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# Implications of autonomic nervous system and central inflammatory parameters for the perception of pain in fibromyalgia patients

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**To all suffering  
fibromyalgia patients**

**Smärta har inte tagit livet AV mig.**

**Smärta har tagit livet IFRÅN mig!**

**En patient.**

## ABSTRACT

Dysfunctions of the autonomic nervous system and of endogenous pain modulation have been reported in fibromyalgia (FM) patients. The dysregulation of the autonomic nervous system, i.e., increased sympathetic activity at baseline and hyporeactivity during exercise and stress, could contribute to muscle ischemia as well as to the exercise intolerance that is typically seen in FM patients.

Isometric contractions are potent stimuli to provoke muscle ischemia, increased muscle pain, heart rate (HR) and blood pressure (BP). In our first two studies we used isometric contractions to investigate the interaction between cardiovascular regulation and pain perception and to assess activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (HPA-axis) in FM patients. Glia cell activation has been suggested as a possible pathophysiological mechanism in FM and can be linked to dysfunction of autonomic nervous system. Increased levels of pro-inflammatory cytokines have been reported in the blood of FM patients, but cytokines have, to our knowledge, never been studied in the cerebrospinal fluid (CSF) in FM. In study three and four, we investigated pro-inflammatory cytokines in the CSF of patients with FM, rheumatoid arthritis (RA) and controls.

In study 1 we assessed the interactions between cardiovascular regulation and pain perception during static muscle contractions in FM patients and healthy controls. We found that systolic and diastolic BP increased during contraction and decreased following contraction in both groups alike. A significant increase in HR was seen during contraction in FM patients, but not in healthy controls. The rated exertion/fatigue and pain intensity increased more during contraction and remained elevated longer following contraction in the patient group. Pressure pain thresholds (PPTs) were lower in patients compared to controls at all times. No group differences in PPT changes over time were found. In conclusion, no indication of an attenuated cardiovascular response to exercise was found in our FM patients. The more pronounced HR increase in patients during contraction was most likely due to de-conditioning. No exercise related change in PPTs was seen in either group, most likely due to insufficient exercise intensity, but the pain induced by contraction was more pronounced in FM patients.

In study 2 we investigated activation of the sympathetic nervous system and the HPA-axis during static contractions in FM patients and healthy controls. BP and HR increased during contraction and decreased following contraction in both groups alike. Compared to baseline, plasma catecholamines increased during contraction in both groups alike but FM patients had lower levels of plasma adrenaline and a non-significant tendency to lower plasma noradrenaline at all times. No baseline group differences in adrenocorticotrophic hormone (ACTH) were found. ACTH increased at exhaustion in controls, but not in FM patients and FM patients had lower ACTH at exhaustion compared to controls. High sensitivity C-reactive protein (CRP) was elevated in FM patients compared to controls. In conclusion, FM patients exhibited a hypoactive sympatho-adrenal system as well as a hypo-reactive HPA-axis during static exercise.

In study 3 we assessed pro-inflammatory cytokines in the CSF and serum in FM patients and headache controls. We reported elevated CSF and serum concentrations of interleukin-8 (IL-8), but not IL-1b, in FM patients. Our conclusion was that the cytokine profile was in accordance with FM symptoms being mediated by abnormal activity in the sympathetic

nervous system rather than dependent on prostaglandin associated mechanisms. The results support the hypothesis of glia cell activation in FM.

In study 4 CSF and serum concentrations of pro- and anti-inflammatory cytokines in our FM cohort were compared to patients with an inflammatory rheumatic disease, i.e., rheumatoid arthritis (RA). We found different CSF cytokine profiles with higher concentrations of the pro-inflammatory IL-1b and lower concentrations of the anti-inflammatory IL-1Ra, IL-4 and IL-10 in the CSF of RA patients, compared to FM. FM patients had higher CSF and serum IL-8 concentrations than RA patients. Our results indicate different profiles of central cytokine release, i.e., IL-1b in patients with inflammatory, prostaglandin associated pain (RA) and IL-8 in patients with dysfunctional, possibly sympathetically mediated pain (FM).

**Keywords:** fibromyalgia, blood pressure, heart rate, pain ratings, exercise, catecholamines, hypothalamo-pituitary-adrenal axis, sympathetic nervous system, autonomic nervous system; glia cells, central inflammation, cytokines, chemokines, lumbar puncture.

## SAMMANFATTNING PÅ SVENSKA

Dysfunktion av autonoma nervsystemet och av kroppsegna smärthämmande system har tidigare rapporterats hos patienter med fibromyalgi (FM). Dysfunktionen av det autonoma nervsystemet i form av ökad basal sympatikusaktivering och hyporeaktivitet i samband med fysisk ansträngning och stress, skulle kunna bidra till sämre muskelgenomblödning och ökad smärta i samband med fysisk aktivitet. Det senare är kännetecknande för FM.

Statiskt muskelarbete är ett potent stimulus för att framkalla muskelsmärta och öka puls och blodtryck. I våra två första studier använde vi oss av statiskt muskelarbete för att studera interaktionen mellan kardiovaskulär reglering och smärtupplevelse och för att kartlägga aktiveringen av sympatiska nervsystemet och HPA (Hypothalamic-pituitary-adrenocortical)-axeln. Gliacellsaktivering har föreslagits som en potentiell orsak till smärta vid FM och gliaceller kan aktiveras i samband med avvikande autonom reglering. Tidigare har ökade koncentrationer av pro-inflammatoriska cytokiner rapporterats i blod hos FM patienter, men inga studier av markörer för gliacellsaktivering i form av pro-inflammatoriska cytokiner i ryggmärgsvätska har kommit till vår kännedom. I studie tre och fyra undersökte vi förekomsten av pro-inflammatoriska cytokiner i ryggmärgsvätska hos patienter med FM, ledgångsreumatism (reumatoid artrit (RA)) och kontroller.

I första studien undersöktes interaktionen mellan kardiovaskulär reglering och smärta under statiskt muskelarbete hos FM patienter och friska kontroller. Systoliskt och diastoliskt blodtryck ökade under statiskt muskelarbete och återgick till ursprungsläget efter statiskt muskelarbete i båda grupperna. En signifikant ökning av pulsen sågs under muskelkontraktionen hos FM patienterna men inte hos friska. FM patienterna rapporterade mer smärta och större grad av utmattning under muskelkontraktionen och dessa symptom kvarstod längre efter kontraktionen jämfört med kontrollerna. FM patienterna hade lägre smärtrösklar för tryck (pressure pain thresholds (PPTs)) före, under och efter kontraktionen jämfört med kontrollerna. Inga gruppskillnader sågs beträffande förändringar i PPTs över tid. Således sågs ingen antydning till minskad kardiovaskulär respons under fysisk ansträngning hos FM patienter. Den mer uttalade pulsökningen hos FM patienterna förklaras sannolikt av sämre kondition i denna grupp. Inga signifikanta förändringar av smärtrösklar för tryck sågs i samband med fysisk ansträngning i någon grupp, troligen på grund av att ansträngningen inte var tillräckligt intensiv. FM patienterna upplevde dock kontraktionen som mer smärtsam än kontrollerna.

I andra studien undersöktes aktiveringen av sympatiska nervsystemet och HPA-axeln i samband med statiskt muskelarbete hos patienter med FM och friska kontroller. Systoliskt och diastoliskt blodtryck ökade lika mycket i båda grupperna under statiskt muskelarbete och återgick till ursprungsläget efter statiskt muskelarbete. I båda grupperna sågs en ökning av katekolaminer i blod i samband med statiskt muskelarbete men FM patienterna hade lägre koncentrationer av adrenalin före och under muskelarbete och en tendens till lägre koncentrationer av noradrenalin före och under kontraktionen jämfört med friska. Utgångsvärden av ACTH (adrenocorticotropic hormone) skilde sig inte mellan grupperna. I slutet av kontraktionen sågs dock ökade koncentrationer av ACTH hos friska, men inte hos FM patienter och FM patienterna hade signifikant lägre nivåer vid utmattning jämfört med friska. FM patienterna hade högre koncentrationer av högkänsligt CRP (C-reactive protein) jämfört med friska. Sammanfattningsvis fann vi en hypoaktivitet i sympatiska nervsystemet och en hyporeaktivitet i HPA-axeln under statiskt muskelarbete hos FM patienter.

I tredje studien undersöktes förekomsten av inflammatoriska cytokiner i ryggmärgsvätska och blod hos patienter med FM och kontroller. Vi fann ökade koncentrationer av interleukin (IL)-8, men inte IL-1b i ryggmärgsvätska och blod hos FM patienter. Profilen av de inflammatoriska cytokinerna hos FM patienter är i överensstämmelse med vad man kan förvänta sig vid en avvikande autonom funktion, men stämmer inte med en prostaglandinmedierad smärta. Vidare är våra resultat förenliga med förekomsten av smärtrelaterad gliacellsaktivering hos FM patienter.

I fjärde studien undersöktes förekomsten av pro- och anti-inflammatoriska cytokiner i ryggmärgsvätska och blod hos patienter med FM och patienter med en inflammatorisk reumatisk sjukdom, ledgångsreumatism (reumatoid artrit (RA)). I enlighet med vår hypotes fann vi olika cytokinprofiler mellan grupperna, med högre koncentrationer av den pro-inflammatoriska cytokinen IL-1b men lägre koncentrationer av de anti-inflammatoriska IL-1Ra, IL-4 och IL-10 i ryggmärgsvätskan hos RA patienter jämfört med FM patienter. Däremot hade FM patienterna högre koncentrationer av IL-8 i ryggmärgsvätska och blod jämfört med RA patienter. Således fann vi olika profiler av cytokiner i ryggmärgsvätska, dvs högre koncentrationer av IL-1b hos patienter med en inflammatorisk, prostaglandinmedierad smärta (RA) och högre koncentrationer av IL-8 hos patienter med en dysfunktionell smärta, möjligen relaterad till en dysfunktion av sympatiska nervsystemet (FM).

## LIST OF PUBLICATIONS

1) Kadetoff D, Kosek E. The effects of static muscular contraction on blood pressure, heart rate, pain ratings and pressure pain thresholds in healthy individuals and patients with fibromyalgia. *Eur J Pain* 2007;11:39-47.

2) Kadetoff D, Kosek E. Evidence of reduced sympatho-adrenal and hypothalamic–pituitary activity during static muscular work in patients with fibromyalgia. *J Rehabil Med* 2010;42:765-772.

3) Kadetoff D, Lampa J, Westman M, Andersson M, Kosek E. Evidence of central inflammation in fibromyalgia - Increased cerebrospinal fluid interleukin-8 levels. *J Neuroimmunol* 2012;242:33-38.

4) Kosek E, Kadetoff D, Westman M, Le Maitre E, Andersson M, Lampa J. Evidence of different mediators of central inflammation in dysfunctional and inflammatory pain: IL-8 in fibromyalgia and IL-1 in rheumatoid arthritis. To be submitted.



## **LIST OF ABBREVIATIONS**

A	Adrenaline
ACR	American College of Rheumatology
ACTH	Adrenocorticotrophic hormone
ANS	Autonomic nervous system
BDNF	Brain-derived neurotrophic factor
BP	Blood pressure
BPM	Beats per minute
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CPM	Conditioning pain modulation
CRH	Corticotrophin-release hormone
CRP	C - Reactive Protein
CSF	Cerebrospinal fluid
DNIC	Diffuse noxious inhibitory controls
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
FIQ	Fibromyalgia impact questionnaire
FM	Fibromyalgia
fMRI	Functional magnetic resonance imaging
HADS	Hospital Anxiety Depression Scale
5-HIAA	5-Hydroxyindoleacetic acid
HNCS	Heterotopic noxious conditioning stimulation
HPA-axis	Hypothalamic-pituitary-adrenocortical axis
HR	Heart Rate
HRV	Heart rate variability

ICD	International Statistical Classification of Diseases and Related Health Problems
IL	Interleukin
MFI-20	Multidimensional fatigue inventory - 20
MSNA	Muscle sympathetic nerve activity
MTX	Methothrexate
MVC	Maximal voluntary contraction force
NA	Noradrenaline
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NS	Nociceptive specific neurons
PSQI	Pittsburg Sleep Quality Inventory
PGE2	Prostaglandin E2
PPTs	Pressure pain thresholds
PSNS	Parasympathetic nervous system
PVN	Paraventricular nucleus
REM	Rapid Eye Movement
SNS	Sympathetic Nervous System
SP	Substance P
TNF	Tumour Necrosis Factor
VAS	Visual Analogue Scale
WDR	Wide dynamic range
WU	Wind up

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## INTRODUCTION

Fibromyalgia (FM) is a common rheumatologic condition characterised by chronic widespread pain and reduced pain thresholds, with hyperalgesia and allodynia (Carville et al. 2008). FM patients have widespread muscular pain, tenderness, fatigue and disturbed sleep. A number of associated symptoms such as paresthesias, subjective swelling, headache, irritable bowel syndrome, irritable bladder and psychological distress are often present in FM (Wolfe et al. 1995; McCain 1993).

The prevalence of FM in the general population is 1,3 - 4,8%, out of which 80 - 90% are women (Wolfe et al. 1995). Mental stress and static muscle contractions have been recognised as the two major risk factors for development of work-related myalgias and cause aggravated pain in myalgia as well as in FM patients. Approximately 80% of FM patients report localised pain for many years before developing the generalised pain (Henriksson et al. 2004).

The pain mechanisms are most likely multifactorial, as suggested by the prevailing bio-psycho-social model. Peripheral mechanisms (i.e., muscle ischemia, neurogenic inflammation), central factors (dysfunctional autonomic nervous system and endogenous pain modulatory mechanisms), and psychological aspects (depression, anxiety, stress) have all been implicated in the pathophysiology of FM (Kosek 2009). The pain in FM is most likely explained by a complex interaction between peripheral and central mechanisms.

### ***Dysfunction of endogenous pain modulation in fibromyalgia***

Important central nervous system mechanisms relevant for FM pain include temporal summation of pain (wind up) and central sensitization (Staud et al. 2001; Woolf and Decosterd, 1999). These pain mechanisms are dependent on N-methyl-D-aspartate (NMDA) and substance P (SP) receptors of wide dynamic range (WDR) and nociceptive specific (NS) neurons in the dorsal horn of the spinal cord (Davies and Lodge, 1987). Wind up (WU) represents an important pain mechanism that can result in short- and long-term changes of neuronal responsiveness, including central sensitization. WU occurs during repetitive nociceptive stimuli leading to the removal of the magnesium block of the NMDA receptor, followed by calcium influx into the cell and triggering of signalling cascades that can result in amplification of nociceptive input and long-term central sensitization. The mechanism responsible for central sensitization seems to strongly depend on stimulus intensity and on the descending pain inhibitory mechanisms (Woolf 1996). Many studies have provided convincing evidence for abnormal WU and central sensitization in patients with FM (Staud et al. 2001; Staud et al. 2004; Price et al. 2002).

Several authors have proposed the dysfunction of endogenous pain modulation as the basis for aetiology and pathogenesis in FM (Smythe 1979; Moldovsky 1982; Yunus 1992). This was supported by findings of abnormal concentrations of transmitter substances implicated in pain regulation in the cerebrospinal fluid (CSF) of FM patients, i.e; decreased levels of serotonin, noradrenalin, dopamine (Russell et al. 1992) and elevated concentrations of SP (Russell et al. 1994), glutamate, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Sarchielli et al. 2007). SP is a neuropeptide that is released by descending pathways and spinal interneurons in addition to being released by primary nociceptive afferents in the dorsal horn of the spinal cord where it exerts an excitatory, sensitizing effect on secondary nociceptive neurons. SP may also coordinate the response to stress by interacting with the hypothalamo-pituitary-axis (HPA-axis) and the sympathetic nervous system (SNS) (Black 2002). SP levels in the CSF of FM patients have been reported to be 2-3 times higher in comparison with healthy controls (Russell 2002). Low levels of the serotonin metabolite (5-

HIAA) in the CSF of FM patients could explain the decrease of deep stages of non rapid eye movement (non-REM phase) sleep and the increase of somatic complaints, depression and perceived pain that are typical for FM (Moldofsky 1982).

Indications of central dysfunction were found in previous studies when standardised static contractions gave rise to increased pressure pain thresholds (PPTs) at the contracting muscle in healthy controls, while a paradoxical decrease in PPTs was seen in FM patients, signifying peripheral sensitisation and/or a dysfunction of endogenous pain modulation in FM (Kosek et al. 1996a). Furthermore, FM patients were reported to have a generalized, multimodal increase in pain sensitivity (Kosek et al. 1996b).

The dysfunction of diffuse noxious inhibitory controls (DNIC)-like mechanisms in FM has been suggested by several authors (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997). DNIC, now called conditioning pain modulation (CPM) (Yarnitsky et al. 2010) is a supraspinally mediated physiological phenomenon that has been defined in animal studies as a powerful and widespread inhibition of WDR neurones in the dorsal horn of the spinal cord by heterotopic noxious conditioning stimulation (HNCS) (LeBars et al. 1979). Submaximal effort tourniquet test at the left forearm (ischemic pain) reduced sensitivity to pressure pain at the right thigh in healthy controls, but not in FM patients (Kosek and Hansson, 1997). Furthermore, stimulation with painful tonic heat at the foot increased electrocutaneous pain thresholds at the forearm in healthy controls, but not in FM patients (Lautenbacher and Rollman, 1997). The results of both studies are in accordance with a dysfunction of CPM in FM, which could contribute to the widespread pain in FM patients.

