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THE PATHOPHYSIOLOGY OF CHRONIC FATIGUE SYNDROME IN ADOLESCENTS

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«For every affection of the mind that is attended with either pain or pleasure, hope or fear, is the case of an agitation whose influence extends to the heart, and there induces changes from the natural constitution, in the temperature, the pulse and the rest, which impairing all nutrition in its source and abating the powers at large, it is no wonder that various forms of incurable disease in the extremities and in the trunk are the consequence, inasmuch as in such circumstance the whole body labours under the effects of vitiated nutrition and a want of native heat.»

From 'Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus', by William Harvey (1578-1657), quoted from (159)

Abbreviations

ACI	Acceleration index
ASBF	Acral skin blood flow
CDC	Centers for Disease Control and Prevention
CFS	Chronic fatigue syndrome
CNS	Central nervous system
DBP	Diastolic blood pressure
DBPV	Diastolic blood pressure variability
EDVI	End diastolic volume index
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
HUT	Head-up tilt-test
LBNP	Lower body negative pressure
LF	Low frequency
MBP	Mean arterial blood pressure
SBP	Systolic blood pressure
SI	Stroke index
TPRI	Total peripheral resistance index
TT	Tympanic temperature

List of appended papers

Paper I. Wyller VB, Thaulow E, Amlie JP. Chronic fatigue and orthostatic intolerance effectively treated by propranolol. J Pediatr 2007, *in press.*

Paper II. Wyller VB, Due R, Saul JP, Amlie JP, Thaulow E. Usefulness of an abnormal cardiovascular response during low-grade head-up tilt-test for discriminating adolescents with chronic fatigue from healthy controls. Am J Cardiol 2007; 99: 997-1001

Paper III. Wyller VB, Saul JP, Amlie JP, Thaulow E. Sympathetic predominance of cardiovascular regulation during mild orthostatic stress in adolescents with chronic fatigue. Clin Physiol Funct Imaging 2007, *in press.*

Paper IV. Wyller VB, Saul JP, Walløe L, Thaulow E. Enhanced sympathetic response during orthostatic stress and attenuated sympathetic responses during isometric exercise may account for clinical symptoms in adolescents with chronic fatigue. Eur J Appl Physiol 2007, *in press.*

Paper V. Wyller VB, Godang K, Mørkrid L, Saul JP, Thaulow E, Walløe L. Abnormal thermoregulatory responses in adolescents with chronic fatigue syndrome: relation to clinical symptoms. Pediatrics 2007, *in press.*

Contents

Preface and acknowledgements

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However, inspiration and access to volunteers would not have been enough. The launching of a research project exploring CFS pathophysiology required the joint effort of a visionary, enthusiastic clinical academic and a precise, realistic natural scientist. My supervisors, Erik Thaulow, Dept. of Paediatrics, Rikshospitalet and Lars Walløe, Dept. of Physiology, University of Oslo, are 'archetypes' in this regard, whereas Phil Saul, Dept. of Pediatrics, Medical University of South Carolina – being both a pediatric cardiologist and a physiologist – personify a synthesis. I am most grateful for their continuous support, supervision and advice.

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Nordstrand, Oslo, February 2007

Vegard Bruun Wyller

1 Introduction

This thesis has two 'starting points' – one in the clinic, related to encounters with chronic fatigued patients (see paper I), and one in the basic sciences, related to a search for methods suited for exploring the pathophysiology of chronic fatigue syndrome. Each of these areas will be thoroughly elaborated on throughout the next two sections, followed by a paragraph addressing epistemological issues, prior findings, and research questions.

1.1 Clinical starting point – an overview of chronic fatigue syndrome

1.1.1 Definitions and terminology

Chronic fatigue syndrome (CFS) is a common and – in many instances – severely disabling disease (2, 318). Different case-definitions exists; most widespread – in research as well as in clinical practice - is the one developed by the US Centers for Disease Control and Prevention, commonly referred to as the CDC-definition (142) (Table 1). Here, the main criterion is persistent or relapsing fatigue of 6 months duration or more, severely affecting daily activities. In addition, patients should report at least 4 of 8 specific accompanying symptoms.

Other case-definitions in current use are the so-called Oxford-definition (360), the Australian definition (248), and the Canadian definition (55). None of these deviate strongly from the CDC-definition, but there are important nuances. More specifically, the Oxford-definition requires the presence of 'mental fatigue' and accepts symptoms that might indicate a psychiatric disorder; the Australian definition does not require a new or definite onset of fatigue; whereas the Canadian definition excludes patients with any symptoms of mental illness.

Table 1. CDC-definition of chronic fatigue syndrome*

Main criteria (patients must adhere to all)
Persistent or relapsing fatigue of 6 months duration or more
Fatigue severly affects daily activities
Fatigue is not explained by any concurrent somatic or psychiatric condition
Fatigue is new or definite in onset
Fatigue is not the result of ongoing exertion
Fatigue is not alleviated by rest
Additional criteria (patients must adhere to at least 4)
Impaired memory and/or concentration
Sore throat
Tender cervical and/or axillary lymph nodes
Muscle pain
Multi-joint pain
New headaches
Unrefreshing sleep
Post-exertional malaise

* Adapted from (142)

The different case-definitions – and their similiarities and differences - have been substantially debated. Two questions are of particular importance in this thesis:

- Are the different definitions more or less interchangeable, or do they define distinctly different subgroups of patients? To put it even more pointedly: Is there a correspondence between a certain case definition and a particular mechanism of disease?
- How valid are these definitions? More specifically: is the CDC-definition valid when applied to adolescents?

These problems will be addressed more in depth later (see 1.3.1).

The complexity is even higher when it comes to terminology. *Chronic fatigue syndrome* (CFS) is the preferred term among most scientists and clinicians, and will also be used in this thesis. *Myalgic encephalomyelitis* (ME) is commonly used among patient organizations (318). Whether CFS and ME designate identical or different (though related) disorders, is widely disputed. It has been maintained that *neurasthenia* – primarily used within the field of psychiatry – is a synonymous term (434). Other less common terms are *post-infectious fatigue syndrome* and *chronic fatigue and immune dysfunction syndrome.* Some argue that even entities such as *gulf war-syndrome* and *multiple chemical sensitivity* should be added to this list (25).

1.1.2 Epidemiology and history

Epidemiological data on CFS are confusingly non-consistent. This is partly explained by the varying case-definitions. However, two US community-based surveys using the CDC-definition found prevalences of 0.23 % and 0.42 % (198, 327), whereas a British primary-care study, using the same case definition, found a prevalence of 2.6 % (438). Incidences have been estimated as high as 0.18 % and 0.37 % (237, 327).
Less is known about the impact of different sociodemografic variables. Most studies have reported the prevalence in women to be about 3 times higher than in men (318). CFS is relatively rare in children younger than 10 years, whereas the vulnerability seems to be much higher in adolescents 10-17 years. An Australian survey found a prevalence of 5.5/100 000 and 48/100 000 in these two age groups, respectively (248), whereas a British study indicates much higher numbers (125). CFS is often regarded as a condition typical of industrialized communities and Caucasian ethnicity, paralleling the attitudes towards neurasthenia in the 19th century (254). Recent epidemiological surveys indicate that CFS is equally common among Blacks and Hispanics as among Whites in the US, excluding race as an important, independent factor (47, 198). However, the prevalence of fatigue syndromes seems to be higher in well-developed countries than in underdeveloped (371), at least partially justifying the notion of CFS as a disease of 'modern civilization'. Still, a Chinese study recently reported a CFS prevalence of 6.4 % (456), whereas the prevalence of chronic fatigue (not CFS) among Indian women was found to be as high as 12 % (302).
Historically, descriptions of *febricula* - a CFS-like condition - can be traced back to the 1750s (362). In a retrospective study of medical records, Jones and Wessely found indications of CFS among British soldiers in the 1850s (200). The term *neurasthenia* was first introduced by the neurologist George Beard and the psychiatrist E Van Deusen in 1869 (435). The first recorded epidemic outbreak of a CFS like condition occurred in 1934 in Los Angeles, USA, among health care professionals from several hospitals (199). Similar outbreaks have been described later on; the most prominent in Akureyri, Iceland (1948); Adelaide, Australia (1949); the Royal Free Hospital, London, UK (1954) and Great Ormond Street Hospital, London, UK (1970). The term *myalgic encephalomyelitis* originated from these events, but an infectious agent was never detected. Retrospectively, it is impossible to determine whether all or some of these medical conditions correspond precisely to the modern definition of CFS.

1.1.3 Clinical features

As indicated in the name, *fatigue* is the dominating complaint in patients with CFS (2, 318). It is important to recognize this symptom as different from common tiredness or sleepiness, experienced by everyone from time to time (Figure 1). The patients use notions like 'overwhelmingly exhausted', 'totally empty of energy' etc., and they

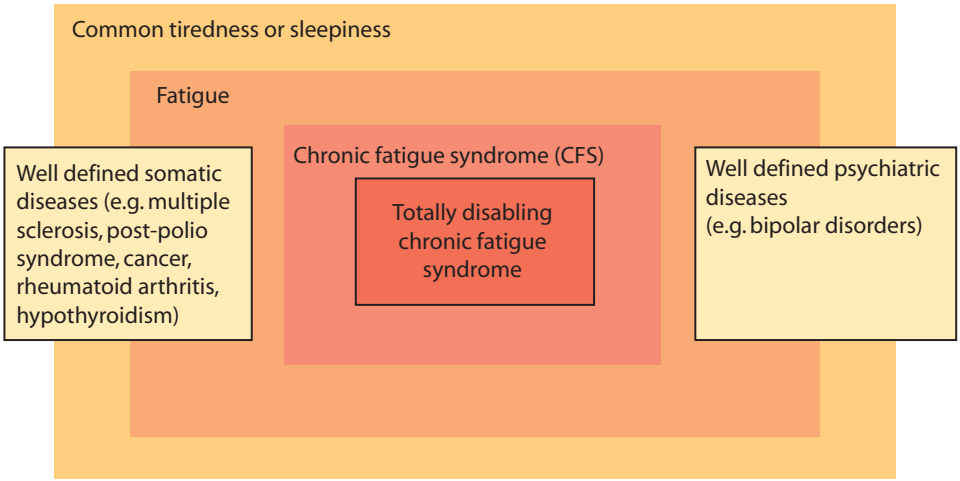


Figure 1. Schematic outline of how CFS should be differentiated from well-defined somatic and mental diseases as well as other subjective complaints (like common tiredness and sleepiness). (Adapted from (100) and slightly modified, with permission.)

describe the fatigue as qualitatively different from earlier experiences (178, 375). Limited exertions, whether mental or physical, disproportionately worsen the sensation of fatigue. Likewise, rest or sleep does not substantially relieve it.

In addition, the patients are to a varying extent bothered by additional symptoms, some of which are required according to the CDC-definition (Table 2) (5, 123, 223, 318, 375). However, none of them are specific to CFS. As far as we know, no comprehensive study has specifically addressed the frequency of each of these accompanying symptoms in a large cohort of CFS patients. In a majority of patients, the symptom intensity is fairly stable, but some report distinct fluctuations (128).

The onset of CFS can be gradual or acute (123, 318). In the latter situation, the patients often report symptoms and signs indicative of a preceding infectious disease, like mononucleosis, influenza or gastroenteritis (20, 345). However, despite intensive research, no infectious agent seems to be specifically related to CFS. In addition, evidence suggests that psychosocial stress might precipitate the disease in some patients as well (180, 345). The relationship between these empirical findings and CFS pathophysiology theories will be further elaborated below (see 1.3.2).

A diagnose of chronic fatigue syndrome requires a thorough clinical evaluation. No single diagnostic test exists. Therefore, several guidelines have been developed, for adults (1, 55, 417), as well as children/adolescents (123). Although not identical, the main messages from these guidelines are common, prompting the practitioner to:

- Identify and recognize the patients' characteristic symptoms, especially their experience of fatigue.

Table 2. List of common accompanying symptoms in CFS, putatively organized according to organ systems

Organ system	*Symptom*
Nervous system	Headache Dizziness Problems with balance Increased sensitivity to light, sounds and smells Subjective temperature sensitivity Impairments of memory and concentration Sleep disturbances
Musculoskeletal system	Muscle pain Multi-joint pain
Circulatory system	Orthostatic intolerance Palpitations Paleness
Digestive system	Abdominal pain Diarrhoea Nausea
Immune system	Tender lymph nodes Sore throat Night sweats

- Rule out differential diagnoses by a standardized and comprehensive (but not exhaustive) set of investigations.

In addition, the practitioner should assess the patients' functional impairments, which might be severe, causing school and work absenteeism, social isolation and eventually a breakdown of normal family life (318). A 4-stage functional classification system has been proposed (1): *Mild* designates mobile patients, who are able to carry out e.g. ordinary housework. *Moderate* means reduced mobility and limited ability to perform daily activities. *Severe* labels patients who use a wheel-chair and whose performance is restricted to some very simple activities, like teeth-brushing. *Very severe* is the category for completely disabled patients, who are bedridden and not able to take care of personal hygiene.

The question of co-morbidity in CFS has been extensively debated. Three problems are of particular interest. First, the CDC-definition of CFS requires the exclusion of somatic and/or psychiatric disorders that might explain the fatigue, like malignancies, rheumatic diseases and chronic infections (142) (see 1.1.1). Whereas this prerequisite

is usually unproblematic in a practical sense, some cases expose an inherent inconsistency of the case definition. For instance, should a retracted course of EBV-infection be labelled 'chronic mononucleosis', 'CFS' or both? Second, there are several clinical similarities between CFS and other so-called 'functional somatic syndromes', like irritable bowel syndrome and fibromyalgia (17). This fact increases the diagnostic challenges, as well as raises further fundamental concerns about the case definition. Third, there are different views concerning the relationship between CFS and well-defined psychiatric diagnoses. The evidence seems conflicting; for instance, some studies report increased prevalence of depression among CFS patients (2, 207), children and adolescents being particularly at risk (148), whereas findings in other studies dispute such a relationship (316, 318). Despite all these challenges, recent evidence supports the notion of CFS as a distinct diagnostic entity (63).

Qualitative research indicates that CFS patients might have problematic relationships with doctors and other health care professionals, feeling unaccepted, marginalized and not prioritized (286). Doctors, on the other hand, report helplessness and scepticism confronted with a condition of undetermined nature (8). These findings underscore how CFS raises fundamental social and ethical challenges within the doctor-patient-relationship. Without neglecting the several complicated aspects of these issues, it seems pertinent to emphasize the doctors' obligation to pay attention to and acknowledge the patients' subjective experience of symptoms, despite the lack of objective signs.

1.1.4 Treatment and prognosis

Various treatments of CFS have been subjected to randomized controlled trials. However, recent reviews conclude that only cognitive behavioral therapy (CBT) and graded exercise therapy (GET) have a scientifically proven beneficial effect (10, 100, 114, 280, 315, 335, 443). Important components of CBT are explanation of patho-physiological theories about CFS, challenging of fatigue-related cognitions and gradual increase of physical activity (318). In this way, simply speaking, the patients learn to acquire control over their symptoms. CBT is also of proven value among adolescents with CFS (401). Its success, however, does not necessarily imply a 'psychological' or 'mental' etiology. GET exposes the patient to an individually adjusted and structured exercise program (318). The aim is a gradual increase of activity level; thus GET might be regarded as a component of CBT. If the patients experience the exercise to be too strenuous, compliance falls. Thus, a very careful and gradual approach seems to be most beneficial (100). How these principles of treatment relate to subgroups of CFS patients remains a question of debate. It is important to note that the severely disabled patients are scarcely represented in the trials.

Other therapeutic approaches that have been subjected to research include gluco-corticoids, mineralcorticoids, antidepressants, anticholinergic agents, antiviral drugs, growth hormone, immunoglobulins, dietary prescriptions and alternative/complementary

therapy. For all, the present evidence is inconclusive or indicates no beneficial effect (100).
Management of CFS patients should also include attention to possible complications, like secondary depression and dietary deficiencies in the severely disabled. Further, patients need appropriate assistance with social and economical issues, as problems related to these areas may constitute important perpetuating factors (37, 352). In children and adolescents with CFS, particular effort should be devoted to their situation at school, establishing courses adjusted to the patients' individual capacity (123).
The long term prognosis of CFS is disputed. A recent review reported a 5 % median full recovery and 40 % median improvement across different primary studies (51). The prognosis of children and adolescents with CFS seems to be considerably better, with full or partial recovery in 60-80 % (21, 337).

1.2 Basic science starting point – an overview of the autonomic nervous system

1.2.1 Structure and function of the autonomic nervous system

The *autonomic nervous system* (ANS) denotes those parts of the nervous system that are related to involuntary and unconscious control of internal organs, in contrast to the somatic nervous system which is devoted to conscious perception and voluntary action (43, 155). Generally speaking, the purpose of ANS is to maintain internal homeostasis by constantly adjusting organ function; at the tissue level, this regulatory task is carried out through an effect on smooth muscles, heart muscles and glands.
The sensory part of ANS mediates information to the central nervous system from receptors in the internal organs. These receptors have different properties; of particular interest in regards to this thesis are the mechanical receptors located in the walls of great veins/heart/pulmonary vessels and aorta/carotid arteries, commonly referred to as *cardiopulmonary receptors* and *baroreceptors,* respectively. The effector part of ANS consists of chains of two neurons which synapse in an autonomic ganglion; thus, they are commonly labeled preganglionic and postganglionic (Figure 2). The transmitter in the ganglia is acetylcholine, acting on nicotinic receptor proteins of the postganglionic neuron.
Based upon both structural and functional characteristics, one can differentiate between two branches of the effector part of ANS: the parasympathetic nervous system and the sympathetic nervous system (43, 155, 353). The former innervates only the visceral organs proper, whereas the latter also has contact with blood vessels all over the body, as well as the skin and the musculoskeletal system (Table 3). Overall, the parasympathetic

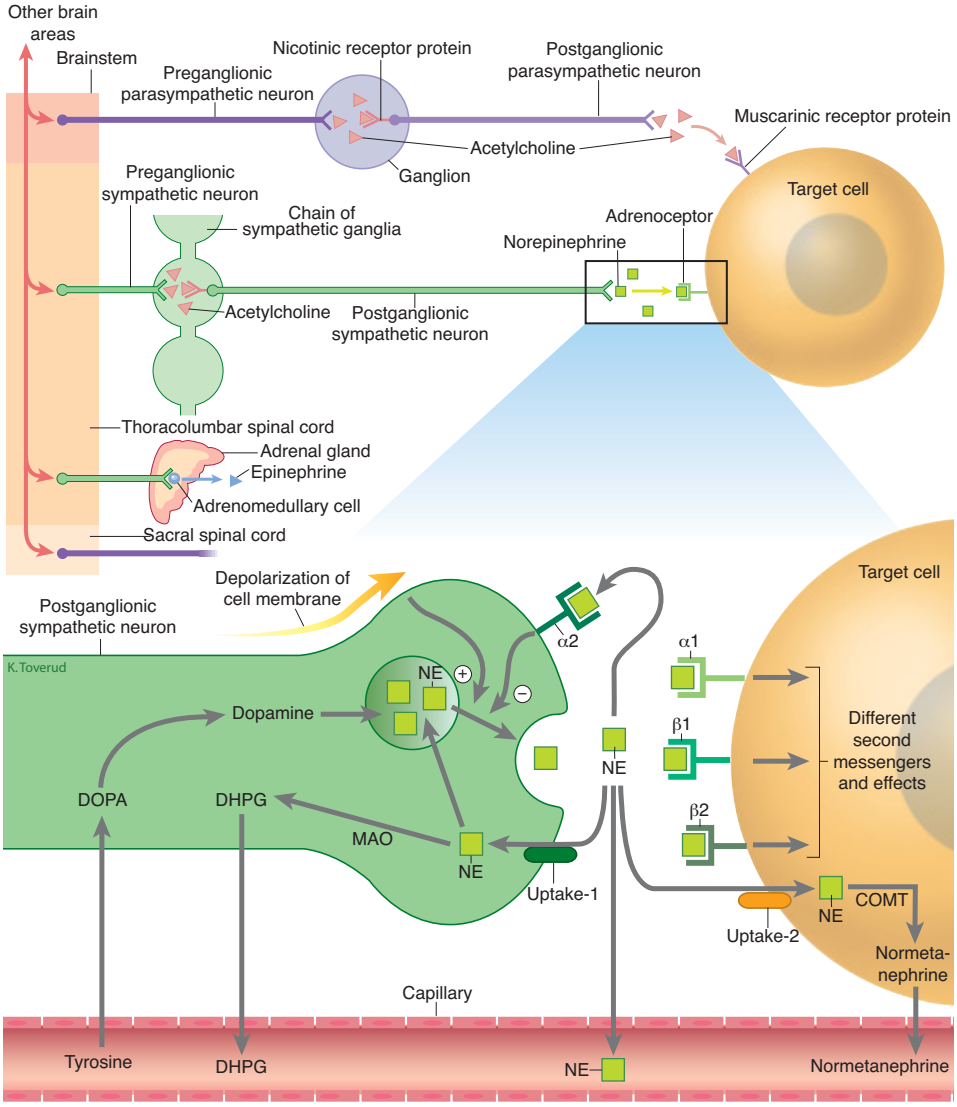


Figure 2. Schematic overview of the autonomic nervous system (upper part), and detailed outline of a noradrenergic sympathetic synapse (lower part, adapted and modified from (155)). NE=norepinephrine, DOPA=dihydroxyphenylalanine, DHPG=dihydroxyphenylglycol, MAO=monoamine oxidase, COMT=catechol-O-methyl-transferase.

Table 3. Main effects of autonomic nerve activity and epinephrine in different organs. Overview*

Organ	*Parasympathetic nerve activity*	*Sympathetic nerve activity*	*Epinephrine*
Heart	Decreased rate	Increased rate Increased contractility	Increased rate Increased contractility
Kidney		Increased renin secretion Vasoconstriction	Vasoconstriction
Lungs	Bronchoconstriction	Bronchodilation	Bronchodilation
Liver		Increased glycogenolysis	Increased glycogenolysis
Adipose tissue			Increased lipolysis
Gastrointestinal tract	Increased motility and secretion	Decreased motility and secretion Vasoconstriction	Decreased motility and secretion Vasoconstriction
Urinary bladder	Detrusor muscle contraction	Sphincter muscle contraction	
Genital organs	Erection	Ejaculation	
Eye	Miosis Accommodation Tear secretion	Mydriasis	
Skeletal muscles		Vasoconstriction Shivering	Vasodilation Shivering
Skin		Piloerection Sweating Vasoconstriction	Vasoconstriction
Lymphatic organs		Undetermined effects	Undetermined effects
Central nervous system			Improved concentration Enhanced emotional experiences

* Adapted from (43, 155, 353)

nervous system is mainly responsible for conservative, vegetative processes, while the sympathetic nervous system is particularly important for 'emergency'-reactions when internal homeostasis is threatened. Accordingly, parasympathetic and sympathetic nervous activity often has reciprocal effects on organ functions. However, this general rule greatly oversimplifies the complex and dynamic interactions between the two divisions of ANS.

The preganglionic neurons of the parasympathetic nervous system originate in the nuclei in the brain stem and the sacral spinal cord (Figure 2) (43, 155). Many of them follow the vagus nerve; hence 'vagal' is commonly, though inaccurately, used as a synonym for 'parasympathetic'. The postganglionic neurons use acetylcholine as the main transmitter, acting upon muscarinc receptor proteins of which there are several variants related to different second messengers.

