The pain profile in fibromyalgia

Painomic studies of pain characteristics
and proteins in blood

Karin Wåhlén

Linköping University Medical Dissertations No. 1757

The pain profile in fibromyalgia

Painomic studies of pain characteristics and
proteins in blood

Karin Wåhlén

Department of Health, Medicine and Caring Sciences,
Linköping University, Sweden
Linköping 2020

© Karin Wåhlén, 2020

Cover design: Karin Wåhlén

All published articles are open access articles and published under the terms of the Creative Commons Attribution License (CC BY, **paper II** and **III**) or CC BY – non commercial (**paper I**), and ownership and copyright of the articles remains with the authors.

Printed in Sweden by LiU-Tryck, Linköping, Sweden, 2020

ISBN 978-91-7929-783-1

ISSN 0345-0082

To mom,
I didn't cure your cancer but I promised you that
I would do something great and here it is.
I love you

MAIN SUPERVISOR

Bijar Ghafouri, Professor

Pain and Rehabilitation Center, and Department of Health,
Medicine and Caring Sciences, Linköping University, Linköping, Sweden

CO-SUPERVISORS

Björn Gerdle, Professor

Pain and Rehabilitation Center, and Department of Health,
Medicine and Caring Sciences, Linköping University, Sweden

Mika Gustafsson, Associate Professor

Bioinformatics, Department of Physics, Chemistry and Biology,
Linköping University, Linköping, Sweden

FACULTY OPPONENT

Manuela Schmidt, Professor

Head of Systems Biology of Pain, Department of Pharmacology & Toxicology
Universität Wien, Wien, Austria

EXAMINATION BOARD

Toomas Timpka, Professor

Department of Health, Medicine and Caring Sciences,
Linköping University, Linköping, Sweden

Karin Enander, Associate professor

Department of Physics, Chemistry and Biology,
Linköping University, Linköping, Sweden

Kristian Borg, Professor

Department of Clinical Sciences,
Karolinska Institutet, Stockholm, Sweden

Alternative members of the board:

Paul Hamilton, Associate professor

Department of Biomedical and Clinical Sciences,
Linköping University, Linköping, Sweden

Åshild Olsen Faresjö, Assistant professor

Department of Health, Medicine and Caring Sciences,
Linköping University, Linköping, Sweden

The included papers in this thesis was supported by the following funders:

- Swedish Research Council
- Swedish Rheumatism Association
- Swedish Council for Working Life and Social Research
- AFA Insurance
- Åke Wiberg Foundation
- Magnus Bergvalls Foundation
- Linköping University Hospital Research Fund
- Region Östergötland (ALF)
- Region Östergötland Research Fund (Sinnescentrum)
- Karolinska Institute Foundation
- ALF-LUA at Sahlgrenska University Hospital
- Stockholm County Council (ALF)
- Health and Medical Care Executive Board of Västra Götaland Region
- Medical Research Council of Southeast Sweden (ALF)

TABLE OF CONTENTS

TABLE OF CONTENTS	1
ABSTRACT	1
SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING	2
LIST OF PAPERS	4
ABBREVIATIONS	6
INTRODUCTION	8
Chronic pain	8
<i>IASP definitions</i>	8
<i>Definition of chronic widespread pain and fibromyalgia</i>	9
<i>Nociplastic pain</i>	9
<i>Symptoms of CWP/FM</i>	9
Biomarkers of CWP/FM	12
Principles of proteomics	12
<i>Two-dimensional gel electrophoresis</i>	13
<i>Matrix assisted laser desorption/ionization-time of flight MS</i>	14
<i>Shot-gun proteomics</i>	14
<i>Multivariate data analysis and bioinformatics</i>	15
<i>Bioinformatics</i>	16
Proteomic studies of CWP/FM	17
The plasma proteome	20
AIMS	21
MATERIALS AND METHODS	22
Description of cohorts	22
<i>Ethical approvals and consents</i>	22
<i>Cohort I</i>	22
<i>Cohort II</i>	22
<i>Resistance exercise intervention program</i>	23
<i>Comparison of cohorts</i>	24