Supporting a central nervous system dysfunction, recent imaging studies showed enhanced transmission and/or processing of nociceptive input (Gracely et al. 2002; Jensen et al. 2009) and an inability to activate descending pain inhibitory mechanisms (Jensen et al. 2009) in FM patients. Gracely et al. (2002) used functional magnetic resonance imaging (fMRI) to investigate pain responses in FM patients and healthy controls during a pressure pain paradigm. The authors reported that FM patients rated higher levels of pain compared to healthy controls in response to the same absolute pressure stimuli and exhibited higher levels of brain activity in thirteen brain regions. In 2004 Cook and collaborators demonstrated decreased thalamic activity and augmented pain processing in response to both painful and non-painful heat stimuli in FM patients (Cook et al. 2004). Results from these two studies provide evidence for altered central pain processing in patients with FM. Jensen et al. (2009) used fMRI to demonstrate that FM patients failed to activate the rostral anterior cingulate cortex (rACC) during pressure pain stimuli, confirming previous behavioural evidence for impaired inhibition of nociceptive input in FM. Result from this study demonstrated that the homeostatic relation between afferent input of nociceptive signals and descending inhibition is out of balance in FM. The pain inhibitory network is always part of normal pain response and constitutes an essential role for regulation of nociceptive input.

### ***Dysfunction of autonomic nervous system in fibromyalgia***

The autonomic nervous system (ANS), and in particular the SNS, is an important component of the human stress response. Chronic pain is a large stress factor. The principal components of the human stress response are the locus ceruleus (located in the brainstem), the norepinephrine-sympathetic system (i.e., the sympathoadrenal system) and the HPA-axis (Chrousos and Gold, 1992). At the peripheral level the ANS consists of two branches: the SNS and the parasympathetic nervous system (PSNS). SNS and PSNS richly innervate many peripheral organs, including heart, skeletal muscles, smooth muscles of the vascular system, several endocrine glands (including the adrenal gland) and other organs (Gilbey and Spyer, 1993; Tsigos and Chrousos, 2002), which means that ANS is involved in main regulatory

systems of the involuntary functions essential to life. SNS and PSNS usually act antagonistically and when one is active the other is inactive. The endocrine response to stress consists of two components of the ANS: the adrenomedullary and the neuronal components of the SNS, which together are referred to as the sympathetic-adrenal-medullary system regulating the secretion of the stress hormones catecholamines, i.e., adrenaline (A) and noradrenaline (NA) (Petzke and Claw, 2000; Charmandari et al. 2005; Lundberg 2005). A is secreted by the medullary part of adrenal gland (situated above the kidney) and mainly activates beta-receptors leading to an increase in heart rate, constriction of cutaneous blood vessels, dilatation of muscular blood vessels, relaxation of the visceral smooth muscles and bronchodilatation. NA is the main transmitter of the sympathoneural component and primarily secreted by the nerve terminals and partially by the adrenal medulla. NA mainly activates alpha-receptors and causes the constriction of blood vessels and increases blood pressure.

Catecholamines are widely used in many investigations as stress biomarkers. A has been associated with mental or emotional stress situations. NA has been more associated with body posture, cold exposure and physical exercise (Goldstein et al. 2003; Lundberg 2005).

Cardiovascular responses are considered indicators of autonomic balance (Martinez-Lavin 2007; Thayer and Brosschot, 2005). The heart is innervated by both SNS and PSNS, which control the heart rate (HR). The Vagal nerve (a primarily parasympathetic nerve) has a tonic inhibitory control over HR in normal resting conditions. The influence of SNS leads to an increase in HR both in normal and stressful conditions. HR is not fixed but instead constantly varies over a wide range due to this balance between the SNS and PSNS (Martinez-Lavin et al. 1998; 2007; Thayer and Brosschot, 2005; Thayer and Lane, 2007). This phenomenon has been called heart rate variability (HRV) and analyzing HRV over time is a commonly used non-invasive method in ANS investigations.

Together with ANS, the other main stress system is the HPA-axis. Following stress exposure, the paraventricular nucleus (PVN) of the hypothalamus releases corticotrophin-release hormone (CRH) stimulating the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. The ACTH, in turn, activates the secretion of cortisol, another stress hormone, into the bloodstream (Charmandari et al. 2005; Chrousos and Gold, 1992; Tsigos and Crousos, 2002). The ANS and the HPA-axis itself reciprocally interact with the HPA-axis at multiple sites, at both central and peripheral levels, activating and potentiating each other. This interplay between the two stress systems is important and fundamental in coordinating the organism's stress response.

Dysregulation of the ANS and HPA-axis has been suggested in FM. Although controversial, there is evidence that the autonomic state of FM patients is characterised by increased sympathetic and decreased parasympathetic tone at baseline with concurrent sympathetic hypo reactivity to various stressors (Vaerøy et al. 1989; Martinez-Lavín et al. 1997; Keleman et al. 1998; Cohen et al. 2000; Martinez-Lavín and Hermosillo 2000; Petzke and Clauw 2000). However, normal concentrations of plasma and urinary catecholamines in FM patients have also been reported (Yunus et al.1992; Mengshoel et al.1995; Giske et al. 2008). Finally, Riva et al (2012a) found attenuated activity in both branches of the ANS in FM patients with lower adrenaline and dopamine levels (attenuated SNS activity of the adrenomedullary component) and reduced activity of the PSNS branch in terms of higher resting HR. Moreover they found decreased HPA-axis activity in FM patients with hypocortisolism (i.e., low cortisol levels) (Riva et al. 2010).

A failure to increase secretion of catecholamines (Van Denderen et al. 1992; Mengshoel et al. 1995; Giske et al. 2008) and cortisol (Van Denderen et al. 1992) in a normal way has been reported in FM patients during exercise. Analysis of HRV indicated a dysregulation of the ANS with increased sympathetic activity at baseline and lower reactivity during exercise and

stress (Cohen et al 2000), which could explain the exercise intolerance in FM. Furthermore, increased muscle sympathetic nerve activity (MSNA) in FM patients has been suggested based on reports of beneficial effects of regional sympathetic blockade (Bengtsson and Bengtsson 1988; Bäckman et al. 1988). However, Elam et al. (1992) found no statistically significant difference in basal MSNA between FM patients and healthy controls at rest, while a less pronounced MSNA (microneurography of n. peroneus) was seen in FM patients compared to controls during an isometric handgrip exercise.

Based on the previous findings, Elvín and collaborators hypothesized that decreased sympathetic activation during exercise could have contributed to their findings of a less efficient regulation of muscle blood flow during exercise leading to increased muscle ischemia and possibly peripheral sensitization in FM patients (Elvin et al., 2006). Moreover ANS dysfunction could also have a negative impact on endogenous pain modulation during exercise in FM patients (Kosek et al. 1996a; Lannersten and Kosek, 2010). FM patients report increased pain during and following isometric contractions which is in accordance with the fact that isometric contractions are a potent stimulus to provoke muscle ischemia, increase muscle pain, HR and BP also in healthy subjects (Kjaer et al. 1991; Kjaer and Secher, 1992). Kjaer et al. (1992) reported increased serum catecholamine and ACTH levels, as well as an increase in BP and HR during a two-legged sustained static knee extension corresponding to 15% maximal voluntary contraction force (MVC) in healthy subjects. However, we are not aware of any previous studies that compared the impact of the same absolute workload on ANS and HPA-axis in FM patients and healthy controls.

### ***The role of glia cells in pain regulation***

The ANS plays a key role in regulating of the immune system, specifically by modulating the magnitude of immune responses to inflammatory stimuli (Tracey 2002). Emerging epidemiological and clinical evidence indicates that dysregulated activity in the sympathetic and parasympathetic divisions of the ANS is associated with increased risk for inflammatory disease, such as rheumatoid arthritis (RA) (Toussiro et al. 1993).

An inflammatory response begins when monocytes and macrophages are activated by tissue damage or pathogens to release pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF-alpha), interleukin (IL)-1b and IL-6. A number of mechanisms suppress macrophage activation and prevent the excessive production of pro-inflammatory cytokines, including humoral mechanisms, such as local release of anti-inflammatory mediators by activated immune cells and the systemic release of glucocorticoids after activation of the HPA-axis (Marsland et al. 2007).

Stress and inflammation can evoke cytokine production in humans. It has been hypothesized that chronic stress and increased sympathetic tone as well as activation of the immune system can induce central inflammatory activity that in turn mediates increased pain sensitivity and mental fatigue (Watkins and Maier, 2005). The immune-to-brain communication pathway triggers the production of a constellation of central nervous system (CNS) mediated phenomena, which is known as the 'sickness response' (Watkins and Maier, 2005). This sickness response is created by immune-to-brain signals activating CNS glia to release glial pro-inflammatory cytokines (Watkins and Maier, 2005).

The activation of glia cells has been implicated in the development and maintenance of chronic pain (Watkins and Maier, 2005). Interactions between activated glia and neurons are of importance for the development of central sensitization and hyperalgesia (Ren and Dubner, 2008; Milligan and Watkins, 2009). Therefore, it has been hypothesized that activation of glia



could be involved in human chronic pain syndromes characterized by pain amplification, such as FM (Staud 2004; Gur and Oktayoglu, 2008).

Furthermore, elevated concentrations of substances involved in nociceptive transmission and central sensitization, such as SP, glutamate, NGF and BDNF, have been reported in the CSF of FM patients (Russell et al. 1998; Giovengo et al. 1999; Sarchielli et al. 2007). Notably, SP, glutamate and BDNF can activate glia cells through receptors localized on microglia and astrocytes (Milligan and Watkins, 2009). Following activation, glia cells release pro-inflammatory cytokines/chemokines such as TNF-alpha, IL-1b and IL-8 as well as BDNF, NGF, glutamate and SP (Sofroniew et al. 2001; Watkins and Maier, 2005; Milligan and Watkins, 2009). Thereby, the activation of glia cells can further increase pain amplification (Watkins and Maier, 2005) and could thus be implicated in the altered pain modulation in FM patients.

An alternative way to activate glia is through blood-borne, pro-inflammatory cytokines released by peripheral immune cells (Watkins and Maier, 2005) and transported across the blood-brain-barrier by a special transport mechanism (Gutierrez et al. 1993; Banks et al. 1995; Quan and Herkenham, 2002). Increased levels of pro-inflammatory cytokines (Gur et al. 2008; Bazzichi et al. 2007) and reduced concentrations of anti-inflammatory cytokines (Uceyler et al. 2006) have been reported in the blood of FM patients. Furthermore, elevated serum IL-8 concentrations were related to increased pain intensity in FM patients (Gur et al. 2002).

The central inflammatory mechanisms have been implicated in patients with inflammatory joint disease, i.e., RA, causing fatigue and possibly leading to increased pain. It is commonly accepted that TNF-alpha and IL-1b are pivotal mediators in the RA pathogenesis (Dinarello et al. 1996; Feldman et al. 2008). Furthermore, elevated CSF concentrations of pro-inflammatory cytokines, including IL-1b and IL-8, have been found in pain conditions such as osteoarthritis (Lundborg et al. 2010) and neuropathic pain (Kotani et al. 2004; Backonja et al. 2008). Kotani et al. (2004) suggested that elevated pro-inflammatory cytokine concentrations in CSF may be useful for identifying patients who are likely to develop intractable post herpetic neuralgia.

However, in animals there is evidence that IL-1b and IL-8 contribute to pain and hyperalgesia by different mechanisms (Verri et al. 2006). Peripherally administered IL-8 induced local hyperalgesia mediated by beta-adrenergic receptors (Bartfai et al. 2001; Oh et al. 2001) that could be blocked by beta-adrenergic receptor antagonists (Sachs et al. 2002) as well as by the adrenergic neurone-blocking agent guanethidine (Cunha et al. 1991) but was not influenced by local cyclooxygenase-2 (COX-2) inhibitors (Sachs et al. 2002; Cunha et al. 1991). On the contrary, administration of IL-1b in peripheral tissues as well as intrathecally stimulates COX-2 activity (Bartfai 2001; Samad et al. 2001) and the IL-1b mediated increase in pain sensitivity can be prevented by administration of COX-2 inhibitors (Cunha et al. 1991; Sachs et al. 2002; Samad et al. 2001), but not by drugs acting at beta-adrenergic receptor antagonists (Cunha et al. 1991; Sachs et al. 2002) or by guanethidine (Cunha et al. 1991). Thus differential mechanisms mediate the hyperalgesic effects of IL-1b (COX-2) and IL-8 (beta-adrenergic receptors) (Cunha et al. 2005; Verri et al. 2006).

It would be expected that RA patients with an inflammatory, COX-2 sensitive pain component, would predominantly have increased CSF IL-1b while FM patients with more sympathetically mediated pain which is COX-2 independent would exhibit the opposite pattern, i.e., increased CSF IL-8 levels. Our findings of increased CSF IL-1b levels in patients with RA compared to surgical controls and to patients with multiple sclerosis (MS) (Lampa et al. 2012) are in accordance with this. However, to our knowledge, no previous studies have

directly compared the CSF patterns of pro- and anti-inflammatory cytokines in patients with inflammatory and non-inflammatory pain conditions.

## **FIBROMYALGIA**

### ***Historical facts***

The term 'fibromyalgia' is relatively new but this condition has been described in the medical literature for centuries (Claw 2009). Sir William Gowers in 1904 described inflammatory changes in the fibrous tissues of muscles of the back and used the word 'fibrositis' (Wallace 1997). Many rheumatic disease referrals in the British army during World War II were for fibrositis. This term was soon spread to the USA. It was renamed to 'fibrositis syndrome' by Smythe's group at the University of Toronto who convincingly associated the pain with systemic symptoms, such as fatigue and sleep disorder (Smythe 1979). The current concept of fibromyalgia was established by Smythe and Moldovsky in the mid-1970s, with the new term 'fibromyalgia', reflecting increased evidence that this disorder represents a pain condition '-algia' rather than inflammation '-itis'. At the University of Illinois, Yunus and his colleagues asserted that 'fibromyalgia' better describe the syndrome (Yunus et al. 1981) and they were the first who correlated symptoms such as headache, irritable colon, numbness, paresthesias and subjective swelling with this disorder. Scientific investigations of fibromyalgia first began in the early eighties. At that time fibromyalgia was variously proposed as a psychiatric disorder ('psychological rheumatism'), a muscle disorder, a sleep disorder or as a generally hyperirritable state.

The reproducible alterations in sleep electro encephalography (EEG) patterns during the non-REM phase and other abnormalities presented by Moldovsky et al. (1975) and then by Bennett (1987) promoted acceptance of the syndrome in the medical society. A committee was formed to formulate a definition of FM for epidemiological and statistical purposes. The definition was published by the American College of Rheumatology (ACR) in 1990 (Wolfe et al. 1990) and brought official recognition to fibromyalgia. The World Health Organization officially recognized the disorder in 1992 (Quintner 1992). In Sweden the diagnosis of FM was recognized in the nineties and received its own code, M79.7, in the International Statistical Classification of Diseases (ICD)-10 in the beginning of the 2000<sup>th</sup>.

### ***Classification criteria***

The classification criteria for FM proposed by the American College of Rheumatology 1990 (ACR-90) (Wolfe et al. 1990) are currently used internationally in research and clinical practice (Table 1). These criteria were originally meant only for research purposes. The use of uniform criteria has been of importance not only for pain research but also for the development of new treatment strategies.

Many voices have been raised advocating a revision of the ACR-90 criteria to make them more appropriate for clinical use. For this purpose new criteria were proposed by the ACR in 2010. The 2010 criteria (Wolfe et al. 2011) addressed a number of problems with the 1990 criteria. They eliminated the tender point count, a physical examination item, substituting the widespread pain index (WPI), a 0 - 19 count of the number of body regions reported as painful by the patient. In addition, the 2010 criteria assessed on a 0 - 3 severity scale a series of symptoms that were characteristic of FM; fatigue, non-refreshed sleep, cognitive problems, and the extent of somatic symptom reporting. The items were combined into 0 - 12 Symptom Severity (SS) score. Finally, WPI and SS scores could be combined into a 0 - 31

‘fibromyalgiansess’ scale, a second measure of polysymptomatic distress (Table 2). However, the newly proposed criteria are not yet internationally accepted for research purposes. In this theses the ACR-90 criteria have been used in all studies.

**Table 1. The 1990 American College of Rheumatology criteria for the classification fibromyalgia (Wolfe et al. 1990).**

**1. History of widespread pain**

*Definition:* Pain is considered widespread when all of the following are present: pain in the left side of the body, pain in the right side of the body, pain above the waist, and pain below the waist. In addition, axial skeletal pain (cervical spine or anterior chest or thoracic spine or low back) must be present. In this definition, shoulder and buttock pain is considered as pain for each involved side. ‘Low back’ pain is considered lower segment pain.

**2. Pain in 11 of 18 tender point sites on digital palpation**

*Definition:* Pain, on digital palpation, must be present in at least 11 of the following 18 sites:

Occiput: Bilateral, at the suboccipital muscle insertions.

Low cervical: bilateral, at the anterior aspects of the intertransverse spaces at C5 – C7.

Trapezius: bilateral, at the mid-point of the upper border.

Supraspinatus: bilateral, at origins, above the scapula spine near the medial border.

Second rib: bilateral, at the second costochondral junctions, just lateral to the junctions on upper surfaces.

Lateral epicondyle: bilateral, 2 cm distal to the epicondyles.

Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle.

Greater trochanter: bilateral, posterior to the trochanteric prominence.

Knee: bilateral, at the medial fat pad proximal to the joint line.

Digital palpation should be performed with an approximate force of 4 kg.

For a tender point to be considered ‘positive’ the subject must state that the palpation was painful. ‘Tender’ is not to be considered ‘painful’.

