
Curtain						Ranger		
Bell						Bird		
Coffee						Shoe		
School						Stove		
Parent						Mountain		
Moon						Glasses		
Garden						Towel		
Hat						Cloud		
Farmer						Boat		
Nose						Lamb		
Turkey						Gun		
Colour						Pencil		
House						Church		
River						Fish		

STROOP COLOUR WORD TEST

Participant Code…… Date ……. Time ……

Part 1: Read out words (Word page)

Participant reads out the words printed in black as quickly as possible. Record the number of correct words read in 45 seconds, and any errors.

"This is a test of how fast you can read words on this page. After I say begin, you are to read down the columns starting with the first one until you complete it and then continue without stopping down the remaining columns in order. If you finish all the columns before I say "Stop", then return to the first column and begin again. Remember do not stop reading until I tell you to "Stop" and read out load as quickly as you can. If you make a mistake, I will say "No" to you. Correct your error and continue without stopping."

Start timing when participant says first word.

RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	BLUE	RED	RED	BLUE
RED	RED	GREEN	BLUE	GREEN
BLUE	GREEN	BLUE	GREEN	RED
RED	BLUE	GREEN	BLUE	GREEN
BLUE	GREEN	RED	GREEN	RED
GREEN	RED	BLUE	RED	BLUE
BLUE	GREEN	GREEN	BLUE	GREEN
GREEN	RED	BLUE	RED	RED
RED	BLUE	RED	GREEN	BLUE
GREEN	RED	BLUE	RED	GREEN
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	GREEN	BLUE	BLUE
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	BLUE	RED	GREEN
GREEN	RED	GREEN	BLUE	BLUE
RED	BLUE	RED	GREEN	RED
GREEN	RED	GREEN	BLUE	GREEN

Number of items: ……

Number of errors: ……

Part 2: Name colours (Colour page)

Participant names the colours of XXXX as quickly as possible. Record the number of correct colours named in 45 seconds and any errors.

"This is a test of how fast you can name the colours (red, green or blue) on this page. You will complete this page just as you did the previous page, starting with the first column. Remember to name the colours out loud as quickly as you can."

BLUE	RED	BLUE	GREEN	RED
RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	GREEN	RED	RED	BLUE
RED	BLUE	GREEN	BLUE	GREEN
GREEN	GREEN	RED	GREEN	RED
RED	RED	BLUE	RED	BLUE
BLUE	BLUE	GREEN	BLUE	GREEN
RED	RED	RED	GREEN	BLUE
BLUE	BLUE	GREEN	BLUE	GREEN
GREEN	GREEN	BLUE	RED	RED
RED	BLUE	RED	BLUE	BLUE
GREEN	GREEN	GREEN	RED	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	GREEN	GREEN	BLUE	BLUE
BLUE	RED	RED	GREEN	RED
RED	BLUE	BLUE	RED	GREEN
GREEN	RED	GREEN	BLUE	BLUE
BLUE	GREEN	BLUE	RED	RED

Number correct: ……

Number errors: ……

Part 3: Name colours of words (Colour-Word page)

Participant names the colours of word as quickly as possible. Record the number of correct colours named in 45 seconds and any errors.

"This Word page is like the page you just finished. I want you to name the colour of the ink the words are printed in, ignoring the word that is printed for each item. For example [point to the first item], this is the first item: what would you say? [If correct ask for second item; if incorrect reiterate naming of colour of ink and repeat first item.] Good. You will do this page just like the others, starting with the first column and then going on to as many columns as you can. Remember, if you make a mistake, just correct it and go on."