The preganglionic neurons of the sympathetic nervous system emanate from the intermediolateral column of the thoracolumbar spinal cord, and end in the preaortic and paravertebral chains of ganglia (Figure 2) (43, 155). Most postganglionic neurons use norepinephrine (noradrenalin) as the main transmitter, but the neurons supplying sweat glands use acetylcholine. In addition, other transmitter substances like ATP and neuropeptide Y have been described but their physiological effects remain largely unknown (109, 308). Norepinephrine is synthesized from the amino acid tyrosine; intermediate products in this process are dihydroxyphenylalanine (DOPA) and dopamine (Figure 2) (155). The transmitter is stored in synaptic vesicles and released by exocytosis upon depolarization of the cell membrane. The receptor proteins binding to norepinephrine, commonly referred to as *adrenoceptors,* are divided into two main categories, labeled alpha and beta. Numerous subtypes are described, having different functional properties. Alpha2-adrenoceptors, which are found presynaptically at sympathetic neurons and also in the central nervous system, are particularly important for negative feedback-regulation, as the binding of norepinephrine to these proteins attenuates sympathetic nervous activity. Released norepinephrine is inactivated by two different uptake-mechanisms (Figure 2) (155). Uptake-1 designates transport by a specific membrane protein back into sympathetic nerve terminals. Here, the transmitter might be recycled into storage vesicles, or degraded by the enzyme monoamine oxidase (MAO) to form dihydroxy-phenylglycol (DHPG). Uptake-2 means transport into non-neural cells, and subsequent degradation is mainly performed by the enzyme catechol-O-methyltransferase (COMT), forming normetanephrine.

The adrenal medulla closely resembles a sympathetic ganglion. The adrenomedullary cells are controlled by preganglionic sympathetic neurons, and are thus themselves analogue with postganglionic neurons (43, 155). However, they secrete *epinephrine* (adrenalin) instead of norepinehrpine, and this chemical compound functions as a hormone, not a neurotransmitter. Although epinephrine binds to both alpha- and beta-adrenoceptors, the affinity differs from that of norepinephrine. This partly explains why increased adrenomedullary activity and generally enhanced sympathetic nerve activity does not produce identical physiological effects (Table 3). The main break-down route of epinephrine is degradation by COMT to metanephrine.

Specific areas in the central nervous system receive afferent impulses from receptors in the internal organs and control efferent impulses in the effector part of ANS (Figure 3). In addition, these areas communicate with other brain centers responsible for emotional and cognitive processes, voluntary movements, conscious perception, and endocrine control. For instance, an area in the reticular substance of the *medulla oblongata* (the rostral ventrolateral medulla, RVLM) indirectly receives inputs from baroreceptors, and directly controls preganglionic sympathetic neurons, thus constituting a vital part of the baroreceptor reflex (se 1.2.2) (155). In addition, RVLM is reciprocally connected with the nearby *raphe nuclei,* with *locus ceruleus* in pons, with the *paraventricular nucleus* in hypothalamus, and with *amygdala* in the limbic system. The raphe nuclei probably have a key role in processing painful stimuli (43). The neurons of locus ceruleus

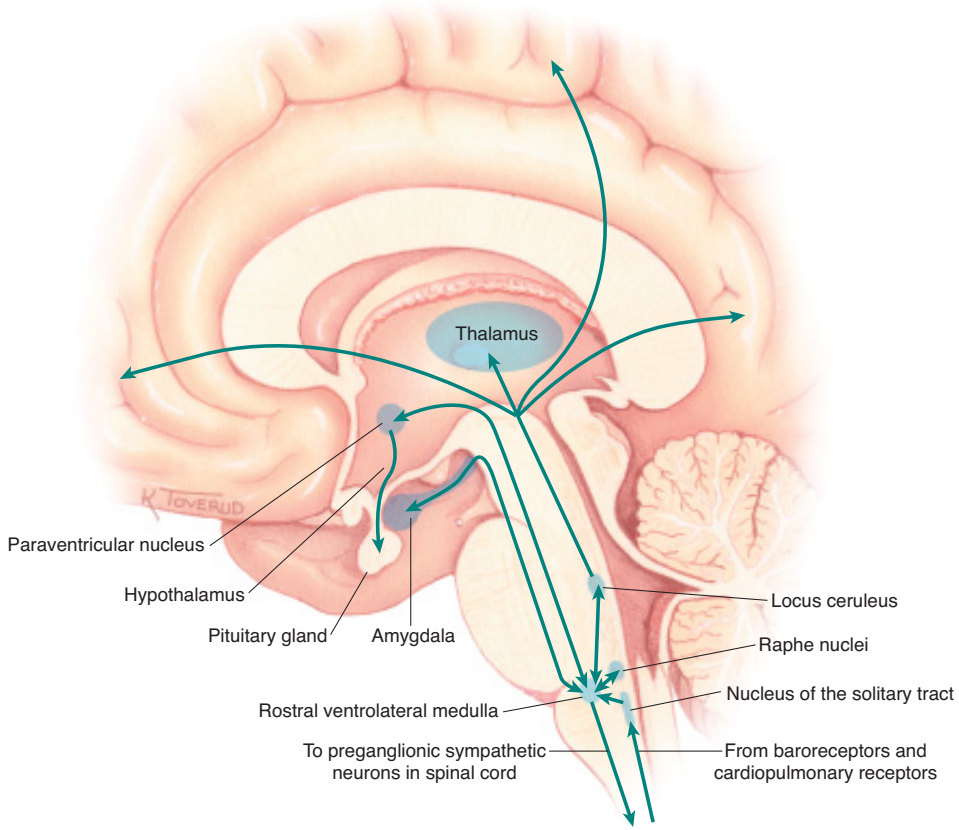


Figure 3. Selected areas and connections within the central nervous system important for autonomic control. The figure is greatly simplified. (Adapted and modified from (43)).

project extensively to all brain areas (160). Being part of the brain stem 'activation system', the locus ceruleus probably participate in the regulation of sleep and consciousness; in addition, this area seems to be important for attention (43). Neurons in the paraventricular nucleus produce the hormones vasopressin and corticotropin-releasing hormone (CRH), which are secreted by axons projecting to the pituitary gland. CRH controls the synthesis of adrenocorticotropic hormone (ACTH), which in turn regulates the secretion of glucocorticoids from the adrenal cortex. Amygdala probably has a key role in emotional memory, relating sensory information to specific emotional states, and orchestrating appropriate behavioral and autonomic responses (43). In other words, amygdala seems to be important for various learning processes, in particular conditioning.

1.2.2 Homeostatic regulatory systems

General considerations

Teleologically speaking, the main purpose of the autonomic nervous system is to maintain short-time homeostasis (155). This is achieved through numerous control circuits, whose common, basic structure is schematically outlined in Figure 4. In this thesis, three of these regulatory systems are of particular interest, namely those controlling arterial blood pressure, blood volume and body temperature.

The performance of all these reflex loops might be characterized by two variables: the *set-point*, designating a theoretical objective for the continuous adjustment of the controlled variable (like arterial blood pressure), and the *sensitivity* (or gain), referring to the response of the effector organs when the controlled variable deviates from the

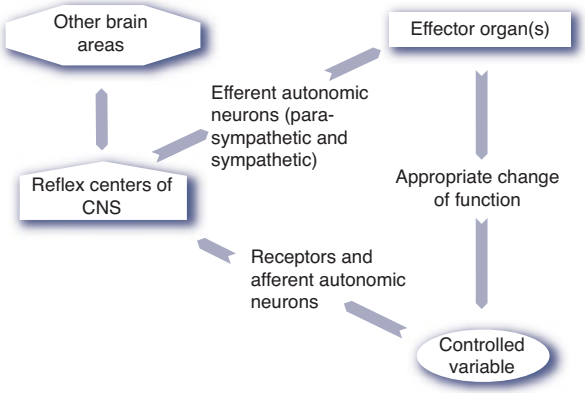


Figure 4. Schematic outline of a homeostatic control circuit involving the autonomic nervous system.

set-point (381). Both set-point and sensitivity might be altered in different physiological and pathophysiological states. For instance, during exercise, the set-point of the baroreceptor reflex is reset to a higher level, probably due to the combined effect of neural inputs from higher brain centers ('central command') and from receptors in the working muscle (269). In addition, there is a decrease of sensitivity which seems to be caused by altering sympathetic and parasympathetic modulation of heart rate (292).
Most control circuits, whether in physiology or engineering, expose dynamic behavior, characterized by the fluctuations of the controlled variable around a mean value corresponding to the set-point (7, 73, 416). This *variability* may be explored by sophisticated mathematical analyses, providing important information concerning the neural control mechanisms, as will be further elaborated below (see 2.2.2).

Arterial blood pressure
The arterial blood pressure is continuously monitored by mechanical receptors – *baroreceptors* - in the aorta and the two common carotid arteries (Figure 5) (113). The afferent neural impulses are transmitted to the *nucleus of the solitary tract* in the brain stem via the glossofaryngeal and vagus nerves, and further mediated to cardiovascular control centers of the reticular substance and to other brain areas (see 1.2.1) (216, 381) (Figure 3). Recent evidence indicates that even cortical brain regions are involved (218).
The efferent part of the reflex loop has two 'limbs': sympathetic and parasympathetic neurons to the heart adjust heart rate and contractility, whereas sympathetic neurons to arterioles adjust total peripheral resistance (155). The relative contribution from the

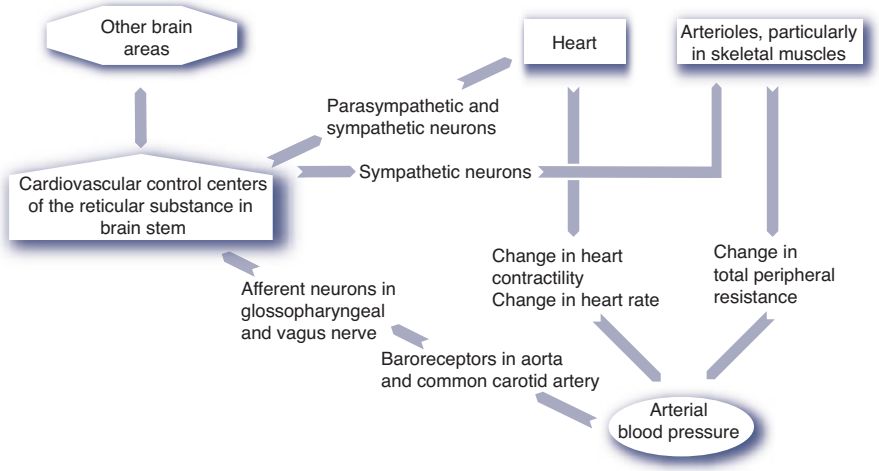


Figure 5. Schematic outline of the baroreceptor reflex controlling arterial blood pressure.

different vascular beds for increasing total peripheral resistance in distinct physiological and pathophysiological states is still largely undetermined; however, skeletal muscle vasoconstriction is usually considered the most important effector response (209, 353). Whether the baroreceptor reflex participates in long-term regulation of blood pressure is unknown. However, ample evidence indicates that baroreceptor resetting occurs shortly after artificial manipulation of blood pressure, which raises questions concerning their role in long term regulation (259, 294).

Blood volume

Blood volume is regulated by several complex control circuits, involving both neural and endocrine mechanisms (Figure 6) (155, 353). Here, the focus is on the autonomic nervous system. The *cardiopulmonary receptors* designate a diverse group of mechanical receptors located in the central, low-pressure part of the circulatory system (172). Their apparent role in circulatory homeostasis may justify the term 'volume receptors'; however, detailed knowledge of their anatomical and functional properties is still lacking. Afferent discharge seems to follow the same route as afferent discharge from the arterial baroreceptors (326). Still, the effector part of the reflex is not identical to the baroreceptor reflex, as one of its important components is efferent sympathetic neurons to the kidneys (155, 164). These neurons control both renal perfusion and the release of the hormone renin. In addition, efferent sympathetic neurons to skeletal muscles adjust total peripheral resistance by controlling arteriolar constriction (12), and supposedly also adjust total blood vessel capacitance by controlling venous constriction. The participation of other vascular beds in the control circuit remains uncertain, but adjustments of splanchnic perfusion probably play an important role (186). Finally, efferent parasympathetic and

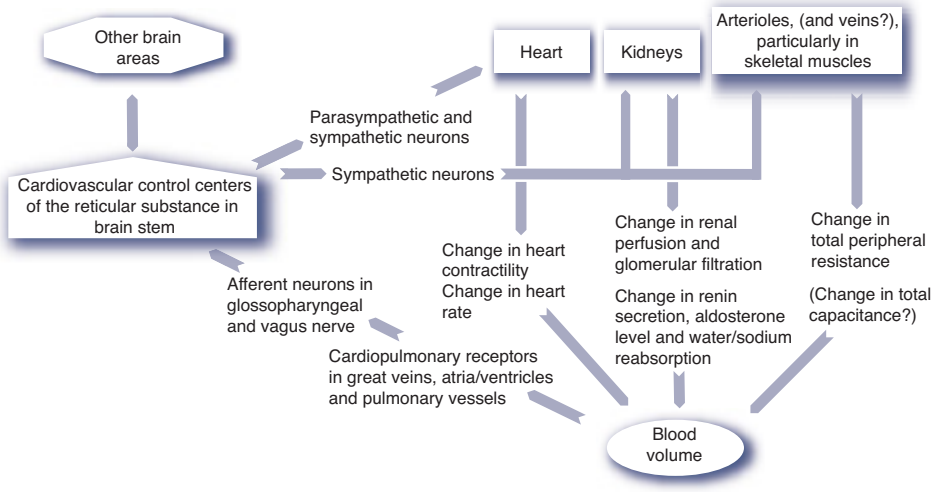


Figure 6. Schematic outline of the cardiopulmonary reflex controlling blood volume.

sympathetic neurons adjust heart rate and contractility (144, 418). However, heart rate control also differs from the baroreceptor reflex, as moderately augmented cardiac filling may – in some situations – increase heart rate (the so-called 'Bainbridge-reflex') (15). To increase complexity further, evidence indicates that the cardiopulmonary and baroreceptor reflexes interact (430).

Body temperature

Discrete regions of the hypothalamus seem to be directly involved in human thermoregulation, having specific populations of temperature-sensitive neurons (43, 155) (Figure 7). In addition, these areas receive sensory information from thermoreceptors in the skin. A region in the posterior hypothalamus seems to be the principal 'conductor' orchestrating the physiological responses to heat or cold (105). Areas in the brain stem, in particular the raphe nuclei, are probably involved in the efferent pathways (54, 278). The effector organs involved with human thermoregulation are sweat glands, skin arterioles, skin arteriovenous anastomoses and skeletal muscles. Sweat glands are innervated by cholinergic sympathetic neurons, which also seem to promote vasodilation in nearby arterioles by a local release of NO and prostaglandins from the endothelial cells (210, 211). However, the main control of skin arterioles, and also of skin arteriovenous anastomoses, is exerted by vasoconstrictive noradrenergic sympathetic neurons (23). Skeletal muscles are controlled by motor neurons of the somatic nervous system; however, enhanced sympathetic outflow and epinephrine secretion promotes shivering (155, 391).

Several other features of human thermoregulation add to its complexity. First, central and peripheral thermoreceptors may give rise to conflicting afferent information (353).

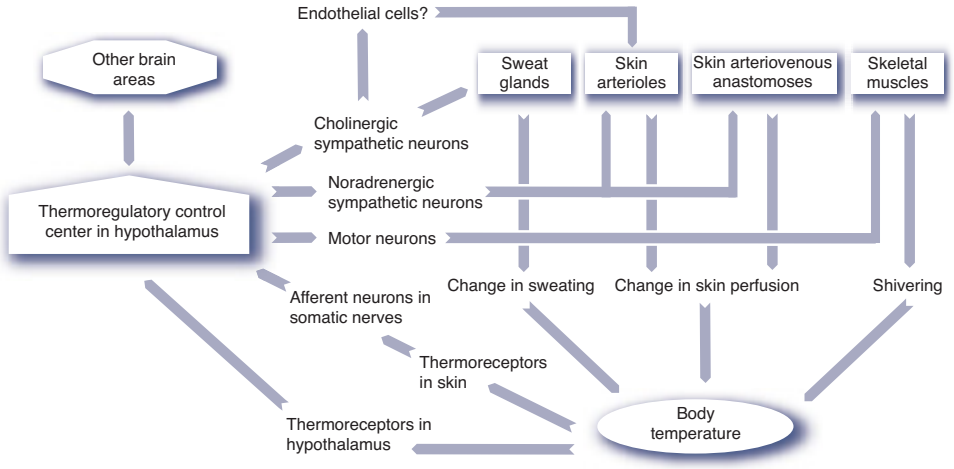


Figure 7. Schematic outline of the thermoregulatory reflex controlling body temperature.

Second, local temperature changes may call for differentiated responses to distinct parts of the body (23). Third, the thermoregulatory and cardiovascular control circuits share effector organs – the skin blood vessels – causing 'conflicts of interest' during for instance strenuous exercise (155). Fourth, thermoregulation also includes appropriate behavioral responses, such as taking on or off clothes, as well as endocrine responses from the adrenals and the thyroid gland adjusting energy metabolism (391).

1.3 The pathophysiology of chronic fatigue syndrome – epistemological issues, prior findings and research questions

The approaching of a scientific problem is inevitably influenced by prior knowledge and attitudes. There is – in other words – no true neutral position (147). A researcher is always restricted by his 'horizon of understanding' even before he starts searching the literature and formulating research questions. Therefore, in this section, I will start out by discussing some fundamental epistemological issues and clarifying my positions in regards to them. Thereafter I will present the body of knowledge concerning CFS pathophysiology and the specific aims of this thesis.

1.3.1 Epistemological considerations and premises

Fatigue is poorly defined both linguistically and biologically, despite how common it is in clinical practice (407). One attempt of classification is to differentiate between 'central' fatigue, originating from the central nervous system (CNS) and 'peripheral' fatigue, originating from peripheral nerves and muscles. However, the evidence justifying such a theoretical construct seems sparse. Even the precise meaning of the word 'fatigue' is obscure, a consequence of its completely subjective nature. Does it describe identical experiences when used in relation to, e.g., CFS, liver disease and marathon running, or is the sensation of 'fatigue' in these situations different (though related), indicating separate biological mechanisms?

As fatigue is a subjective experience, it cannot be objectively measured. Several psychometric instruments have been developed (108, 228). However, the lack of a 'gold standard' will always raise questions of validity, both in regards to the instruments themselves, and the empirical research based upon them. Thus, from a very restrictive point of view, one might argue that fatigue is not 'researchable' within the paradigm of the natural sciences at all.

Recent theories of fatigue focus primarily on mechanisms within CNS. Evidence indicates the important role of corticotropin-releasing hormone (CRH), some cytokines (IL-1beta, IL-6), and the neurotransmitters serotonin (5-hydroxytryptamine), dopamine

and norepinephrine (56, 250, 407). These mediators may be influenced from processes – including diseases – outside the CNS, like chronic inflammatory conditions, but they are also strongly related to nerve activity in limbic and cortical brain centers. Thus, fatigue is dependent upon both 'mental' and 'somatic' processes, as can be demonstrated empirically (97, 173).

Exploring mechanisms of causality is a general aim within the natural sciences (147). However, given the complex nature of fatigue, cause-effect-relationships are difficult to establish. For instance, is the increased serotonin concentration within CNS, which has been reported in fatigued subjects (407), a cause or a consequence of fatigue? Similar uncertainties apply to most interpretations of empirical findings within this field. Further, the causes of fatigue can be multifactorial in each individual, and a 1:1-relation between cause and effect seems both empirically and theoretically unlikely (436).

The challenges described above in relation to fatigue in general, also apply to CFS research. In addition, there is the problem of case definition, as mentioned in 1.1.1. No firm evidence supports a specific relationship between certain accompanying symptoms and pathophysiological mechanisms; thus, the symptom criteria in the CDC-definition may seem somewhat arbitrary (31, 436). Among children and adolescents, the validity of this definition is even less established (123, 265). On the other hand, grouping together conditions that do have different etiology or pathophysiology, although we do not know them yet, might obscure important scientific findings. As of now, this dilemma awaits its solution.

In conclusion, research surrounding CFS pathophysiology is challenged by:

- The subjective nature of fatigue, including the problems of how to measure it.
- The limited knowledge concerning the biological mechanisms of fatigue, including undetermined cause-effect-relationships, the possibility of multi-factoriality, and the likelihood of body-mind-interaction.
- The uncertain validity of the CFS case definition, especially within the pediatric population.

In this thesis, the positions taken towards these challenges are as follows. First, although fatigue is a subjective experience, we maintain that it nevertheless constitutes a *phenomenon* which merits systematical, scientific investigation. That fatigue – and CFS – *exists* and constitutes a *clinical entity* is an assertion of high 'face validity', supported by empirical findings (178, 405). Furthermore, scientific investigations in this field should certainly include, but not be restricted to, the use of hermeneutic methods. In our view, the relative absence of measurable entities does not exclude a natural science approach *a priori.* Second, given the limited mechanistic knowledge, investigations could hardly be governed by hypotheses of causality. Rather, the aim must be *phenomenologic:* to *explore* and *describe* aspects of the pathophysiology, and thereby – hopefully – be able to *present* hypotheses for further research. Third, the complexity of the matter, and in particular the possibility of body-mind-interactions, suggests that the results of the study should be interpreted within a *biopsychosocial*

framework, despite its biological starting point (117). There is a widespread acceptance that fatigue cannot be understood within a traditional, dichotomous, Cartesian-inspired classification of medical phenomena as either 'physical' or 'psychological' (318, 436). Fourth, the ambiguity of the CDC-definition, specifically emphasized in a recent, authoritative review (63), suggests a modification of it. We therefore omitted the bicriterion (4 of 8 accompanying symptoms, see 1.1.1), and included patients solely based upon the fulfillment of the main criterion, as will be further outlined below (see 2.1.1).

1.3.2 Prior research on CFS pathophysiology

Prior research surrounding the pathophysiology of CFS has been conducted along several tracks, reflecting the great uncertainty about the condition as well as the different scientific traditions among the researchers. This has resulted in a vast amount of papers; a PubMed search using 'chronic fatigue syndrome and pathogenesis' as criterion generated more than 1600 hits. Still, there is at present no coherent theory, and CFS is often labeled 'mysterious' or 'controversial' (155). In this brief review, all aspects of the pathophysiology will be considered, but with an emphasis on research relating CFS to the autonomic nervous system.

Genetics
Twin studies indicate a moderate heritability of CFS (63, 404). In a recent comprehensive attempt to integrate clinical and epidemiological data with genomic and proteomic profiles (429), findings suggest that chronic fatigue is related to polymorphisms of genes involved in CNS control of autonomic and endocrine effector systems, including the genes for monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (154, 373). Further analyses of gene expressions in mononuclear blood cells revealed very complex results, indicating altered activation of genes controlling both common metabolic pathways (gluconeogenesis, lipid metabolism) and signal transduction pathways involved in immune and neuroendocrine responses (124, 439). Other studies have reported a similar complicated picture (215).