Included instruments to assess clinical characteristics in CWP/FM	26
<i>The Hospital Anxiety and Depression Scale</i>	26
<i>Pain intensity</i>	26
<i>Pain sensitivity</i>	27
<i>Fibromyalgia Impact Questionnaire</i>	27
<i>Quality of Life instrument</i>	27
<i>Pain Catastrophizing Scale</i>	28
<i>The Multidimensional Fatigue Inventory</i>	28
<i>Chronic Pain Acceptance Questionnaire</i>	28
Proteomics.....	28
<i>Sample collection</i>	28
<i>2-DE</i>	28
<i>Shotgun proteomics</i>	31
<i>Summary of the material and methods used in the studies</i>	33
Statistics.....	35
<i>Univariate statistics</i>	35
<i>Multivariate data analysis</i>	35
<i>PCA</i>	35
<i>OPLS-DA</i>	35
<i>OPLS</i>	36
Bioinformatics.....	36
Methodology considerations.....	36
RESULTS AND DISCUSSION	38
<i>Cohort I (paper I and II)</i>	38
<i>Cohort II (paper III and IV)</i>	42
<i>Exercise in FM and physical functioning and proteins</i>	44
<i>The plasma proteome of CWP/FM</i>	45
<i>Clinical characteristics in CWP/FM and correlation to plasma proteins</i> .47	
CONCLUSION	49
FUTURE PERSPECTIVES	50
REFERENCES.....	52
ACKNOWLEDGEMENTS.....	63

“Den som föga lär åldras som en ox”

Från en poster jag fick av mormor när jag tog studenten 😊

ABSTRACT

Chronic widespread pain (CWP), including fibromyalgia (FM), is a complex pain condition, where little is known about the molecular mechanisms contributing to its pathophysiology. To date, there are no established biomarkers for CWP/FM. This thesis has investigated potential molecular mechanisms and biomarkers in blood for chronic pain in women with CWP/FM. Furthermore, investigations are made to evaluate whether common pain characteristics such as pain intensity, sensitivity, and psychological distress in CWP/FM are correlated with specific proteins in blood.

The pain profile of CWP/FM, which includes the plasma proteome and clinical characteristics, is analyzed using proteomics, advanced multivariate statistics, and bioinformatics. The results from **paper I, III, and IV** indicate that there are prominent systemic changes related to immunity, inflammation, and metabolic processes in women with CWP/FM compared to healthy controls. Furthermore, **paper II and III** show that in CWP/FM, pain intensity is related to protein profiles involved in immunity processes, psychological distress with metabolic and immunity processes, and pain sensitivity with inflammatory processes.

In **paper IV**, the plasma proteome is investigated before and after a 15 weeks resistance exercise intervention in FM and healthy controls. Both at baseline and post exercise in FM and controls, prominent protein alterations are found that are involved in immunity, stress, mRNA stability, and muscle structure development. Exercise seems to influence clinical characteristics and circulating proteins in FM. Furthermore, specific plasma proteome profile is found related to grade of chronification, pain sensitivity, and improved muscle force of the quadriceps muscle.

To summarize, the results from this thesis suggest that in CWP/FM there might be a dysregulation in the biological processes involved in the immune system and metabolic processes, which are tightly linked to several proteins in the complement system and blood coagulation cascade. These results shed light on potential ongoing mechanisms involved in the pathophysiology of the complex pain condition CWP/FM. This type of biomarker research has a large potential in increasing knowledge about mechanisms involved in CWP/FM and can hereby open for better clinical understanding and management of this and other chronic pain states. The clinical value of collecting a blood sample and measuring stable pain mechanism markers in combination with evaluation of anamnesis and clinical examination would in the future help clinicians and patients receive a faster and more precise diagnosis and ultimately better treatment strategies.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Långvarig smärta har blivit vanligare bland befolkningen och är idag ett stort hälsoproblem. Ca 1 av 10 drabbas av generaliserad smärta (engelska: *chronic widespread pain*, CWP) dvs smärta spridd över hela kroppen. Fibromyalgi (FM), som ingår i CWP drabbas ca 1-4% av befolkningen och är vanligare bland kvinnor än män. Det är vanligt att personer med CWP/FM utöver sin spridda smärta även upplever trötthet, sömnsvårigheter, depression, ångest, ökad smärtkänslighet och stelhet av muskler och leder. Symtomen har i sin tur stor påverkan på arbetsförmåga, hälsa och livskvalitet. Idag är dem bakomliggande orsakerna för CWP/FM ofullständigt kartlagda och det finns inga markörer som på ett objektivt sätt kan fastställa diagnos.