For classification purposes, patients will be said to have fibromyalgia if both criteria are satisfied. Widespread pain must have been present for at least 3 months. The presence of a second clinical disorder does not exclude the diagnosis of fibromyalgia.

**Table 2. Revised ACR criteria: American College of Rheumatology 2010 preliminary diagnostic criteria (Wolfe et al. 2011).**

**Criteria:**

A patient satisfies diagnostic criteria for fibromyalgia if the following three conditions are met: 1) Widespread Pain Index  $\geq 7$  and Symptom Severity Score  $\geq 5$  or Widespread Pain Index between 3 - 6 and Symptom Severity Score  $\geq 9$ .

2) Symptoms have been present at a similar level for at least 3 months

3) The patient does not have a disorder that would otherwise explain the pain

**Ascertainment:**

1) Widespread Pain Index (WPI): Note the number of areas in which the patient has had pain over the last week. In how many areas has the patient had pain? Score will be between 0 and 19:

Shoulder girdle, Lt.	Hip (buttock, trochanter), Lt.	Jaw, Lt.	Upper back
Shoulder girdle, Rt.	Hip (buttock, trochanter), Rt.	Jaw, Rt.	Lower back
Upper arm, Lt.	Upper leg, Lt.	Chest	Neck
Upper arm, Rt.	Upper leg, Rt.	Abdomen	
Lower arm, Lt.	Lower leg, Lt.		
Lower arm, Rt.	Lower leg, Rt.		

2) **Symptom Severity Score:** Fatigue; Waking unrefreshed; Cognitive symptoms;

For the each of the three symptoms above, indicate the level of severity over the past week using the following scale: 0 = No problem; 1 = Slight or mild problems: generally mild or intermittent; 2 = Moderate: considerable problems, often present and/or at a moderate level; 3 = Severe: pervasive, continuous, life-disturbing problems.

The Symptom Severity Score is the sum of the severity of the 3 symptoms (fatigue, waking unrefreshed, and cognitive symptoms) plus the sum of the number of the following symptoms occurring during the previous 6 months: headaches, pain or cramps in lower abdomen, and depression (0–3). The final score is between 0 and 12.

## ***Symptoms***

FM patients suffer from chronic widespread migrating pain in the whole body with variations in intensity that often depends on physical activity, cold exposure, mental exertion, psychological stressors, lack of sleep and systemic infections. FM patients report that in addition to pain (77,8 %), weakness (73 %) and fatigue (77,3 %) constitute a serious problem to perform household work and gainful employment (White et al. 1999). They are frequently forced to perform their tasks in a slower pace and to reduce the amount of daily work (Henriksson and Burckhardt, 1996). Chronic pain, neuro-endocrine abnormalities, non-restorative sleep, deconditioning, poor coping abilities and depression can all contribute to fatigue in FM patients. The etiology of the fatigue in FM is proposed to be multifactorial. Sleep disturbance is frequent and according to Wolfe et al (1990) approximately 75% of FM patients report that they are easily awakened, easily aroused by external disturbances or negative thoughts, and feel tired in the morning. Morning stiffness is present in approximately 70% of FM patients and does not differ from descriptions of morning stiffness given by patients with rheumatoid arthritis. FM patients also frequently report a sense of swelling, most often diffusely localized to hands and feet. In more advanced cases of FM, cognitive dysfunctions such as difficulties to concentrate, memory disturbances, stress intolerance and reduced simultaneous capacity are often present (White et al. 1999).

## ***Subtypes of fibromyalgia***

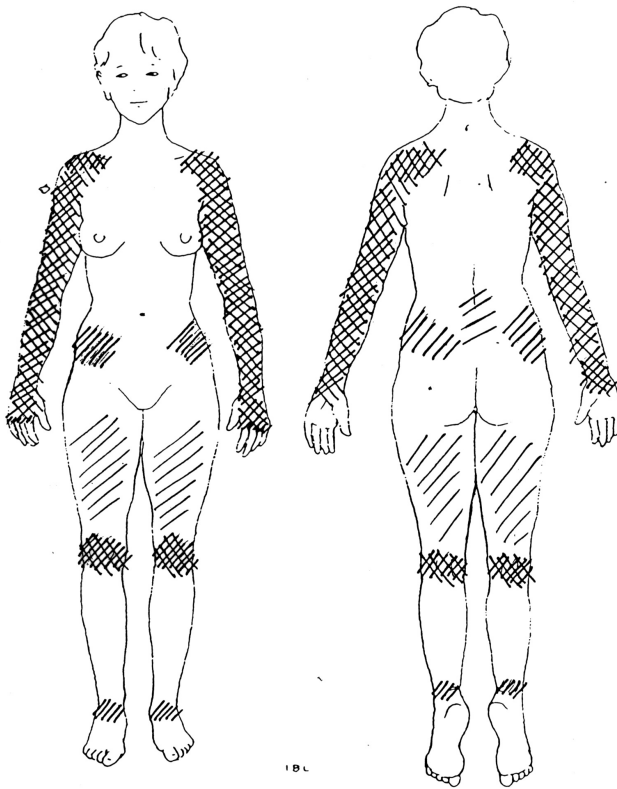
It is recognized that FM patients are a heterogeneous group and this may contribute to controversial results in different studies. Among the various suggestions Giesecke et al. (2003) suggested three different subtypes of FM based on differences in pressure-pain thresholds and psychological factors;

- 1) extreme tenderness but lack of any associated psychological or cognitive factors,
- 2) moderate tenderness and normal mood,
- 3) a group in whom mood and cognitive factors may be significantly influencing the symptom report.

Recent evidence suggests a role of functional genetic polymorphisms influencing the serotoninergetic and catecholaminergic systems for the pathogenesis of FM (Buskila et al. 2007; Ablin et al. 2006). Improved understanding of relevant genetic factors may be valuable for better subgrouping of FM patients in the future which in turn may improve the design of studies and provide a basis for more specific pharmacological treatment approach.

## ***Pain drawings***

Harold Palmer was in 1949 the first to suggest that pain drawing sketches could be used to distinguish 'functional pain' from 'organic pain' (Palmer 1949). The pain drawing sketch is a visual tool, a body contour of a human frontal and dorsal projection, in which the patient can express her or his pain by using symbols to describe the pain experience regarding the quality of pain and/or mark the affected body areas (Margolis et al. 1986). This method allows the patient to describe the pain experience in an uncomplicated way, even independent of language, and this is easily learned. The method has gained interest and has become widely used in clinical practice as an assessment tool of patient's subjective pain experience. In FM the pain drawing sketch became an important and useful method to assess if the area of widespread pain fulfilled the ACR classification criteria in individual patients. Pain drawings were used in all studies in this thesis to make sure that patients fulfilled the ACR 1990 FM criteria. A typical pain drawing is shown in fig. 1.



*Fig. 1. A “typical” pain drawing of a female FM patient.*

## **RHEUMATOID ARTHRITIS**

RA is a chronic systemic autoimmune disorder characterized by pain and inflammation of the joints (Harris 1990). Although severity of disease may fluctuate over time, there is often progressive destruction of the articular and periarticular joint structures subsequently leading to deformity and disability (Pincus 1995). The etiology of RA remains poorly understood, however it seems clear that both genetic susceptibility and environmental factors, such as smoking, play important roles in the pathogenesis of the disease.

About 1% of the world's population is afflicted by RA, women three times more often than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. RA patients often suffer not only from pain but also from chronic fatigue (Norheim et al. 2011) and report reduced quality of life.

Clinical diagnosis of RA is made on the basis of symptoms, physical exams, radiological and laboratory findings, although the ACR and the European League Against Rheumatism (EULAR) published classification criteria for the primary use in clinical trials.

Various treatments are available. Pharmacological treatments such as anti-inflammatory drugs, NSAIDs, ASA, COX1 and COX2 blockers, are used to suppress the symptoms, while disease-modifying antirheumatic drugs (DMARDs) are required to inhibit or halt the underlying immune process and prevent long-term damage. The last decades, a new group of drugs, biologics, have nearly revolutionized the treatment of RA. These drugs exert their

effects by antagonizing pro-inflammatory cytokines (i.e., TNF-alpha, IL-1b or IL-6)(Nam et al. 2010; Neurath et al. 2011).

Classification criteria for RA were presented and established in 1987 (Arnett et al. 1988)(Table 3). Because they were developed in a cohort of patients with established disease, their usefulness for the diagnosis and classification of recent-onset RA came under increasing criticism. Studies of early, undifferentiated arthritis showed poor performance of the 1987 criteria in separating patients who developed definite RA from those who had nonserious self-limiting arthritis or other forms of inflammatory joint disease (van der Helm-van Mil et al. 2008; Saraux et al. 2001). Moreover, the 1987 criteria failed to take in account a number of tests commonly used by rheumatologists for diagnostic purposes. In addition, during the last decade it has become clear that early identification and treatment of the disease is a corner stone for preventing later joint damage and loss of function, and consequently, a revision of the 1987 criteria was in order (Combe 2011). In 2010 the new 2010 ACR/EULAR classification criteria for RA were published as a result of collaboration between the two major international societies (Aletaha et al. 2010)(Table 4).

**Table 3. The 1987 revised criteria for the classification of rheumatoid arthritis (traditional format)(Arnett et al. 1988)\***

**Criterion**

1. Morning stiffness
2. Arthritis of 3 or more joint areas
3. Arthritis of hand joints
4. Symmetric arthritis
5. Rheumatoid nodules
6. Serum rheumatoid factor
7. Radiographic changes

**Definition**

1. Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
3. At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint.
4. Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry).
5. Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician.
6. Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in 4 % of normal control subjects.
7. Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

\* For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is *not* to be made.

PIP = proximal interphalangeal joint; MCP = metacarpophalangeal joint; MTP = metatarsophalangeal joint

**Table 4. 2010 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) for classifying rheumatoid arthritis (Aletaha et al. 2010).**

<b>A. Joint involvement</b>	
1 large joint	0
2–10 large joints	1
1–3 small joints (with or without involvement of large joints)	2
4–10 small joints (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	5
<b>B. Serology (at least 1 test result is needed for classification)</b>	
Negative RF and negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high-positive ACPA	3
<b>C. Acute-phase reactants (at least 1 test result is needed for classification)</b>	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
<b>D. Duration of symptoms</b>	
< 6 weeks	0
≥6weeks	1

Patients eligible for classification: at least one joint with definite clinical synovitis (swelling); no alternative diagnosis better explaining the synovitis.

Classification criteria: sum the scores for categories A through D. A score  $\geq 6/10$  classifies the patient as having definite RA. CRP = C-reactive protein, ACPA = anticitrullinated peptide antibodies.

## **AIMS OF THE THESIS**

Aberrations of cardiovascular regulation and dysfunction of endogenous pain modulation have been reported in FM patients. Dysregulation of the autonomic nervous system, with increased sympathetic activity at baseline and hypo reactivity during exercise and stress (Cohen et al. 2001; Petzke and Claw, 2000), could contribute to muscle ischemia as well as to the exercise intolerance that is typically seen in FM patients. In healthy controls, isometric contractions are potent stimuli to provoke muscle ischemia, increased muscle pain, HR and BP (Kjaer et al. 1991; Kjaer and Secher, 1992). Therefore we used isometric contractions in our first two studies to investigate the interactions between cardiovascular regulation and pain perception and to assess activation of the SNS and the HPA-axis during static exercise in FM patients. In addition to pain regulation, the ANS is also implicated in the stress response and affects the functioning of the immune system. Glia cells form a component of the immune system in the CNS and activation of glia cells has been implicated in the development and maintenance of chronic pain (Watkins and Maier, 2005). Glia cell activation has also been suggested as a possible pathophysiological mechanism in FM (Staud 2004; Gur and Oktayoglu, 2008). Immune activation in FM is supported by findings of increased levels of pro-inflammatory cytokines in the blood of FM patients (Bazzichi et al. 2007; Salemi et al. 2003). Markers of immune and glia cell activation in the CNS have, to our knowledge, never been studied in FM patients. In our two following studies we investigated pro-inflammatory cytokines in the CSF in FM patients in comparison with headache controls, and RA patients, respectively.

### ***SPECIFIC AIMS***

#### **Study 1**

The purpose of this study was to assess the interactions between cardiovascular regulation, subjective perception of pain and exertion/fatigue and the sensitivity to pressure pain in FM patients and healthy controls before, during and following standardized isometric contractions of m. quadriceps femoris.

The following questions were addressed:

- 1) Do FM patients differ from healthy controls in BP and HR regulation during and following standardized isometric contractions?
- 2) Do FM patients differ from healthy controls in ratings of exertion/fatigue and pain intensity during and following standardized isometric contractions? Are the changes in exertion/fatigue and pain intensity related to the changes in BP and HR?
- 3) Do PPTs change at the contracting muscle and at a distant site during and following standardized isometric contractions in FM patients and healthy controls, respectively?

We hypothesized that:

- FM patients will have a reduced BP and HR response to static exercise
- FM patients will have higher ratings of exercise induced pain and fatigue



- Contrary to controls, FM patients will fail to increase PPTs during contraction due to a failure to recruit endogenous pain inhibitory mechanisms

## **Study 2**

The objective of this study was to assess activation of SNS and the HPA-axis during standardized isometric contractions in FM patients and healthy controls. The same absolute contraction force was used in both groups in order to avoid the potential confounding factors related to possible differences in fitness levels.

The following questions were addressed:

- 1) Do FM patients differ from healthy controls in sympatho-adrenal activity during static muscular work?
- 2) Do FM patients differ from healthy controls in HPA-activity during static muscular work?

We hypothesized that:

- FM patients will have a relative hypo-reactivity of the sympathetic-adrenal and HPA-axis during static contractions
- FM patients will have a decreased release of noradrenaline during isometric contractions as a sign of SNS hypo-reactivity

## **Study 3**

Activation of glia cells resulting in intrathecal elevations of cytokines and chemokines has been hypothesized in chronic pain syndromes such as FM. The aim of this study was to investigate the profiles of two principally different pro-inflammatory cytokines/chemokines, IL-1b and IL-8 in the CSF of FM patients to assess potential central NS inflammation in FM.

This study addressed the following question:

Do FM patients differ from controls regarding CSF concentrations of IL-1b and IL-8?

We hypothesized that FM would have increased CSF IL-1b and IL-8 concentrations in accordance with a central inflammatory response.

## **Study 4**

The objective of this study was to compare the CSF cytokine/chemokine profiles of FM patients and patients with a COX-2 mediated inflammatory pain, i.e. RA.

This study addressed the following questions:

Do FM patients differ from RA patients regarding the CSF concentrations of pro-inflammatory cytokines/chemokines TNF-alpha, IL-1b, IL-6 and IL-8?

Do FM patients differ from RA patients regarding the CSF concentrations of the anti-inflammatory IL-1Ra, IL-4 and IL-10?

We hypothesized that:

- FM patients will have higher CSF IL-8 levels (ANS dysfunction but not a COX-2 sensitive pain condition)
- RA patients will have higher CSF IL-1b and IL-6 levels (COX-2 sensitive pain condition)
- FM patients will have higher CSF concentrations of the anti-inflammatory IL-1Ra, IL-4 and IL-10 compared to RA patients

## MATERIALS AND METHODS

### SUBJECTS

#### Patients

##### *Fibromyalgia patients*

All participating FM patients in study 1 and 2 were outpatients at the Department of Rehabilitation Medicine, Karolinska University Hospital, Stockholm, Sweden. Seventeen female FM patients, average age of 38,8 years (range 22 - 56) participated in study 1 and sixteen of them also in study 2 (average age 38,2 years, range 22 - 56 years). The participating FM patients, all females, in study 3 and 4 were recruited from the Department of Rehabilitation Medicine, Danderyds Hospital, Stockholm, Sweden. Fifteen FM patients (average age 46.2 years, range 25 - 60 years) participated in both studies. All FM patients fulfilled the ACR-1990 classification criteria for fibromyalgia (Wolfe et al. 1990). All patients had normal laboratory tests regarding erythrocyte sedimentation rate, hematology count, liver enzymes, creatinine kinase, thyroid function, rheumatoid factor and antinuclear antibodies. No medications were taken on a regular basis and no analgesics had been used on the day of assessment. None of the FM patients had other known painful conditions or diseases. The patients were recruited consecutively.

##### *Rheumatoid arthritis patients*

Fourteen female patients (average age 51.1 years, range 36 – 59 years) participated in study 4. They were outpatients at the Department of Rheumatology, Karolinska University Hospital, Stockholm, Sweden, and fulfilled the 1987 and the 2010 ACR criteria for RA (Arnett et al. 1988; Aletaha et al. 2010) and none fulfilled the ACR-1990 criteria for FM. The average number ( $\pm$  standard deviation) of swollen and painful joints was  $4.9 \pm 3.8$  and  $4.2 \pm 3.9$ , respectively. The average disease activity score for 28 joint count (DAS28) was  $3.55 \pm 1.3$ . Twelve patients (86 %) had antibodies to citrullinated peptide antigens (ACPA) and ten patients (71 %) had positive rheumatoid factor (RF). Seven patients were on methotrexate (MTX) monotherapy, two were on MTX combined with etanercept or infliximab, one was on adalimumab monotherapy, three were on sulfasalazine and one was on hydroxychloroquine. Three patients were also on low-dose prednisone (all below 7.5 mg/d). No NSAIDs or analgesics were administered within 24 hours before CSF sampling and pain and fatigue assessments.