Infections
CFS often has an acute onset with symptoms strongly resembling an infection (see 1.1.3). Therefore, a substantial amount of research has tried to detect a possible infectious agent. In the 1980s, much attention was given to the Epstein-Barr virus (EBV), as infectious mononucleosis may have a prolonged course or – in the worst case – develop into CFS (52, 441). However, no specific role of EBV has been established (226); rather, an EBV-infection should be regarded as one of many possible precipitating and eventually perpetuating factors (2). The same view applies to several other microorganisms that may similarly elicit severe fatigue and a prolonged recovery in a

subset of patients; examples include cytomegalovirus, parvovirus B19, *Brucella*-species, *Toxoplasma gondii, Coxiella burnetii, Mycyplasma*-species and *Chlamydia pneumoniae* (61, 62, 243, 284). However, it should also be noted that common, non-specific infections (like upper respiratory tract infections) are not likely to trigger CFS (437).
A possible pathogenetic role of enteroviruses has been thoroughly debated. Using the PCR technique, Gow and co-workers reported enteroviral RNA in skeletal muscle biopsies in a majority of adult CFS patients; however, enteroviral RNA was also detected among some controls (163). In a recent review, Chia concluded that enteroviruses might have a pathogenetic role in CFS patients, possibly causing chronic inflammatory changes in skeletal muscle (61).

Immunity
The significance of immune system disturbances in CFS patients has been a matter of controversy. Based on a systematic review of studies addressing T-cell function, B-cell function, NK-cell function, immunoglobulins and cytokines, Lyall and co-workers concluded in that there is no consistent pattern of immunological abnormalities in CFS patients, although they found a trend towards changes in T-cell activity (255). Recent studies have reported a reduced level of the cytokine TGF-beta1, which normally inhibits antibody production, increased levels of IL-6 (53), which stimulates the acute phase response, and alterations in the 2-5A synthetase/ribonuclease L pathway (94, 403), which participate in intracellular defense against viruses. The latter abnormality also seems to correlate with exercise performance (289). More generally, there is evidence of a bias towards Th2 immune responses (humoral) at the cost of Th1 immune responses (cellular) in CFS (63, 301, 372). This is consistent with frequent reports of reduced NK cell activity (2, 249), as these cells are important effectors in the Th1 immune reaction. However, a twin study did not report significant differences between CFS patients and their healthy siblings (344).
Studies of autoimmunity have yielded conflicting results. Recently, researchers reported the presence of autoantibodies specifically directed against the muscarinic cholinergic receptor protein (410) as well as autoantibodies against certain common cellular antigens (428). An association between CFS and distinct HLA antigens has also been reported (191).

Oxidative stress
One study found increased levels of methaemoglobin and other indicators of oxidative stress, correlating strongly with the patients' complaints (328). Similar findings have later been reported by others, providing evidence of free radical attacks on cell membrane phospholipids in CFS patients (212). These results could be explained by immune activation in general, but could also be attributed to a persistent viral infection. Increased oxidative stress has, in turn, been proposed as an explanation for altered skeletal muscle excitability as well as muscle pain and postexertional malaise among CFS patients (197).

Skeletal muscle function

Several early studies concluded that CFS patients have perfectly normal muscle strength, endurance and recovery, as reviewed in the Australian CFS guidelines (64). However, others report that patients are weaker than sedentary controls as judged from maximum voluntary contraction (143, 304), and that their performances are further attenuated 24 hours later, indicating delayed recovery (143). Neurophysiological experiments suggest that one probable explanation is an altered activation of cortical motor areas in the central nervous system of CFS patients; this phenomenon being even more pronounced when the isometric exercise induced a subjective experience of fatigue (351, 367). Related findings of altered cortical excitability are reported during non-fatiguing movements (380), and also immediately prior to motor performances, the latter indicating disturbances of attention (162). Interestingly, attention deficit has also been suggested in an earlier neurophysiological study of different design (314), whereas altered planning of motor activities is adherent with recent findings by functional magnetic resonance imaging (91).

In 1991, Behan and co-workers reported intracellular lipid excess and morphological changes of mitochondria in skeletal muscle biopsies from CFS patients; these findings could perhaps be attributed to an intracellular infectious process, but are also similar to alterations found in hereditary mitochondrial myopathies (19). However, these findings were not reproduced (234, 312). On the contrary, it was concluded that despite inactivity, skeletal muscle biopsies from CFS patients exhibit less fiber atrophy and related changes than expected. A defect of oxidative metabolism and subsequent enhancement of anaerobic glycolysis has been reported (447), but was not confirmed in recent research (197). Still, Vecchiet and co-workers demonstrated increased pain sensitivity in skeletal muscles and related morphological abnormalities (426). These findings may also explain the altered activation of central motor areas due to negative feedback, especially since exercise seems to lower pain threshold in CFS patients (442).

Neuroimaging

Neuroimaging studies in CFS patients have yielded conflicting results. Using brain MRI, some investigators have reported subtle alterations of subcortical white matter, correlating with the patients' complaints (77) and a reduction in total gray matter volume (90), whereas others did not find any differences between CFS patients and healthy controls (80, 167). Functional MRI and SPECT techniques indicate an alteration in information processing (90, 236), planning of motor activities (91), cortical perfusion in general (457) and brain stem perfusion (83). However, a twin study indicated that the resting regional blood flow pattern in the brain is similar between patients and their healthy twins (244). A few PET scan studies have been undertaken in CFS patients. Tirelli and co-workers documented glucose hypometabolism in the frontal cortex and brain stem (414), whereas Siessmeier and co-workers found alterations of brain glucose metabolism among half of the included patients, though no clear pattern could be defined (368). Recently, two independent groups have reported a decreased number and/or

affinity for the receptor protein 5-HT_{1A} in hippocampus (70) and the serotonin transporter protein in the cingulate gyrus (449). These results are in accordance with results suggesting blunted serotonine activation of HPA axis (106), but contradict earlier indications of increased serotonergic neurotransmission (69).

Sleep

CFS patients regularly complain of altered sleep patterns, in particular difficulties initiating and maintaining sleep (224). Several researchers have provided evidence of sleep disturbances in CFS (131, 261, 415), but no consistent pattern has emerged (2), and some trials even failed to demonstrate any significant alterations in EEG signals during sleep (13).

Cognitive function

Cognitive tests of CFS patients have revealed disturbances of memory, attention and information processing, including those patients devoid of any psychiatric comorbidity (93). Albeit the evidence is not uniform, a recent review concluded that CFS patients do have modest, but significant, cognitive impairments (272). Some reports indicate that cognitive performance deteriorates further during exercise (26, 232) but conflicting results exist (78). Morris and co-workers exposed CFS patients to different mental and bodily stressors, and reported an improved speed in planning tasks during concomitant administrations of the alpha2-adrenoceptor agonist clonidine in high doses (277). There are, however, also reports of a discrepancy between perceived and actual cognitive performance among CFS patients (271), and twin studies showed that the healthy twins had similarly reduced information processing abilities when compared to the CFS patients (256).

Psychology and psychiatry

The possible relationships between CFS and psychiatric disorders have been – and still are – matters of great controversy. Partly, this can be explained by the ambiguity inherent in the different case definitions of CFS. Specifically, many CFS patients fulfill the diagnostic criteria for a somatization disorder. However, whether such a diagnosis is made «is, to a considerable degree, dependent on the examiner's attributions of chronic fatigue syndrome symptoms and is of limited use in understanding chronic fatigue syndrome» (2). Also, the prevalence of panic disorders and generalized anxiety disorders is much higher among CFS patients than within the general population, both among adults (130, 235) and adolescents (149), suggesting related pathophysiologies. Finally, depression is also common among CFS patients, but recent evidence confirms that depression and CFS are two distinct entities (2, 63, 318, 422).

Although CFS often has an infection-like onset, research suggests that critical life events (e.g. loss of spouse), severe physical stressors (trauma, surgery) and perceived chronic difficulties – in particular those described as dilemmas - may precipitate the disorder (180, 345, 412). Besides, some studies report that certain personality traits, like

perfectionism and conscientiousness, predispose for CFS (319, 440), but evidence is conflicting (448).
Psychological and social issues are often regarded as important perpetuating factors in CFS (318). Certain illness perceptions, such as a poor sense of personal control over symptoms and a strong focus on bodily sensations, are correlated to increased impairments in several studies (182, 187, 310). Likewise, CFS patients express a fear of physical exercise that does not correspond to their physical disability (288, 369), they perceive their cognitive performance as poorer than it is in reality (271), and they sleep better than what they subjectively report (13). Patients' attributions also seem to come into play, as a one-sided focus on somatic processes is related to a poorer outcome (187).
These inappropriate cognitions may be strengthened by social interaction with family, friends and health care professionals (318, 352). Reduced self-esteem is a common complaint among adults (440) as well as adolescents (149), and a lack of social support, which is often experienced by CFS patients, may further worsen the situation (317). Finally, the social role of being ill is – despite obvious undesirable consequences - also potentially rewarding, causing an unconscious cycle of reinforcement (318).

Endocrinology
The hypothalamus-pituitary-adrenal axis (HPA axis) has been extensively explored among CFS patients, and there seems to be a general agreement concerning some subtle alterations, although the results are far from uniform (63, 68, 299, 318). Most researchers in this field report low basal levels of cortisol in urine, plasma and saliva as well as enhanced negative feedback, possibly due to increased sensitivity or number of glucocorticoid receptors in the brain (68); some recent studies, however, failed to reproduce these findings (190, 208). The normal circadian rhythm of HPA activity is also disturbed, particularly attenuating cortisol secretion during the morning hours (63, 95, 103, 415). As for challenge tests, most studies indicate blunted HPA axis responses to exercise, hypoglycemia and the administration of stimulating pharmaceuticals (68, 295, 355); however, high doses of the alpha2-adrenoceptor agonist clonidine increase plasma levels of cortisol under conditions of high arousal (277). The underlying mechanisms for these disturbances as well as their functional consequences remain unresolved; however, a relationship to the documented immune abnormalities is an obvious possibility (63).
Several researchers have focused on other endocrine systems. There are some reports of increased levels of dehydroepiandrostenedione (DHEA) (71), whereas others found the opposite (68). Studies addressing endogenous opoid tone have also reported conflicting results (68, 190). The GH and prolactin systems seem to be intact in CFS patients (68, 103, 295), whereas melatonin levels appear to be higher than normal (222).
Reports concerning catecholamines are sparse; existing evidence indicates increased basal levels of epinephrine, but normal plasma levels of norepinephrine (208, 413).

Compared with controls, CFS patients have lower plasma levels of tyrosine and higher plasma levels of tryptophan after strenuous physical exercise, indicating disturbances of the noradrenergic and serotoninergic transmitter systems in the CNS (150).
Demitrack and colleagues found reduced plasma levels of the norepinephrine breakdown product 3-methoxy-4-hydroxyphenylglycol (MHPG) and increased levels of the serotonine metabolite 5-hydroxyindoleacetic acid (5-HIAA), but the levels within cerebrospinal fluid were similar to those found in the healthy controls (96). The significance of this finding is unclear, as MHPG in plasma derives from many sources (156).

Circulatory homeostasis
The first papers on cardiovascular disturbances in CFS patients emerged in the 1990s, reporting neurally mediated hypotension during head-up tilt tests (35, 339). Subsequently, variants of haemodynamic instability during orthostatic challenge – most commonly neurally mediated hypotension or orthostatic tachycardia - have been described by many researchers in adult as well as pediatric patients (231, 306, 340, 386, 387). Similar baseline abnormalities have also been reported (231), as well as a relationship between symptom severity and a decline in stroke volume and baroreceptor sensitivity during tilt (306, 307). More sophisticated analyses of cardiovascular variability indicate a sympathetic predominance in the modulation of heart rate and total peripheral resistance during rest, orthostatic challenge and moderate exercise (81, 92, 282, 370, 383). Recently, a shortened QT interval was reported among CFS patients, further indicating increased sympathetic and attenuated parasympathetic cardiac neurotransmission (283). A twin study, however, showed that CFS patients and their healthy twins had similar haemodynamic responses to tilt-table testing (313), and a recent population-based study failed to demonstrate orthostatic instability among CFS patients (201), challenging the assumption that cardiovascular dysregulation is an important aspect of CFS pathophysiology. Moreover, some studies of variability have been inconclusive (390, 458) or simply negative (111, 453).
Hypovolemia has been proposed to underlie these regulatory disturbances, and was indeed indicated in one study, although not to the level of statistical significance (126). Accordingly, Streeten and co-workers provided evidence of reduced erythrocyte volume (398) and impaired lower-limb venous innervation (396) in CFS patients, whereas Rowe and colleagues found a strong association between CFS and the connective tissue disease Ehlers-Danlos syndrome (338). Taken together, these results suggest that relative hypovolemia might be a cause of hemodynamic disturbances in CFS. However, two controlled trials of volume expansion treatment (fludrocortisone) in CFS were not successful (309, 341).
Hemodynamic disturbances have also been documented in other organ systems. Brain stem hypoperfusion was an early finding (83). A general reduction in cerebral blood flow upon standing has been reported as well (409), but was not confirmed in subsequent experiments (325), thus weakening a hypothesis of reduced brain perfusion as a direct

cause of the fatigue sensation. McCully and co-workers found normal oxidative metabolism in working skeletal muscle, but subtle alterations in blood flow after dynamic exercise, possibly due to sympathetically induced vasoconstriction (267). Abnormal dilatation of renal and other intraabdominal veins has also been reported (408). Finally, there is evidence of altered skin circulation, as CFS patients are more sensitive to the vasodilative effect of locally applied acetylcholine (203, 378).

Temperature homeostasis

Adolescents with CFS seem to have an altered circadian rhythm of core body temperature (415). Two studies in adult patients, however, found normal circadian variation (175, 176). Pazderka-Robinson and colleagues reported increased skin temperature and lower electrodermal activity among CFS patients; the latter indicating attenuated sweating (305). There is also evidence of altered skin circulation, as outlined above (203, 378).

The autonomic nervous system

Pagani and co-workers were among the first to suggest a relationship between autonomic dysfunction and unexplained chronic fatigue, based upon research on cardiovascular variability (298). Subsequently, the same group put forward a hypothesis of autonomic dysfunction in CFS, characterized by enhanced sympathetic activity at rest and reduced responsiveness to excitatory stimuli (297). Freeman and Komaroff reached similar conclusions based upon thorough autonomic testing that indicated an increased sympathetic control of cardiovascular variables at rest, but decreased sympathetic modulation of the same variables during orthostatic challenge (137). They proposed postviral autonomic neuropathy or cardiovascular deconditioning as possible underlying mechanisms for the observations. The possible significance of autonomic disturbances and the need for comprehensive research projects have been recognized in subsequent papers (151, 159, 282). However, a unifying theory relating autonomic dysfunction to other pathophysiologic aspects of CFS is still lacking.

Concluding remarks

Based upon this review, the following concluding statements seem to be justified:

- There is a hereditary component in CFS.
- Several different infectious agents may precipitate the disorder, but there is no specific relationship.
- There is a shift towards a Th2 immune response at the cost of a Th1 immune response.
- There is increased oxidative stress.
- Muscle weakness can be explained by alteration in CNS; evidence concerning distinct muscle pathology is uncertain.
- Neuroimaging studies indicate reduced perfusion and metabolism in some brain areas, as well as alterations in serotoninergic neurotransmission.
- There are alterations of cognitive function, in particular memory, attention and information processing.

- Psychological stress may precipitate CFS, whereas certain personality traits constitute predisposing factors.
- Diverse psychological and social mechanisms contribute to the perpetuation of the disorder.
- The activity and responsiveness within the HPA axis is decreased.
- There are alterations of cardiovascular regulation, particularly during orthostatic stress, indicating increased sympathetic neurotransmission; relative hypovolemia may be an underlying mechanism.
- There are disturbances in skin circulation.
- Autonomic dysfunction might constitute an important aspect of CFS pathophysiology.

1.3.3 Aims and research questions

As evident from the review above, the scientific knowledge concerning CFS pathophysiology is regrettably non-coherent and bewildering. In other words, there is a great need for a unifying theory, bringing together empirical results which seem unrelated at first glance.
As outlined in 1.2, the autonomic nervous system possesses strong 'integrative properties', not only controlling organ functions and internal homeostasis, but also constituting an important substrate for body-mind-interactions. Thus, the reported evidence of autonomic dysfunction seems to be a promising starting point when searching for a unifying CFS theory. Consequently, the general aim of this thesis is to further explore the putative role of autonomic dysfunction in CFS pathophysiology. More specifically, we asked the following questions:

A. When comparing adolescent CFS patients with healthy controls, how is the expression of symptoms indicative of altered cardiovascular and thermoregulatory autonomic control?
B. During supine rest, when comparing adolescent CFS patients with healthy controls:
    a. How is the autonomic modulation of cardiovascular variables?
    b. What are the levels of catecholamines and metanephrines?
    c. How is the autonomic modulation of thermoregulatory variables?
C. During orthostatic stress, when comparing adolescent CFS patients with healthy controls:
    a. How is the autonomic modulation of cardiovascular variables?
    b. How is the autonomic modulation of cardiovascular variables when isometric exercise is performed in addition?
D. During local cold stress, when comparing adolescent CFS patients with healthy controls:
    a. What are the levels of catecholamines and metanephrines?
    b. How is the autonomic modulation of thermoregulatory variables?

2 Material and methods

2.1 Material

2.1.1 CFS patients

In 2001, a 13-year old boy suffering from long-lasting fatigue was referred to the Dep. of Pediatrics at Rikshospitalet-Radiumhospitalet Medical Centre (a national tertiary referral hospital) (see paper I). The clinical experience gained during the management of this patient and a couple of other related cases resulted in the establishment of the present research project as well as an outpatient service for children and adolescents suffering from unexplained, chronic fatigue. In the latter, the patients were examined according to an extensive, pre-defined program (Table 4), as recommended in the literature (123). The program was revised in 2005, and due to resource constraints, a significant portion of it was from this point on delegated to the referring unit. Still, all patients underwent a thorough assessment at the referral centre.
A diagnose of CFS was made if the fatigue had persisted for more than 3 months, and if the tests did not reveal any somatic or psychiatric disease which could explain it. Thus, we omitted the bicriteria in the CDC case definition and also required a shorter duration of fatigue than is required of adults, complying with recent recommendations (123, 136, 265) (see 1.1.1). Patients were consecutively asked to participate in the research project if they otherwise fulfilled criteria as outlined in Table 5. Both patients and their parents/next-of-kin received oral and written information about the purpose and content of the experiments. Their right to withdraw at any time without risking poorer medical care was specifically emphasized. A written consent was obtained before inclusion. All participants received a payment of NOK 200.
Before revising the clinical examination program, 15 patients were enrolled, constituting the basis for the analyses in paper IV and V. After the program was revised, another 14 patients were included. However, due to concurrent alterations in experimental protocols (see 2.2.1), 2 patients from the first cohort had to be excluded from the analyses in paper II and III. Thus, these papers are based upon 27 patients.

Table 4. Routine examinations in children and adolescents suffering from unexplained chronic fatigue

Pediatric clinical assessment

Blood samples*
- Full blood count
- ESR/CRP
- Electrolytes
- Renal function test
- Liver function test
- Iron status
- Glucose
- Thyroid function test
- Antibodies towards EBV, CMV, *Borrelia*, *Mycyplasma, Chlamydia*
- Autoantibodies/complement activity

Urine samples*
- Dipstick
- Total protein
- Catecholamine breakdown products

Imaging
- Chest X-ray
- Abdominal ultrasound
- MRI of brain and spinal cord

Cardiologic examinations
- 24-hours ECG recording
- Exercise tolerance test (treadmill)†
- Echocardiography†

Miscellaneous
- EEG
- Psychiatric assessment‡

* Only the most important analyses in blood and urine are shown here
† These tests were removed from the routine program after revisions in 2005.
‡ Due to resource constraints, only approximately one half of the patients were offered extensive assessment from an experienced child psychiatrist affiliated with the Dep. of Pediatrics, Rikshospitalet-Radiumhospitalet Medical Centre. The rest of the patients were referred to psychiatric teams in their local communities.

Table 5. Criteria for inclusion and exclusion of participants

Participants	*Inclusion criteria*	*Exclusion criteria*
CFS patients	12-18 years of age > 3 months of chronic fatigue* Severe disability resulting from fatigue†	Well-defined somatic or psychiatric disease that might explain the fatigue
Healthy controls	12-18 years of age	Any known chronic disease Regular use of pharmaceuticals (including contraceptive pills)

* The patients included in paper IV and V had all been fatigued > 6 months. This was, therefore, stated as an inclusion criterion, thus adhering better to the CDC-definition. Likewise, in paper II and III, we reported inclusion based upon > 4 months of fatigue, as no patients had shorter disease duration.
† 'Severe disability' usually implied school absenteeism more than once a week. However, some patients were able to follow normal school progression, but only if they desisted from all other social activities. These patients were considered equally disabled.

2.1.2 Healthy controls

In the period between August 2003 – June 2005, schools in the Oslo/Bærum area were asked to recruit healthy controls for the study. Four schools responded positively, and specific classes were selected in order to achieve a similar age distribution among patients and controls. These classes received both oral and written information, and individuals then volunteered to participate. During the course of the study, the emerging proportion of males vs. females among the patients was used as a guide for the recruitment of volunteer controls, thus assuring a similar distribution of gender within the two groups.
Prior to final inclusion, the volunteers and their parents/next-of-kin received additional written information about the purpose and content of the experiments. Their right to withdraw at any time was specifically emphasized. A written consent was obtained. All participants received a payment of NOK 200.
In total, 57 healthy controls were included, constituting the basis for the analyses in paper V. Paper IV builds upon 56 controls, as one had to be excluded due to experimental failure. However, parts of the experimental protocol were altered during the study period, as will be further outlined below (see 2.2.1). Therefore, some controls had to be excluded from the analyses in paper II and III, and the total number in these papers is only 33.

2.2 Methods

2.2.1 Experimental protocols

For each participant, all experiments were performed during a single day, according to the program outlined in Table 6. During the course of the study, we alternately summoned CFS patients and controls, in order to minimize the risk of systematic errors. One week prior to the experiments, the participants were instructed not to drink beverages containing alcohol or caffeine, not to take any drugs, and not to use tobacco products. On the day of the experiments, they were supposed to have fasted overnight. Two light meals, consisting of a maximum of 2 pieces of bread and 2 glasses of water/juice, were offered at certain hours, as specified in Table 6.

Neuroendocrine responses to strong cooling of one hand
All participants were instructed to apply an ointment containing the local anesthetic lidocaine (Emla®) on the skin of the antecubital fossa one hour prior to arrival in the morning. They rested supine for about 15 minutes, and a catheter was then placed in an antecubital vein (Figure 8). After supine rest for another 15 minutes, blood samples were collected on ice-cold vacutainer tubes containing gluthation-EGTA and heparin for measurements of plasma catecholamines (norepinephrine, epinephrine, dopamine)

Table 6. Overview of experimental day

Time	*Experiment/Activity*	*Reported in paper*
07.30 am	Check in	
08.00 am	Neuroendocrine responses to strong cooling of one hand	IV, V
08.45 am	Light meal	
09.00 am	Questionnaire	II, IV, V
09.30 am	Head-up tilt-test (HUT)	II, III
11.00 am	Valsalva-maneuver	Not reported
11.30 am	Lower body negative pressure (LBNP) with handgrip	IV
01.00 pm	Light meal	
01.30 pm	Cardiovascular responses to mental exercise	Not reported
01.45 pm	Cardiovascular responses to moderate cooling of one hand	V
03.30 pm	End of experiments	

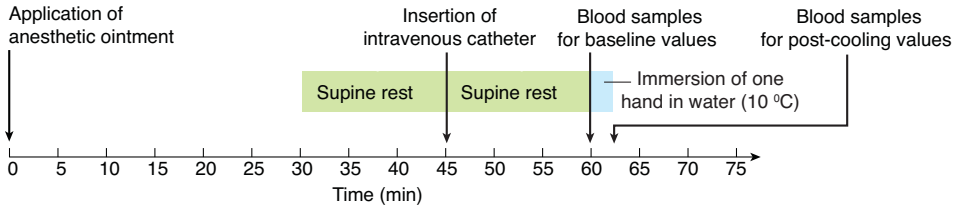


Figure 8. Neuroendocrine responses to strong cooling of one hand: schematic outline of the experiment.

and metanephrines (normetanephrine, metanephrine, 3-methoxytyramine), respectively. The opposite hand was then immersed in cold water (10 °C) for 2 minutes, after which new blood samples were collected immediately. Samples were then centrifuged at 4 °C, and plasma was separated for storage at –80 °C until assayed.