Syftet med denna avhandling är att undersöka hur proteiner i blod skiljer sig åt mellan kvinnor med CWP/FM och friska kvinnliga kontroller. Vidare har syftet varit att undersöka om kliniska variabler, till exempel smärtintensitet, smärtkänslighet och psykologisk belastning (ångest/depression), har ett samband med proteinmönster i blod. Slutligen har effekten av ett 15 veckors träningsprogram och dess påverkan på proteiner i blod samt kliniska variabler hos kvinnor med FM och friska kontroller analyserats.

I **studie I, III, och IV** studerades proteinmönstret i blod mellan CWP/FM och kontroller. Resultatet visade att flertalet proteiner kunde urskilja CWP/FM gruppen från kontrollerna. Dessa proteiner var involverade i flertalet förlopp i kroppen bland annat inflammation, metabolism och immunförsvar.

I **studie II och III** undersöktes om det funna proteinmönstret i blod hade något samband med smärtintensitet, smärtkänslighet och psykologisk belastning hos denna specifika CWP/FM grupp. Resultatet visade att det fanns ett specifikt proteinmönster kopplat till respektive undersökt variabel. Smärtintensitet var relaterat till proteiner involverade i immunförsvar; smärtkänslighet var relaterat till proteiner inom inflammation; och psykologisk belastning var relaterat till proteiner inom metabolism och immunförsvar. I **studie IV** studerades proteinmönstret i blod före och efter 15 veckors styrketräning hos FM samt friska kontroller. Resultaten visade att det fanns förändringar i specifika proteiner involverade i flertalet förlopp i kroppen bland annat stress, processer för muskelstrukturutveckling samt immunförsvar. Vidare sågs även ett specifikt mönster av proteiner som kunde relateras till smärtans varaktighet (dvs hur länge man levt med smärta), smärtkänslighet samt förbättrad muskelstyrka i lårmuskeln. Styrketräning visade sig ha en positiv effekt på flera av de rapporterade symtomen hos FM gruppen och till viss del påverkades även proteinmönstret.

Sammanfattningsvis har studierna i denna avhandling visat att i denna specifika CWP/FM grupp finns skillnader i proteinmönstret i blod jämfört med friska kontroller. Det funna proteinmönstret är främst involverat i olika förlopp inom blodkoagulation samt olika komponenter kopplade till immunförsvaret.

Genom identifieringen av dessa specifika proteinmönster i blodet hos CWP/FM kan det bidra till ökad förståelse av bakomliggande sjukdomsmekanismer hos denna patientgrupp. Detta kan i framtiden bidra till underlättandet av diagnostisering, behandling, rehabilitering och slutligen skapa kliniska verktyg i form av smärtnärkörer vid utredning av olika långvariga smärttillstånd.

LIST OF PAPERS

The thesis is based on the following papers, which are referred to by their Roman numerals:

Paper I

Wåhlén K., Olausson P., Carlsson A., Ghafouri N., Gerdle B., and Ghafouri B., *Systemic alterations in plasma proteins from women with chronic widespread pain compared to healthy controls: a proteomic study*. J Pain Res, 2017. 10: p. 797-809.

Paper II

Wåhlén K., Ghafouri B., Ghafouri N., and Gerdle B., *Plasma Protein Pattern Correlates With Pain Intensity and Psychological Distress in Women With Chronic Widespread Pain*. Front Psychol, 2018. 9(2400): p. 2400.