BLUE	RED	BLUE	GREEN	RED
RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	GREEN	RED	RED	BLUE
RED	BLUE	GREEN	BLUE	GREEN
GREEN	GREEN	RED	GREEN	RED
RED	RED	BLUE	RED	BLUE
BLUE	BLUE	GREEN	BLUE	GREEN
RED	RED	RED	GREEN	BLUE
BLUE	BLUE	GREEN	BLUE	GREEN
GREEN	GREEN	BLUE	RED	RED
RED	BLUE	RED	BLUE	BLUE
GREEN	GREEN	GREEN	RED	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	GREEN	GREEN	BLUE	BLUE
BLUE	RED	RED	GREEN	RED
RED	BLUE	BLUE	RED	GREEN
GREEN	RED	GREEN	BLUE	BLUE
BLUE	GREEN	BLUE	RED	RED

Number correct: ……

Number errors: ……

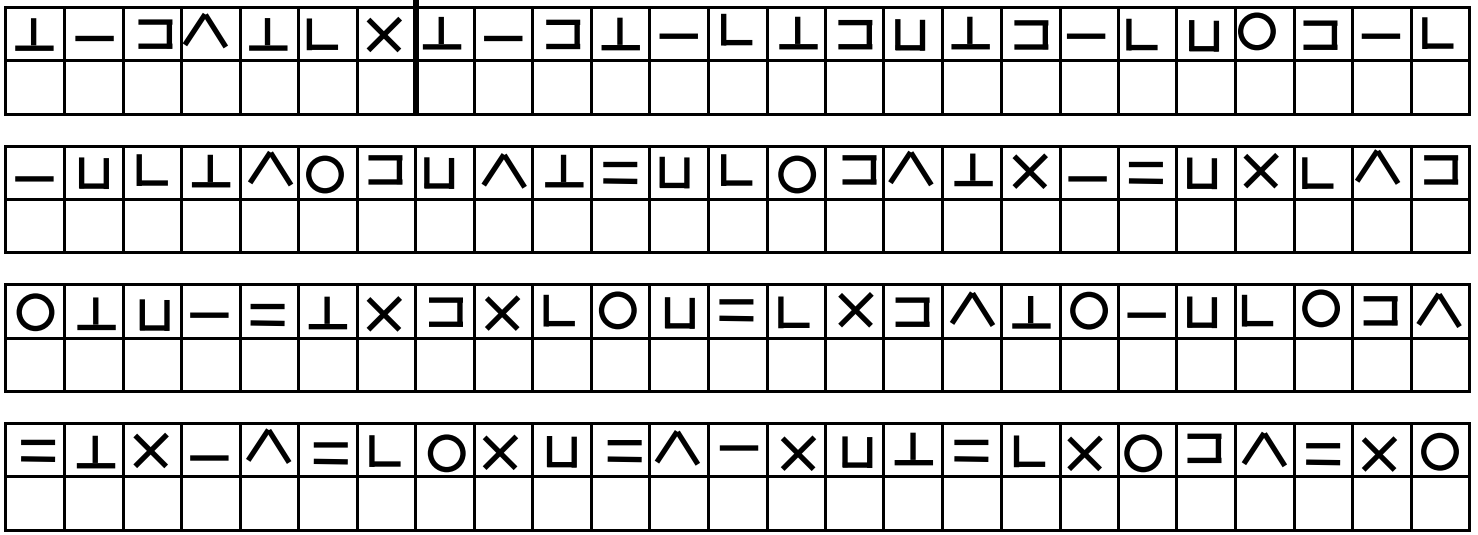
Participant Code…… Date …… Time ……

DIGIT SYMBOL (DSST) - VERSION 1

Time for **two minutes**. If completed before two minutes note time taken to complete.

Instruction: Copy the symbol from the top row to the bottom row.

SAMPLES



Time taken or number completed in two minutes: …… Errors: …….

Participant Code…… Date …… Time ……

DIGIT SYMBOL (DSST) - VERSION 2

Instruction: Copy the appropriate symbol below each digit according to this key.

SAMPLES

2	1	3	7	2	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3

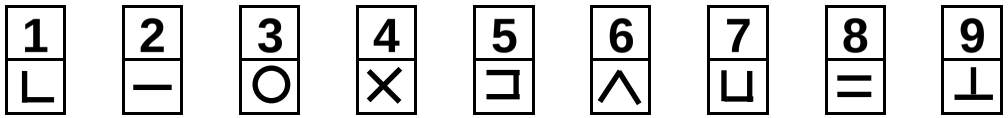
6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7

9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

Time taken or number completed in two minutes: …… Errors: …….
Participant Code…… Date …… Time ……

DIGIT SYMBOL (DSST) - VERSION 3

Instruction: In this sheet, somebody else already copied the symbols below each digit. However, this person made a few mistakes. Try to find and mark these mistakes – you do not need to correct them.