Metanephrines were extracted from the plasma using solid-phase ion exchange columns (Bond Elute-Accucat, Varian Medical Systems, California, USA) and a commercial mobile phase (Chromsystems, München, Germany) (240). Both catecholamines and metanephrines were quantified by high performance liquid-chromatography with a reverse phase column and glassy carbon electrochemical detector (Agilent Technologies, Colorado, USA). The intra- and interassays variations were 10.7 % and 14.5 % for norepinephrine, 23.5 % and 10.5 % for epinephrine, 7.2 % and 6.8 % for dopamine, 12.6 % and 4.3 % for normetanephrine, 11.7 % and 3.0 % for metanephrine, and 15.3 % and 11.2 % for 3-methoxytyramine.

Additional endocrine analyses were also carried out, including cardiac peptides, sex hormones, renin, aldosteron, HPA axis hormones, vasopressin and insulin. The results are not reported in this thesis.

Questionnaire

A questionnaire was developed based upon the translation of the Autonomic Symptom Profile, a validated instrument for assessing orthostatic intolerance and other variants of autonomic dysfunction (402). Some items were slightly modified in order to fit our particular age group. Furthermore, the questionannire included a translated an slightly modified version of the Fatigue Severity Scale (228), as well as questions focusing on functional consequences of CFS based upon personal clinical experience with patients. Finally, we added questions related to the CDC case definition and to demographic characteristics. The subjects answered the questions during an interview. Only some results from this extensive questionnaire are reported in this thesis.

During the course of the study, the questionnaire was slightly revised based upon personal experience of its feasibility. Thus, a few items were not answered by all participants.

Head-up tilt-test

Conventional procedures of head-up tilt-test (HUT) involve tilting to 60°-80° for 30-45 minutes (22). Heart rate (HR) is usually continuously recorded from the electrocardiogram (ECG), whereas arterial blood pressure is measured at predefined intervals (eg. every second minute) using ausculatory or oscillometric techniques. Thus, the hemodynamic recordings are not very accurate. Correspondingly, the test focuses on 'hard' endpoints, such as syncope or near-syncopal events. However, the tolerance for orthostatic stress is lower in adolescents than in children and adults (347, 444), and conventional HUTs are burdened by a high rate of false positive results in this age group, making the test less informative (89).

Our first HUT protocol implied 30 minutes of 60° tilt, and was performed in 2 CFS patients and 22 healthy controls. Of these participants, 2 patients (100 %) and 9 controls (41 %) experienced a syncope or a near-syncopal event (defined as more than 10 mm Hg fall in either systolic or diastolic blood pressure, combined with orthostatic symptoms). Faced with these results, we considered the protocol to be both uninformative and possibly unethical, and therefore decided to revise it.

In the revised HUT protocol, which was successfully undertaken by 27 CFS patients and 33 healthy controls, the subjects lay supine on an electronically operated tilt table with foot-board support (Model 900-00, CNSystems Medizintechnik, Graz, Austria). They were attached to the Task Force Monitor® (Model 3040i, CNSystems Medizintechnik, Graz, Austria); a combined hardware and software device for non-invasive recording of cardiovascular variables (133, 166). A 5 minute baseline recording was obtained. Subjects were then head-up tilted 20° over approximately 10 seconds (Figure 9). They were remained at 20° for 15 minutes, and then tilted back to horizontal position after which the recording was continued for another 5 minutes, making the total experimental period 25 minutes. Subjects were asked not to speak or move during the recording period.

Instantaneous HR was obtained from the R-R interval of the ECG. Photoplethysmography on the right middle finger was used to obtain a non-invasive, continuous recording of arterial blood pressure. This method correlates satisfactorily with invasive pressure measurements (300), and has also been validated in adolescents

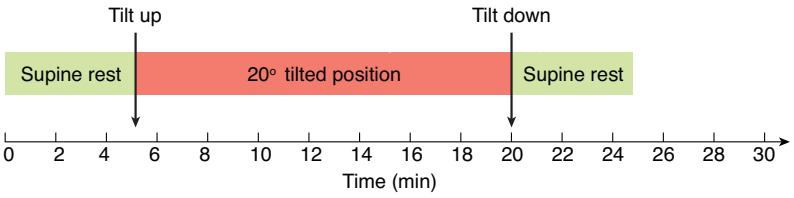


Figure 9. Head-up tilt-test (revised protocol): schematic outline of the experiment.

and children (356). Approximately every minute, the recorded value was calibrated against conventional oscillometric measurement of arterial blood pressure on the subjects' left arm. The method of impedance cardiography, in which a small electrical potential is applied between electrodes placed on the neck and upper abdomen, was used to obtain a continuous recording of the temporal derivate of the transthoracic impedance (dZ/dt) (98). All recorded signals were transferred online to the built-in recording computer of the Task Force Monitor®, running software for real-time data acquisition.

Lower body negative pressure with handgrip

The technique of lower body negative pressure (LBNP) is a well-established tool for studies of cardiovascular adjustments during orthostatic stress (382), whereas handgrip is a common test for studies of cardiovascular adjustments during isometric exercise (161, 205). In our laboratory, we have developed a combined experimental set-up in order to study complex regulatory adjustments (184).

The participants lay supine with their lower body in a plastic chamber, in which air could be evacuated very rapidly, thus reaching a pre-defined negative pressure within milliseconds (185). In order to prevent air leak, rubber devices were used to make a tight seal around the subjects' waist. They were lightly dressed, and the ambient temperature was kept between 23 and 26 °C. They were familiarized with the test procedure in two pilot experiments. By means of an electronic device (AB-detector, Gøteborg, Sweden), the force of the subjects' left-sided handgrip was continuously displayed to them. They were first asked to perform maximum isometric work for about 10 seconds. Based on the mean value, the 30 % level was calculated, and they were then asked to use a couple of minutes to become familiar with this amount of force.

Five minutes were used for baseline registration of cardiovascular variables (Figure 10). Then, LBNP of –20 mm Hg was applied instantaneously. After 6 minutes of LBNP, the subjects were asked to perform left-sided handgrip with 30 % of maximum force

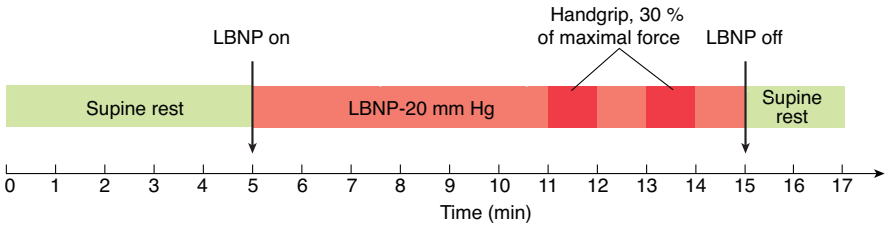


Figure 10. Lower body negative pressure with handgrip: schematic outline of the experiment.

Instantaneous HR was obtained from the ECG. Photoplethysmography on the right middle finger was used to obtain a non-invasive, continuous recording of arterial blood pressure (2300 Finapres, Ohmeda, Madison, WI, US) (300, 356). A continuous recording of maximum blood flow velocity in the ascending aorta was obtained by a bidirectional ultrasound Doppler velocimeter (SD-100, GE Vingmed Ultrasound, Horten, Norway), operating in pulsed mode at 2 MHz (119). Using a handheld transducer, the ultrasound beam was directed from the suprasternal notch towards the aortic root. The sample volume range was adjusted so that measurements were made 1-2 cm above the aortic valve. A continuous recording of mean blood flow velocity in the right brachial artery (BABF) was obtained by another bidirectional ultrasound Doppler velocimeter (SD-50, GE Vingmed Ultrasound, Horten, Norway), operating in pulsed mode at 10 MHz (251). A continuous recording of acral skin laser flux, which is a measure of acral skin blood flow (ASBF), was obtained by a laser-Doppler instrument (DRT4, Moore instruments, Milway, Devon, UK), with the probe firmly attached with adhesive strips to the pulp of the right index finger (24). The BABF and ASBF results are not reported in this thesis. All recorded signals, including the pressure in the LBNP-chamber and the force of the handgrip, were transferred online to a recording computer running a program for real-time data acquisition (developed by Morten Eriksen, Dep. of Physiology, University of Oslo, Oslo, Norway).

Cardiovascular responses to moderate cooling of one hand

In our laboratory, the general design of this experiment has been used in several other studies concerning normal physiology (23). The participants were lightly dressed and lay supine in a climatic chamber. Ambient temperature was maintained around 26 °C, assuring that subjects were within their thermoneutral zone. The left hand was immersed in a stirred thermostat-controlled water bath (CB 29-20e, Heto-Holtan, Åbyhøj, Denmark) with a temperature of 35 °C. 30 minutes were used for acclimatization and another 5 minutes for baseline registration, after which the water temperature was gradually lowered to 19 °C over approximately 50 minutes (Figure 11).

A continuous recording of acral skin laser flux, which is a measure of acral skin blood flow (ASBF), was obtained by a laser-Doppler instrument (DRT4, Moore instruments, Milway, Devon, UK) (24). The probes were firmly attached with adhesive strips to the pulp of the flexor surface of the distal phalanges of the right and left index fingers. In this position, the probes mainly detect blood flow in arteriovenous anastomoses (23). Tympanic temperature (TT) was continuously monitored by an electronic probe (D-

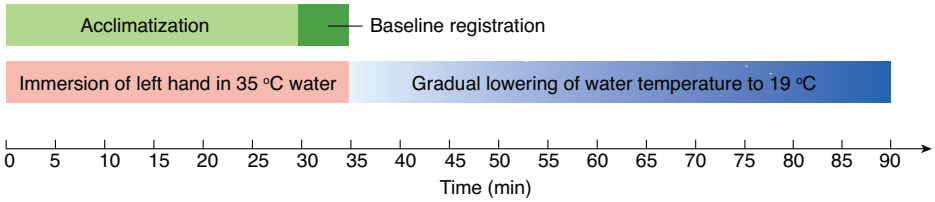


Figure 11. Cardiovascular responses to moderate cooling of one hand: schematic outline of the experiment.

TM1, Exacon, Roskilde, Denmark) inserted in the outer auditory canal and isolated from the ambient air by a piece of cotton. TT corresponds well with core body temperature (331). Instantaneous heart rate (HR) was obtained from the ECG, whereas photoplethysmography on the right middle finger was used to obtain a non-invasive, continuous recording of arterial blood pressure (2300 Finapres, Ohmeda, Madison, WI, US) (300, 356). All recorded signals, including the temperature of the water bath, were transferred online to a recording computer running a program for real-time data acquisition (developed by Morten Eriksen, Dep. of Physiology, University of Oslo, Oslo, Norway).

2.2.2 Data analyses

Conventional cardiovascular variables

In all experiments using photoplethysmographic registration of blood pressure, the beat-to-beat mean arterial pressure (MAP) was calculated by numerical integration of the recorded continuous blood pressure signal within subsequent RR-intervals. Accordingly, beat-to-beat systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken as the highest and lowest value, respectively, of the recorded continuous blood pressure signal within subsequent RR-intervals.

In the HUT experiment, beat-to-beat stroke volume (SV) was calculated from the impedance signal (133). This method has been validated both in adults (98, 342) and children (38, 343). In the LBNP experiments, beat-to-beat SV was calculated by multiplying the value obtained by numerical integration of the recorded instantaneous maximal blood flow velocity in the ascending aorta during each R-R interval by the area of the aortic valve orifice. This area was determined by 2D ultrasound imaging in a separate session. This method has been validated in adults (119), but not in children. Cardiac output (CO) was calculated as SV times HR. Total peripheral resistance (TPR) was calculated as MAP divided by CO. Furthermore, in the HUT experiments, the impedance signal was used to estimate the beat-to-beat end diastolic volume of the left ventricle (EDV), based upon the calculation of SV and ejection fraction (263), and

acceleration index (ACI), defined as the highest positive slope of the impedance signal (d^2Z/dt^2_{max}). ACI corresponds to the maximal acceleration of blood flow during the ejection phase, and is regarded as an indirect marker of cardiac contractility (213).
All variables based upon volume measurements, i.e. CO, SV, TPR and EDV, were normalized according to body surface area (BSA), using the following formula: BSA = 0.00235 x (height in cm)$^{0.423}$ x (weight in kg)$^{0.515}$.

Heart rate and blood pressure variability
Spontaneous *variability* is a characteristic feature of all cardiovascular variables, as described above (see 1.2.2), reflecting the «homeodynamic interplay between perturbations to cardiovascular function and the dynamic responses of the cardiovascular regulatory systems» (7). Analyses of variability might be performed in the time domain, where the extent of variability within a recording of certain length is expressed using common statistical terms (411). For instance, when analyzing variability of RR-intervals (inverse HR) in an ECG recording, SDNN is the standard deviation of all RR-intervals, RMSSD is the square root of the mean square differences of successive RR-intervals, and pNN50 is the proportion of successive RR-intervals with a difference greater than 50 ms.
Alternatively, analyses might be carried out in the frequency domain, in which the signals are regarded as a mixture of sine waves of different amplitudes and frequencies (7). This *spectral analysis* approach requires more sophisticated mathematical procedures; the most common can be classified as either non-parametric methods (e.g. Fast Fourier Transform) or parametric methods (e.g. Autoregressive modelling) (57, 73). In any case, the extent of variability is expressed as the *power* of different frequencies. Spectral analyses of short-time recordings of heart rate or blood pressure usually reveal three distinct peaks in the power spectrum (411). By convention, spectral power densities are computed in three frequency bands corresponding to these peaks, assigned very low frequency (VLF, <0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz), respectively.
The RR-interval power spectral density in the HF-band might be considered an index of the parasympathetic modulation of heart rate, whereas RR-interval power spectral density in the LF region is due to the combined effect of cardiac parasympathetic and sympathetic activity (73, 346). The LF/HF-ratio has often been regarded as a measure of 'sympathovagal balance' in the modulation of HR, particularly during orthostatic stress (112, 296). Blood pressure variability in the LF band (also known as Mayer waves) is primarily a consequence of variations in efferent sympathetic neural activity to peripheral resistance vessels, whereas blood pressure variability in the HF band is due to a combination of the mechanical influence of ventilation and the effects of heart rate variability on blood pressure (73, 258).
In this thesis, variability analyses were performed on the HR and DBP recordings from the HUT experiments, using an adaptive autoregressive algorithm (see paper III) (132). This technique yields a time-varying spectrum in which spectral power densities are

computed from consecutive segments of the biosignals (16, 57). The lengths of these segments vary, as the biosignals are continuously monitored for departure from stationarity, and the segment boundaries are placed accordingly. This approach is advantageous in a situation where the variability is supposed to change as the consequence of an intervention, e.g. the transition from supine to tilted position.

Mathematical processing of beat-to-beat recordings
Plots of the beat-to-beat recorded cardiovascular variables were inspected for each participant. Obvious artifacts, e.g. due to temporary instrumental failure, were removed and replaced by linear interpolation between values appraised to be valid. For the main analyses in paper II-V, the median values within defined time periods of each recording were computed, thus reducing the influence of possible false outliers even more.
In addition, in paper II-IV, the beat-to-beat recordings were converted to 4 Hz time series of equal length by linear interpolation. These time series were filtered by assigning the median value within a sliding window to each time point, thus removing both minor artifacts and stochastic variability. Furthermore, all time series were normalized, taking the mean value of the first time period as zero. Coherent averaging was then performed by calculating the arithmetical mean for each time point (333).
In paper V, the beat-to-beat recordings of ASBF Left were searched for vasoconstrictor events, defined to occur at the water temperature where the median value computed over 30 consecutive heart beats was permanently less than 50 % of the median value prior to cooling.

Statistical analyses
In all experiments, data plots were routinely inspected to assess their empirical distribution. If approximating normality, the mean was taken as an estimate of the expectancy of the population under study, and 95 % confidence intervals for the mean was calculated according to parametric methods (see paper II and IV). If the empirical distribution of data deviated from normality, the median and non-parametric 95 % confidence intervals were used for the same purposes (see paper III and V).
In all experiments, non-parametric statistical test were regarded as most 'robust', and therefore used to explore the differences between the two groups of participants (188). In order to reduce the methodological problem of performing multiple statistical comparisons, we reserved the statistical tests for variables that were considered most central to our research questions. Generally, a $p < 0.05$ was considered statistically significant.

2.2.3 Ethical and legal considerations

The principle of *informed consent* is considered vital in all research involving human beings (121). Complying with this principle requires that the participants are fully

informed about all aspects of the experiments, including any possible side-effects, and that they are able to make a totally independent decision concerning whether or not to participate. Therefore prior to inclusion, both patients and controls received thorough information about the experiment orally and in written form.

In practice, and particularly when children are involved, the ideal of informed consent are impossible to achieve (229). First, being 'fully informed' is, at least theoretically, an endless task; in addition, there are no means to assess whether the information has been perceived correctly by each participant. Second, no process of decision is totally free of obligations; furthermore, children are not considered fully competent of making decisions about their own life.

These obstacles were partly overcome by providing thorough information and requiring a written statement of consent from the participants' parents/next-of-kin. Still, we maintain that medical research should also adhere to the principle of *primum non nocere* (374). In other words, experiments on humans - and children in particular - cannot be justified solely by the participants' free, informed decision if the experiments impose too great a risk of harmful effects.

Prior to the experimental phase of this study, none of the experiments were considered to carry any element of risk. However, the following parts were addressed as potentially unpleasant: pain during insertion of intravenous catheter and immersion of hand in cold water, and dizziness or near-syncope during HUT and LBNP. If near-syncope occurred, the protocols demanded the immediate termination of the experiment, in order to minimize the risk of manifest syncope.

Our initial HUT protocol resulted in a high incidence of near-syncope and even syncope, and was therefore abandoned as described above (see 2.2.1). During the revised experiment, one CFS patient complained of unpleasant dizziness; however, near-syncope or syncope was never observed. LBNP precipitated a harmless cardiac arrhythmia in a healthy control; the experiment was terminated immediately, and the participant was referred to an ordinary cardiologic assessment. Two other subjects (one CFS patient, one control) reported dizziness or other unpleasant experiences during LBNP. The use of anesthetic ointment seemed effective in reducing the pain related to catheter insertion.

The study was approved by the Regional committee for ethics in medical research and the Norwegian Data inspectorate. The authors of the papers are all qualified according to the Vancouver guidelines. None of them possesses any conflicts of interest related to the subject matter of this thesis.

3 Results

3.1 Subjects

27 CFS patients and 33 healthy controls were included in the experiments reported in paper II and III, whereas 15 CFS patients and, respectively, 56 and 57 healthy controls were included in paper IV and V (Table 7). In all experiments, the two groups had a comparable distribution of gender, age, weight, height, and body surface area. All participants, except one control, were of Caucasian ethnicity. The CFS patients were sedentary, had a high Fatigue severity scale sum score, and reported a substantial amount of functional impairment; however, no one was permanently bedridden. About 60 % fit the CDC case definition of CFS.

3.2 Experimental results

The following research questions were presented in 1.3.3:

A. When comparing adolescent CFS patients with healthy controls, how is the expression of symptoms indicative of altered cardiovascular and thermoregulatory autonomic control?
B. During supine rest, when comparing adolescent CFS patients with healthy controls:
    a. How is the autonomic modulation of cardiovascular variables?
    b. What are the levels of catecholamines and metanephrines?
    c. How is the autonomic modulation of thermoregulatory variables?
C. During orthostatic stress, when comparing adolescent CFS patients with healthy controls:
    a. How is the autonomic modulation of cardiovascular variables?
    b. How is the autonomic modulation of cardiovascular variables when isometric exercise is performed in addition?

Table 7. Subject characteristics

Variable	*Paper II and III*		*Paper IV and V*	
	Control	*CFS*	*Control**	*CFS*
Number	33	27	57	15
Female gender	19 (58 %)	18 (68 %)	34 (60 %)	10 (67 %)
Adherence to the CDC-criteria		15 (56 %)		9 (60 %)
School absenteeism once a week or more[†]	0 (0 %)	23 (85 %)	0 (0 %)	10 (77 %)
Not able to attend leisure activities once a week or more[†]	0 (0 %)	24 (96 %)	0 (0 %)	13 (100 %)
Permanently bedridden	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Mean (range)	Mean (range)	Mean (range)	Mean (range)
Age (years)	15 (13-18)	15 (13-18)	16 (13-18)	15 (12-18)
Weight (kg)	61 (44-77)	56 (37-92)	62 (44-99)	59 (43-92)
Height (cm)	172 (149-195)	169 (145-197)	172 (149-195)	171 (160-192)
Body surface area (m^2)	1.7 (1.4-2.0)	1.6 (1.3-2.3)	1.7 (1.4-2.2)	1.7 (1.4-2.2)
Duration of fatigue (months)		30 (4-132)		31 (6-60)
Fatigue severity scale, sum score[‡]	1.5 (1.0-2.3)	4.5 (2.9-5.0)	1.5 (1.0-3.6)	4.6 (3.8-5.0)
Reported physical exercise (hours/week)	6.7 (2-14)	0.5 (0-6)	6.2 (1-20)	0.7 (0-7)

* One female control included in paper V did not participate in the experiments of paper IV, due to the precipitation of cardiac arrhythmia during LBNP (see 2.2.3). Thus, in paper IV, some of the values (e.g. the numbers of female participants) deviate slightly from what is presented here.
[†] Due to revisions in the questionnaire (see 2.2.1), these items were not answered by all participants, influencing the calculated percentage.
[‡] The fatigue severity scale was slightly modified from the original publication, using a 1-5 Likert response scale for each item (228).

D. During local cold stress, when comparing adolescent CFS patients with healthy controls:
    a. What are the levels of catecholamines and metanephrines?
    b. How is the autonomic modulation of thermoregulatory variables?

Below, the results of the experiments are summarized in accordance with these questions

3.2.1 Symptoms of altered cardiovascular and thermoregulatory autonomic control

Palpitations, dizziness, skin flushing, pale skin and an increased tendency to sweat were prominent symptoms in the patient described in paper I. Table 8 summarizes the questionnaire results reported in paper II, IV and V. As for cardiovascular autonomic control, there is evidence that CFS patients more frequently than controls feel dizzy

Table 8. Symptoms of altered cardiovascular and thermoregulatory autonomic control – summary of results comparing CFS patients to healthy controls

Variables	*Questionnaire*
Cardiovascular autonomic control	
Experience fainting when rising quickly	(↑)
Feeling dizzy just after a heavy meal	(↑)
Feeling dizzy after standing upright for a long period	↑
Feeling dizzy during moderate physical exercise	↑
Feeling dizzy taking a hot bath or shower	↑
Thermoregulatory autonomic control	
Experience sudden changes in skin colour	↑
- Sudden redness of skin	↓
- Sudden paleness of skin	↑
Experience shivering hands	↑
Sweating more than others	↑
Feeling unusually warm after standing upright for a long period	↑
Feeling unusually warm after moderate physical exercise	↑
Feeling unusually warm after a hot bath or shower	(↑)

Arrow=significant difference, direction indicating how CFS patients deviate from controls. Arrow in parentheses=non-significant tendency.