Paper III

Wåhlén K., Ernberg M., Kosek E., Mannerkorpi K., Gerdle B., and Ghafouri B., *Significant correlation between plasma proteome profile and pain intensity, sensitivity, and psychological distress in women with fibromyalgia*. Scientific Reports, 2020. 10(1): p. 12508.

Paper IV

Wåhlén K., Yan H., Welinder C., Ernberg M., Kosek E., Mannerkorpi K., Gerdle B., and Ghafouri B., *Plasma proteomics in exercise intervention in fibromyalgia identified the grade of chronification as an important factor that benefit the outcomes*.

Manuscript.

Other publications not included in the thesis but related to pain:

Gerdle B., **Wåhlén K.**, and Ghafouri B., *Plasma protein patterns are strongly correlated with pressure pain thresholds in women with chronic widespread pain and in healthy controls-an exploratory case-control study*. Medicine (Baltimore), 2020. 99(22): p. e20497.

Peolsson A., Karlsson A., Ghafouri B., Ebbers T., Engstrom M., Jonsson M., **Wåhlén K.**, Romu T., Borga M., Kristjansson E., Bahat H.S., German D., Zsigmond P., and Peterson G., *Pathophysiology behind prolonged whiplash associated disorders: study protocol for an experimental study*. BMC Musculoskelet Disord, 2019. 20(1): p. 51.

Sørensen L.B., Gazerani P., **Wåhlén K.**, Ghafouri N., Gerdle B., and Ghafouri B., *Investigation of biomarkers alterations after an acute tissue trauma in human trapezius muscle, using microdialysis*. Sci Rep, 2018. 8(1): p. 3034.

Shimada A., Castrillon E.E., Baad-Hansen L., Ghafouri B., Gerdle B., **Wåhlén K.**, Ernberg M., Cairns B.E., and Svensson P., *Increased pain and muscle glutamate concentration after single ingestion of monosodium glutamate by myofascial temporomandibular disorders patients*. Eur J Pain, 2016. 20(9): p. 1502-12.

ABBREVIATIONS

Abbreviation	Description
2-DE	Two-dimensional gel electrophoresis
ACR	American College of Rheumatology
BFB	Bromophenol blue
BMI	Body mass index
CON	Controls
CPAQ	Chronic Pain Acceptance Questionnaire
CWP	Chronic widespread pain
DDA	Data dependent analysis
DIA	Data independent analysis
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FIQ	Fibromyalgia Impact Questionnaire
FM	Fibromyalgia
HADS	The Hospital Anxiety and Depression Scale
HCD	Higher-energy collisional dissociation
HPLC	High pressure liquid chromatography
IAA	Iodoacetamide
IASP	International Association for the Study of Pain
LC-MS/MS	Liquid chromatography- mass spectrometry
MALDI-TOF	Matrix assisted laser desorption/ionization – time of flight
MFI	Multidimensional Fatigue Inventory
MVDA	Multivariate data analysis
MW	Molecular weight
NRS	Numeric rating scale
OC	Orthogonal component
OD	Optical density
OPLS	Orthogonal partial least square analysis
OPLS-DA	Orthogonal partial least square – discriminant analysis

p(corr)	correlation coefficient, expressed as p(corr), which range from -1 to +1
PC	Principle component
PCA	Principle component analysis
PCS	Pain Catastrophizing Scale
pI	Isoelectric point
PPT	Pressure pain thresholds
QC	Quality control sample
QoL	Quality of Life
RT	Room temperature
STRING	Search tool for retrieval of interacting genes/proteins
VAS	Visual Analogue Scale
VIP	Variable influence on projection

INTRODUCTION

Chronic pain

IASP definitions

In 1979, the International Association for the Study of Pain (IASP) defined pain as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” [1]. Over four decades later, in May 2020, and after two years of intense work by the multinational Presidential Task Force, including debates and reviews of comments from clinicians, researchers, and the public, IASP released a new definition of chronic pain: “An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” [2]. This new definition has a supplement, which is more clearly and easier to interpret and includes the etymology of the word “pain”. Per definition, for pain to become chronic or persistent, it has to be present for at least three months and/or beyond over normal healing time. As stated in both definitions, pain is an experience of sensations and emotions and is always subjective.