#### Controls

##### *Healthy controls*

The healthy controls in study 1 and 2 were recruited by local advertisement at the Karolinska University Hospital, Stockholm and Karolinska Institute. Seventeen healthy, female, age matched controls (average age 37.4 years, range 22 – 53 years) participated in study 1 and sixteen of them also in study 2, (average age 38,3 years, range 22 - 53 years). Another fifteen healthy females (average age 44.4 years, range 25 – 61 years) participated in study 3 and 4. They were assessed in the same way as the FM patients except that no lumbar puncture was performed (for ethical reasons). The subjects were recruited by advertising at public places at Danderyds Hospital, Stockholm.

### *CSF controls*

Patients with non-inflammatory neurological symptoms (NINS) were used as CSF controls in study 3. CSF samples from 11 female patients (average age 41.9 years, range 32 - 60 years) that had been investigated for headache at the Department of Neurology at Karolinska University Hospital were analyzed. The routine blood tests, clinical CSF analysis and brain MR showed no signs of inflammatory disease or other pathology in this cohort. No medications were taken on a regular basis and no analgesics had been used on the day of assessment. None of the headache controls had FM symptoms.

All four studies were approved by the local ethical committee and all subjects gave their informed consent to participate. The studies followed the guidelines according to the Declaration of Helsinki.

## **MATERIAL**

### *Pressure pain thresholds*

PPTs were assessed using pressure algometry. The pressure algometer (Somedic Sales AB, Hörby, Sweden) used in the studies had a probe area of 1cm<sup>2</sup>. The rate of pressure increase was kept to approximately 40 - 60 kPa/s in study 1 and 2, and approximately 30 kPa/s in study 3. The reason we choose a higher rate of pressure increase in study 1 and 2 was that endogenous pain modulation during static muscle contractions was assessed and we wanted to avoid long durations of pressure stimulation in case of high PPTs during contractions.

### *Assessment of muscle strength*

Maximal isometric strength was assessed using a dynamometer (0 - 500 N)(PIAB test system, Mecmesin Ltd, West Sussex, UK) in study 1 and 2.

### *Assessment of blood pressure and heart rate*

BP and HR was assessed by an automatic digital blood pressure monitor UA-767 (DBPM)(A and D Instruments Ltd., Oxford, UK) in study 1 and 2.

## **LABORATORY ANALYSIS (study 2)**

### *Plasma catecholamines (adrenaline and noradrenaline).*

Samples were collected in a 5 ml Na-heparine tube and immediately placed on ice. The samples were centrifugated for 10 min at 2000g/+10° C. The plasma was pipetted into a collection tube (5 ml polypropylene) and frozen at -70° C. The samples were analyzed by high performance liquid chromatography using 1,2-difenyletylendiamin as pre-column fluorescence derivatization reagent.

### *Plasma adrenocorticotropin hormone (P-ACTH)*

Samples were collected in a 5 ml EDTA tube (siliconized). The samples were centrifuged for 10 min at 2000g/+10° C. The plasma was pipetted into a collection tubes (5 ml polypropylene) and frozen at -20° C. The analysis was performed by chemoluminescence (Advantage Instrument, Nichols, Masison, NJ, USA).

### *Serum cortisol, high sensitive C-reactive protein (CRP), creatine kinase (CK) and myoglobin*

Samples were collected in a 5 ml serum tube and centrifugated for ten minutes at 2000g/+22° C. The analysis of s-cortisol was performed by immunoflourescence (AutoDelfia, Wallac, Turku, Finland). High sensitive S-CRP was analysed by latex-enhanced immunonephelometry (Behring Nephelometer II, Dade Behring, Deerfield, IL, USA). S-CK was analysed using enzymekinetik LX instrument (Beckman Coulter, Brea, CA, USA). S-Myoglobin was analysed by electrochemoluminescence (Elecys 2010, Roche, Basel, Schweiz).

### ***LABORATORY ANALYSIS (study 3 and 4)***

CSF was sampled in polypropylene tubes. CSF samples were immediately centrifuged, supernatants frozen and stored in -80°C until use. Cytokine/chemokine concentrations in serum and CSF were analyzed with Enzyme-Linked ImmunoSorbent Assay (ELISA) (R&D, high sensitivity Quantikine). Sensitivity, expressed as the minimum detectable dose (MDD), for the ELISA kits were as follows: IL-1beta 0.057 pg/ml; IL-1ra 6.26 pg/ml; IL-4 0.11 pg/ml; IL-5 0.29 pg/ml; IL-6 0.039 pg/ml; IL-8 3.5 pg/ml; IL-10 0.09 pg/ml; TNF-alpha 0.106 pg/ml. The CSF samples from the NINS patients were taken from a repository and analyzed in the same way.

### ***PAIN RATINGS AND QUESTIONNAIRES***

Pain drawings on a figurine were used to assess the localization of pain in all studies.

Borg category ratio - 10 (CR-10) scale (Borg 1982) was used to rate exertion/fatigue and pain intensity before, during and following muscle contractions in study 1 and 2. The scale is anchored with the numbers 0 (nothing at all) through 10 (extremely strong) and a dot symbolizing maximal. There is no upper limit to the scale and participants can use fractions to describe level of sensation. Visual analogue scale (VAS) was used for pain ratings in study 3 and 4. The scale was 100 mm and anchored by the words “no pain” and “worst imaginable pain”.

Fibromyalgia Impact Questionnaire (FIQ) (Bennett 2005) was used to rate the impact of FM (study 3). Hospital Depression and Anxiety Scale (HADS), a scale developed for ratings of depression and anxiety in patients suffering from somatic diseases (as opposed to psychiatric patients) (Bjellanda et al. 2002), was used in study 3. Multidimensional Fatigue Inventory (MFI-20) (Lin et al., 2009) was used to rate fatigue (study 3 and 4). Pittsburg Sleep Quality Inventory (PSQI) (Buysse et al., 1989) was used to assess sleep disturbances and quality of sleep (study 3 and 4). Short Form -36 (SF-36) (Contopoulos-Ioannidis et al. 2009) was used to assess health related quality of life (study 3 and 4).

## PROCEDURE

### Study 1

The subjects were comfortably seated in a chair and familiarized with the equipment and the procedure. They were asked to rate exertion/fatigue and pain intensity using the Borg scale. Baseline BP and HR were determined. The sites for pressure algometry assessments were identified and marked (m. quadriceps femoris dx; midway between the groin and the apex of the patella and m. deltoideus dx; lateral part 10 cm below acromion). The algometer was demonstrated and the subjects were instructed to push the button exactly at the moment when the pressure sensation became painful. Two assessments were performed in sites not included in the study to ensure adequate subject performance. PPTs were determined three times at each site with a minimum of 30 s interstimulus interval. The average value was used as the baseline PPT for each site. The maximum isometric knee extensor strength at 90 degrees of knee flexion was determined during a 15 s contraction, three times, with one minute of rest in-between trials. The best value noted on the dynamometer was used to calculate % MVC during the following isometric contraction period. The subjects rested for 10 min following the MVC assessments.

The subjects were then instructed to perform a standardized isometric contraction (39.2 N) with m. quadriceps femoris dx. The contraction was performed in a sitting position, with the hip and knee held in approximately 90° flexion pushing against a resistance ( $4 \text{ kg} \times 9.81 \text{ m/s}^2 = 39.2 \text{ N}$ ) attached horizontally to the right ankle. The contraction was held until exhaustion or for a maximum of 15 min. During the contraction period PPTs were assessed every 30 s alternating between the contracting m. quadriceps femoris and the resting m. deltoideus. BP, HR and ratings of exertion/fatigue and pain intensity were registered following 2 min of contraction and at exhaustion (end of contraction). Immediately following contraction, i.e., at relaxation, and at 5, 10 and 15 min following contraction BP, HR, PPTs (at both sites) as well as ratings of exertion/fatigue and pain intensity were reassessed.

### Study 2

The tests were performed at standardized times in the morning (9 patients and 9 controls) and in the early afternoon (7 patients and 7 controls) in a quiet laboratory room. An intravenous cannula (Venflon) was inserted into the left antecubital vein. The subjects were then comfortably seated in a chair and familiarized with the equipment and the procedure. Following 10 min of rest they were asked to rate global pain intensity on the Borg CR-10 scale. The sites for pressure algometry assessments were identified and marked (m. quadriceps femoris dx; midway between the groin and the apex of the patella and m. deltoideus dx; lateral part 10 cm below acromion). The algometer was demonstrated and the subjects were instructed to push the button exactly at the moment when the pressure sensation became painful. A minimum of two assessments were performed in sites not included in the study to ensure adequate subject performance. PPTs were always determined twice at each site. The average value was calculated and used for further analysis (as the actual PPT). Following additional 20 min of rest the first blood sample was drawn (plasma (p)-A, p-NA, p-ACTH, p-cortisol, p-myoglobin, p-CK, p-high sensitivity CRP, followed by determination of BP, HR and ratings of exertion/fatigue and pain at the thighs (baseline values).

The subjects were then instructed to perform a standardized isometric contraction with m. quadriceps femoris bilaterally. The contraction was performed in a sitting position, with the

hips and knees held in approximately 90° flexion pushing against a resistance (8 kg x 9.81 m/s<sup>2</sup> = 78.5 N) attached horizontally to the ankles. The contraction was held until exhaustion or for a maximum of 20 min. Following 2½ min of contraction a second blood sample was drawn (p-A, p-NA, p-ACTH, p-cortisol), followed by determination of BP, HR, ratings of exertion/fatigue and pain at the thighs and assessment of PPTs. A third blood sample was drawn at exhaustion (during contraction)(p-A, p-NA, p-ACTH, p-cortisol), followed by determination of BP, HR, ratings of exertion/fatigue and pain at the thighs. After 30 minutes of rest in the sitting position the fourth blood sample was drawn (p-cortisol, p-myoglobin, p-CK) followed by determination of BP, HR, PPTs and ratings of exertion/fatigue and pain. Finally, the maximal isometric knee extensor strength (MVC) at 90° of knee flexion was determined during a 15 s contraction, three times, with one minute of rest in-between trials. The best value noted on the dynamometer was used to calculate % MVC during the isometric contraction period. We refrained from assessing maximal strength at the beginning of the session in order to avoid interference with the baseline laboratory analysis.

### **Study 3**

On the first day FM patients and healthy controls completed all questionnaires and pressure algometry was performed. The algometer was demonstrated and the subjects were instructed to push the button exactly at the moment when the pressure sensation became painful. A minimum of two assessments were performed in sites not included in the study to ensure adequate subject performance. PPTs were assessed bilaterally once at 4 different “tender points” according to the FM ACR-1990 criteria (supraspinatus, elbows, gluteus and knees) and the average was calculated as the individual PPT and used for further analysis. The subjects returned the following morning for venous and lumbar (FM patients only) puncture. The lumbar punctures from CSF controls (NINS patients) were taken for diagnostic purposes. However these patients had given their permission for these samples to also be used for research. No corresponding serum samples were available for analysis in this group.

### **Study 4**

On the first day RA and FM patients and healthy controls completed all questionnaires. The subjects returned the following morning for venous and lumbar (patients only) puncture. The concentrations of TNF-alpha, IL-1b, IL-1Ra, IL-4, IL-6, IL-8 and IL-10 were assessed in CSF and serum levels of TNF-alpha, IL-1b, IL-6 and IL-8 were analyzed.

## STATISTICS

### Study 1

Group differences in contraction force and duration of contraction were analyzed by Student's t-test. BP, HR and PPTs were analyzed by a two-way ANOVA with repeated measures on one factor. The between factor was GROUP with two levels (patient and control) and the within factor was TIME with seven (BP, HR) or ten levels (PPTs). As the correlation between each pair of repeated measures was not the same (test of sphericity), an ANOVA model with a completely general covariance structure was applied, instead of the common structure with constant variance and constant covariance. In case of a significant interaction, simple effects were examined, i.e., effects of one factor holding the other factor fixed. Some of the variables were transformed to a log scale because of their skewed distributions. Differences between groups in the ratings of exertion/fatigue, pain and of baroreceptor control of HR were analyzed by Mann Whitney U-test with corrections for ties. The Friedman's test was used for within group comparisons over time, with post hoc analysis using the Wilcoxon's signed rank test. Correlations between ratings of exertion/fatigue, ratings of pain intensity and cardiovascular parameters were analyzed by Spearman rank order correlation coefficient.

### Study 2

Group differences in contraction force and duration of contraction were analyzed by Student's t-test. BP, HR, PPTs and plasma levels of A, NA, ACTH and cortisol were analyzed by a two-way repeated measures of variance ANOVA. The within-subjects variable was TIME with three or four levels (before contraction, during contraction (2½ min. and at exhaustion) and following contraction) and the between-subjects variable was GROUP with two levels (patient and control). Differences between levels of the within factor TIME were evaluated by the Fisher LSD test if the sphericity condition was tenable, otherwise specific contrasts were applied. When more than three pairwise comparisons were performed, the p-values were corrected according to the Bonferroni procedure. When the two-factor interaction was significant, simple main effects were examined, i.e., effects of one factor holding the other factor fixed. For example, test of group effect (fibromyalgia versus controls) at different time points (before, during (2½ min. and at exhaustion) and following contraction). The degrees of freedom of the F-tests associated with the TIME factor were corrected according to Greenhouse-Geisser if the sphericity condition did not hold. Since the distribution of some of the variables were positively skewed, appropriate power transformations were carried out before analyses ( $\log X$  and  $-1/X$ ). Between group differences in CRP, CK and myoglobin were analyzed by Mann-Whitney U test with correction for ties and within group differences by Wilcoxon's signed rank test. Differences between groups in ratings of pain and exertion/fatigue were analyzed by Mann-Whitney U-test with corrections for ties. The Friedman's test was used for within group comparisons of ratings over time, with post hoc analysis using the Wilcoxon's signed rank test. For the omnibus tests,  $p < 0.05$  was considered statistically significant, whereas the Bonferroni method was applied for post hoc contrasts, i.e.  $0.05$  divided by the number of contrasts performed. Software used: Statistica 7.1, StatSoft<sup>®</sup>, Inc. Tulsa OK, USA.



### **Study 3**

Group differences were analyzed by Mann-Whitney U-test. Differences between IL-1b and IL-8 concentrations between serum and CSF in FM patients were analyzed by Wilcoxon's Signed Ranks Test. Correlations were analyzed by Spearman's correlation coefficient.  $P < 0.05$  was considered as a statistically significant difference. Means and standard deviations are presented in the text.

### **Study 4**

Overall group differences were analyzed by Kruskal-Wallis Test and post hoc group differences were assessed by Independent Samples Mann-Whitney U-test. Wilcoxon's Signed Rank Test was used for paired analysis. Correlations were analyzed by Spearman's correlation coefficient.  $P < 0.05$  was considered as a statistically significant difference. Means and standard deviations are presented in the text.

## RESULTS

### Study 1

#### *Maximal voluntary contraction force and contraction time*

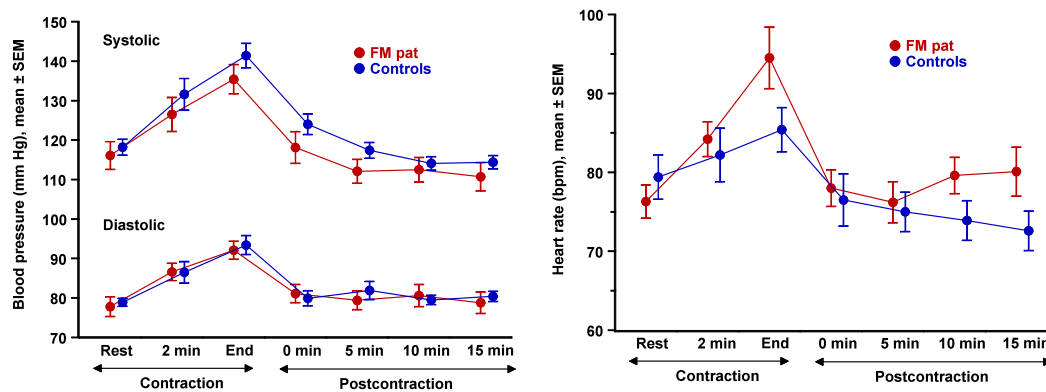
The mean maximal voluntary contraction force (MVC) was 99.7 Nm in patients and 127.7 Nm in controls ( $p < 0.001$ ). On average, the contraction corresponded to 15% MVC in patients and 10% MVC in controls ( $p < 0.02$ ). The average contraction time to exhaustion was 8 min in patients and 10 min in controls ( $p < 0.001$ ). Only three controls and none of the FM patients performed the contraction for 15 min.

#### *Blood pressure and heart rate (Fig. 2)*

There was no statistically significant difference between groups in systolic ( $p < 0.24$ ,  $dF = 1$ ,  $F$  value = 1.41) or diastolic BP ( $p < 0.86$ ,  $dF = 1$ ,  $F$  value = 0.03). The change over time was statistically significant for systolic ( $p < 0.0001$ ,  $dF = 6$ ,  $F$  value = 23.71) and diastolic ( $p < 0.0001$ ,  $dF = 6$ ,  $F$  value = 20.05) BP, but no statistically significant GROUP/TIME interaction was seen for systolic ( $p = 0.35$ ,  $dF = 6$ ,  $F$  value = 1.17) or diastolic BP ( $p = 0.24$ ,  $dF = 6$ ,  $F$  value = 1.43). In both groups alike, systolic and diastolic BP increased compared to baseline at 2 min of contraction ( $p < 0.001$ ), continued to increase during the contraction period (difference between 2 min and exhaustion)( $p < 0.001$ ), decreased immediately following contraction (difference between exhaustion and relaxation)( $p < 0.001$ ) and a further slight decrease was seen during the post contraction period for the systolic (difference between relaxation and 15 min)( $p < 0.001$ ) but not diastolic blood pressure.