Table 9. Cardiovascular, neuroendocrine and thermoregulatory variables during supine rest – summary of results comparing CFS patients to healthy controls

Variables	*Prior to HUT*	*Prior to LBNP*	*Prior to strong cooling*	*Prior to moderate cooling*
Cardiovascular variables				
HR	(↑)	↑		↑
SBP	n.d.	n.d.		
MBP	n.d.	↑		(↑)
DBP	n.d.	↑		
SI	↓	(↓)		
TPRI	↑	n.d.		
EDVI	↓			
ACI	(↓)			
HRV, LF_{norm}	n.d.			
HRV, HF_{norm}	n.d.			
HRV, LF/HF	n.d.			
HRV, time indices	n.d.			
DBPV, LF_{norm}	n.d.			
DBPV, HF_{norm}	n.d.			
Neuroendocrine variables				
Plasma norepinephrine			↑	
Plasma epinephrine			↑	
Plasma dopamine			n.d.	
Plasma metanephrines			n.d.	
Thermoregulatory variables				
TT				↑
ASBF Left				(↓)
ASBF Right				(↓)

Arrow=significant difference, direction indicating how CFS patients deviate from controls. Arrow in parentheses=non-significant tendency. n.d.=no difference. HR=heart rate, SBP=systolic blood pressure, MBP=mean arterial blood pressure, DBP=diastolic blood pressure, SI=stroke index, TPRI=total peripheral resistance index, EDVI=end diastolic volume index, ACI=acceleration index, HRV=heart rate variability, LF_{norm}=low frequency, normalized units, HF_{norm}=high frequency, normalized units, DBPV=diastolic blood pressure variability, TT=tympanic temperature, ASBF=acral skin blood flow.

after standing upright, during moderate physical exercise and during a hot bath. Less convincing evidence indicates an increased tendency to faint and to feel dizzy after heavy meals. As for thermoregulatory autonomic control, there is evidence that CFS patients more often experience sudden changes in skin color, a sudden paleness of skin, shivering hands, sweating, and feeling unusually warm after standing upright and after moderate physical exercise. A sudden redness of skin is rarer among CFS patients than controls. Less convincing evidence indicates that CFS patients tend to feel unusually warm after a hot bath.

3.2.2 Cardiovascular, neuroendocrine and thermoregulatory variables during supine rest

Examination of the patient reported in paper I revealed supine tachycardia. Table 9 summarizes the results during supine rest reported in paper II-V. As for cardiovascular variables, there is firm evidence of increased HR and decreased SI among CFS patients, and also evidence of increased MBP, DBP, and TPRI and decreased EDVI. Less convincing evidence indicates decreased ACI among CFS patients. As for neuroendocrine variables, there is evidence of increased plasma norepinephrine and epinephrine. As for thermoregulatory variables, there is evidence of increased TT, whereas less convincing evidence indicates decreased ASBF Left and Right.

3.2.3 Cardiovascular responses to orthostatic stress alone and combined with isometric exercise

Examination of the patient reported in paper I revealed orthostatic tachycardia and hypotension that normalized during treatment with propranolol. Table 10 summarizes the results reported in paper II-IV. When orthostatic stress is applied in isolation, there is firm evidence of a greater increase in HR, MBP, DBP, and TPRI and a greater decrease in SI among CFS patients. There is also evidence of altered heart rate variability, with a greater increase in LF_{norm} and LF/HF-ratio and a greater decrease in HF_{norm} among CFS patients. Less convincing evidence indicates a greater increase in ACI among CFS patients. When isometric exercise is perfomed in addition to orthostatic stress, there is evidence of a smaller increase in HR and DBP and a smaller decrease in SI among CFS patients. There is less convincing evidence of a smaller increase in MBP and TPRI in this situation.

Table 10. Changes in cardiovascular variables during orthostatic stress – summary of results comparing CFS patients to healthy controls

Variables	*During HUT*	*During LBNP*	*During LBNP and handgrip*
Cardiovascular variables			
Δ HR	↑	↑	↓
Δ SBP	↑	n.d.	n.d.
Δ MBP	↑	↑	(↓)
Δ DBP	↑	↑	↓
Δ SI	↓	(↓)	↑
Δ TPRI	↑	(↑)	(↓)
Δ EDVI	n.d.		
Δ ACI	(↑)		
Δ HRV, LF_{norm}	↑		
Δ HRV, HF_{norm}	↓		
Δ HRV, LF/HF	↑		
Δ HRV, time indices	n.d.		
Δ DBPV, LF_{norm}	n.d.		
Δ DBPV, HF_{norm}	n.d.		

Arrow=significant difference, direction indicating how CFS patients deviate from controls. Arrow in parentheses=non-significant tendency. n.d.=no difference. HR=heart rate, SBP=systolic blood pressure, MBP=mean arterial blood pressure, DBP=diastolic blood pressure, SI=stroke index, TPRI=total peripheral resistance index, EDVI=end diastolic volume index, ACI=acceleration index, HRV=heart rate variability, LF_{norm}=low frequency, normalized units, HF_{norm}=high frequency, normalized units, DBPV=diastolic blood pressure variability.

3.2.4 Cardiovascular, neuroendocrine and thermoregulatory responses to local cold stress

Table 11 summarizes the results reported in paper V. During strong cooling, evidence indicates similar changes in the neuroendocrine variables among both CFS patients and controls. During moderate cooling of the left hand, there is evidence of a greater decrease in TT and a smaller decrease in ASBF Left among CFS patients, and there is less convincing evidence of a smaller decrease in ASBF Right among CFS patients.

Table 11. Changes in cardiovascular, neuroendocrine and thermoregulatory variables during local cold stress – summary of results comparing CFS patients to healthy controls

Variables	*During strong cooling*	*During moderate cooling*
Cardiovascular variables		
Δ HR		n.d.
Δ MBP		n.d.
Neuroendocrine variables		
Δ Plasma norepinephrine	n.d.	
Δ Plasma epinephrine	n.d.	
Δ Plasma dopamine	n.d.	
Δ Plasma metanephrines	n.d.	
Thermoregulatory variables		
Δ TT		↓
Δ ASBF Left (cooled)		↑
Δ ASBF Right (non-cooled)		(↑)

Arrow=significant difference, direction indicating how CFS patients deviate from controls. Arrow in parentheses=non-significant tendency. n.d.=no difference. HR=heart rate, MBP=mean arterial blood pressure, TT=tympanic temperature, ASBF=acral skin blood flow.

3.2.5 Comparison of CFS patients with sedentary controls

As discussed more in depth below, some of the results among CFS patients could theoretically be explained by sedentary deconditioning (see 4.2.4). To explore this possibility, we extracted those controls that reported 4 hours or less of physical activity per week ('sedentary controls', n=26), and compared this subgroup with CFS patients in regards to the most important variables from paper IV and V.

The two groups had an equal distribution of gender, age, weight and height (Table 12). At rest, the values among sedentary controls were almost identical to the values of the control group as a whole (Table 13). When comparing sedentary controls with CFS patients, tests of significance revealed the same results as when all the controls were compared with CFS patients, cf. Table 9. During orthostatic stress, the sedentary controls had a smaller increase in HR, DBP and TPRI than the undivided control group, thus amplifying the differences in regards to the CFS patients, cf. Table 10. During orthostatic stress combined with isometric exercise, the sedentary controls had a greater increase in HR than the control group as a whole, thus heightening the differences with the CFS patients, but a smaller increase in TPRI. During moderate cooling, the sedentary controls and the undivided control group responded almost identically; thus, they differed from CFS patients to the same extent, cf. Table 11.

3.2.6 Quality of data during LBNP with handgrip

As described above, two identical runs of LBNP with handgrip were carried out with each participant (see 2.2.1), making possible an assessment of the quality of the obtained data. For each pair of observations of HR, DBP and SI among the healthy controls, the difference was calculated and plotted against the mean, as suggested by Bland & Altman (28). The differences were appraised to be independent of the mean value, and conventional normality plots indicated an approximate normal distribution of these variables, justifying parametric methods. Thus, 95 % empirical range of the mean

Table 12. Characteristics of sedentary controls (physical exercise ≤ 4 hours/week) vs. CFS patients

Variable	*Sedentary controls*	*CFS*
Number	26	15
Female gender	17 (65 %)	10 (67 %)
	Mean	
Age (years)	16	15
Weight (kg)	64	59
Height (cm)	172	171
Reported physical exercise (hours/week)	3.3	0.7

Table 13. Sedentary controls compared with CFS patients for selected variables (cf. paper IV and V)

Variable	*Controls (n=57)**	*Sedentery controls (n=26)**	*CFS (n=15)**	*p-value, CFS vs sedentary controls*[†]
At rest				
HR (beats/min), mean	65.7	66.7	75.8	0.03
DBP (mm Hg), mean	65.0	65.7	70.1	0.06
TPRI (mm Hg/l/min/m^2), mean	11.1	11.0	11.6	0.59
Norepinephrine (pmol/L), median	1156	1263	1646	0.06
Epinephrine (pmol/L), median	171	164	216	0.03
ASBF Left (a.u.), median	322.2	331.6	249.9	0.19
ASBF Right (a.u.), median	271.2	273.5	122.3	0.17
TT (^oC), median	35.96	36.00	36.41	0.06
Changes during orthostatic stress (LBNP)				
Δ HR (beats/min), mean	6.6	5.0	12.4	0.004
Δ DBP (mm Hg), mean	2.2	2.0	4.8	0.03
Δ TPRI (mm Hg/l/min/m^2), mean	2.7	2.4	3.9	0.10
Changes during orthostatic stress + isometric exercise (handgrip)				
Δ HR (beats/min), mean	8.9	11.5	2.6	0.002
Δ DBP (mm Hg), mean	8.4	8.0	5.3	0.03
Δ TPRI (mm Hg/l/min/m^2), mean	2.2	1.6	1.1	0.36
Changes during moderate cooling				
Δ ASBF Left (a.u.), median	–267.6	–271.7	–132.7	0.03
Δ ASBF Right (a.u.), median	–39.0	–41.5	1.4	0.10
Δ TT (^oC), median	0.08	0.04	–0.22	0.19

* For some variables, the totals are slightly lower, cf. paper IV and V.

[†] Wilcoxon-Mann-Whitney's test, 2-sided

differences could be calculated from the standard deviations and gives an impression of agreement between the two situations, whereas the mean differences with corresponding 95 % confidence intervals reveal possible biases (28, 40). The results are displayed in Table 14. Except for HR during LBNP, the mean differences are small and the confidence intervals include zero.

Table 14. Mean differences (95 % confidence intervals) [95 % empirical range]* of cardiovascular variables in healthy controls (n = 55)† during two consecutive runs of LBNP

Variables	*Baseline*	*During LBNP*	*During LBNP and handgrip*
HR (beats/min)	–0.5 (–1.7 - 0.7) [–9.2 - 8.3]	2.5 (0.6 - 4.4) [–11.5 - 16.6]	1.0 (–0.9-2.8) [–12.7 - 14.6]
DBP (mm Hg)	–0.03 (–1.8 - 1.7) [–12.8 - 12.8]	1.3 (–-0.2 - 2.9) [–10.4 - 13.1]	1.7 (–0.5 - 3.9) [–14.5 - 17.9]
SI (ml/m^2)	–0.3 (–1.6 - 1.1) [–10.6 - 10.1]	–0.5 (–1.8 - 0.7) [–9.7 - 8.7]	0.1 (–1.2 - 1.5) [–9.7 - 10.0]

* Based upon parametric methods (40).
† One subject underwent only one single run of LBNP, and was therefore excluded from this analysis (see 2.2.1).

4 Discussion

4.1 Answers to the research questions

4.1.1 Symptoms of altered cardiovascular and thermoregulatory autonomic control

In general, our questionnaire results confirm that CFS patients have symptoms of altered cardiovascular and thermoregulatory autonomic control. As far as we know, these clinical phenomena have never before been systematically explored and charted.
Dizziness, which is reported more frequently among CFS patients, is an ambiguous term; unknowingly people often (incorrectly) use it to describe vertigo (446). However, when it occurs in circumstances known to challenge cardiovascular regulation (upright position, physical exercise, hot surroundings, heavy meals), the most likely underlying mechanism is a reduced brain perfusion due to either hypotension or cerebral vasoconstriction. The increased tendency to faint among these patients supports this conclusion.
The experience of feeling unusually warm is also difficult to interpret in terms of underlying mechanisms. This symptom may accompany hypotension, and should in that case be interpreted as a sign of altered cardiovascular control (402). However, the 'hot flush' characteristically affecting menopausal women is caused by cutaneous vasodilation and thereby increased skin temperature, possibly due to altered noradrenergic control of skin blood vessels (9). A sudden paleness of skin, on the other hand, directly indicates cutaneous vasoconstriction due to increased sympathetic outflow. Accordingly, increased tendency to sweat and shiver indicates enhanced sympathetic outflow to sweat glands and skeletal muscles, respectively (155).

4.1.2 Cardiovascular, neuroendocrine and thermoregulatory variables during supine rest

Our results show substantial alterations of cardiovascular, neuroendocrine and thermoregulatory variables in CFS patients at rest. As far as we are aware, such an extensive investigation of baseline values has not been undertaken before; however, there are scattered reports of similar findings (208, 231, 305, 370, 413).

As arrhythmia was ruled out during ordinary clinical examinations, the increased resting HR among CFS patients indicates enhanced sympathetic or attenuated parasympathetic cardiac nerve activity, possibly a combination (155). Alternatively, this finding could be explained by increased levels of circulating epinephrine. Decreased SI in CFS patients might suggest low contractility due to myocardial dysfunction; however, a more immediate explanation is reduced filling, supported by the observed tendency towards decreased EDVI. The latter, in turn, might signal reduced blood volume or increased venous capacitance, but could also be directly related to high HR and correspondingly low filling time (116).

The resistance in the arterioles of skeletal muscles is the main contributor to TPRI in most physiological states (353). Thus, a tendency towards increased TPRI among CFS patients indicates skeletal muscle vasoconstriction, which – in turn – is strongly suggestive of increased sympathetic nerve activity, as circulating catecholamines have a vasodilative effect in skeletal muscles (155).

Arterial blood pressure and body temperature are the controlled variables in the reflex circuits ensuring cardiovascular and temperature homeostasis (see 1.2.2). Therefore, a tendency towards increased MBP and DBP as well as TT among CFS patients directly suggests altered *set-point values* for the cardiovascular and thermoregulatory control systems. According to Goldstein, such homeostat resetting is a hallmark of *distress* (155). All other factors being equal, the maintenance of cardiovascular homeostasis in this situation requires increased sympathetic modulation of the heart and arterioles. Similarly, maintenance of thermoregulatory homeostasis requires enhanced sympathetic outflow to skin blood vessels and skeletal muscles, causing vasoconstriction and shivering.

A high level of norepinephrine in the antecubital vein plasma of CFS patients might suggest increased sympathetic nerve activity to forearm skin and skeletal muscle (156). Likewise, a high plasma level of epinephrine is most likely a result of increased sympathetic nerve activity to the adrenals. However, because high levels of plasma catecholamines could result from either increased spill-over or reduced removal, which in turn depends upon both sympathetic nerve activity, the capacity of different reuptake- and breakdown-pathways, and local blood flow (155, 156), there are several alternative explanations as well.

4.1.3 Cardiovascular responses to orthostatic stress

Abnormal cardiovascular responses to orthostatic stress, as documented in this thesis, are perfectly in line with a number of other reports (35, 231, 306, 339, 340, 383, 386, 387). However, an exeriermental set-up combining orthostatic stress and isometric exercise has not been undertaken before; thus, our findings of attenuated cardiovascular responses among CFS patients in this particular situation appear to be novel.

The greater increase in HR in relation to orthostatic stress in CFS patients suggests enhanced sympathetic and/or attenuated parasympathetic cardiac nerve activity (155). This notion is further supported by the analyses of heart rate variability, in which the greater increase in LF_{norm} and LF/HF-ratio and greater decrease in HF_{norm} among CFS patients indicates a sympathetic predominance in the modulation of the sinoatrial node (258, 296, 346). As outlined above, a greater decrease in SI might be ascribed to the HR influence on filling time (116); reduced contractility due to myocardial dysfunction seems unlikely, as ACI tended to be higher in CFS patients than in controls.

Following the reasoning from 4.1.2, a greater increase in TPRI among CFS patients suggests a stronger sympathetically mediated vasoconstriction in skeletal muscles. Furthermore, a greater increase in MBP and DBP might be a consequence of bringing the set-point to an even higher level, thus redefining the range of homeostasis (155).

The cardiovascular responses to combined orthostatic stress and isometric exercise might be considered a 'mirror image'. The smaller increase of HR among CFS patients is consistent with attenuated sympathetic cardiac responses (184). Similarly, a tendency towards a smaller increase of TPRI suggests attenuated sympathetic outflow to skeletal muscle arterioles. Again, the differences in SI among the two groups might be a consequence of the different HR responses, whereas a smaller increase of MBP and DBP among CFS patients suggests an inability to raise the set-point in this particular situation.

4.1.4 Cardiovascular, neuroendocrine and thermoregulatory responses to local cold stress

Mechanisms of thermoregulation have hardly been explored among CFS patients, and we are not aware of any study that has applied experimental methods similar to ours. Thus, our findings regarding responses to local skin cooling appear to be unique to this thesis.

The similar neuroendocrine response to strong cooling among CFS patients and controls indicates that this stressor causes an identical general enhancement of sympathetic nerve activity in both groups. Thus, CFS patients have preserved reflex arches and response abilities within the sympathetic nervous system.

Blood flow through arteriovenous anastomoses (AVAs) is the main determinant of ASBF (23, 251). These vessels are sympathetically innervated. Thus, a smaller decrease of ASBF Left among CFS patients during cooling of the left hand (and a similar tendency towards smaller decrease of ASBF Right) indicates attenuated sympathetic nerve activity to the AVAs. Alternatively, this result might be explained by a defect in local vasoconstrictor mechanisms. Furthermore, in CFS patients, TT decreased during the moderate cooling experiment, suggesting a normalization of the thermoregulatory set-point value. As outlined above, attenuated sympathetic outflow to skin blood vessels would be an expected consequence of such resetting (see 4.1.2), fitting neatly with the observed ASBF data.

4.1.5 Concluding remarks

Taken as a whole, we have provided firm evidence of altered sympathetic nerve activity among CFS patients, supporting the hypotheses of sympathetic dysfunction as essential in CFS pathophysiology (137, 151, 159, 282, 297). More specifically, and in relation to our research questions, we conclude that:

A. CFS patients have symptoms of altered cardiovascular and thermoregulatory autonomic control. Some of the thermoregulatory symptoms directly suggest enhanced sympathetic nerve activity to the sweat glands, the skeletal muscles and the skin vessels.
B. During supine rest, CFS patients seem to have increased sympathetic nerve activity to the heart, the skeletal muscle arterioles and the adrenals; the latter causing increased plasma levels of epinephrine. In addition, there is evidence of increased set-point values for arterial blood pressure and body temperature among CFS patients.
C. During orthostatic stress, CFS patients seem to have enhanced sympathetic nerve activity to the heart and the skeletal muscle arterioles, as well as a greater increase in the arterial blood pressure set-point. However, when orthostatic stress is combined with isometric exercise, CFS patients seem to have attenuated sympathetic cardiovascular outflow and a smaller increase in the arterial blood pressure set-point.
D. During local cold stress, CFS patients and healthy controls have similar sympathetic responses to strong cooling. However, during moderate cooling, CFS patients seem to have attenuated sympathetic outflow to skin arterioles, combined with a normalization of the set-point value for body temperature.

4.2 Possible explanations of altered sympathetic nerve activity in CFS

Altered sympathetic nerve activity, as documented among CFS patients in this thesis, might stem from several physiological or pathological states. In the following, hypovolemia, oxidative stress, the postural orthostatic tachycardia syndrome, sedentary deconditioning, gravitational deconditioning, and disturbances of CNS autonomic control will be discussed thoroughly in relation to our findings.

4.2.1 Hypovolemia

Two prior studies have indicated absolute (126) or relative (396) hypovolemia in CFS patients (see 1.3.2). A reduction in central blood volume is registered by cardiopulmonary receptors, causing altered afferent neural transmission to the brain stem cardiovascular control center (155). The reflex response consists of increased sympathetic outflow to the heart, to the arterioles of the kidneys and skeletal muscles, and possibly to other vascular beds as well (including the splanchnic circulation), as outlined in detail above (see 1.2.2). As for alterations in arterial blood pressure, present evidence is somewhat conflicting; e.g., Zollai et al. reported increased SBP, MBP and DBP after blood donation (460), whereas Triedman et al reported the opposite (418). However, when hypovolemia is experimentally induced by diuretics or simulated by low-level LBNP, most studies seem to find unaltered blood pressure values (141, 144, 219, 431). Hypovolemia tends to increases the levels of plasma norepinephrine, but not plasma epinephrine (140, 144).

When orthostatic stress is applied in a hypovolemic state, the cardiovascular sympathetic responses are generally augmented as compared to normovolemia (141, 193, 219, 220, 418). Arterial blood pressure, however, tends to stay unaltered (141, 219, 418). No studies specifically address the influence of hypovolemia upon combined orthostatic stress and isometric exercise. However, a couple of studies indicate an upper limit of vasoconstriction mediated by sympathetic nerve activity (140, 354). Such a 'saturation' phenomenon implies that high resting sympathetic activity will influence negatively the sympathetic vasoconstrictor responses to added stressors. Furthermore, norepinephrine has negative feedback-effects on sympathetic neurotransmission within the cardiovascular system; the functional importance is probably most pronounced during conditions of high sympathetic outflow (419). Whether these mechanisms apply to sympathetic control of all organs remains to be demonstrated.

Whether hypovolemia influences thermoregulation has been only scarcely explored in experimental studies. However, there is some evidence of an opposite causal relationship. For instance, a recent study concluded that hyperthermia attenuates the baroreflex-

controlled increase in cardiovascular sympathetic activity during transient hypotension (451). However, local cooling or heating of skin did non influence the baroreflex response (450). Others have found that local cold stress might provoke sympatho-inhibition during orthostatic stress (247), and that heat stress enhances the skeletal muscle sympathetic response during orthostatism (87).
In this thesis, hypovolemia might explain our assumption of increased sympathetic outflow to the heart and the skeletal muscle arterioles when CFS patients are at rest, as well as their enhanced sympathetic cardiovascular responses during orthostatic stress. Furthermore, hypovolemia is also compatible with attenuated sympathetic responses to combined orthostatic stress and isometric exercise, due to the 'saturation phenomenon' as described above. However, hypovolemia does not explain increased levels of epinephrine nor increased set-point values for arterial blood pressure and body temperature at rest. The further increase of blood pressure set-point during orthostatic stress is also inexplicable, as well as the observed response to moderate cooling. Finally, volume expansion treatment (fludrocortisone) in CFS has no documented effect (309, 341). In conclusion, hypovolemia seems to be an unlikely cause of the autonomic alterations documented in this thesis.

4.2.2 Oxidative stress

Several studies have documented increased oxidative stress among CFS patients, especially in the skeletal muscles (197, 425). This state may increase afferent neural activity, and thereby enhance resting sympathetic outflow to skeletal muscle arterioles through a reflex mechanism (155, 324). During orthostatic stress, such a mechanism might possibly cause enhanced sympathetic responses; interestingly, the attenuated responses to added isometric exercise are also compatible with such an explanation (264).
However, oxidative stress in the skeletal muscles does not readily explain the altered cardiac autonomic control, the set-point alterations, the increased baseline epinephrine level nor the skin responses to moderate cooling. Overall, we believe that increased oxidative stress does not comply with the results presented in this thesis.