The World Health Organization (WHO) has developed the International Classification of Disease (ICD), to standardized reporting of diseases and different health conditions. For long time, chronic pain has not been regarded as a disease, but as a secondary symptom related to other diseases. IASP has proposed a new systematic classification of chronic pain, divided into two categories to be included in ICD-11: chronic primary and secondary pain. According to ICD-11 chronic primary pain syndromes include chronic widespread pain, complex regional pain syndrome, chronic primary headache or orofacial pain, visceral pain, and musculoskeletal pain. Chronic secondary pain includes pain related to other diseases such as cancer-related pain, postsurgical or posttraumatic pain, neuropathic pain, secondary headache and orofacial pain, visceral pain, and musculoskeletal pain [3]. ICD-11 is approved by WHO and is now implemented in the different countries; a Swedish version will be available in 2022. In the European population, the prevalence of chronic pain of at least moderate intensity is approximately 20% and several risk factors underlying chronic pain are related to demographics (age, sex, ethnicity, sociodemographic background and occupational status), life style and behavior (smoking, alcohol, physical activity, diet), clinical factors (pain, mental health, medications, weight, sleep disorders), and other factors (attitudes and beliefs towards pain, history of trauma) [4].

One of the most common types of chronic pain is musculoskeletal pain which is classified as localized, regional, or widespread [5, 6]. In the category of

widespread pain; chronic widespread pain (CWP) and the subgroup fibromyalgia (FM) is included.

Definition of chronic widespread pain and fibromyalgia

Over the last few decades, the American College of Rheumatology (ACR) has released several criteria to assess a clinical diagnosis of CWP and FM. The first definition was published in 1990 [7], which is still commonly accepted and used in research. The ACR defines CWP by presence of generalized pain in a minimum of three of four body quadrants, and additional pain along the axial skeletal plane. In addition, the pain needs to be present for at least three months, a condition also referred to as chronic by the IASP definition. Additionally, for a FM diagnosis, generalized allodynia/hyperalgesia needs to be present i.e. pain in a minimum of 11 of 18 tender points assessed by palpation (force of 4 kg) [7]. For location of tender points see Figure 1.

The most recent ACR updates of the CWP/FM criteria (2010/2011 and 2016) do not require a medical examination to assess tender points [8-10] although these updates still requires the presence of generalized pain and symptoms for at least three months. However, a physician-based questionnaire is used to define painful body regions and symptom severity using the widespread pain index and the symptom severity scale [8-10].

The clinical assessment of CWP and FM consists of clinical examinations by physicians of tender points (depending on which criteria are applied), pain duration, pain intensity, medical history including screening for serious comorbidities and patient self-reports from standardized questionnaires [11, 12].

Nociplastic pain

Recently, IASP have defined three categories of pain mechanisms such as nociceptive pain, neuropathic pain, and nociplastic pain. FM is given as an example of a nociplastic pain condition [13], and is defined as: *“Pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain.”* (<https://www.iasp-pain.org/terminology>) [13]. No definite criteria for nociplastic pain have yet been presented by IASP but Gerdle et al suggested that widespread pain and increased pain sensitivity are important clinical characteristics [14].

Symptoms of CWP/FM

In the general adult population, the prevalence of CWP has been reported to be around 10% [15, 16], and for FM the number ranges between 1-4% depending on which ACR criteria used [16-18]. Except for presence of widespread pain with allodynia or hyperalgesia, individuals diagnosed with CWP/FM often experience symptoms such as fatigue, sleep disturbance, depression and/or anxiety and

stiffness of muscles and joints [19-21]. Beyond these symptoms other factors could play a role in this pain condition such as female sex, obesity and increasing age [22, 23]. Hence, women are overrepresented among CWP/FM even though the newer ACR criteria result in identifying more men [17, 24].