There was no statistically significant difference in HR between groups ( $p = 0.28$ ,  $dF = 1$ ,  $F$  value = 1.21). HR changed over time ( $p < 0.001$ ,  $dF = 6$ ,  $F$  value = 11.19) and the GROUP/TIME interaction was significant ( $p = 0.0081$ ,  $dF = 6$ ,  $F$  value = 3.57). Compared to baseline, HR increased in patients at 2 min of contraction ( $p < 0.001$ ), continued to increase during the contraction period (difference between 2 min and exhaustion)( $p < 0.004$ ), decreased immediately following contraction (difference between exhaustion and relaxation)( $p < 0.001$ ) and remained stable during the 15 min post contraction assessments. In controls, no statistically significant changes from baseline were observed during the contraction period, HR decreased immediately following contraction (difference between exhaustion and relaxation)( $p < 0.03$ ) and remained stable during the 15 min of post contraction assessments. There was a statistically significant difference in HR increase from baseline between FM patients and healthy controls at exhaustion ( $p < 0.02$ ), but not at 2 min of contraction.

The global index of the baroreflex control of HR was calculated as the ratio of the change in HR to a change in systolic blood pressure (HR during – baseline / SBP during - baseline), with low values indicating a high degree of baroreflex control (Raven et al. 2000). The average baroreceptor control corresponded to 1.6 in FM patients and 0.7 in healthy controls ( $p < 0.007$ ) at two minutes of contraction and 1.1 in FM patients and 0.4 in controls at exhaustion ( $p < 0.003$ ).



**Fig. 2** BP and HR (mean  $\pm$  SEM) in 17 FM patients (red) and 17 healthy controls (blue) before, during and following an isometric contraction of the right m.quadriceps femoris until exhaustion. In both groups alike, the systolic and diastolic BP increased at 2 min of contraction (rest to 2 min) ( $p < 0.001$ ), continued to increase during the contraction period (2 min to end) ( $p < 0.001$ ) and decreased immediately following contraction (end to 0 min) ( $p < 0.001$ ). A further slight decrease was seen during the postcontraction period (0 to 15 min) for the systolic ( $p < 0.001$ ) but not diastolic blood pressure in both groups. HR increased in patients at 2 min of contraction (rest to 2 min) ( $p < 0.001$ ), continued to increase during the contraction period (2 min to end) ( $p < 0.004$ ), decreased immediately following contraction (end to 0 min) ( $p < 0.001$ ) and remained stable during the 15 min post contraction assessments. In controls, no statistically significant changes from baseline were observed during the contraction period, HR decreased immediately following contraction (end to 0 min) ( $p < 0.03$ ) and remained stable during the 15 min of post contraction assessments (0 to 15 min). There was a statistically significant difference in HR increase from baseline between FM patients and healthy controls at exhaustion ( $p < 0.02$ ), but not at 2 min of contraction.

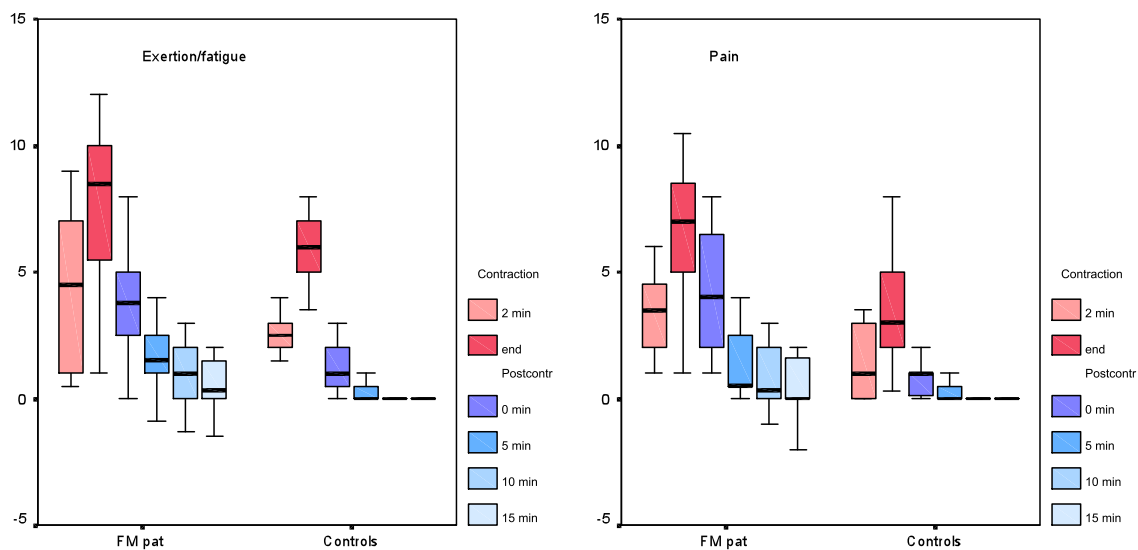
### **Ratings of exertion/fatigue and pain intensity (Fig. 3)**

FM patients had higher ratings of exertion/fatigue ( $p < 0.003$ ) and pain ( $p < 0.001$ ) at all times compared to controls. Compared to baseline, ratings of exertion/fatigue in the right thigh increased in both groups at 2 min of contraction (difference between baseline and 2 min) ( $p < 0.001$ ), continued to increase until exhaustion (difference between 2 min and exhaustion) ( $p < 0.001$ ) and decreased immediately following contraction (difference between exhaustion and relaxation) ( $p < 0.001$ ) in both groups. Following contraction the ratings of exertion/fatigue remained elevated compared to baseline in FM patients and controls, respectively, at relaxation ( $p < 0.001$ ;  $p < 0.001$ ), 5 min ( $p < 0.001$ ;  $p < 0.01$ ) and in FM patients also at 10 min ( $p < 0.03$ ). The ratings of exertion/fatigue had returned to baseline 10 min following contraction in controls and 15 min following contraction in FM patients. There was no difference in the increase from baseline of ratings of exertion/fatigue between FM patients and controls at 2 min of contraction. However, the increase from baseline in ratings of exertion/fatigue was more pronounced in FM patients compared to controls at exhaustion ( $p < 0.05$ ) at relaxation ( $p < 0.005$ ), and at 5 min ( $p < 0.005$ ) and 10 min ( $p < 0.03$ ) following contraction.

Compared to baseline, ratings of pain intensity in the right thigh increased in both groups at 2 min of contraction ( $p < 0.001$ ), continued to increase until exhaustion (difference between 2 min and exhaustion) ( $p < 0.001$ ) and decreased immediately following contraction (difference between exhaustion and relaxation) ( $p < 0.001$ ), although at relaxation they were still elevated compared to baseline ( $p < 0.001$ ). The ratings of pain intensity returned to baseline at 5 min following contraction in controls, but remained elevated compared to baseline throughout the observation period in FM patients (5 min;  $p < 0.001$ ; 10 min;  $p < 0.01$ ; 15 min;  $p < 0.05$ ). The increase in pain ratings compared to baseline was more pronounced in FM patients than in controls during (2 min;  $p < 0.005$ ; exhaustion;  $p < 0.001$ ) and following (relaxation;  $p <$

0.001: 5 min;  $p < 0.005$ : 10 min;  $p < 0.05$ ) contraction. No statistically significant difference in the increase of pain ratings from baseline was seen between the groups at 15 min following contraction.

There was a positive correlation between the increase in HR and the increase in ratings of exertion/fatigue at exhaustion in healthy controls ( $k=0.57$ ;  $p < 0.02$ ). No other statistically significant correlations were seen between ratings of exertion/fatigue or pain intensity and cardiovascular parameters. A statistically significant correlation was seen between ratings of exertion/fatigue and pain intensity in both groups at 2 min of contraction (FM;  $k=0.62$ ;  $p < 0.01$ : Controls;  $k=0.79$ ;  $p < 0.001$ ) and at exhaustion in patients ( $k=0.84$ ;  $p < 0.001$ ) but not in controls ( $k=0.25$ ; NS).



**Fig. 3.** Ratings of exhaustion/fatigue and pain intensity (Borg CR-10 scale) in 17 FM patients and 17 healthy controls before, during and following isometric contraction of m.quadriceps femoris dx until exhaustion. Compared to controls, FM patients had higher ratings of exhaustion/fatigue ( $p < 0.003$ ) at all times. There was no statistically significant difference in the increase from baseline of ratings of exertion/fatigue between FM patients and controls at 2 min of contraction. However, ratings of exertion/fatigue had increased more from baseline in FM patients compared to controls at exhaustion (end) ( $p < 0.05$ ), at relaxation (0 min) ( $p < 0.005$ ), at 5 min ( $p < 0.005$ ) and at 10 min ( $p < 0.03$ ) following contraction. No statistically significant difference in the increase of ratings of exertion/fatigue from baseline was seen between groups at 15 min following contraction. Compared to controls, FM had higher ratings of pain ( $p < 0.001$ ) at all times. The increase in pain ratings from baseline was more pronounced in FM patients compared to controls at 2 min ( $p < 0.005$ ), at exhaustion (end) ( $p < 0.001$ ), at relaxation (0 min) ( $p < 0.001$ ), and at 5 min ( $p < 0.005$ ) and 10 min ( $p < 0.05$ ) following contraction. No statistically significant difference in the increase of pain ratings from baseline was seen between the groups at 15 min following contraction.

### ***Pressure pain thresholds***

#### ***M. Quadriceps femoris***

There was a statistically significant difference in PPTs between groups ( $p < 0.0001$ ,  $dF = 1$ ,  $F$  value = 37.04), but no statistically significant effect over time ( $p = 0.30$ ,  $dF = 9$ ,  $F$  value = 1.26) nor a significant interaction between GROUP/TIME ( $p = 0.97$ ,  $dF = 9$ ,  $F$  value = 0.30). PPTs were lower in FM patients compared to controls at all times ( $p < 0.001$ ) at m. quadriceps femoris. (Data not shown).

### *M. Deltoideus*

There was a statistically significant difference in PPTs between groups ( $p < 0.0001$ ,  $dF = 1$ ,  $F$  value = 22.11), a statistically significant change over time ( $p = 0.0001$ ,  $dF = 9$ ,  $F$  value = 5.57), but no statistically significant interaction between factors GROUP/TIME was seen ( $p = 0.47$ ,  $dF = 9$ ,  $F$  value = 0.98). PPTs were lower in FM patients compared to controls ( $p < 0.001$ ) at all times. Compared to baseline, PPTs increased at 5 min of contraction in both groups alike ( $p < 0.001$ ) and remained at this level during the rest of the contraction period and during the 15 min post-contraction assessments. (Data not shown).

### *Comparison of subgroups matched according to % MVC*

Ten FM patients were matched with 10 controls performing contractions with the same ( $\pm 1$ ) % MVC (FM patients 10.9% MVC, controls 10.6% MVC, NS). The duration of contraction was 8.7 min in patients and 9.7 min in controls (NS). There were no statistically significant group differences in SBP, DBP nor HR at 2 min of contraction or at exhaustion (data not shown). There were no statistically significant group differences in the increase from baseline in ratings of exertion/fatigue at 2 min or at exhaustion. Ratings of pain intensity had increased more from baseline in the patient group compared to controls at 2 min ( $p < 0.03$ ) and at exhaustion ( $p < 0.05$ ).

## **Study 2**

### ***Maximal voluntary contraction force and contraction time***

The mean MVC corresponded to 240.2 Nm in patients and 345.4 Nm in controls ( $p < 0.002$ ). On average, the contraction corresponded to 11 % MVC in patients and 8 % MVC in controls ( $p < 0.01$ ). The average contraction time to exhaustion was 12 min in patients and 16 min in controls ( $p < 0.001$ ). Only two controls and one patient performed the contraction for 20 min.

### ***Ratings of exertion/fatigue and pain intensity***

FM patients rated higher global pain intensity (median: 3, range 0 - 6.2) compared to controls who were all pain free ( $p < 0.001$ ). FM patients also had higher ratings of exertion/fatigue ( $p < 0.001$ ) and pain in the thighs ( $p < 0.001$ ) at all times compared to controls.

Compared to baseline, ratings of exertion/fatigue in the thighs increased in both groups at 2½ min of contraction (difference between baseline and 2½ min)( $p < 0.001$ ) and continued to increase until exhaustion (difference between 2½ min and exhaustion)( $p < 0.001$ ) in both groups. Following contraction (30 min) the ratings of exertion/fatigue remained elevated compared to baseline in FM patients ( $p < 0.003$ ), but had returned to baseline in controls. The increase from baseline in ratings of exertion/fatigue was more pronounced in FM patients at all times compared to controls (2½ min;  $p < 0.001$ , exhaustion;  $p < 0.007$  and following contraction  $p < 0.001$ ). The median ratings of exertion/fatigue at baseline, during contraction (2½ and exhaustion) and 30 min following contraction were; 1.5, 7.5, 10.0 and 2.75 in FM patients and 0, 2.8, 5.0 and 0 in controls.

Compared to baseline, ratings of pain intensity in the thighs increased in both groups at 2½ min of contraction ( $p < 0.001$ ) and continued to increase until exhaustion (difference between 2½ min and exhaustion)( $p < 0.001$ ). The ratings of pain intensity remained elevated in FM patients at 30 min following contraction ( $p < 0.001$ ), but had returned to baseline in controls (no controls reported any pain at this time). The increase from baseline in ratings of pain intensity was more pronounced in FM patients at all times compared to controls ( $p < 0.001$ ). The median ratings of pain at baseline, during contraction (2½ and exhaustion) and 30 min

following contraction were; 1.5, 6.0, 7.0, and 2.0 in FM patients and 0, 1.5, 3.0 and 0 in controls.

### ***Blood pressure and heart rate***

There was no statistically significant difference between groups in systolic ( $p < 0.77$ ,  $dF = 1$ ,  $F$  value = 0.09) or diastolic ( $p < 0.74$ ,  $dF = 1$ ,  $F$  value = 0.11) BP. The change over time was statistically significant for systolic ( $p < 0.0001$ ,  $dF = 3$ ,  $F$  value = 70.77) and diastolic ( $p < 0.0001$ ,  $dF = 3$ ,  $F$  value = 74.96) BP, but no statistically significant group/time interaction was seen for systolic ( $p < 0.99$ ,  $dF = 3$ ,  $F$  value = 0.03) or diastolic ( $p < 0.63$ ,  $dF = 3$ ,  $F$  value = 0.63) BP. In both groups alike, systolic and diastolic BP increased compared to baseline at 2½ min of contraction ( $p < 0.001$ ), continued to increase during the contraction period (difference between 2½ min and exhaustion)( $p < 0.001$ ) and had returned to baseline 30 min following contraction. (Data not shown).

There was no statistically significant difference in HR between groups ( $p < 0.34$ ,  $dF = 1$ ,  $F$  value = 0.94). HR increased over time ( $p < 0.0001$ ,  $dF = 3$ ,  $F$  value = 65.13) but there was no statistically significant group/time interaction ( $p < 0.30$ ,  $dF = 3$ ,  $F$  value = 1.23). In both groups alike, HR increased compared to baseline at 2½ min of contraction ( $p < 0.001$ ), continued to increase during the contraction period (difference between 2½ min and exhaustion)( $p < 0.001$ ) and had returned to baseline 30 min after contraction. (Data not shown).

### ***Pressure pain thresholds (PPTs)***

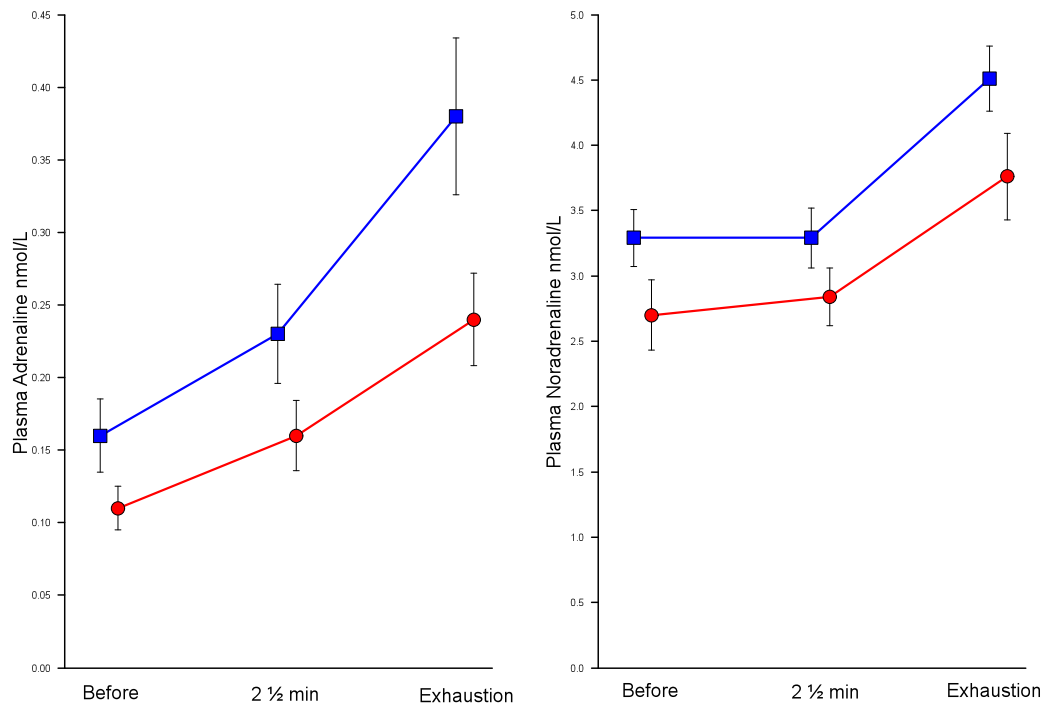
PPTs were lower in FM patients compared to controls at m. quadriceps dx. ( $p < 0.0001$ ,  $dF = 1$ ,  $F$  value = 41.36) and at m. deltoideus dx. ( $p < 0.0001$ ,  $dF = 1$ ,  $F$  value = 42.59). There was no statistically significant time effect at m. quadriceps ( $p < 0.19$ ,  $dF = 2$ ,  $F$  value = 1.72) nor at m. deltoideus ( $p < 0.07$ ,  $dF = 2$ ,  $F$  value = 2.83), nor a significant interaction between group/time (quadriceps:  $p < 0.78$ ,  $dF = 2$ ,  $F$  value = 0.25, deltoideus:  $p < 0.62$ ,  $dF = 2$ ,  $F$  value = 0.48). (Data not shown).