4.2.3 Postural orthostatic tachycardia syndrome (POTS)

As exemplified in paper I, there appears to be a relationship between the postural orthostatic tachycardia syndrome (POTS) and CFS, both in adults (127) and adolescents (383), although this relationship is questioned in some later reports (201). POTS designates a condition characterized by an increase in HR of more than 30 beats/minute during a head-up tilt test, accompanied by unpleasant symptoms (such as lightheadedness, palpitations and nausea) (384). Hypotension may occur, but is not a prominent feature.

The pathophysiology of POTS is undetermined, but seems to involve efferent sympathetic neurons and/or adrenergic synapses. At rest, POTS patients tend to have an increased heart rate (33, 157, 383), and detailed studies of norepinephrine kinetics confirmed increased sympathetic cardiac activity (158). Some researchers have reported increased arterial blood pressure as well (33, 158). Furthermore, there are reports of enhanced resting sympathetic outflow to skeletal muscles (145) together with increased muscle blood flow (389), pointing towards a vasoconstrictor defect. However, the evidence is conflicting (33, 158).
The level of plasma norepinephrine in upper limb venous blood is usually elevated (157, 359); some studies also report increased levels of epinephrine (157). In lower limb venous blood, however, norepinephrine levels tend to be lower than normal; furthermore, the transmitter turnover during different sympathoexcitatory stimuli is impaired, suggesting reduced neuronal reuptake and a 'patchy' sympathetic denervation predominantly affecting the legs (194, 195). However, a reuptake failure does not necessarily imply neuronal degeneration, and a mutation in the norepinephrine reuptake protein has indeed been documented in a pair of twins suffering from POTS (358).
A low level of LBNP does not seem to affect HR or MBP in POTS patients (158). However, in some studies, orthostatic stress is associated with attenuated sympathetic nerve activity to skeletal muscles (145) and blunted arterial vasoconstriction (385), causing 'pooling' in the dependent limb. Accordingly, similar results have been obtained during pharmacologically induced hypotension (33), further indicating some sort of sympathetic denervation, although the evidence is conflicting (279). Thus, the marked HR increase in regards to more severe orthostatic stress is regarded as a physiological response to reduced cardiac filling due to a peripheral vasoconstrictive defect. Recent evidence indicates that the mechanisms causing reduced cardiac filling might vary, suggesting a subgrouping of POTS patients; however, the mechanism for the HR response, which constitutes the hallmark of the disease, seems to remain identical (388).
Our results do not seem to support a close relationship between POTS and CFS pathophysiology. Our findings of increased TPRI at rest and enhanced TPRI during orthostatic exercise among CFS patients is neither consistent with sympathetic denervation nor any other vasoconstrictive defects. This notion is further supported by the neuroendocrine responses to strong cooling, indicating preserved reflexes and response abilities within the sympathetic nervous system; importantly, a similar test in POTS patients showed attenuated responses (194). Finally, the set-point alterations of arterial blood pressure and body temperature do not seem to be a feature of POTS.

4.2.4 Sedentary deconditioning

The CNS patients included in this study reported a significantly lower level of physical activity than the healthy controls (Table 7). Sedentary deconditioning and reduced aerobic capacity should therefore be considered a possible cause of the experimental

results, as endurance training has a substantial influence on cardiovascular regulation (291). Recent evidence indicates that this is partly explained by alterations of CNS autonomic control, but other mechanisms are also involved; for instance, physical exercise increases blood volume, which in turn exerts a tonic load on cardiopulmonary receptors (168).

Regular exercise causes a substantial decrease in resting HR, which is probably a combined result of increased SV due to hypertrophy, and central attenuation of cardiac sympathetic drive (290). Furthermore, there is evidence of lowered TPR and muscle sympathetic nerve activity, contributing to a small but significant decrease in resting blood pressure (82, 165, 291). Plasma levels of norepinephrine are reduced (82), as are possibly also the levels of epinephrine (291, 329). Thus, physical exercise is established as an important therapy in patients with hypertension (82).

Although lowering basal activity, endurance training seems to enhance the responsiveness within the sympathetic nervous system, at least in some situations. For instance, Grassi et al. documented a greater increase in HR and muscle sympathetic nerve outflow during drug induced hypotension after a period of physical training (165). Kjær reported enhanced adrenal responsiveness in trained subjects compared to sedentary controls during non-exercise stimulation (221). However, the evidence is somewhat conflicting, as other researchers have documented attenuated sympathetic responses to dynamic exercise after a training period (322). Interestingly, reduced responsiveness among the well-trained seems to be even more pronounced during isometric exercise; several studies have reported both attenuated HR increase (290) and attenuated sympathetic muscle nerve activity (377) in handgrip experiments. Unfortunately, there are contradicting findings in this area as well (323).

A detailed study by Levine et al. compared the responses to LBNP among subjects having different fitness levels (241). The responses to low level LBNP was rather similar in high-fit, medium-fit and low-fit individuals; however, compared to the low-fit group, the medium-fit tended to have a greater increase in HR and a smaller increase in TPR. More or less identical results have been reported by others (246, 285), indicating that moderate endurance training does not significantly influence the responses to orthostatic stress. However, intense endurance training is associated with reduced orthostatic tolerance, possibly due to structural alterations of muscle veins causing increased pooling (291).

Physical training is reported to enhance both the sweating and the vasodilator responses to a new bout of exercise (134). Accordingly, training might prevent the attenuation of thermoregulatory responses that are associated with bed-rest or reduced gravity (86, 363). However, the evidence is somewhat conflicting (181). Conversely, there is also evidence of reduced thermogenic threshold and elevated thermogenic sensitivity among endurance-trained subjects when exposed to a cold environment (196, 348).

Our findings of increased blood pressure, HR, TPRI and catecholamines at supine rest among CFS patients seem to fit with a hypothesis of sedentary deconditioning. Likewise, attenuated sympathetic skin response to moderate cooling might be explained by this

mechanism. However, the enhanced sympathetic responses to orthostatic challenge among CFS patients and the equal responsiveness to strong cooling among the two groups do not seem to fit this model. Furthermore, the attenuated responses to isometric exercise in CFS directly contradict what would be expected if sedentary deconditioning was an important underlying cause. Finally, our subgroup analysis comparing CFS with those controls that were least physically active (see 3.2.5) did not support such a hypothesis. However, it should be noted that even the most sedentary controls were more physically active than the CFS patients.

4.2.5 Gravitational deconditioning

A discrete, though related deconditioning phenomenon takes place during conditions of reduced gravity (179). This has been extensively studied in astronauts following space-flights. However, similar processes can also be observed during prolonged bed-rest.

Resting HR tends to increase in individuals subjected to gravitational deconditioning (75). This is probably caused by attenuated parasympathetic modulation of the sinoatrial node (274), but there are also indications of reduced cardiac sympathetic activity, although not to the same extent (75). Resting blood pressure values are unchanged (76, 192), whereas increased resting TPR is reported by most researchers, as reviewed by Waters et al (433). Accordingly, gravitational deconditioning is related to increased baseline sympathetic muscle outflow (242). Little is known about possible baseline alterations in the nervous control of skin circulation. Resting plasma level of norepinephrine tends to be lower (76, 160, 433), whereas epinephrine level seems to remain unchanged (160, 433).

Gravitational deconditioning attenuates the normal responses to orthostatism (75). Thus, strong orthostastic stress might precipitate syncope, whereas moderate orthostatic stress does not seem to influence blood pressure levels. The reduced orthostatic tolerance is explained partly by a combination of hypovolemia and increased venous compliance, causing a substantial reduction in central venous pressure (75, 192). Another important underlying mechanism is probably a blunted increase in TPR, as has been demonstrated both in healthy subjects following bed-rest (365), in astronauts following spaceflights (48), and in animal models (179). Accordingly, Kamiya et al reported an attenuated increase in skeletal muscle sympathetic outflow during orthostatic stress (204). However, the evidence is not uniform, as enhanced TPR responses have also been found by some researchers (76). Furthermore, the HR increase connected to orthostatic stress has been reported to be less pronounced after gravitational deconditioning due to a reduced responsiveness of the baroreflex (75); however, enhanced HR responses have been documented in other studies (433).

Ample evidence indicates that gravitational deconditioning influences the mechanisms involved with heat loss, in particular during physical activity (85, 239). Also, a higher

core temperature has been observed (273). The causes of this phenomenon seem to be impaired sweating as well as vasodilator responses; more specifically, evidence indicates both an increased threshold and reduced gain (273). Hypovolemia seems to be an important underlying explanation, but other mechanisms involving post-synaptic alterations of effector tissue are probably involved as well (86, 135). Recently obtained evidence suggests that prolonged bed-rest might cause attenuated cold-induced vasoconstriction and shivering responses as well (270). In the study of Mekjavic et al., the vasoconstrictor defect was attributed to the mechanical effects on vessels during microgravity, whereas reduced skeletal muscle function due to atrophy and attenuated central activation may account for less shivering. Accordingly, gravitational deconditioning seems to attenuate the response to the cold pressor test (432).
Gravitational deconditioning might explain our observations of increased HR and TPR among CFS patients at rest, and possibly also the increased body temperature and attenuated vasoconstrictor responses to moderate cooling. However, the neuroendocrine values at rest and during strong cooling, the changes in blood pressure set-point at rest and during orthostatic challenge, and finally the enhanced sympathetic cardiovascular response during orthostatic stress does not support gravitational deconditiong as an important underlying mechanism. In addition, none of the CFS patients were permanently bedridden (Table 7), and even very limited gravitational stimuli seem sufficient for preserving normal cardiovascular reflexes (406).

4.2.6 Disturbances of CNS autonomic control

As outlined above (see 1.2.2), all homeostatic control circuits operate according to a defined *set-point.* In some situations, the set-point level is altered, thus redefining the homeostatic range (155). For instance, both dynamic and isometric exercise is related to resetting of the baroreflex, whereas orthostasis normally is not. Fever is the most thoroughly studied example of set-point resetting within the thermoregulatory system, caused by the effect of inflammatory mediators on the hypothalamic thermoregulatory control areas (332). More generally, set-point changes should be considered vital for the CNS control and modulation of organ physiology. However, the neuronal substrates responsible for such changes remain largely unknown. A possible mechanism is relative alterations in activity among different brain centers. Thus, the set-point of a given control circuit is not localized to a single anatomical structure, but depends upon the dynamic interplay within neuronal networks (155).
The evidence presented in this thesis directly suggests altered resting set-point levels for blood pressure and body temperature among CFS patients. However, there are alternative explanations. The increased blood-pressure during orthostatic stress might be attributed to an overshooting-mechanism, causing a discrepancy between the real and the 'desired' value. Likewise, the attenuated blood pressure increase during isometric exercise might indicate an inability to raise the blood pressure to the desired level,

analogous to what occurs during some variants of orthostatic hypotension (159). Still, both findings are more readily explained by a resetting disturbance; in particular, the permanently increased blood pressure level during orthostatic stress does not seem to fit well with an overshooting-mechanism, which would be anticipated as a more transient phenomenon.
As homeostat resetting is a specific CNS phenomenon (155), our findings among CFS patients indicate a disturbance of CNS autonomic control. In turn, such a disturbance might explain other findings. An increased blood-pressure set-point at rest implies increased sympathetic outflow to the heart and the skeletal muscles, causing higher HR and TPR (see 1.2.2). Further elevation of the set-point during orthostatic stress elicits enhanced sympathetic cardiovascular activity, explaining a further increase in HR and TPR. A restricted set-point elevation during isometric exercise, on the other hand, has the opposite effect on sympathetic efferents, resulting in attenuated HR and TPR-responses. Furthermore, an increased body temperature set-point at rest implies increased sympathetic nerve activity to the sweat glands, the skeletal muscles and the skin vessels, in accordance with the patient's symptoms and their tendency towards reduced perfusion of arteriovenous anastomoses. Normalization of the set-point during moderate cooling reduces the sympathetic outflow to arteriovenous anastomoses, resulting in higher acral skin blood flow.

4.2.7 Concluding remarks

When seen as a whole, we conclude that the altered sympathetic nerve activity demonstrated among CFS patients most likely is caused by disturbances of CNS autonomic control characterized by abnormal homeostat resetting. In other words, altered CNS autonomic responsiveness seems to be an important aspect of CFS pathophysiology. Using Goldstein's terminology, CFS might be regarded a disease of 'homeodynamics' (155).
Interestingly, this statement seems perfectly in line with Friedman's publication from 1945 describing 'neurocirculatory asthenia', in which he concludes (138): «Changes in the rate, rhythm, and force of cardiac contractions were observed […] to be proceeded by, or associated with, excitation of the sympathetic nervous system. […] Evidence was obtained which suggested that the excitation […] resulted from hypothalamic discharge.»
This conclusion has important implications. The homeostatic control circuits are intimately related to the concept of *stress* (see 5.1.1). According to Goldstein, *distress* defines a specific kind of stress, characterized by homeostatic resetting for one thing (155). Increased plasma levels of epinephrine, also demonstrated among our CFS patients, is another hallmark of distress. Thus, a possible relationship between CFS and stress/distress merits further consideration, as will be comprehensively discussed in section 5.

4.3 Methodological considerations and study limitations

4.3.1 Recruitment

The uncertainties related to the CDC definition of CFS and our reasons for not adhering strictly to it have been extensively discussed elsewhere (see 1.3.1). Theoretically, the main disadvantage of our chosen approach seems to be the risk of grouping together conditions that have different etiology or pathophysiology, possibly obscuring important scientific findings. In addition, only a subgroup of the patients underwent expert psychiatric assessment at our institution (see Table 4), further increasing the possibility of heterogeneity. In other words: we were at risk of performing a type II-error. However, we did find statistical significant differences between patients and controls, even though the sample sizes were quite small. This fact seems to constitute an indirect justification of our inclusion criteria, bringing the validity of the CDC definition into question.
A logical consequence seems to be increased generalizability of our results. In fact, they might apply to all patients having long lasting, unexplainable and disabling fatigue, regardless of additional symptoms. However, this generalization is narrowed for two other reasons; we only studied adolescents, and the patients were recruited from a national referral center. Thus, further research projects should be population based and include a wider age group.
The possibility of sedentary deconditioning among CFS patients has been thoroughly outlined above (see 4.2.4). Although existing evidence does not support deconditioning as an explanation for the observed abnormalities, more sound proof might emerge from research comparing CFS patients with controls who have the same level of physical activity, and this should therefore be considered in the planning of new research.

4.3.2 Experimental protocols

Generally, the dynamic function of the autonomic nervous system might be assessed in three different ways (155):

- By microneurography, an invasive technique recording the electrical bursts in autonomic neurons.
- By neurochemical methods, implying invasive measurement of transmitter substances and their metabolites.
- By recording end-organ adjustments, which might be performed invasively as well as non-invasively.

Except for the experiment assessing neuroendocrine responses to strong cooling, we used the latter approach, applying non-invasive techniques exclusively. This is a substantial advantage, as all invasive procedures affect autonomic function in itself, introducing a possible source of error. Besides, non-invasive methodology is usually

considered ethically unproblematic. However, the shortcomings are obvious: end-organ recordings provide only *indirect* information, resulting in interpretational caveats and uncertainties, as illustrated by the comprehensive discussion on cardiovascular variability-indices (112, 258). Ideally, therefore, the results reported in this thesis should be confirmed by supplemental approaches such as microneurography and neurochemical methods, as further discussed below (see 5.2.5).

As a compensation for relying predominantly upon one methodological approach, we tested the same autonomic reflex mechanism by using two different experimental protocols: the head-up tilt-test and the lower body negative pressure-test. The similar results in these two experiments strengthen the validity of our conclusions. Furthermore, alternating between patients and controls during the study period reduces the risk of systematic errors (see 2.2.1).

Some particular problems related to the handgrip-part of the LBNP-experiment increase the uncertainties of the obtained results. Substantial evidence indicates that the cardiovascular responses during isometric exercise are independent of muscle mass, but depend upon the relative tension of the contraction (189, 445). As the CFS patients produced significantly less force during handgrip than did the healthy controls, one possible explanation for the hemodynamic differences is simply that the patients did not reach 30 % of their maximum handgrip capacity. Furthermore, the cardiovascular adjustments during isometric exercise were not studied in isolation. Thus, we are unable to tell whether or not orthostatic stress is a prerequisite for the observed reduced sympathetic response during handgrip in the CFS group. Finally, the experimental protocol demanded the repetition of the isometric exercise after a one-minute resting period (see Figure 10), and long-lasting metabolic changes in the muscles during the first bout might have influenced the cardiovascular responses during the second bout.

Other limitations of this study related to experimental set-up include:

- The participants were allowed to eat and drink on two occasions during the experimental day (see Table 6). The intake of food and fluid might have considerable influence on cardiovascular function (202, 366). However, since the meals were standardized and offered at predefined times, they should not contribute to differences between patients and controls.
- Baseline blood volume/plasma volume was not measured, leaving the question of hypovolemia in CFS patients unresolved.
- Plasma catecholamines were not measured during orthostatic stress or moderate cooling. A combined setup might have yielded additional information.
- Normetanephrine is the product of extraneuronal breakdown of catecholamines, whereas dihydroxyphenylethylene glycol (DHPG) mainly results from intraneuronal metabolism (155, 156). Concomitant measurement of this metabolite would have given better insight into sympathetic nerve function.
- Respiratory activity has been shown to change during orthostatic stress, and could therefore influence cardiovascular variability (79); however, ventilation was not measured nor controlled in the HUT experiment.

4.3.3 Quality of data

The technique of impedance cardiography applied during the HUT experiment might have limited validity, as indicated in several reports (38, 263, 303). The evidence supporting the validity of indices derived from the impedance signal, such as EDVI and ACI, is even weaker, emphasizing the necessity for cautious interpretation. Otherwise, as outlined above, the validity of the recording methods used in the different experiments have been firmly established among healthy adults (see 2.2.1). However, apart from the photopletysmographic technique of blood pressure measurements (356), none of the methods have been thoroughly assessed when used with children and adolescents, underscoring the need for further research projects addressing this issue.
The study design was aimed at minimizing influence of random error; most important, all experiments were carried out by the same researcher. A more formal analysis of random error within the LBNP experiment was made possible because each subject was exposed to two identical consecutive experimental runs (see 2.2.1). As outlined in 3.2.6, in healthy controls the 95 % empirical range of the mean differences is about 20-30 units wide for HR, DBP and SI, signaling quite low agreement between the two experimental runs, and a correspondingly high degree of random error (see Table 14). The quality of derived variables, such as TPRI, is even more reduced, as they are influenced by random errors from different sources. However, the main consequence of this phenomenon is increased risk of performing type II-errors. As we did find significant differences among patients and controls, the presence of random errors seem to have had limited impact.
Table 14 in 3.2.6 also visualizes a possible bias, as the mean difference of HR during LBNP was significantly higher than zero. Thus, there seems to be a slight trend towards higher HR in the second experimental run of LBNP as compared to the first one, indicating that they were not totally independent of each other. A likely explanation might be long lasting hormonal responses. However, as the protocols were identical among patients and controls, this bias should not interfere with the observed differences between the two groups.

5 Towards a unifying theory of the chronic fatigue syndrome

In the previous section, we concluded that the altered sympathetic nerve activity demonstrated among CFS patients is most likely caused by disturbances of CNS autonomic control characterized by abnormal homeostat resetting (see 4.2.7). Thus, the pathophysiology of CFS seems to be intimately linked to the concept of *stress/ distress.*

The first part of this section introduces a conceptual framework defining stress/distress and rooting these conditions in mechanisms of biology and psychology. Thereafter, the possible link between stress/distress and CFS will be thoroughly discussed in relation to other research findings as reviewed in the introductory part of this dissertation (see 1.3.2). The aim is to develop a unifying, still comprehensive, theory on the pathophysiology of CFS. Notably, this intellectual process is *inductive,* moving from an empirical to a theoretical level. It will be followed by an analogous *deductive* process, resulting in hypotheses that may be subjected to further research.

5.1 A conceptual framework

5.1.1 The stress theory of Goldstein

The autonomic nervous system – and the sympathetic branch in particular – has a key role in the *response to stress.* 'Stress' might be considered an ambiguous term; however, it is generally accepted as denoting a fundamental aspect of both physiology/ pathophysiology and psychology. Goldstein defines stress as «a condition in which expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal

or external environment, and this discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses» (155).
In Goldstein's view, stress is intimately related to the concept of homeostasis and the regulatory circuits described above (see 1.2.2) (155). In fact, stress occurs when homeostasis is threatened by any external perturbation. Thus, situations like orthostasis, exercise and cold exposure can all be considered 'stressful', eliciting a stress response, of which the characteristics – the 'pattern' - is determined by the control circuit. For instance, during orthostasis, the gravitational force will temporally disturb both arterial blood pressure and cardiac filling. However, the body has an 'expectation' of the ideal cardiac filling and arterial blood pressure levels, commonly referred to as the set-points. In this case, such a discrepancy between the ideal and real values will elicit a stress response characterized by a sympathetically induced increase in heart rate, contractility and total peripheral resistance. It is important to note, however, that most stress responses are not restricted to increased sympathetic (or reduced parasympathetic) nerve activity; other important effector systems are the somatic nervous system (regulating behavior), the adrenomedullary system (regulating plasma epinephrine), the hypothalamo-pituitary-adrenocortical system (HPA axis, regulating plasma glucocorticoids), the renin-angiotensine-aldosterone-system, and endogenous opioids.
It is evident that stress, as defined in this way, requires *perception.* In other words, stress and stress responses always involve the central nervous system. However, *consciousness* is not a prerequisite (155). In the example of orthostasis, the cardiovascular control center, by means of the baroreceptors and cardiopulmonary receptors, perceives a mismatch between the ideal and actual situation, but this mismatch is never experienced by the conscious 'self'. However, involvement of consciousness does not exclude a condition from being stressful, corresponding to our common usage of these terms. For instance, a trauma might be perceived – both consciously and unconsciously – a threat to homeostasis. Furthermore, involvement in a traffic accident might be a consciously *anticipated* homeostatic catastrophe even before any trauma has occurred, eliciting a patterned stress response that includes behavior.
A similar distinction is also made in regards to the term 'expectations'. According to the definitions, expectations or the 'ideal levels' might be genetically programmed not involving consciousness, as is presumably the set-point of the arterial baroreceptor reflex, or they might be 'deduced from circumstances', denoting a conscious, mental process. A third alternative, of particular importance in this thesis, is that expectations might depend upon *prior learning*. In everyday language, 'learning' is usually understood as an intellectual, highly conscious and voluntary activity. However, numerous important learning processes, also in humans, can be classified as *conditioning*, not involving consciousness or will (43). In *classical conditioning,* first described by Pavlov, a neutral stimulus (e.g. bell ringing) is repeatedly paired with a stimulus (e.g. presentation of food) that reflexively elicits a response (e.g. salivation). Associative learning ensures that after a while, the neutral stimulus itself will give rise to the response. In *operant (instrumental) conditioning,* a certain behavior is followed by positive

reinforcement (reward) or negative reinforcement (punishment), which in turn – by associative learning - increases or decreases the likelihood of the behavior. Thus, whether a situation is perceived as stressful, and how we eventually respond to it, is highly dependent upon the unconscious influences of our earlier experiences.
Distress can be defined as a specific kind of stress, characterized by a *conscious interpretation* of a homeostatic threat in a broad sense (155). Thus, there is a close analogy – or even an overlap – between a physiological and a psychological perspective, the latter often defining distress as an inability to cope or a lack of controllability. Furthermore, distress is related to *aversiveness* and therefore, by the means of the learning processes described above, promotes withdrawal, escape and avoidance behavior. A third characteristic of distress is *observable signs* reflecting the emotional state of the organism, often produced by changes in sympathetic nerve activity; one example is facial paleness which accompanies fear and facial flushing when one is angry. Fourth, distress evokes a *patterned endocrine response* causing elevated plasma levels of steroid hormones and epinephrine due to adrenocortical and adrenomedullary activation, respectively. Some have proposed a sequence of endocrine responses, causing different hormone levels in the early and late phases of distress (183). Finally, *homoeostat resetting* (i.e., change of set-points in the control circuits) is by Goldstein regarded as a hallmark of distress, causing a redefinition of homeostasis (155). However, in contrast to earlier views, the response of the sympathetic nervous system is not uniform rather it is tailored to the homeostatic challenges of the specific circumstances.
According to Goldstein, «situations evoking distress typically involve a complex interplay of classically and operantly conditioned behaviors» (155). For instance, distress might be percipitated by a neutral stimulus if it is associated with an event threatening homeostasis (classical conditioning), leading to behavior that ensures avoidance of the stimulus (operant conditioning). Repeated exposure to a stimulus evoking distress may cause reduced responsiveness, a phenomenon known as habituation; however, the organism might concurrently develop increased responsiveness to other potentially stressful stimuli (dishabituation).