A diagnosis of CWP/FM is often accompanied with other comorbidity diseases such as irritable bowel syndrome, rheumatoid diseases, migraines, or chronic fatigue syndrome [25, 26]. Furthermore, FM might be considered a subgroup at one end of the pain spectrum. Compared to CWP patients, FM patients experience more severe symptoms with higher pain intensities that affect daily life management [27, 28].

The general treatment of CWP/FM is symptomatically based and includes both pharmacological options (e.g., standard analgesics and antidepressants if needed) and non-pharmacological options. The non-pharmacology strategies are often addressed in a multimodal and person centred setting, including psychological/behavioural therapies, education, workplace interventions and different exercise interventions.

Figure 1. Location of tender points according to ACR criteria 1990. 1 and 2) Low cervical, bilateral at the anterior aspects of C5-C7. 3 and 4) Second rib, bilateral at the second costochondral junctions. 5 and 6) Greater trochanter: bilateral, posterior to the trochanteric prominence. 7 and 8) Knees: bilateral, at the medial fat pad proximal to the joint line. 9 and 10) Occiput: bilateral, at the suboccipital muscle insertions. 11 and 12) Trapezius, bilateral, at the midpoint of the upper border. 13 and 14) Supraspinatus: bilateral, above the scapula spine near the medial border. 15 and 16) Lateral epicondyle: bilateral, 2 cm distal to the epicondyles. 17 and 18) Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle.

IASP has assigned 2020 the “Global Year to the Prevention of Pain” (<https://www.iasp-pain.org/GlobalYear>). This theme focuses on two prevention strategies: exercise and nutritional management. This theme reflects the importance of exercise interventions for chronic pain management and as it is the first line treatment option for CWP/FM [29]. Many reports and studies have noted that CWP/FM leads to physical inactivity [30], reduced muscle strength [31, 32], and fear-avoidance behaviours towards exercise, with potential worsening of symptoms, especially pain intensity [33, 34]. In addition, exercise has been

shown to have positive effects on pain intensity, muscle strength, fatigue, quality of life and physical function, all common facets of CWP/FM [35-40].

Although, CWP/FM is a complex pain condition, its etiology behind it is poorly understood. Today, the diagnosis is based on clinical assessments and symptoms and there is a lack of mechanisms-based diagnosis in CWP/FM. One reason for this is the fact that the pathophysiology and molecular mechanisms behind CWP/FM are not fully elucidated. A pathophysiology and molecular mechanism approach to CWP/FM would provide more useful information with respect to diagnosis and treatment.

Peripheral and central sensitization

Pain is transmitted via complex peripheral and central signaling pathways, including the central nervous system (CNS) and is interpreted by the individual's physical, emotional and mental states [41, 42]. In brief, the activation of nociceptors can be initiated by tissue damage and local inflammation, a process that involves several compounds such as prostaglandins, bradykinin, serotonin, and cytokines [43]. The afferent fibers transmit the signals to the dorsal horn and activates secondary neurons, which are further transmitted to the CNS. The pain signal is processed by differed areas in the brain which is sometimes referred to as the "the pain matrix", and include areas of the limbic system and the thalamus [44]. In peripheral sensitization, the threshold for activating nociceptors are lowered, leading to hyper responsiveness. The IASP taxonomy defines central sensitization as: "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input", which in chronic pain states are expressed as hypersensitivity [45]. Indirectly, central and peripheral sensitization are implied to be involved in the concept of allodynia (a stimuli that provoke pain which under normal conditions are not painful) and hyperalgesia (a painful stimuli that provokes more pain then during normal conditions) [45]. Neurobiological factors such as peripheral and central sensitization as well as other factors of the biopsychosocial model for instance psychological and social factors appears to be involved in development of and maintenance of chronic pain.

Biopsychosocial model

As all individuals are unique, the experience of pain is unique. Pain is described in biological terms as central and peripheral sensitization as well as influenced by numerous factors that could be explained to some extent by the concepts of the biopsychosocial model. This model considers the biological, psychological and social factors that can influence pain [46]. Biological factors such as diseases severity, nociception, inflammation, genetics, and brain function, could all be possible biomarkers. Examples of psychological factors are catastrophizing thoughts, anxiety/depression, coping, and stress. Social factors include

economics, social interactions, cultural factors, and lifestyle [46]. These three factors contribute themselves and in combination to an individual's experience of pain. This thesis investigated two of the factors associated with CWP/FM - biological and psychological factors.