### ***Plasma catecholamine levels (Fig. 4)***

FM patients had reduced levels of p-A at all times compared to controls ( $p < 0.04$ ,  $dF = 1$ ,  $F$  value = 4.40). P-A increased over time ( $p < 0.0001$ ,  $dF = 2$ ,  $F$  value = 33.70), but there was no statistically significant time/group interaction ( $p < 0.88$ ,  $dF = 2$ ,  $F$  value = 0.13). P-A increased in both groups alike at 2½ min compared to baseline ( $p < 0.002$ ) and continued to increase until exhaustion (difference between 2½ min and exhaustion) ( $p < 0.001$ ). The average increase from baseline in A ( $\pm 95\%$  confidence interval (CI)) was  $0.050 \pm 0.038$  nmol/l in FM patients and  $0.064 \pm 0.042$  nmol/l in controls at 2½ min and  $0.129 \pm 0.054$  nmol/l in FM patients and  $0.211 \pm 0.088$  nmol/l in controls at exhaustion. The average increase from 2½ min. to exhaustion was  $0.079 \pm 0.057$  nmol/l in FM patients and  $0.147 \pm 0.074$  nmol/l in controls.

There was a non-significant tendency of lower p-NA levels in FM patients compared to controls ( $p < 0.08$ ,  $dF = 1$ ,  $F$  value = 3.25). P-NA increased over time ( $p < 0.0001$ ,  $dF = 2$ ,  $F$  value = 53.95), but there was no significant time/group interaction ( $p < 0.46$ ,  $dF = 2$ ,  $F$  value = 0.78). There was no statistically significant difference between p-NA at 2½ min compared to baseline, but p-NA increased at exhaustion in both groups alike compared to baseline ( $p < 0.001$ ) and compared to 2½ min ( $p < 0.001$ ).

The average increase ( $\pm$  95% CI) in NA from baseline to exhaustion was  $1.034 \pm 0.396$  nmol/l in FM patients and  $1.212 \pm 0.302$  nmol/l in controls and the average increase from 2½ min. to exhaustion was  $0.919 \pm 0.345$  nmol/l in FM patients and  $1.225 \pm 0.406$  in controls.



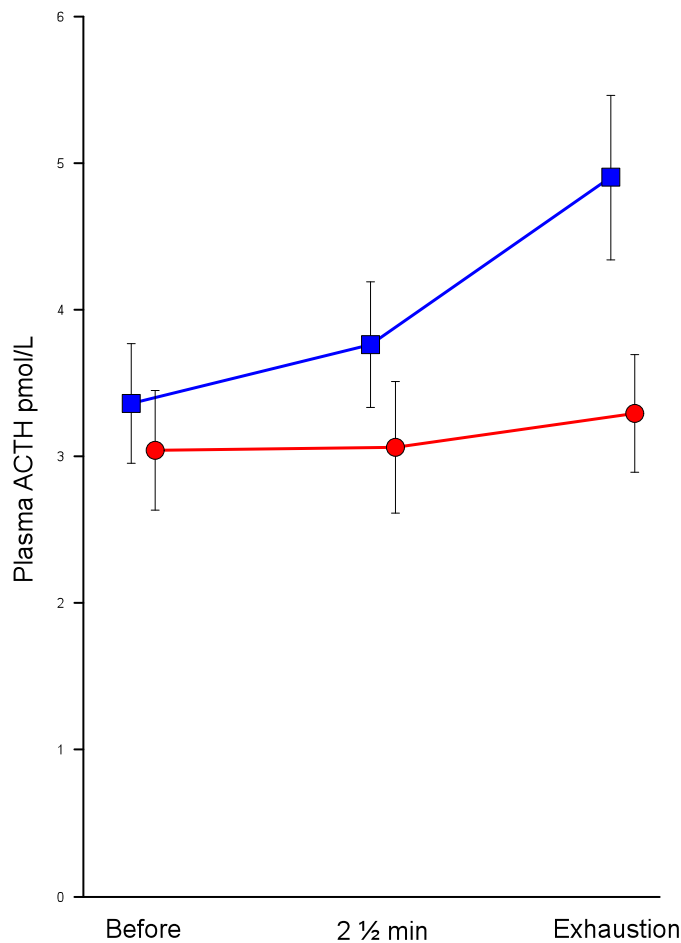
**Fig 4.** Plasma adrenaline and noradrenaline concentrations in 16 FM patients and 16 healthy controls before and during (at 2½ min. and at exhaustion) a two-legged isometric knee extension until exhaustion. Adrenaline; FM patients had lower concentrations of p-A at all times compared to controls ( $p < 0.04$ ). Compared to baseline, p-A increased in both groups alike at 2½ min. of contraction ( $p < 0.002$ ) and continued to increase until exhaustion ( $p < 0.001$ ). Noradrenaline; There was a non-significant tendency of lower p-NA levels in FM patients compared to controls at all times ( $p < 0.08$ ). There was no statistically significant difference between p-NA at 2½ min. compared to baseline, but p-NA had increased at exhaustion in both groups alike compared to baseline ( $p < 0.001$ ) and compared to 2½ min. ( $p < 0.001$ ).

### ***Plasma adrenocorticotrophic hormone and cortisol***

The ACTH samples of two patients were lost due to technical failure. Analysis of ACTH were therefore based on 14 FM patients and 16 controls. No statistically significant group difference was found in the concentration of p-ACTH ( $p < 0.18$ ,  $dF = 1$ ,  $F$  value = 1.90). P-ACTH increased during contraction ( $p < 0.0001$ ,  $dF = 2$ ,  $F$  value = 19.31) and a significant group/time interaction was found ( $p < 0.002$ ,  $dF = 2$ ,  $F$  value = 7.12). In healthy controls, there was a tendency to an increase in p-ACTH at 2½ min compared to baseline ( $p < 0.09$ ), and p-ACTH had increased at exhaustion compared to baseline ( $p < 0.001$ ) and compared to 2½ min ( $p < 0.001$ ), respectively. No statistically significant change in p-ACTH was found in FM patients ( $p < 0.23$ ). There was no statistically significant difference in p-ACTH between patients and controls at baseline ( $p < 0.64$ ), nor at 2½ min ( $p < 0.29$ ), but FM patients had

lower p-ACTH levels at exhaustion compared to controls ( $p < 0.02$ ) (Fig. 5). The average increase of ACTH ( $\pm 95\%$  CI) in controls was  $2.106 \pm 1.194$  pmol/L from baseline to exhaustion and  $1.138 \pm 0.621$  pmol/L from  $2\frac{1}{2}$  min. to exhaustion.

We did not find any statistically significant differences in p-cortisol between subjects assessed in the morning compared to those assessed in the afternoon. There was no statistically significant difference in p-cortisol between groups ( $p < 0.81$ ,  $dF = 1$ ,  $F$  value = 0.06) and no statistically significant time effect was found ( $p < 0.19$ ,  $dF = 3$ ,  $F$  value = 1.64) nor a significant time/group interaction ( $p < 0.92$ ,  $dF = 3$ ,  $F$  value = 0.16). (Data not shown).



**Fig. 5.** Plasma ACTH levels in 14 FM patients and 16 healthy controls before and during (at  $2\frac{1}{2}$  min. and at exhaustion) a two-legged isometric knee extension until exhaustion. In controls, there was a tendency to an increase in p-ACTH at  $2\frac{1}{2}$  min compared to baseline ( $p < 0.09$ ), and p-ACTH had increased at exhaustion compared to baseline ( $p < 0.001$ ) and compared to  $2\frac{1}{2}$  min ( $p < 0.001$ ), respectively. No statistically significant change in p-ACTH was seen in FM patients during contraction and FM patients had lower concentrations of p-ACTH at exhaustion compared to controls ( $p < 0.02$ ).



### ***Plasma high sensitivity C-reactive protein, creatine kinase and myoglobin***

The mean P-high sensitivity CRP was higher in FM patients (2.66 mg/l) compared to controls (1.16 mg/l) ( $p < 0.02$ ). There were no statistically significant group differences at baseline in average CK concentrations (patients: 0.88 microkat/l; controls: 1.30 microkat/l) or myoglobin concentrations (patients: 26.81 microg/l; controls: 29.06 microg/l). No significant changes were seen when CK values were compared before and following the contraction in patients (0.88 microkat/l and 0.87 microkat/l) or controls (1.30 microkat/l and 1.16 microkat/l). The same was found for myoglobin values before and following contraction (patients: 26.81 microg/l and 28.62 microg/l (NS); controls: 29.06 microg/l and 37.19 microg/l (NS)).

### **Study 3**

#### ***Subject characteristics (Table 5)***

FM patients had an average duration of FM corresponding to 2.9 years (range 1 – 10 years) and an average duration of pain corresponding to 10.3 years (range 2 – 30 years). They rated ongoing pain intensity as 65.8 mm (range 42 – 87 mm), while all healthy controls were pain free ( $p < 0.001$ ). PPTs were lower in FM patients compared to healthy controls ( $p < 0.001$ ). Compared to healthy controls, FM patients had higher ratings of fatigue ( $p < 0.001$ ), sleep disturbance ( $p < 0.001$ ), depression ( $p < 0.001$ ) and anxiety ( $p < 0.001$ ) and lower ratings of quality of life ( $p < 0.001$ ).

#### ***Serum concentrations of cytokines/chemokines (Table 6)***

FM patients had higher average serum concentrations of IL-8 compared to healthy controls ( $p < 0.02$ ), but lower serum IL-1b ( $p < 0.007$ ), IL-5 ( $p < 0.001$ ) and TNF-alpha ( $p < 0.04$ ) concentrations. No statistically significant group differences were found regarding serum concentrations of IL-6 and IL-10. Since previous publications have found decreased TH1 to TH2 interleukin ratios in depressed patients (Kim et al. 2007; Li et al. 2010), we wanted to investigate whether the TH2 interleukin IL-5 was influenced by depression in FM patients. We found a statistically significant negative correlation between serum IL-5 and ratings of depression (HAD-D) ( $r = -0.518$ ;  $p < 0.05$ ) in our FM patients. IL-6 levels, also previously shown to be elevated in depressed patients, did not correlate with ratings of depression in our FM patients ( $r = 0.243$ ;  $p = 0.4$ ).

#### ***Cerebrospinal fluid concentrations of IL-1b and IL-8 (Fig. 6)***

One CSF FM sample was lost due to technical failure and therefore 14 samples were analysed. FM patients had significantly higher CSF IL-8 levels ( $62.3 \pm 26.3$  pg/ml) compared to NINS patients ( $16.0 \pm 8.6$  pg/ml) ( $p < 0.001$ ) (Fig. 7). There was no statistically significant difference in CSF IL-1b levels between FM patients ( $2.6 \pm 2.0$  pg/ml) and NINS patients ( $6.4 \pm 6.1$  pg/ml) (Fig 6).

**Table 5. Descriptive data for FM patients (n = 15) and healthy controls (n = 15).**

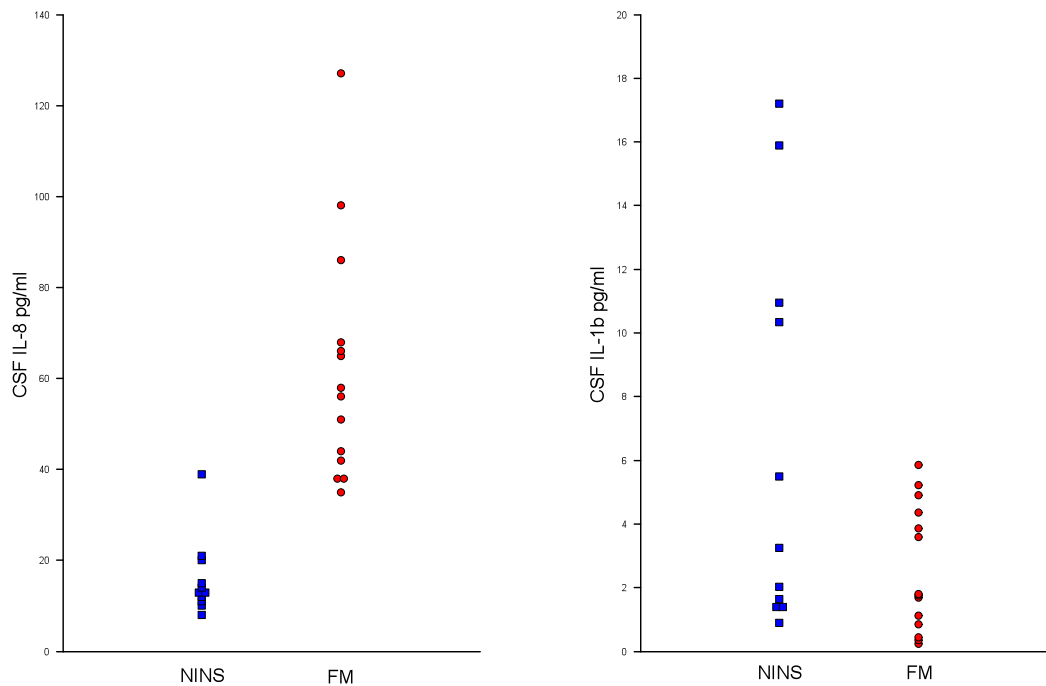
SD = standard deviation, NA = not applicable, NS = non significant, VAS = visual analogue scale, PPTs = pressure pain thresholds, MFI-20 = Multidimensional Fatigue Inventory 20 items, PSQI = Pittsburg Sleep Quality Inventory, HADS = Hospital Depression and Anxiety Scale, FIQ = Fibromyalgia Impact Questionnaire, SF-36 = Short Form -36; phys = physical components; ment = mental components (original 0–100 scoring algorithms based on the summated ratings method).

<b>Average &amp; SD</b>	<b>FM patients</b>	<b>Healthy controls</b>	<b>Group differences</b>
<b>Age</b>	46.2 ± 11.1	44.4 ± 10.7	NS
<b>Pain dur. (years)</b>	10.3 ± 8.2	NA	-
<b>FM dur. (years)</b>	2.9 ± 2.7	NA	-
<b>Pain (mm VAS)</b>	65.8 ± 13.2	0 ± 0	P < 0.001
<b>PPTs (kPa)</b>	99.5 ± 36.7	471.6 ± 104.5	P < 0.001
<b>Fatigue (MFI-20)</b>	18.1 ± 1.4	5.1 ± 1.0	P < 0.001
<b>Sleep (PSQI)</b>	13.2 ± 3.7	1.8 ± 1.7	P < 0.001
<b>Depression (HADS)</b>	6.9 ± 3.4	0.5 ± 1.1	P < 0.001
<b>Anxiety (HADS)</b>	9.8 ± 4.0	1.9 ± 1.8	P < 0.001
<b>FIQ (%)</b>	72.5 ± 12.5	NA	-
<b>SF-36phys</b>	26.4 ± 7.6	97.5 ± 2.7	P < 0.001
<b>SF-36ment</b>	40.3 ± 21.2	90.4 ± 6.3	P < 0.001

**Table 6. Serum cytokine and chemokine concentrations (pg/ml) in FM patients (n = 15) and healthy controls (n = 15).**

Groups differences are shown. P < 0.05 is regarded as a statistically significant difference. Sd = standard deviation. IL = interleukin.

<b>Mean &amp; SD</b>	<b>FM patients</b>	<b>Healthy controls</b>	<b>Group diff.</b>
Serum IL-1b	0.59 ± 0.08	0.83 ± 0.24	p < 0.007
Serum IL-5	9.36 ± 3,37	28.89 ± 15.59	p < 0.001
Serum IL-6	1.44 ± 0.76	1.09 ± 0.55	NS
Serum IL-8	21.36 ± 5.54	15.53 ± 4.83	p < 0.02
Serum IL-10	33.44 ± 61.18	16.33 ± 11.76	NS
Serum TNF-alpha	2.77 ± 1.62	4.41 ± 2.29	p < 0.04



**Fig. 6.** Cerebrospinal fluid (CSF) concentrations of interleukin-8 (IL-8) and IL-1b in 14 female fibromyalgia (FM) patients and 11 age- matched patients with non-inflammatory neurological symptoms (NINS). FM patients had significantly higher CSF concentrations of IL-8 compared to NINS patients ( $p < 0.001$ ), but no statistically significant group difference was found for CSF IL-1b.

***Relationships between serum and CSF levels of IL-1b and IL-8 in FM patients***

CSF concentrations of IL-1b and IL-8 were higher than corresponding serum concentrations (IL-1b; CSF  $2.6 \pm 2.0$  pg/ml, serum  $0.6 \pm 0.1$  pg/mg,  $p < 0.005$  and IL-8; CSF  $62.3 \pm 26.3$  pg/ml, serum  $21.4 \pm 5.5$  pg/ml,  $p < 0.001$ ). CSF concentrations of IL-1b or IL-8 did not correlate with serum concentrations.