5.1.2 Supplemental stress theories

The cognitive activation theory of stress (CATS), developed by Ursin and co-workers and based on experimental research with both animals and humans (118, 421), does not seem to differ in any vital aspects from the theories formulated by Goldstein (155) (see 5.1.1). However, the emphasis is somewhat different. CATS primarily focuses on conditions that are consciously regarded a threat to homeostasis; thus, the term ‘stress’ in this theory seems to correspond to Goldstein’s use of ‘distress’. Furthermore, CATS is concerned with the neuropsychological arousal elicited in stressful situations, and how expectancies might modulate both the arousal itself and the subsequent compensatory behavioral and autonomic/endocrine responses. In this regard, there are

two possible types of expectancies: stimulus expectancy, which modulates arousal and responses in terms of classical conditioning, and response outcome expectancy, which influence would be that of operant conditioning. In relation to the latter, CATS defines 'coping' as an expectancy of possessing the ability to respond adequately; thus 'coping' is not related to the response itself, nor to the factual outcome of a given situation (421). 'Hopelessness', on the other hand, occurs if the response abilities are expected to be insufficient. 'Helplessness' is defined as a condition in which the response abilities are expected to be irrelevant; i.e., the subject does not possess any means of preventing a certain outcome.

When applied to illnesses, CATS presumes that a persistent state of hopelessness and helplessness will result in sustained arousal, which on the neurobiological level might be related to sensitization of certain neuronal circuits within the CNS (118, 421). Hypothetically, this sustained arousal may cause a vast range of subjective bodily complaints, as well as be responsible for detrimental effects on different organ systems, such as an increased tendency for gastric ulcers. A serous objection to this theory, as mentioned by the researchers themselves, is the problem of measuring sustained arousal due to the constant interference of normal homeostatic reflexes; thus, the theory might have limited scientific value as it seems difficult to test experimentally.

A related, though not identical concept has been promoted by McEwen, using 'allostasis' to denote the constantly changing activity in the adaptive systems of the body in response to internal or external demands (268). Thus, 'allostasis' has a more general meaning than homoeostasis; it seems to include both behavioral responses, as well as alterations of the homeostatic levels (i.e., 'homeodynamics' according to Goldstein (155)). 'Allostatic load' is a general term for chronic altered activity in the adaptive systems, possibly contributing to disease processes; for instance, hypertension is regarded a 'shut down-failure'. Compared with CATS, this theory seems to be less concerned with the cognitive aspects of stress, and focused more on the possible harmful effects of chronic altered stress responses.

5.1.3 The concept of CFS as a disorder of sustained arousal

That CFS might be regarded a 'stress disorder' has been proposed earlier (72, 169), and similar models have recently been developed for other diseases of unknown, but possibly related origin, such as fibromyalgia (424). In this thesis, we have provided evidence of altered sympathetic nerve activity among CFS patients, indicating disturbances of CNS autonomic control characterized by abnormal homeostat resetting (see 4.2.6). Thus, our results seem to support an intimate relationship between CFS and stress/distress. More specifically, we present the following postulate as a starting point for further elaboration:

Chronic fatigue syndrome is a condition of disproportionate compensatory CNS responses to common stressors. Dependent on the theoretical framework, this condition might be labeled 'chronic distress' (155), 'sustained arousal' (421), or 'allostatic load shut-down failure' (268).

Without intending to signal a preference for any particular theory, in the following we will consequently use the term 'sustained arousal'.

5.2 The CFS sustained arousal theory

In the following, we discuss the CFS sustained arousal theory in relation to prior research on pathophysiology, as presented more in detail above (see 1.3.2). In accordance with Prins, we find it convenient to differentiate between predisposing factors, precipitating factors, and factors that might be regarded as perpetuating or as a mere consequence of the disease process (318). Thereafter, we propose a unifying model for the CFS pathophysiology, and discuss its implications for further research.

5.2.1 Predisposing factors

Genetics

The functioning of the sympathetic nervous system is highly dependent upon several genes; at some loci, even single nucleotide polymorphism may considerably influence its abilities, probably explaining - among other things – why individuals respond differently to pharmacological antihypertensive treatment (459). In other words, our stress reactions are to some extent genetically determined (155).

A couple of twin studies indicate that several abnormalities described among CFS patients are a consequence of inheritance, and not of the current disease process (244, 256, 313, 344). Still, there is increasing evidence of a specific hereditary component to this disease (63, 404). Interestingly, recent research indicates an association to polymorphisms of genes involved in CNS control of autonomic and endocrine effector systems, including the genes for monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (154, 373). Thus, it is tempting to suggest a possible relationship between a specific genetic background, a certain stress responsiveness, and an increased risk of developing CFS.

Personality

Some studies report that certain personality traits, like perfectionism and conscientiousness, predispose for CFS (319, 440). Concurrently, it is widely accepted that personality constitutes an important factor in how we respond to both physical (238) and mental (32) stressors; on a more theoretical level, personality seem to influence both the perception of stressful stimuli and the available coping strategies (155, 421). The influence of CNS noradrenergic systems on personality is generally recognized (102). Novel evidence suggests a relationship between certain alleles at the MAO-A genetic locus and specific behavioral traits, such as 'straightforwardness', 'conscientiousness', 'novelty seeking' and 'reward dependence' (334, 364). Likewise, polymorphisms of the alpha2-adrenoreceptor gene may have related implications (74). Unfortunately, the MAO-A gene was excluded from parts of the extensive genetic analysis of CFS patients recently performed by CDC researchers (154). Thus, putative genetic risk factors of CFS may be partly mediated through its effect on personality, making personality an interactive, or perhaps even confounding, variable.

Earlier experiences of stress

In humans as well as animals, the responses to a certain stress factor are highly dependent upon the unconscious influences of prior experience (118, 155) (see 5.1). Experiences in early developmental phases seem to be of particular importance (59). In other words, the CNS centers controlling these responses are amenable to learning. The mechanisms of such learning might be that of classical conditioning, creating – for instance - an association between stress responses and symbolic cue that does not threaten homeostasis. Eventually, operant conditioning might establish a link between a stressor and a certain coping strategy.

The additive effect of repeated exposure to different physical and psychosocial stressors is highly unpredictable. Still, if CFS is regarded as a condition of disproportionate stress responsiveness, one should expect a high incidence of potentially stressful situations among CFS patients prior to the outbreak of the disease. Some preliminary evidence seems to support this notion (412). Also, the close similarities between CFS and the Gulf war-syndrome, affecting veterans from the 1991 war in the Persian gulf, are notable in this regard (27), as well as the 'neurocirculatory asthenia' described among World war II soldiers (138). However, comprehensive research is still lacking.

Cultural issues

Although disputed, the prevalence of fatigue syndromes seems to be higher in well-developed countries than in underdeveloped (371), at least partially justifying the notion of CFS as a disease of 'modern civilization'. In this context, the question is whether certain aspects of the present Western culture might expose the population to a specific kind of stress or distress, increasing the risk of developing CFS in some individuals. Naturally, this problem seems more related to philosophy than physiology. Still, it is

not a novel perspective; in the 1800s, neurasthenia was partly attributed to the strain on the nervous system caused by industrialization (400).
Individualism seems to be a hallmark of postmodern culture (46). Western man has to a great extent emancipated himself from religious, moral, social and political authorities and traditions, and is – as never before – free to follow his own will and desire (122). This is often regarded as a positive situation of increased freedom, but it is also related to potential side effects, as recognized among medical academics (281) as well as religious leaders (321). Paradoxically, his freedom also dooms the postmodern individual to *elect* everything; from his clothes, friends and leisure activities to his career, moral standards and religious beliefs. To put it succinctly: he is free and forced to elect and construct his own self. John Donne seems to be outdated: in our time, man *is* (or at least behaves as) an island, entire of itself...
It seems reasonable that this cultural phenomenon might promote an experience of stress in some individuals. First, the unlimited freedom of choice might be perceived as a threatening one: a vast amount of choices implies a vast amount of possible *wrong* choices as well. In other words, man is burdened by an overwhelming responsibility; the success in life, or eventually the lack of success, is entirely dependent upon his own decisions. Second, whereas social support might strengthen our coping abilities (423), there is a short step from individualism to *loneliness* and *helplessness,* reducing our potential to handle stressors appropriately (420).
There is at present no firm empirical evidence linking these cultural phenomena to the development of CFS. Interestingly though, some researchers do find that *dilemmas* – which normally flourish in a setting of unlimited possible choices - may be related to the disorder (180).

5.2.2 Precipitating factors

Infection
There are intriguing similarities between CFS and post-polio syndrome (44). In the latter, fatigue is a common complaint, as well as cognitive impairments such as reduced memory, concentration and attention. The clinical features of 'epidemic' outbreaks of CFS (see 1.1.2) show strikingly similarities to epidemics of polio, and similar disturbances in the HPA axis, cardiovascular regulation and EEG activity have been demonstrated in both groups of patients. Post-polio syndrome, like CFS, seems to be aggravated by physical as well as emotional stress (45). Autopsies of polio victims reveal lesions in multiple brain stem areas, among others the reticular formation, paraventricular nucleus and locus ceruleus (44). Thus, Bruno and coworkers put forward a common fatigue theory based upon brain stem dysfunction, in particular influencing the 'activation system' in the reticular formation
A similar way of reasoning was presented by Dickinson, putatively relating CFS to infectious damage of the reticular activation system of the brain stem (101). Notably,

the microorganisms that seem most strongly associated with CFS outbreaks – Epstein-Barr virus and enteroviruses (2, 61) – commonly affect the central nervous system (446). Thus, in our setting, one might speculate that certain infectious agents may specifically distort the neuronal areas responsible for the compensatory responses to common stressors. To our knowledge, no research has addressed this possibility.
Alternatively, the relationship between CFS and infections may be regarded as non-specific (2). Here, it should be emphasized that long-lasting infections, as well as other states of chronic inflammations, constitute considerable stressors, provoking patterned responses from the CNS (357). Due to a combination of predisposing factors, some individuals might be particularly prone to long-lasting stress responses. Moreover, in such a situation, the responses may easily become unconsciously associated with non-stressful stimuli through the mechanisms of classical conditioning, resulting in sustained arousal (169).
It should be emphasized that altered stress responses may have profound influence upon the immune system, as further outlined below. Thus, the findings of viral reactivation and increased antibody levels in CFS might be an *effect* of the disease process rather than a cause (50, 120). Eventually, one might speculate that viral reactivation leads to inflammation that further enhances the stress response, thus establishing a vicious circle.

Strenuous physical exercise
Strenuous physical exercise, such as during high level competition, represent a great challenge to homeostasis, and therefore requires a comprehensive compensatory response (155). Post-exertional malaise is an almost uniform complaint of all CFS patients, and also included in the CDC definition (142, 318). Surprisingly, we have not found any report specifically addressing strenuous physical exercise as a possible precipitating factor. There is, however, striking similarities between CFS and the overtraining syndrome, which is described by some researchers as a stress related disorder (6). In addition to fatigue and exercise intolerance, overtrained athletes have increased sympathetic cardiovascular modulation (18) increased oxidative stress (129), a predominance of Th2 vs. Th1 immune response (230), and a blunted HPA axis responsiveness (6). Thus, there is strong cause to believe that CFS and overtraining might have some pathophysiologic mechanisms in common.

Severe psychosocial stress
According to some reports, CFS might be precipitated by critical life events (e.g. loss of spouse) (345, 412). In addition, the similarities with asthenia resulting from combat experience should be underlined in this paragraph as well (27, 138). Such events constitute severe stressors, eliciting emotional and behavioral as well as autonomic and endocrine responses (155). As for unspecific infections, the dynamics of such a response depends on predisposing factors as well as the influence of classical and operant conditioning; in susceptible individuals, the end result may be sustained arousal.

5.2.3 Consequences of the disease process and perpetuating factors

Molecular biology

Binding of catecholamines to the beta-adrenoceptors of white blood cells activates mainly the cAMP/protein kinase A-pathway, in turn controlling the activity of several important transcription factors (115). Therefore, a profound change in gene expression of peripheral mononuclear cells, as have been documented in recent CFS studies (124, 439), is – in principle - fully coherent with a hypothesis of enhanced sympathetic and adreno-medullary activity as a central pathogenetic component.

The detailed mechanisms possibly relating altered sympathetic nerve activity to the observed alterations in gene transcription remain to be explored. However, recent reports may shed light on some possible links; e.g., activation of the estrogen receptor, as has been reported among CFS patients (124), can be achieved through catecholaminergic stimulation of the cAMP/protein kinase A-pathway (293). Furthermore, acute psychosocial stress in healthy subjects has been shown to have significant effects on the transcription of several genes in circulating leukocytes (276).

Immunity and infection

Various kinds of stress seem to have profound effects on the immune system; main findings include attenuated cellular immunity (including decreased NK cell activity and decreased T-cell responsiveness), as well as a tendency towards reactivation of latent herpesviruses, including the Epstein-Barr virus (50, 152). Similar changes have been reported in the aftermath of strenuous physical exercise (233). Some of these effects might be attributed to increased sympathetic nerve activity and heightened levels of catecholamines. For instance, during acute psychological stress, norepinephrine and epinephrine seem responsible for recruiting different subset of NK-cells (34). During exercise, increased levels of catecholamines are probably important for leukocyte redistribution and functional alteration (349, 361). When taken together, increased sympathetic nerve activity seems to promote a shift towards Th2 immune responses at the cost of Th1 immune responses.

Intriguingly, such a shift corresponds neatly with the recent findings among CFS patients (see 1.3.2) (63, 301, 372). Furthermore, it has been reported that psychosocial stress in CFS patients attenuates the cytokine production induced by the infusion of lipo-polysaccarides (171). One underlying mechanism might be diminished responsiveness to catecholamines, as was indeed demonstrated by Kavelaars and co-workers who reported reduced cytokine production by monocytes from CFS patients when stimulated with the beta2-adrenoreceptor agonist terbutaline (208). This finding, in turn, may be explained by receptor downregulation due to permanently increased plasma levels of catecholamines.

Thus, immune dysfunction in CFS may be regarded an epiphenomena rather than a casual factor, as has been pointed out by others (66). Furthermore, the findings of increased activity of intracellular microorganisms in CFS patients, most convincingly

reported for enteroviruses (61) and *Mycoplasma*-species (284), might be fully explained by reactivation of latent infections due to immunological alterations (225).
It has not escaped our attention that the immune dysfunction of CFS may also contribute to patients' complaints and therefore should be regarded as a perpetuating factor. For instance, diverse cytokines have effects on CNS; e.g., IL-1 induces fever through an influence on the hypothalamus (301), and IL-6 may provoke fatigue (170). Catecholamines stimulate secretion of the latter, also within the CNS, by binding to the beta-adrenoceptor of antigen presenting cells (115). Interestingly, Gupta and co-workers did find experimental evidence of a correlation between IL-6 production and patients' complaints of fatigue (170).

Oxidative stress
Increased levels of catecholamines promote oxidative stress by an auto-oxidation mechanism resulting in reactive intermediate products, which in turn may give rise to free oxygen radicals (99). This mechanism probably contributes significantly to myocyte damage after myocardial infarction, and explains some of the detrimental effects of enhanced sympathetic nerve activity related to this condition. Interestingly, the cardiac cell disturbances – which are partly reversible - are characterized by increased lipid content and mitochondrial swelling (99, 257), similar to what has been reported in skeletal muscles of CFS patients (19). In fact, recent evidence suggests a direct link between increased levels of catecholamines and skeletal muscle damage (49, 260, 287). Thus, the increased oxidative stress documented in CFS patients (212, 328) may be a consequence of increased sympathetic outflow and increased catecholamine levels, probably contributing to symptoms such as muscle pain and postexertional malaise (197). There is also potential for a vicious circle, as pain and tissue damage caused by oxidative stress may enhance sympathetic outflow further through simple reflex mechanisms.

Skeletal muscle function
Since stress elicits a concomitant autonomic and behavioral response, there should be an intimate relationship between CNS autonomic centers and CNS areas involved in skeletal muscle control. Thus, in rats, experimental evidence indicates that adrenoreceptor stimulation elicits behavioral activation through a coordinated influence on motor areas and areas responsible for motivation and arousal (393, 395). Norepinephrinergic neurons in locus ceruleus, as well as epinephrinergic neurons in other sites, probably play important roles in this process (392, 394). Accordingly, in other species, the activity in these neurons increases in response to salient events (320), and also in relation to the decision to act (67). A related finding among rats is the presence of neurons in the paraventricular nucleus of hypothalamus and other nearby areas that seem to exert concomitant influence of motor neurons and sympathetic preganglionic neurons (214).

Therefore, if CFS is a state of sustained arousal and permanently altered CNS control system, disturbances of skeletal muscle nerve control is an expected finding, and has indeed been documented in several reports (91, 162, 314, 351, 367, 380). Such disturbances may be reinforced through the mechanisms of operant conditioning, explaining the 'kinesiophobia' documented in CFS patients (288, 369); i.e., repeated experiences of insufficient motor responses to common stressors will weaken the perceived coping abilities, possibly resulting in a state of 'helplessness' (421). These CNS mechanisms may also be directly involved in the development of fatigue sensations: in healthy subjects, fatigue induced by isometric exercise is related to an increase of electrical activity in brain motor areas prior to a repeated motor performance, possibly being a result of altered concentration or attention (350).
However, peripheral interaction between stress responses and motoric systems might also contribute to the skeletal muscle dysfunction in CFS. Catecholamines strongly influence the excitability of striated muscle cell membrane (177), probably explaining why psychological distress alters EMG records of skeletal muscle (330); these findings also constitute a possible explanation for the altered skeletal muscle excitability reported by Jammes and co-workers in CFS patients (197). Moreover, the reported pathology of skeletal muscles in CFS patients (197) may increase afferent neural activity, and thereby enhance resting sympathetic outflow through a reflex mechanism (324). Concurrently, such enhanced activity may contribute to muscle damage (49, 260, 287), thus establishing a vicious circle. Skeletal muscle atrophy due to inactivity might of course set up a negative self-reinforcing process as well.

Neuroimaging
The postulated disturbances of CNS autonomic control should be reflected in neuroimaging studies. Indeed, there are reports of altered perfusion (83), metabolism (414) and transmitter activity (70, 449) in brain areas responsible for stress responses. However, only a few of the functional neuroimaging studies among CFS patients have explored brain activity during mental stress, and none of them have investigated CNS responses to bodily stress, like exercise or orthostasis.

Sleep
There are indications of sleep disturbances among CFS patients (131, 261, 415), although firm evidence is lacking. Importantly, sleep is controlled by brain stem areas that also participate in stress responses. For instance, norepinephrinergic neurons in the locus ceruleus probably play a key role in the regulation of sleep rhythms (43), causing different gene expressions in cortical neurons during sleep and wakefulness (65). Interestingly, in patients with cardiac failure, sleep disturbances seem to be correlated with enhanced sympathetic nerve activity (452), and similar findings have recently been reported among CFS patients (174). Thus, we propose that distorted sleep among CFS patients is a consequence of the disease process, possibly contributing to their general feeling of malaise.

Cognitive function

According to Goldstein, distress implies *consciousness* (155) and must therefore involve cortical brain areas (43); likewise, conscious mental processes are presupposed in the CATS theory of stress (118). Consequently, given our postulate of CFS as a disorder of sustained arousal, it should follow that there are disturbances of cognitive functions. Although evidence is conflicting, there seems to be a general agreement of some cognitive impairment in CFS, particularly affecting memory, attention and information processing (93, 272). Altered attention directly points to a disturbance of the reticular substance, which is important for the modulation and 'gating' of afferent impulses from all kinds of receptors (43). In situations perceived as a threat to homeostasis, the reticular substance automatically focuses our attention to the sensory information of particular relevance, and concurrently inhibits transmission of irrelevant information. Thus, it is reasonable that a state of sustained arousal will have severe impacts upon one's attention abilities.

Reports of further weakening of cognitive abilities during stressful situations, such as exercise (26, 232), is in line with a sustained arousal theory of CFS as well. The reported beneficial effect of cognitive and endocrine effects of clonidine under conditions of high arousal among CFS patients were interpreted as being indicative of supersensitive postsynaptic alpha2-adrenoceptors in the brain (277), seeming to contradict our postulate. However, as central alpha2-stimulation primarily has a sympathoinhibitory effect (155), these results are equally adherent with a hypothesis of enhanced, centrally driven sympathetic neurotransmission in CFS.

Cognitive dysfunction in CFS may also interact complexly with mechanisms of conditioning. For instance, there is evidence of discrepancies between perceived and actual cognitive abilities among these patients (271), a phenomenon that seems to be fully explicable within the CATS theory (118, 421). For instance, a limited cognitive impairment might cause repeated experiences of insufficient coping strategies, thus negatively influencing the *perceived* coping abilities through operant conditioning. Importantly, this perceived impairment is a stronger predictor of outcome than the real impairment, resulting in a self-fulfilling prophecy. Such processes seem to be of general importance, influencing healthy individuals as well. For instance, well-trained subjects have a lower increase of epinephrine during bouts of exercise than sedentary controls (221). Experimental evidence indicates that the mechanism underlying this different response is a training-status dependent change in perceived exertion, rather than the magnitude of exertion itself.

Dysfunctional cognitive processes analogue to those described above might underlie similar reports of discrepancies between perceived and real abilities in other areas (13, 288, 369). Furthermore, they give a clue as to why patients' attributions seem to be related to actual outcome (187), and why cognitive therapy is proven effective (10, 100, 114, 280, 315, 335, 443). Finally, they contribute to vicious circles: sustained arousal inhibits cognitive functions, whereas cognitive impairments weaken coping strategies and thereby contribute to sustained arousal.

Psychology and psychiatry

The state of *anxiety,* neuroanatomically related to limbic brain areas (43), elicits strong stress responses. Thus, it is reasonable to suppose similarities in the pathophysiologies of anxiety disorders and CFS. Indeed, the enhancement of central noradrenergic transmission seems to be a key feature of the former, explaining findings of increased plasma catecholamines (60). In panic disorder as well as post traumatic stress disorder, there is evidence of an alteration in alpha2-adrenoceptor sensitivity (14, 39). Amygdala, which is reciprocally connected with locus ceruleus and nearby brain stem centers, seems to be the most important brain area for conditioned fear responses, whereas other parts of the limbic system (e.g. anterior cingulate cortex) have a modulating role (60). Gupta recently proposed that there is similar amygdalar fear conditioning in CFS patients (169), whereas Naschitz and colleagues reported a specific pattern of cardiac autonomic control in CFS patients that closely resembles what has been previously observed in anxiety disorders (283).