Biomarkers of CWP/FM

CWP/FM is complex and the pathophysiological and molecular mechanisms behind it are not fully elucidated. There is a lack of mechanism-based diagnosis in CWP/FM. Some physiological measurements are already used to assess and confirm diagnosis of CWP/FM e.g., ACR criteria hyperalgesia/allodynia, pressure pain thresholds and questionnaires and self-reported pain intensities. Valid objective biological markers (i.e., biomarkers) for diagnosis and individual treatment is lacking.

The definition of a biomarker is *“a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic process, or pharmacologic responses to a therapeutic intervention”* [47]. The application of a biomarker is numerous: for diagnosis, state of disease, screening, drug development/-targets, and discovery of pathophysiological and normal mechanisms [47]. Depending on the target area, a molecular biomarker could be a protein, metabolite, lipid, or a gene specifically detectable in a biofluid or tissue. Effective biomarker should be highly sensitive, specific, easily interpreted, easily accessed (preferable through low invasive techniques), and be inexpensive.

Principles of proteomics

The proteome is defined as a complete set of proteins in any given cell, tissue, or biofluid in a specific organism at a specific time point [48]. The study of the proteome is called proteomics, a terminology first used for over 25 years ago [49]. Compared to the genome, the proteome is constantly interchangeable depending on protein abundance, localization, interaction, function, and post-translational modifications (PTMs) such as glycosylation, acetylation or phosphorylation. In addition, the proteome can be affected by environmental interactions with the genome as well as with other factors such as disease states [49].

To date (October 2020), the human proteome organization (HUPO) has found 17,874 proteins which represents 90.4% of the total human proteome (www.hupo.org downloaded 2020-10-29). Studying the human proteome could provide information about unknown mechanisms in complex disease states and in the long run provide information about the underlying pathophysiology and develop new drug targets for specific diseases.

The omics family is constantly growing as the development of new techniques are moving forward and different areas are being explored from the more traditional flow from gene to protein e.g., genomics, transcriptomics, and proteomics, to smaller molecules and lipids, which are covered by metabolomics and lipidomics. Protein modifications can be studied by phosphomics or glycomics and even interaction between proteins, genes, and other small molecules could be achieved through the study of interactomics.

A search in PubMed using “pain” resulted in 850,484 articles and a search in PubMed using “omics” resulted in 14,837 articles. Hence, combining these two search terms to “painomics” generated only five articles. This thesis explores proteomics, more specifically the plasma proteome of women with CWP/FM and related clinical characteristic, which are included in the concept of **painomics**.

Proteomics are known for being exploratory and hypothesis-generating research, as hundreds to thousands of proteins are analyzed simultaneously. There are two well established proteomic methods that are used in biomarker research: two-dimensional gel electrophoresis (2-DE), and liquid chromatography-mass spectrometry (LC-MS/MS) shotgun proteomics.

Two-dimensional gel electrophoresis

For almost three decades ago, the methodology of high-resolution 2-DE was described by O’Farrel and Klose, investigating the proteome of *Escherichia coli* and mouse tissues and serum, respectively [50, 51]. This method is a gel-based protein separation technique which separates proteins in two dimensions: first according to a protein’s isoelectric point (pI) and then in the second dimension according to a protein’s molecular weight (MW) [52, 53].