**Study 4**

***Subject characterization (Table 7)***

FM patients rated higher ongoing pain intensity (VAS) ( $p < 0.001$ ) compared to RA patients. Furthermore, compared to RA patients and healthy controls, FM patients reported higher ratings of fatigue (RA;  $p < 0.002$ , controls;  $p < 0.001$ ), sleep disturbance (RA and controls;  $p < 0.001$ ) and lower ratings of quality of life (RA and controls;  $p < 0.001$ ). Compared to healthy controls, RA patients had higher ratings of fatigue ( $p < 0.001$ ), sleep disturbance ( $p < 0.001$ ) and lower quality of life (SF-36: mental  $p < 0.014$ , physical  $p < 0.001$ ).

**Table 7. Descriptive data for FM patients (n = 15), RA patients (n = 14) and healthy controls (n = 15).** SD = standard deviation, NA = non applicable, NS = non significant, VAS = visual analogue scale, MFI-20 = Multidimensional Fatigue Inventory 20 items, PSQI = Pittsburg Sleep Quality Inventory, SF-36 = Short Form-36; phys = physical components; ment = mental components (original 0–100 scoring algorithms based on the summated ratings method). Overall group differences are shown. Statistically significant differences between FM and RA patients are marked † and significant differences between controls and patients are marked ‡. P < 0.05 is regarded as a statistically significant difference.

Average & SD	FM patients	RA patients	Healthy controls	Group differences
Age	46.2 ± 11.1	51.1 ± 7.2	44.4 ± 10.7	NS
Duration FM/RA (years)	2.9 ± 2.7 †	8.4 ± 8.7 †	NA	P < 0.028
Pain (mm VAS)	65.8 ± 13.2 †	26.8 ± 18.1 †	NA	P < 0.001
Fatigue (MFI-20)	18.1 ± 1.4 †‡	14.0 ± 4.2 †‡	5.1 ± 1.0	P < 0.001
Sleep (PSQI)	13.2 ± 3.7 †‡	6.6 ± 3.0 †‡	1.8 ± 1.7	P < 0.001
SF-36phys	26.4 ± 7.6 †‡	62.4 ± 18.6 †‡	97.5 ± 2.7	P < 0.001
SF-36ment	40.3 ± 21.2 †‡	72.5 ± 21.6 †‡	90.4 ± 6.3	P < 0.001

### ***Serum concentrations of cytokines/chemokines (Table 8)***

There were statistically significant (Kruskal-Wallis Test) overall group differences for TNF-alpha ( $p < 0.001$ ), IL-1b ( $p < 0.001$ ) and IL-8 ( $p < 0.001$ ), but only a tendency for IL-6 ( $p = 0.054$ ). Post hoc comparisons revealed significantly lower TNF-alpha and IL-1b serum concentrations in both patient groups compared to healthy controls ( $p < 0.05$ ). RA patients had lower serum concentrations of TNF-alpha ( $p < 0.006$ ) and IL-1b ( $p < 0.001$ ) compared to FM patients. In contrast, FM patients had higher serum IL-8 concentrations compared to RA patients ( $p < 0.001$ ) and healthy controls ( $p < 0.02$ ), and RA patients had lower serum IL-8 levels compared to healthy controls ( $p < 0.002$ ). Although the overall group difference was not statistically significant post hoc comparisons, as expected, revealed higher serum IL-6 levels in RA patients compared to healthy controls ( $p < 0.029$ ).

**Table 8. Serum cytokine and chemokine concentrations (pg/ml) in FM patients (n = 15), RA patients (n = 14) and healthy controls (n = 15).**

Overall groups differences are shown. Statistically significant differences between FM and RA patients are marked † and significant differences between controls and patients are marked ‡. P < 0.05 is regarded as a statistically significant difference. Sd = standard deviation.

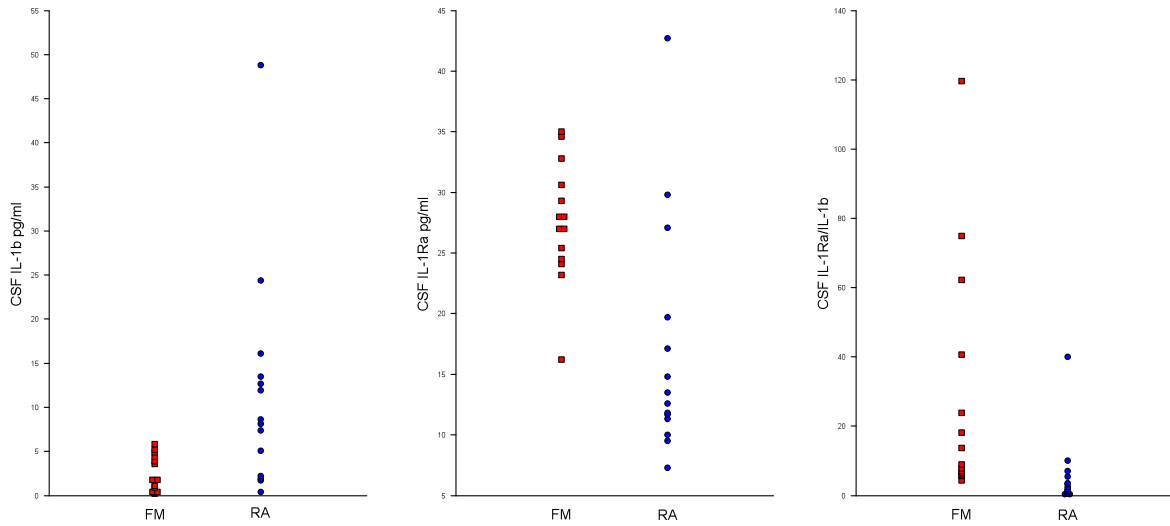
Serum levels Means ± Sd	FM	RA	Controls	P-values
<b>TNF-alpha</b>	2.77±1.62†‡	1.41±0.96†‡	4.41±2.29	P < 0.001
<b>IL-1b</b>	0.59±0.08†‡	0.02±0.06†‡	0.83±0.24	P < 0.001
<b>IL-6</b>	1.44±0.76	7.50±16.07‡	1.09±0.55	P = 0.054
<b>IL-8</b>	21.36±5.54†‡	10.42±6.68†‡	15.53±4.83	P < 0.001

#### ***Cerebrospinal fluid concentrations of cytokines/chemokines***

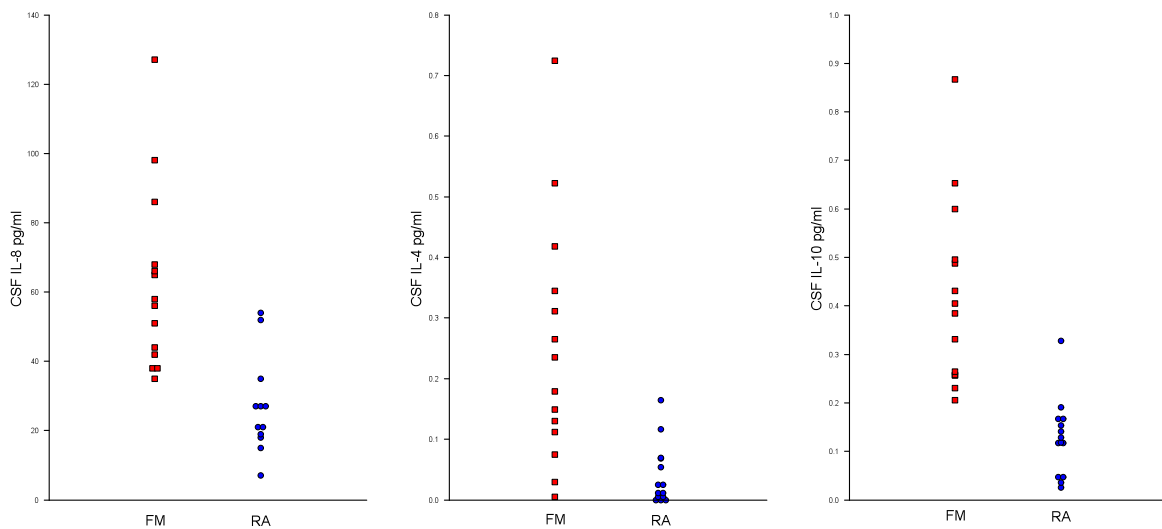
One FM CSF sample was lost due to technical failure and therefore 14 samples were analyzed. Compared to FM patients, RA patients had significantly higher CSF IL-1b (p < 0.002), but lower IL-1Ra (p < 0.002) and lower IL-1Ra/IL-1b (p < 0.001)(Fig 7). The reverse was true for IL-8 (p < 0.001), IL-4 (p < 0.001) and IL-10 (p < 0.001), with higher levels in FM patients compared to RA patients (Fig. 8). Although not statistically significant, FM patients tended to have higher CSF TNF-alpha concentrations (FM; 0.38 ± 0.22 pg/ml, RA; 0.26 ± 0.09 pg/ml)(p = 0.056). There were no significant group differences in CSF IL-6 (FM; 1.80 ± 0.69 pg/ml, RA; 1.60 ± 0.73 pg/ml).

#### ***Relationships between serum and CSF levels of cytokines/chemokines***

CSF concentrations of IL-1b and IL-8 were higher than corresponding serum concentrations in FM (IL-1b p < 0.002; IL-8 p < 0.001) and RA (IL-1b p < 0.007; IL-8 p < 0.004) patients. TNF-alpha was higher in serum compared to CSF in FM (p < 0.001) and RA patients (p < 0.001). There were no statistically significant correlations between serum and CSF concentrations for any of the assessed cytokines/chemokines.



**Fig. 7.** Cerebrospinal fluid (CSF) concentrations of interleukin-1 beta (IL-1b), IL-1Ra and the IL-1Ra/IL-1b ratio in 14 female fibromyalgia (FM) patients and 14 female age- matched patients with rheumatoid arthritis (RA). Compared to RA patients FM patients had lower CSF IL-1b ( $p < 0.002$ ), higher IL-1Ra ( $p < 0.002$ ) and higher IL-1Ra/IL-1b ratio ( $p < 0.001$ ).



**Fig. 8.** Cerebrospinal fluid (CSF) concentrations of interleukin-8 (IL-8), IL-4 and IL-10 in 14 female fibromyalgia (FM) patients and 14 female age- matched patients with rheumatoid arthritis (RA). Compared to RA patients FM patients had higher CSF IL-8 ( $p < 0.001$ ), higher IL-4 ( $p < 0.001$ ) and higher IL-10 ( $p < 0.001$ ).

## DISCUSSION

In conclusion we found a normal cardiovascular regulation in FM patients during and following isometric contraction. FM patients had a hypo-activity in the sympatho-adrenal system and a hyporeactivity in the HPA-axis, which could contribute to exercise intolerance that is characteristic of FM. Furthermore, we found elevated CSF and blood concentrations of the pro-inflammatory cytokine IL-8 indicating a central inflammatory response in FM patients. When FM patients were compared to RA patients, elevated CSF IL-8 concentrations were found in FM patients while RA patients had elevated CSF IL-1b. Our findings indicate that FM and RA patients differ in their cytokine profiles; i.e., increased CSF IL-1b in patients with inflammatory, prostaglandin associated pain (RA) and elevated CSF IL-8 in patients with dysfunctional, possibly sympathetically mediated pain (FM).

### *Cardiovascular regulation and endogenous pain modulation during static contractions in fibromyalgia patients.*

Based on previous studies we hypothesized that FM patients have a reduced BP and HR response to static exercise due to SNS hypo-reactivity (Petzke and Clauw, 2000). The SNS influences muscle blood flow as well as endogenous pain modulation. Therefore, we expected higher exercise induced pain ratings in FM patients and an exercise related decrease in PPTs at the contracting muscle due to sensitization/central disinhibition, while an increase in PPTs was expected in controls due to activation of endogenous pain inhibition. To our surprise, we found evidence of normal BP and HR regulation in FM patients (study 1 and 2). The exercise related increase in pain intensity was, as expected, higher in the patient group than in controls in both studies. However, although FM patients had lower PPTs compared to controls at all times, we did not find any statistically significant changes in PPTs specifically related to the contraction period in either group. The increase in PPTs at m. deltoideus in both groups from baseline and during as well as 15 min following contraction seen in study 1 could not be reproduced in study 2 and the time pattern is atypical for exercise induced analgesia since PPTs usually decrease following the end of contraction (Kosek et al. 1996b; Lannersten and Kosek, 2010). Previous studies have reported increased PPTs at the contracting muscle as well as at resting muscles in healthy individuals, but not in FM patients, during static contractions corresponding to 20-30% MVC (Kosek et al. 1996b; Staud et al. 2005; Lannersten and Kosek, 2010). In our studies (1 and 2) we used the same absolute contraction force and aimed for longer average contraction time (> 5 min) to allow for longer assessments of ANS parameters. Therefore, the average % MVC was lower in our studies (study 1: FM 15%, Controls 10%; study 2: FM 11 %, controls 8 %). We believe that the % MVC used in our studies was too low to activate endogenous pain inhibitory mechanisms, explaining the lack of major changes in PPTs during contraction in both groups.

Considering that our patients performed a contraction corresponding to a higher % MVC than our controls, theoretically, a diminished BP and HR response to a certain % MVC could have remained undetected. Therefore, we also analyzed a subgroup of ten patients and ten controls performing the same ( $\pm 1$  %) MVC. Supporting normal BP and HR regulation in FM patients, we found no statistically significant differences in BP and HR between these subgroups and only pain ratings were elevated in FM patients compared with controls.

Rowell (1991) suggested that the HR increase during low intensity static contractions mainly depends on vagal withdrawal and that the SNS becomes activated first when the HR exceeds 100 beats per minute (bpm), which was not the case in our studies. In our first study, HR increased during contraction in FM patients, but not in controls, leading to higher HR at

exhaustion in patients compared to controls. Our results are at variance with the findings of reduced HR in FM patients compared to healthy controls performing the same amount of dynamic exercise (Van Denderen et al. 1992). One possible explanation for the greater HR response in our FM patients in study 1 is that FM patients were less physically fit which was supported by the lower strength, the lesser endurance and a less pronounced baroreflex control of HR in the patient group (since baroreflex control of HR increases in trained subjects (Raven et al. 1984; Stevens et al. 1992; Raven and Pawelczyk, 1993; Raven et al. 2000)). No statistically significant group difference in HR response was seen during a bilateral contraction of m. quadriceps femoris with a lower % MVC (study 2).

Exertion/fatigue at exhaustion correlated to HR in controls and to rated pain intensity in FM patients, indicating that nociceptive afferent input had a more pronounced influence on perception of exertion/fatigue in the FM patients.

### ***Activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis during static exercise in fibromyalgia patients.***

We choose to examine our patients and controls during the same absolute contraction force in order to avoid two potential confounding factors, one related to the effects of physical fitness on ANS parameters and the second related to the difficulty to correctly assess the true % MVC (Miller et al. 1996). Previously, it has been shown that trained individuals exhibit higher A and NA (Peronett et al. 1981) and ACTH (Galbo 1986) release during the same relative exercise intensity compared with sedentary subjects. However, lower release of A and NA (Peronett et al. 1981) and ACTH (Leal-Cerro et al. 2003) have been reported following the same absolute exercise in trained individuals compared to untrained. Since FM patients are often physically unfit, the finding of lower levels of catecholamines and ACTH in FM patients compared to controls during the same absolute workload would signify a true hypo-activity in the SNS and the HPA-axis. Furthermore, several authors have shown that superimposed twitches during attempted maximal efforts were more pronounced in FM patients than in control subjects (Bäckman et al. 1988; Jacobsen et al. 1991) raising the possibility that sub maximal voluntary effort, motivational factors and/or reflex inhibition due to pain could explain the lesser MVC in FM patients, rather than a real decrease in muscle strength (Miller et al. 1996). Therefore, FM patients and controls performed static contractions using the same absolute contraction force in our study.

To our knowledge, this is the first study comparing catecholamine secretion during the same absolute workload in FM patients and healthy controls. We found reduced plasma A levels and a tendency to reduced plasma NA levels at baseline in FM patients compared to controls which could be explained by reduced physical fitness in FM patients since catecholamine concentrations increase in trained individuals (Galbo 1986). During static contractions plasma A and NA levels increased in both groups alike, but remained lower in FM patients compared to controls at all times (A  $p < 0.04$ ; NA  $p < 0.08$ ) indicating a normal reactivity of the SNS during static contractions, but a hypo activity of SNS at all times. Our results tally those of previous studies where a reduced A, but not NA, response was reported in FM patients during repetitive static contractions corresponding to 30% MVC (Giske et al. 2008) and during dynamic exercise (Van Denderen et al. 1992), respectively. Hypothetically, the long-term basal increase in sympathetic tone (Cohen et al. 2001) could contribute to a down-regulation of catecholamines and explain the low catecholamine concentrations at baseline and during exercise in our FM patients.

No statistically significant group differences were found in plasma ACTH at baseline. Compared to baseline, ACTH increased at exhaustion in controls but not in FM patients



indicating a hypo-reactivity of HPA-axis during static contractions. Normally, ACTH secretion increases during physical activity in relation to intensity and duration (Galbo 1986). Leal-Cerro et al. (2003) reported a lower release of ACTH following the same absolute workload in trained individuals compared to untrained. Furthermore, no difference was found in plasma ACTH between athletes and sedentary controls following repeated concentric knee extensions, even though the total workload was higher in athletes (Minetto et al. 2007). Therefore, the lower ACTH concentration at exhaustion in our FM patients compared to controls, was not likely explained by reduced physical fitness in the patient group. We did not find any statistically significant group differences in baseline cortisol levels, nor any changes during or following contraction. The latter is in accordance with previous studies in healthy controls (Kjear et al. 1991) and is most likely explained by insufficient exercise intensity to induce a cortisol increase (Hill et al. 2008).