SAMPLES

2	1	3	7	2	4	8
—	⊥	○	⊔	—	∧	=

2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4
—	L	○	—	∧	×	—	=	⊐	—	○	L	⊥	—	∧	○	L	×

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
L	○	×	—	⊔	∧	○	⊐	=	—	⊔	⊐	×	∧	○	⊔	—	×	L	⊥	⊐	=	×	⊔	∧

6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
∧	—	⊐	L	∧	—	=	×	⊔	×	○	⊐	⊥	×	=	○	⊥	—	∧	L	⊐	×	∧	L	⊔

9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6
⊥	—	=	×	⊔	L	×	∧	=	⊐	⊥	⊔	∧	=	⊐	—	○	×	=	∧	○	⊔	—	=	∧

Time taken or number completed in two minutes: …… Errors: …….

DIGIT SPAN – FORM 1

Participant Code…… Date ……. Time ……

Digits Forward	Pass/Fail	Score 2, 1 or 0
9-8-3		
1-3-7		
7-2-4-6		
8-7-3-4		
9-6-2-8-5		
5-4-3-9-7		
2-8-6-4-1-9		
5-8-3-6-1-9		
2-4-8-7-5-9-1		
2-8-4-1-7-9-3		
6-5-8-1-9-2-4-7		
3-8-2-9-1-5-4-7		
5-6-2-8-7-4-1-3-9		
4-2-5-6-8-9-1-3-7		
	Total Forward	**Max = 14**

Digits Backward	Pass/Fail	Score 2, 1 or 0
1-9		
6-3		
2-4-5		
8-6-1		
5-3-2-7		
9-4-1-5		
2-8-6-1-4		
5-3-9-4-1		
3-1-4-9-8-7		
2-4-5-6-8-1		
2-9-3-6-5-4-7		
3-9-1-2-8-4-6		
2-8-5-7-1-9-6-3		
5-6-9-1-8-2-7-3		
	Total Backward	**Max = 14**

Trail-Making Test

Participant Code…… Date …… Time ……

Administration:
Part A
Participant instructed to draw a line between the numbers in order as quickly as possible.
This should be timed. Errors should be corrected.

Time to complete (secs)…….

Number of errors…….

Part B
Participant instructed to draw a line switching between number and leeter in order.
Start with the number 1.
This should be timed. Errors should be corrected.

Time to complete (secs) …….

Number of errors…….

Spatial Span

Participant Code…… Date ……. Time ……

Administration:
Start Presentation and load "SpatialSpan.exp" experiment (if not already loaded). Run scenario and enter participant code. Follow the instructions on screen and observe participant during the first trials. Scores will be displayed at the end of the run (until you press "continue").

Forward Score: ……

Backward Score ……

Participant Code…… Date ……. Time ……

Rey AVLT word recall & recognition (Long term memory)

*After approx. 30 mins, recall of words from the **first list only** (the one that was read 5 times), **before** going on to the Recognition trial.*

Again, write down all words that are given.

A7

AVLT WORD RECOGNITION CHECKLIST (A/B) – FORM 1

Instructions: For each word, please indicate whether you think the word came from list A (the list we repeated multiple times), from list B (the list you only heard once), or was on neither of these two lists.

BELL	HOME	TOWEL	BOAT	GLASSES
WINDOW	FISH	CURTAIN	HOT	STOCKING
HAT	MOON	FLOWER	PARENT	SHOE
BARN	TREE	COLOUR	WATER	TEACHER
RANGER	BALLOON	DESK	FARMER	STOVE
NOSE	BIRD	GUN	ROSE	NEST
WEATHER	MOUNTAIN	CRAYON	CLOUD	CHILDREN
SCHOOL	COFFEE	CHURCH	HOUSE	DRUM
HAND	MOUSE	TURKEY	STRANGER	TOFFEE
PENCIL	RIVER	FOUNTAIN	GARDEN	LAMB