Epidemiologic research also seems to support a relationship between CFS and anxiety disorders, demonstrating much higher prevalence of panic disorders and generalized anxiety among CFS patients than in the general population, both in adults (130, 235) and adolescents (149).

Endocrinology

It is universally accepted that stress has profound endocrine consequences, mainly influencing plasma levels of adrenal hormones (155). During acute distress, enhanced sympathetic outflow to the adrenal marrow releases epinephrine, whereas the activation of the HPA axis ensures a release of glucocorticoids from the adrenal cortex. However, several other endocrine mechanisms are involved as well; for instance, in one study, marital conflict was related to increased plasma levels of epinephrine, norepinephrine, growth hormone and ACTH (217).

The observations of increased plasma levels of epinephrine among CFS patients in this thesis and in other reports (208, 413) comply with our postulate of sustained arousal. The documented attenuation of the HPA axis might seem more conflicting. However, whereas acute stress is related to increased levels of glucocorticoids, chronic stress might have the opposite effect, as thoroughly reviewed by Yehuda (454). The mechanism seems to be one of habituation, involving the adrenal cortex and the pituitary, as well as the hypothalamus. As for the latter, there is evidence of increased cortisol receptor density, possibly amplifying the negative feedback control and reducing plasma cortisol concentration (455). It is important to note, however, that a possible alteration of HPA responsiveness may be highly dependent on both the quality and the dynamics of the stressors. Thus, in experimental settings, HPA responsiveness is more affected when animals are exposed to repetitions of the same stimulus, whereas a regimen that constantly changes the nature and severity of the stimulus may not cause a blunted response. In some experiments, the attenuation of the HPA axis also blunted the response to a novel, acute stressor. In humans, similar findings have been reported among Vietnam

soldiers (36), parents of fatally ill children (139) and subjects having highly stressful occupational tasks (427). Furthermore, there are reports of reduced HPA responsiveness in posttraumatic stress disorder (454), although the evidence at this point is somewhat conflicting (245, 311).
When viewed as a whole, we conclude that attenuated HPA responsiveness is coherent with our postulate of CFS as a disorder of sustained arousal. Furthermore, blunted HPA activity during new, acute stressors may contribute to patients' complaints. Of interest here is a study by Streeten, reporting a high prevalence of fatigue among patients with hypocortisolism and 'delayed orthostatic hypotension' (397). The characteristics of the latter group are not well outlined in terms of physiology, but there seem to be several similarities to the cardiovascular responses demonstrated among CFS patients in this thesis.

Circulation
As discussed thoroughly above, the findings of cardiovascular disturbances in CFS patients reported in this thesis directly suggest sustained arousal. This complies with studies reporting higher resting blood pressure as well as increased sympathetic modulation of heart rate and total peripheral resistance among healthy humans during acute and chronic distress, both in test situations (252, 253) and during sleep (174). In mice, there is even experimental evidence of subtle myocardial damage during conditions of chronic psychosocial stress (84).
We found only two studies that were specifically aimed at exploring the cardiovascular autonomic responses during exercise in CFS patients (81, 376). Neither the experimental design nor the analytic methods were highly sophisticated. Nevertheless, their results are in line with ours: Cordero and co-workers found attenuated parasympathetic modulation of heart rate during dynamic exercise (81), whereas Soetekouw and colleagues reported attenuated cardiovascular responses during isometric exercise (376). As outlined above, different stressors elicit discrete, patterned CNS responses (155). Therefore, these reports – indicating opposite sympathetic responses to dynamic and isometric exercise - are not surprising in a state of presumed dysfunctional CNS control, and should not be interpreted as conflicting evidence.
Cardiovascular disturbances may readily explain some complaints among CFS patients, as demonstrated in this thesis. Also, it should be noted that vicious circles may be established within the cardiovascular system as well. As outlined above, the fatigue-induced inactivity causes deconditioning characterized by slight hypovolemia, which in turn amplifies the stress responses to orthostasis and supposedly contributes to a state of sustained arousal (see 4.2.1).

Thermoregulation
Increased body temperature, as demonstrated in this thesis, is consistent with a state of sustained arousal, as is evident in both animal (42, 104) and human research (41). The

mechanism seems to be the heightening of the set-point and a subsequently increased sympathetic outflow to thermoregulatory effector organs, complying with our results discussed above (see 4.1.5).
Recent reports have demonstrated increased acetylcholine-induced vasodilatation among CFS patients (203, 378). This result can have many explanations (379), but does not rule out enhanced sympathetic neurotransmission to skin arterioles. The vasodilative effect of acetylcholine is not caused by a direct effect on vascular smooth muscles, but is mediated through increased NO production in the nearby endothelial cells (155). NO is a potent vasodilator; in addition to having a direct effect on smooth muscle cells, this chemical compound also antagonizes the effect of norepinephrine. Thus, in subjects with high resting sympathetic tone, an increased NO production should cause a greater relative increase in blood flow as compared to subjects with low resting sympathetic tone, fitting neatly with the reported experimental results (203, 378).

Fatigue
A CFS theory should, of course, also address the hallmark of this disease, namely the experience of disabling fatigue. Although still sparse, there is evidence suggesting a possible relationship to the state of sustained arousal. For instance, strenuous, fatiguing physical exercise causes a long-lasting increase in plasma norepinephrine and sympathetic heart rate modulation during the recovery period (146, 399). However, more firm evidence seems to have emerged from animal studies. In rats, painful and inescapable stimuli alter the serotonin (5-HT) neurotransmission in different brain stem and limbic areas, such as the raphe nucleus (266), the amygdala (4) and the hippocampus (3). Interestingly, the nature of the stress influences the responses; the less controllability, the greater the increase in serotoninergic outflow (30). Furthermore, the experience of incontrollable stress strongly influences the neurotransmitter as well as the behavioral response to novel stressors (29). Comparable experiments have demonstrated that chronic stress alters the brain level of CRH and norepinephrine as well (58, 275, 407). Concurrently, neurons utilizing these transmitters mutually influence each other. For instance, serotoninergic neurons from the dorsal raphe nucleus activate CRH neurons in the paraventricular nucleus (107, 336), whereas these CRH neurons in turn seem capable of activating noradrenergic neurons in the locus ceruleus (227).
Interestingly, alterations in the levels of these three neurotransmitters have also been regarded as a potential element of a neuronal substrate underlying the experience of fatigue (407). In particular, an increased level of serotonin has been attributed to the experience of fatigue after exercise (11, 88), and was indeed demonstrated among CFS patients in one study (69). Thus, in conclusion, there is scientific evidence, although still immature, suggesting a possible causal relationship between sustained arousal and the experience of fatigue.

5.2.4 A unifying model of CFS pathophysiology

In summing up the above discussion, we present the following unifying model for the pathophysiology of CFS (Figure 12):

A. *Predisposing factors* of CFS are factors that increase the risk of developing sustained arousal; thus they are related to the CNS mechanisms concerning stress responses. The most important are:
    a. Genetic polymorphisms whose products are involved in stress-induced neurotransmission, such as MAO and COMT polymorphism.
    b. Personality traits that influence the interpretation of, experience of, and response to stressful situations, such as perfectionism.
    c. Prior experiences of stressful situations that modulate CNS stress responses through classical and operant conditioning, such as combat experience.
    d. Cultural phenomena that might be perceived as stressful or interfere with coping abilities, such as 'choice overload' and loneliness in postmodern societies.
B. *Precipitating* factors of CFS are factors that elicit strong CNS stress responses, eventually developing into sustained arousal due to either neuronal damage or the mechanisms of classical and operant conditioning. The most important are:
    a. Infections involving microorganisms such as Epstein-Barr virus and enteroviruses.
    b. Strenuous physical exercise, such as high-level competition.
    c. Severe psychosocial stress, such as loss of a spouse.
C. *Consequences* of sustained arousal and *perpetuating factors* are phenomena that are caused by sustained arousal and eventually contribute to its persistence. The most important are:
    a. Transcriptional activation in leukocytes due to peripheral catecholaminergic stimulation.
    b. A Th2 vs Th1 immune response predominance due to peripheral catecholaminergic stimulation. In turn, this might lead to:
        i. Altered cytokine production, contributing to fatigue and thermoregulatory disturbances.
        ii. Reactivation of intracellular microorganisms.
    c. Oxidative stress due to peripheral catecholaminergic stimulation. In turn, this might lead to:
        i. Muscle damage, contributing to pain and postexertional malaise.
        ii. Enhanced afferent neurotransmission, amplifying stress responses (vicious circle).

d. Skeletal muscle dysfunction, due to both altered CNS neurotransmission in motor areas and peripheral catecholaminergic stimulation. In turn, this might lead to:
    i. Muscle atrophy, amplifying stress responses when moving (vicious circle).
e. Altered perfusion, metabolism and transmitter activity in all brain areas involved in stress responses, due to altered CNS neurotransmission.
f. Sleep disturbances, due to altered CNS neurotransmission in brain stem areas. In turn, this might lead to:
    i. General feeling of malaise.
g. Cognitive impairments, due to altered CNS neurotransmission in cortical areas. In turn, this might lead to:
    i. Discrepancies between perceived and real abilities due to mechanisms of conditioning, contributing to feelings of helplessness.
    ii. Weakened coping abilities, amplifying stress responses (vicious circle).
h. Anxiety-like pathophysiology, due to altered CNS neurotransmission in limbic areas.
i. Attenuated HPA axis activity, due to alterations in hypothalalamic, pituitary and adrenal regulatory mechanisms. In turn, this might lead to:
    i. Blunted HPA axis responsiveness to acute stressors, contributing to patients' complaints.
j. Circulatory abnormalities due to altered set-points of homeostatic control circuits and subsequently altered peripheral catecholaminergic stimulation. In turn, this might lead to:
    i. Orthostatic intolerance and related symptoms.
    ii. Sedentary deconditioning, amplifying stress responses during orthostatis (vicious circle).
k. Thermoregulatory abnormalities due to altered set-points of homeostatic control circuits and subsequent altered peripheral catecholaminergic stimulation. In turn, this might lead to:
    i. Symptoms of thermoregulatory disturbances.
l. Fatigue, due to altered CNS neurotransmission in brain stem/limbic areas.

It is important to emphasize that this model allows *heterogeneity of causal factors* among individuals. In other words: sustained arousal is seen as a 'final common pathway', whereas the combination of predisposing, precipitating and perpetuating factors may vary from patients to patient. Thus, in some subjects, the most important causal factors may be, for instance, a certain genetic trait combined with acute infections,

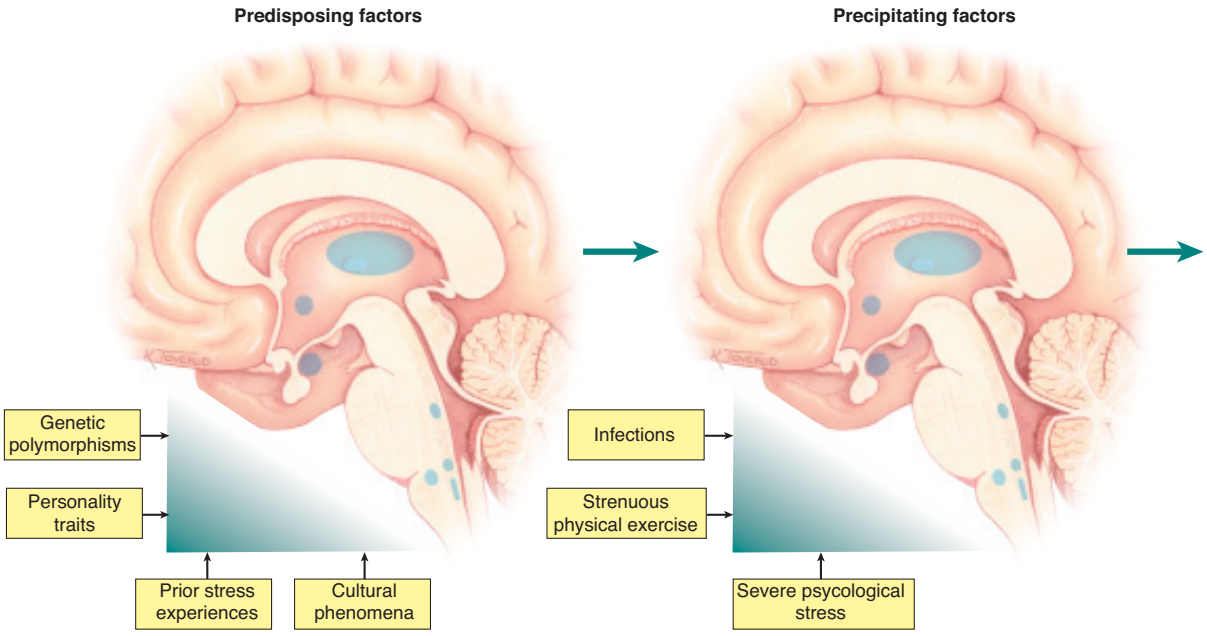
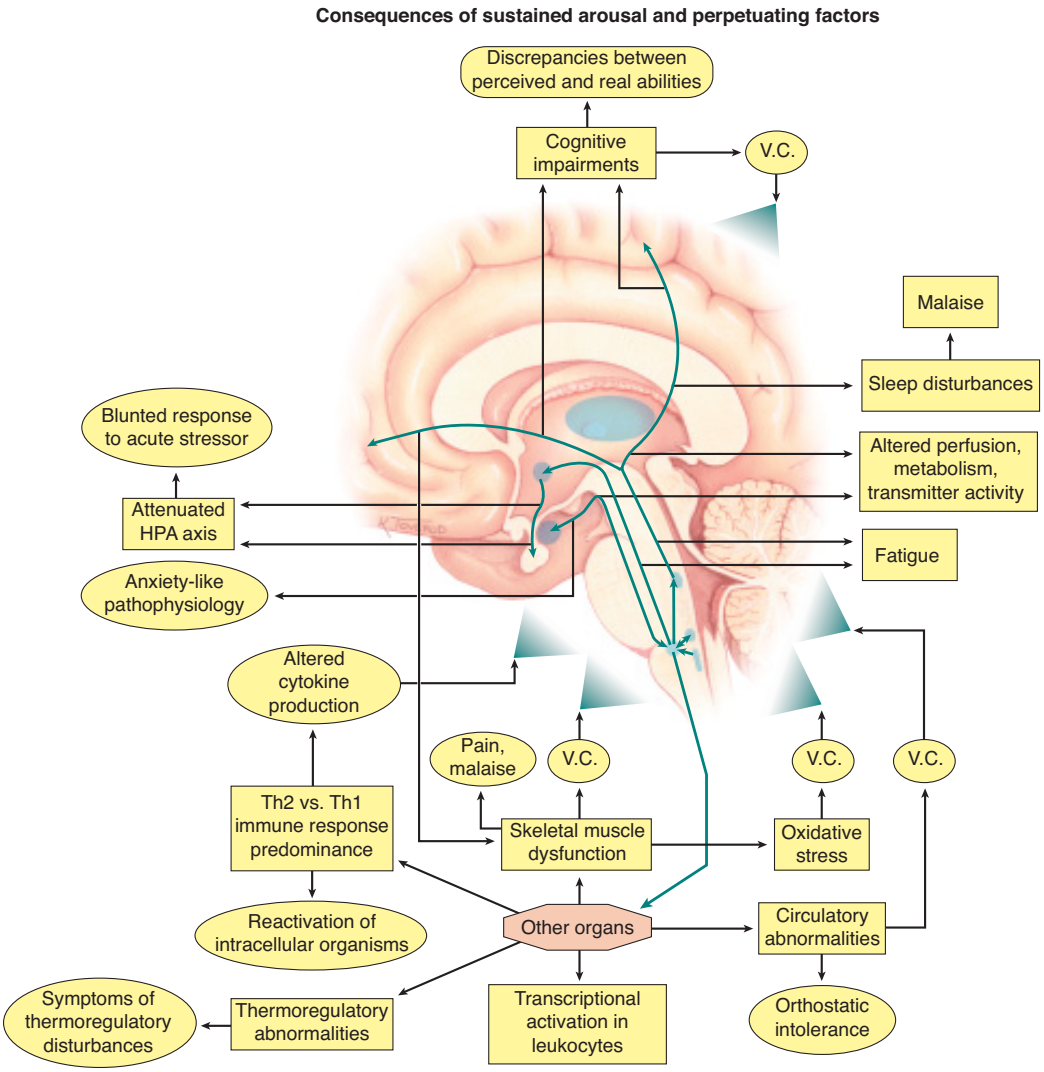


Figure 12. The sustained arousal theory of CFS. The figure should be regarded a simplified overview putatively relating current research findings to disturbances of CNS autonomic control, cf. figure 3. V.C.=Vicious circle.

whereas in others, prior experience combined with severe psychosocial stress might have the greatest impact. This may result in a confusingly non-uniform clinical picture. The proposed CFS sustained arousal theory seems attractive for different reasons. First, it is constructed upon a synthesis of the present knowledge concerning pathophysiology, and unifies findings from different research traditions; it even seems to share some concepts with theories proposed within the field of psychoanalysis (110). Second, it is based upon and coherent with contemporary stress theories, which in turn is firmly



rooted in empirical research. Third, it seems to possess a kind of 'face validity' when appraised in relation to patients' uniform experience of an inability to respond properly to the physical and mental challenges of daily life (178, 375). Fourth, it allows for the deduction of testable hypotheses that might be subjected to further research, as outlined below (see 5.2.5).

However, there are also some objections. The CFS sustained arousal theory implies a biopsychosocial construct that – although widely accepted by most experts - has been

met with skepticism from patient organizations as well as some practitioners and researchers (318, 436). At a more general level, our suggested model challenges a stable, 1:1 relationship between cause and effect, and thereby seems to oppose a more mechanistic approach to biology and medicine. As stated by Manu: «… more than any other issue in contemporary medicine, chronic fatigue syndrome reflects the unresolved conflict between the mechanistic and the biopsychosocial construct of illness» (262). The model proposed here infer heterogeneity of causality as well as clinical presentation, and allow symptom severity to be relative to the extent of stressor exposure. Thus, the model views CFS as a kind of *maladaption*, in many respects analogous to the phenomenon of 'loudspeaker feedback': When a microphone is placed in the vicinity of a loudspeaker, even small noises will be amplified through positive feedback, resulting in an earsplitting sound and functional 'breakdown'. However, there are no specific failures of the different electronic devices *per se;* no 'pathologies' of cables or connections. The problem results from maladaption; the equipment set-up is inappropriate *relative* to the initial noise recorded by the microphone. The problem might be solved by lowering surrounding sounds. In terms of CFS, reducing all external stimuli that might provoke a stress response. However, a more definite solution would be to change the abilities within the feedback loops, by moving the microphone away from the loudspeaker or reducing the gain of the electronic amplifier device; the latter would correspond to a 'down-tuning' of the stress responses within the brain.

5.2.5 Suggestions for further research

Based upon the CFS sustained arousal theory explained above, we will in the following present a few hypotheses and shortly outline possible methods for testing them. The list is not complete, but indicates some natural directions for further research.

1) Certain polymorphisms of genes whose products are involved in stress-induced neurotransmission are more common among CFS patients than healthy controls. Leukocyte DNA must be collected from well-defined populations of patients and controls. A search for polymorphisms should be governed by updated knowledge of the genetic basis for stress responses. Commercial kits for such genomic studies are generally easily available.

2) CFS patients have experienced more stressful situations prior to disease onset than patients suffering from other long-lasting disease. A prospective, population-based design would be preferable due to possible recall bias, but is inconvenient on the practical side. Stressful situations should be operationalized, and might include for instance prematurity, early long-time kindergarden affiliation, other serious diseases, trauma, death of a family member and abuse.

3) Acute Epstein-Barr virus infection elicits a more comprehensive stress response than other viral infections of similar clinical presentation. Patients with verified acute EBV infection and patients with acute viral pharyngitis of other origin could undergo experiments (e.g. head-up tilt-test) to assess their autonomic nerve activity when exposed to stressors. Subjects from the two groups should be matched according to their CRP level, to ensure an equally extensive inflammatory reaction.

4) When exposed to physical stress (such as orthostasis or physical exercise), perfusion, metabolism and transmitter activity in brain areas responsible for stress responses differs among CFS patients and controls. Of particular interest are the serotoninergic and norepinephrinergic pathways in the brain stem and limbic structures. Modern neuroimaging technology makes such studies feasible.

5) Cognitive therapy specifically designed to abolish sustained arousal are more effective in the treatment of CFS than unspecific cognitive therapy. To our knowledge, the effectiveness of different cognitive treatment approaches has not been subjected to research. The design should be randomized and controlled.

6) The characteristics of the baroreflex and the cardiopulmonary reflex differ among CFS patients and controls during rest and orthostatic stress due to set-point alterations. A logical continuation of our own research is to explore further the characteristics of the homeostatic control circuits responsible for volume and blood pressure regulations in CFS patients and controls. A possible analytic tool would be the mathematical technique of transfer function analyses, which could be applied to already recorded data from the LBNP and HUT experiments (416).

7) The blood volume and the central venous pressure are comparable among CFS patients and controls, but the renin-angiogensin-aldosteron activity is not. As the renal secretion of renin is controlled through sympathetic efferents, one should expect increased activity in a state of sustained arousal, but no alterations of blood volume or central venous pressure. Such an experiment would require invasive recordings.

8) When comparing CFS patients to controls, acute physical stress (orthostasis, physical exercise) and mental stress will cause a greater increase in directly recorded sympathetic outflow to skin and skeletal muscles as well as plasma turnover of epinephrine and norepinephrine. Measurements of cardiovascular and thermoregulatory variables, as performed in this thesis, provide only indirect information on sympathetic nerve activity, and are therefore prone to several interpretational caveats. To the best of our knowledge, no study has applied the technique of microneurography which allows direct recordings of sympathetic skin and skeletal muscle activity. Likewise, only a few researchers have performed catecholamine measurements (208, 413), none of them using recently available, advanced methods of kinetics.

9) Pharmaceutical inhibition of brain centers eliciting the stress response will improve the functional abilities of CFS patients and normalize the hemodynamics in stressful situations. The alpha2-adrenoceptor agonist *clonidine* is an effective antihypertensive drug due to central inhibition of sympathetic outflow. Anxiolytic drugs, like *benzodiazepines,* also exert an inhibitory effect on the relevant brain stem centers. As far as we know, their effect in CFS patients has not been subjected to systematic trials. Such trials should focus on the changes of functional capacities, but should also assess whether the stress responses have normalized, possibly using cardiovascular function as an indicator.

Finally, it should be pointed out that the CFS sustained arousal theory – if proven correct – might have implications beyond its present application. Related diseases, such as fibromyalgia, might be fully explicable within the same theoretical framework (421). Furthermore, similar models seem valuable for providing new insight into totally different areas, for instance hypertension and the mechanisms underlying placebo effects. Finally, it might even contribute to a deeper understanding of body-mind interactions in general.
Thus, we have come full circle, recalling the introductory quotation of Harvey: *«For every affection of the mind that is attended with either pain or pleasure, hope or fear, is the case of an agitation whose influence extends to the heart…»* Or, to cite his contemporary Blaise Pascal (1623-62):

«Le coeur a ses raisons que la raison ne connaît point.»

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