Our results are in accordance with reduced ACTH (but not cortisol) response in FM patients to a stress test (induced hypoglycemia)(Adler et al. 1999). The lower ACTH secretion at exhaustion in our FM patients could be explained by reduced CRH response to exercise. In fact, chronically hypoactive CRH neurons (inducing a compensatory up-regulation of pituitary CRF receptors and augmented response to exogenous CRH) have been suggested in FM (Geenen et al 2002). A reduced CRH response in FM could hypothetically be caused by high concentrations of SP in the CSF (Russel et al. 1994) since SP is a potent inhibitor of CRH, and/or could be mediated by decreased sympathetic stimulation (Geenen et al. 2002).

Living with chronic pain has major impacts on many aspects of life and can therefore be regarded as a stressor. Despite this, hypoactive SNS and HPA-axis has been reported in many chronic pain syndromes, including FM (Petzke and Clauw, 2000; Tanriverdi et al. 2007; Riva et al. 2012a,b). It has been hypothesized that chronic pain is initially accompanied by hyperactivity of SNS and HPA-axis, which in time leads to down-regulation and under-activation of these systems (Miller et al. 2007; Van Houdenhove et al. 2009; Riva et al. 2012a,b). Recently, Riva et al. (2012a) found attenuated activity of the adrenal-medullary component of the SNS reflected by significantly lower levels of urinary catecholamines (A and dopamine, but not NA) in FM patients. In the same study a decreased HPA activity was reported in FM patients and the authors suggested that this could have been a result of “exhaustion” of the HPA-axis. Further supporting this hypothesis the same group found hypercortisolism in patients with regional myalgias, (often reported to precede FM), but hypocortisolism in FM patients (Riva et al. 2010).

The high sensitive CRP was twice as high in FM patients compared to controls. High sensitivity CRP is a sensitive, but nonspecific, marker of inflammation reflecting increased production of interleukin-1 (IL-1) and interleukin-6 (IL-6) (Vigushin et al 1993). However, in study three, we found lower IL-1b and TNF-alpha concentrations in our FM patients compared to controls. We speculate that the increased high sensitivity CRP levels might have been due to other pro-inflammatory factors not assessed in our studies, since we have found no evidence that elevated IL-8 concentrations are associated with high CRP (Pine et al. 2011). Salemi et al. (2003) reported increased concentrations of IL-1, IL-6 and TNF-alpha in the skin of FM patients and Uceyler et al. (2006) reported reduced blood levels of the anti-inflammatory cytokines IL-4 and IL-10 in patients with chronic widespread pain. The autonomic imbalance with relative decrease in parasympathetic activity has been described by Cohen et al. (2001) in FM patients and could hypothetically alter the balance between pro- and anti-inflammatory cytokines in favour of the former through the reduction of the anti-inflammatory vagal reflex (Tracey 2002). Over-production of pro-inflammatory cytokines in the CNS has been shown to contribute to central sensitization causing enhanced pain and

fatigue (Watkins and Maier, 2005), which stimulated us for further research regarding central inflammatory processes in FM.

### ***Cytokines in the cerebrospinal fluid and blood of healthy controls, fibromyalgia patients and patients with rheumatoid arthritis***

Glia cell activation resulting in elevated concentrations of intrathecal cytokines/chemokines thus giving rise to central inflammation has been proposed as an important pathophysiological mechanism in human chronic pain states such as FM (Milligan and Watkins, 2009). The activation of glia leads to increased pain sensitivity (allodynia/hyperalgesia) and pain related behavior in animal studies (Hutchinson et al. 2008). Data from animal studies indicate that the two pro-inflammatory cytokines IL-1b and IL-8 contribute to pain and hyperalgesia in an additive manner following tissue injury or peripheral inflammation and that this is mediated by different mechanisms (Cunha et al. 1991; Sachs et al. 2002). Furthermore, increased concentrations of pro-inflammatory cytokines/chemokines, including IL-1b and IL-8 have been reported in the CSF of human pain patients suffering from neuropathic and nociceptive pain (Kikuchi et al. 1999; Kotani et al. 2004; Lundborg et al. 2010). Therefore, we wanted to assess the concentrations of these two pro-inflammatory interleukins in CSF of FM patients. We found elevated CSF IL-8, but not IL-1b concentrations in FM patients compared to CSF controls, i.e., patients with non-inflammatory neurological symptoms (headache). Compared to healthy controls, FM patients had higher serum IL-8, but lower serum IL-1b concentrations. The CSF concentrations of IL-8 were more than 3 times higher compared to serum concentrations. Therefore, our results indicated a central inflammatory response in FM patients involving the pro-inflammatory cytokine IL-8, but not IL-1b.

This finding led us to suspect that different cytokine profiles may be implicated in neuroinflammatory responses in patients with different types of pain. Cytokines are potent immunoregulatory factors and may be directly involved in the disordered immunoregulation found in chronic rheumatic disease. TNF-alpha and IL-1b have been implicated in the pathogenesis of RA as mediators of chronic inflammation (Almonte et al. 1992). RA is an inflammatory, prostaglandin associated pain condition and RA patients usually respond favourably to NSAID treatment regarding pain relief. FM on the other hand, is most often regarded as so called dysfunctional pain, i.e., pain due to a dysfunction of endogenous pain modulation (Kosek et al. 1996a; Jensen et al. 2009; Lannersten and Kosek, 2010). The aberrations of endogenous pain modulation could be related to a dysfunction of the ANS and some researches regard FM as a sympathetically maintained pain syndrome (Martinez-Lavin 2007). FM patients do not respond to NSAIDs, or corticosteroids, which would support the notion of FM as a non-prostaglandin associated pain condition (Carville et al. 2008). If, as suggested, these two pain conditions, RA and FM, have different pain mechanisms and if central inflammation is present in these conditions, due to glia cell activation or other mechanisms, we would expect the central inflammation to be mediated by different profiles of cytokines/chemokines in these two conditions. Therefore, we wanted to use our FM cohort to compare the profiles of pro- and anti-inflammatory substances in CSF of FM and RA patients.

Consistent with our a priori hypothesis we found different patterns of CSF cytokines in RA and FM patients, with elevated IL-1b and reduced IL-1Ra, IL-4 and IL-10 in RA patients and increased CSF IL-8 in FM patients. There were no statistically significant group differences in CSF TNF-alpha or IL-6. The CSF concentrations of IL-1b and IL-8 were significantly higher than serum concentrations in both groups indicating a central inflammatory response involving these cytokines. On the contrary, TNF-alpha concentrations were higher in serum compared to CSF in both groups. Compared to RA patients, FM patients had higher serum TNF-alpha, IL-1b and IL-8 concentrations, but only the serum IL-8 was higher in FM patients

compared to healthy controls. As expected, RA patients had higher serum IL-6 compared to healthy controls (but not compared to FM patients) and there was a positive correlation between serum IL-6 levels and disease activity assessed by DAS28 ( $r = 0.585$ ,  $p = 0.028$ ), which is in accordance with previous reports (Nishimoto et al. 2004; 2007; Madhok et al. 1993). Our results indicate different cytokine patterns in an inflammatory painful condition (RA) and a dysfunctional pain syndrome (FM), which is in accordance with reports from animal studies showing that various pro-inflammatory cytokines/chemokines can contribute to pain by different mechanisms.

The two pro-inflammatory cytokines IL-1b and IL-8 contribute to pain and hyperalgesia in an additive manner following tissue injury or peripheral inflammation (Cunha et al. 1991; Sachs et al. 2002). Peripheral as well as central (intrathecal) IL-1b stimulates COX-2 activity inducing augmented production of prostaglandins such as PGE2 (Bartfai 2001; Samad et al. 2001). Increased production of PGE2 following peripheral inflammation has been reported at the site of inflammation but also in the CNS (Bartfai 2001; Samad et al. 2001; Bianchi et al. 2007). Notably, the increased pain sensitivity induced by peripheral IL-1b was prevented by peripheral administration of COX-2 inhibitors (Cunha et al. 1991; Sachs et al. 2002). In analogy, the increased pain sensitivity induced by central IL-1b was prevented by central application of COX-2 inhibitors (Samad et al. 2001). Furthermore, the IL-1b induced hyperalgesia was not affected by beta-adrenergic receptor antagonists (Cunha et al. 1991; Sachs et al. 2002) or by the adrenergic neurone-blocking agent guanethidine (Cunha et al. 1991). In contrast, peripherally administered IL-8 induced local hyperalgesia (Bartfai 2001; Oh et al. 2001) that was mediated by beta-adrenergic receptors and could be blocked by beta-adrenergic receptor antagonists (Cunha et al. 1991; Sachs et al. 2002) as well as by guanethidine (Cunha et al. 1991), but was not influenced by local administration of COX-2 inhibitors (Cunha et al. 1991; Sachs et al. 2002). In analogy, centrally administered CINC-1 (the rat analogue of human IL-8), reduced mechanical pain thresholds (Yamamoto et al. 1998; Ahn et al. 2005) and increased pain related behaviour in rats (Ahn et al. 2005). The hyperalgesia could be prevented by central pretreatment with beta-adrenergic receptor antagonists, but was not influenced by COX-2 inhibitors (Ahn et al. 2005). Furthermore, activation of the sympathetic nervous system has been shown to increase the production of IL-8 (Elenkov et al. 2000) in peripheral tissues through a beta-adrenergic receptor dependent mechanism (Elenkov et al. 2000; Black 2002). Finally, stress related release of the IL-8 analogue CINC from the hypothalamic-pituitary region has been shown in animal experiments (Sakamoto et al. 1996; Matsumoto et al. 1997). Taken together, these studies suggest the involvement of sympathetic nervous system in the regulation of IL-8 release as well as in IL-8 induced hyperalgesia in animals. In accordance with our hypothesis and with the data from animal studies, we found different profiles of central cytokines, i.e., IL-1b in patients with inflammatory, prostaglandin-associated pain (RA) and IL-8 in patients with dysfunctional, possibly sympathetically mediated pain (FM).

The release of pro-inflammatory cytokines/chemokines by glia cells can be triggered by stress, immune activation and/or afferent nociceptive input (Milligan and Watkins, 2009). It is not fully understood by which mechanisms the peripheral release of pro-inflammatory cytokines/chemokines can be reflected in the CNS, but peripheral unilateral injections of pro-inflammatory cytokines (IL-1b, TNF, IL-6, IL-8) gave rise to bilateral hyperalgesia (Cunha et al. 1992). Cytokines released by peripheral immune cells can be transported across the blood-brain-barrier (BBB) by special transport mechanism (Gutierrez et al. 1993; Banks et al. 1995; Quan and Herkenham, 2002) or by crossing at leaky areas in the BBB through the circumventricular organs (Watkins et al. 1995). However, in our study the concentrations of IL-1b were more than 4 times higher in the CSF compared to serum in FM (and a factor 500

higher in RA due to the very low serum levels). The same was true for IL-8, with CSF IL-8 concentrations three times (FM) and more than twice (RA) higher in the CSF compared to serum. Furthermore, there were no statistically significant correlations between the CSF and serum concentrations of IL-1b or IL-8 in either group. Therefore, a direct transport of the IL-1b and IL-8 across the BBB is less likely in our patients. Peripheral cytokines can have an indirect impact on CNS by activating endothelial cells of blood vessels and also can effect the CNS indirectly by activating the brain endothelium to produce pro-inflammatory cytokines (Reijerkerk et al. 2011), such as IL-1b (Szelenyi 2001) or IL-8 analogue (Zidovetzki et al. 1999; Chen et al. 2011) thus permitting the signal to be transduced from the blood stream into CNS without the need to cross the BBB (Szelenyi 2001).

Glia cells are known to produce IL-1b and IL-1Ra in response to inflammatory stimuli (Guo et al. 2007; Pinteaux et al. 2002) and also have the potential to produce IL-8 (Watkins and Maier, 2009). IL-1Ra is an endogenous, competitive antagonist of the IL-1 receptor, and can modulate the biologic actions of IL-1 by preventing signal transduction (Dinarello 1996; Arendt et al. 1998). IL-1 may contribute to joint damage in RA and therefore inhibition of IL-1 is an important strategy for decreasing inflammation in RA patients. The administration of human IL-1Ra, anakinra, a competitive antagonist of IL-1 that blocks the actions of IL-1 without any detectable agonist activity, may provide symptomatic improvement in RA patients (Fleischman et al. 2006). Interestingly, we found not only elevated IL-1b but also decreased IL-1Ra in the CSF of our RA patients, stressing the potential importance of IL-1 mediated central effects in RA.

The concentrations of TNF-alpha were higher in serum than in CSF in both groups. The lack of significant up-regulation of this pro-inflammatory cytokine in CSF compared to serum is in line with previous data demonstrating low levels of TNF-alpha in CSF (Vladic et al. 2002). Our results suggest a different regulation of cytokine production in the CNS, less dependent on TNF-alpha compared to what has been reported in peripheral tissues (Feldmann and Maini, 2008). TNF-alpha is known as a major cytokine that orchestrate and influence other peripheral cytokines but TNF-alpha maybe have another role in CNS cytokine regulation. However, all RA patients in the present study were on DMARD medications, which might have influenced the serum levels of pro-inflammatory cytokines (Eklund et al. 2007; Chen et al. 2011), which is indicated by the fact that RA patients had lower TNF-alpha, IL-1b and IL-8 serum concentrations than controls.

## CONCLUSIONS

Our results indicate a normal cardiovascular regulation during static contractions in FM patients. Exertion/fatigue at exhaustion correlated to HR in controls and to rated pain intensity in FM patients, indicating that afferent nociceptive input had a more pronounced influence on perception of exertion/fatigue in the patients. We found evidence of a hypo-active sympatho-adrenal system and hypo-reactive HPA-axis in FM, which could contribute to the exercise intolerance that is typical for FM patients.

We found evidence of a low-grade peripheral inflammatory response in our FM patients with elevated sensitive CRP and elevated serum IL-8 levels. IL-8 was elevated in CSF in FM patients indicating a central inflammatory response in FM.

Furthermore, in accordance with our hypothesis, we found different CSF cytokine profiles with higher IL-8 in CSF of FM compared to RA and higher IL-1b and lower IL-1Ra, IL-4 and IL-10 in the CSF of RA patients, compared to FM. Our results indicate different profiles of central cytokine release, i.e., increased IL-8 in patients with dysfunctional, possibly sympathetically mediated pain (FM) and increased IL-1b in patients with inflammatory, prostaglandin associated pain (RA).

## SPECULATIONS

Our results indicate a central inflammatory response and suggest that a bi-directional communication between CNS and peripheral tissues involving the immune system and glia could be of outmost importance in chronic pain conditions such as FM and RA . Increased understanding of these mechanisms could open up for truly new treatment approaches.

### *Future research*

Future proof-of-concept studies using antagonists to pro-inflammatory cytokines/chemokines, such as IL-8, are needed to facilitate development of efficient new biological drugs in FM, in analogy with the success seen in RA. Biomarkers for dysfunctional pain regulation would be clinically valuable tools for diagnostic purposes and for treatment follow-up in patients with complex chronic pain conditions such as FM.

**“It is an exciting time in pain investigation and it is highly probable that the manipulations of cytokines will play an important role in the future management of fibromyalgia (Wallace 2006)”.**

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## APPENDIX

### *Cytokines, brief orientation*

Cytokines (Greek "cyto-", cell; and "-kinos", movement) are a number of substances secreted by specific cells of the immune system. They carry signals locally between cells, and thus have an effect on other cells. Cytokines are secreted by immunocompetent cells, such as immune cells in the blood or glia cells in the CNS, in addition some cytokines have been reported to be released also by neurons. In the periphery T helper cells (Th cells) become activated during the immune response. Th cells are a sub-group of lymphocytes, a type of white blood cell, that play an important role in the immune system, particularly in the adaptive immune system. Th cells provide signals for orchestrating of immune response. There are two groups of cytokines Th1 (pro-inflammatory) and Th2 (anti-inflammatory). TNF-alpha, IL-1b, IL-6, IL-8 belong to the Th1 family and IL-4, IL-5 and IL-10 belong to the Th2 family. Pro-inflammatory cytokines promote inflammation and anti-inflammatory cytokines reduce inflammatory responses. The balance between these two groups is very important for the regulation of immune responses.

Tumor necrosis factor (TNF-alpha) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by other cell types as well. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, to induce apoptotic cell death, to induce sepsis (through IL1 & IL6 production), to induce cachexia and inflammation. TNF-alpha is known as a major cytokine that orchestrate and regulate other interleukins.

IL-1b also known as catabolin is a cytokine protein produced by activated macrophages. This cytokine is an important mediator of the inflammatory response. IL-1b is a major inducer of central COX-2 up-regulation in the CNS, elevating prostaglandin E2 (PGE2) levels in the cerebrospinal fluid. Both TNF-alpha and IL-1b are implicated in RA pathogenesis.

IL-8 is known as neutrophil chemotactic factor, with the primary function to recruit neutrophils who phagocytose the antigen. This cytokine serves as a chemical signal that attracts neutrophils at the site of inflammation.

It has been demonstrated that neurons interact with activated glia cells during development of central sensitization and hyperalgesia (Watkins and Maier, 2005; Milligan and Watkins, 2009). Following activation, also glia cells release pro-inflammatory cytokines.