TOTAL CORRECT HITS FROM LIST A ……

TOTAL CORRECT HITS FROM LIST B ……

FALSE POSITIVES: LIST A …… LIST B ……

MISSES: LIST A …… LIST B ……

THE REY AUDITORY-VERBAL LEARNING TEST
(AVLT A/B) – FORM 1
Scoring sheet

Participant Code……

List A	A1	A2	A3	A4	A5	List B	B	A6	A7
DRUM						**DESK**			
CURTAIN						**RANGER**			
BELL						**BIRD**			
COFFEE						**SHOE**			
SCHOOL						**STOVE**			
PARENT						**MOUNTAIN**			
MOON						**GLASSES**			
GARDEN						**TOWEL**			
HAT						**CLOUD**			
FARMER						**BOAT**			
NOSE						**LAMB**			
TURKEY						**GUN**			
COLOUR						**PENCIL**			
HOUSE						**CHURCH**			
RIVER						**FISH**			
score						score			

TOTAL A1 – A5 …… **A6 ……** **A6-A5 (R.I.) ……**

B …… **A7 ……** **B-A1 (P.I.) ……**

Visual Patterns Task

Participant Code…… Date ……. Time ……

Total score: ……. Max. Number of Targets: ……

Instructions for use:

- Start Presentation and open VPT experiment (if not open already)
- Start scenario and enter participant code (if a response file for the participant had been created earlier, the new responses will be appended to this file)
- Follow the instructions on the screen; help participants for questions
- Record total score and max number of targets from status window

- **Verbal Fluency**

Participant Code…… Date ……. Time ……

Write down all words that are given. Mark every 15s quarter of the test. Time for 60 seconds in total.

F	**A**	**S**
Total: …….	Total: …….	Total: …….

Attention Network Task

Participant Code…… Date ……. Time ……

Participant number: ……

Instructions for use:

- Double-click on “Attention.ebs” shortcut
- Press F7 to start
- Enter a participant number (can be date, but must be between 1 and 32768), and record above
- Enter session number (usually 1; 2 or more for repeat starts)
- Follow the instructions on the screen; help participants for questions

E MRC study ethics approval

NHS
Health Research Authority
NRES Committee North East - Newcastle & North Tyneside 2
Room 002
TEDCO Business Centre
Rolling Mill Road
Jarrow
NE32 4BW

Telephone: 0191 428 3565
Facsimile: 0191 428 3432

4 May 2012

Professor Julia L Newton
Professor of Ageing and Medicine
Clinical Deanery, Level 3
Medical School, Framlington Place
Newcastle University
Newcastle upon Tyne
NE2 4HH

Dear Professor Newton

Study title:	**Understanding the pathogenesis of autonomic dysfunction in chronic fatigue syndrome (CFS) and its relationship with cognitive impairment**
REC reference:	**12/NE/0146**

Thank you for your letter of 3 May 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **Favourable** ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement	v 1	28 March 2012
Evidence of insurance or indemnity	Zurich Municipal (for Newcastle University)	11 July 2011
GP/Consultant Information Sheets	v 1	11 March 2012
Investigator CV	Julia Newton	
Other: Caldicott & Data Protection Sheet		
Other: Email notification re Indemnity (Newcastle University)		03 April 2012
Other: Email notification re MRC Grant		12 December 2011
Other: Funding support letter		19 May 2011
Other: Email notification re Clinical Radiation Expert		02 April 2012
Other: R&D Registration Form		
Other: Response to Peer Review Comments		(not dated)
Protocol	v 1	16 March 2012
REC application	(IRAS 3.4)	21 March 2012
Referees or other scientific critique report	MRC	
Summary/Synopsis	v 2	28 March 2012
Response to Request for Further Information	J Newton	03 May 2012
Participant Consent Form (+ copy with tracked changes)	v 5	01 May 2012
Participant Information Sheet: Patients (+ copy with tracked changes)	v 5	01 May 2012
Participant Information Sheet: Controls (+ copy with tracked changes)	v 5	01 May 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/NE/0146	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

pp

Professor Philip M Preshaw
Chair

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Enclosures: 'After ethical review – guidance for researchers'

Copy to: Ms Amanda Tortice – Joint Research Office, Newcastle upon Tyne Hospitals NHS Foundation Trust