In the first dimension, which is referred to as isoelectric focusing (IEF), denaturated complex protein mixtures are separated according to their pI , which is refers to a protein’s net charge of zero. This process is performed on a thin polyacrylamide gel that contains an immobilized pH gradient (IPG) at a pH between 3 and 12 with lengths of 7, 18 and 24 cm. By applying a voltage for several hours, proteins are electrophoretic separated, making them migrating to their respective pI within their specific pH range [53, 54]. To keep the proteins denaturated and reduced and to aid transferring of proteins, the IPGs are pre-treated before the second dimension with dithiothreitol (DDT) and this is followed with alkylation using iodoacetamide (IAA). In the second dimension, the IPG is mounted onto a large SDS-polyacrylamide gel; the applied voltage then causes the proteins to migrate into the gel and separate according to their molecular weight. Higher MW proteins migrate to the top of the gel and low MW proteins will migrate to the bottom of the gel [53, 55]. The second dimension could either be performed in a horizontal or vertical setup. The vertical setup has the capability to run six to 12 gels simultaneously, whereas horizontal setup can only run one gel at a time. When the migration of the denaturated proteins is achieved, different staining

protocols can be applied to visualize the current proteome pattern. The fluorescent SYPRO Ruby dye has been used throughout this thesis [56]; however, there are other staining dyes available such as silver nitrate and Coomassie Brilliant Blue. The advantages of SYPRO Ruby are minimal background staining, high dynamic range, and highly compatible with downstream protein identification methods such as mass spectrometry [57].

Following protein staining, gels are scanned with a charged coupled device camera. If SYPRO Ruby is used, visualization of the stained protein pattern is performed by ultraviolet (UV) illumination scanning. The scanned gels are then processed by computer programs with different algorithms for protein spot detection and quantification such as PDQuest, MELANIE, Progenesis Workstation, Z3, ProteomeWeaver, or DeCyder [58]. In this thesis, the PDQuest software has been used, and the quantified data retrieved from the program are based on the optical density of individual detected protein spots, which is further used in the statistical analysis.

Before protein identification, proteins of interest are excised from the gel and subjected to proteolytic digestion. This is achieved by commercially available proteolytic enzymes, where Trypsin and Lys-C are commonly used. The digested peptides are then extracted from the gel and downstream protein identification using matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) MS and/or LC-MS/MS can be used. The principle for these two techniques is described briefly below.

Matrix assisted laser desorption/ionization-time of flight MS

In part, this thesis uses MALDI-TOF MS to identify proteins. The MALDI-TOF MS, consists of a high energy laser (N_2), which is pulsed on the root of the crystalized peptide matrix. The high energy pulses result in the peptides to evaporate and ionize. The ionized peptide then travels in an electric field away from the target steel plate into a flight tube under high vacuum. In the flight tube, the time it takes for the peptide to travel from the plate to the target detector is used to calculate the mass-to-charge ratio (m/z), which corresponds to the size of each peptide. Since small ions travel faster due to lower m/z , they will hit the detector before larger ions [59]. This generates a list of several m/z peaks, which then are searched against known protein databases, such as the Swissprot database using the Protein Prospector MS-Fit search engine (<http://prospector.ucsf.edu>).

Shot-gun proteomics

The principle of LC-MS/MS based proteomics is described below.

The digested sample containing a complex mix of peptides, is initially separated on a column by a reversed-phase high pressure liquid chromatography (HPLC) system. The column can be of various length, diameters, and packed materials (e.g., C18 and silica). The peptides entering the column will bind according to their hydrophobicity and will further be eluted by an increasing concentration of

organic solvent - e.g., acetonitrile (ACN) - to create a gradient. When the optimum concentration of ACN arrives, the adherence between the column surface and the peptide will break, which results in eluting the peptides. The more hydrophile peptides will be eluted first, as hydrophobic peptides adhere and bind stronger to the column.

Electrospray ionization (ESI) is used to ionize peptides. By applying high voltage, the eluted peptides are sprayed out at the end of the capillary tip, which generates positively charged droplets. These charged droplets containing the peptides are then delivered into the mass analyzer for further analysis [60]. First, a pre-scan, also referred to as MS scan 1 (MS1) of charged peptides (precursor ions), is performed in the orbitrap, and the most intense peaks (depending on the set-up mass range), are further fragmented using collision-induced disassociation (CID) or higher energy collisional dissociation (HCD) – that is, the charged peptide in gaseous form is collided and fragmented into different ions (daughter ions), which refers to MS scan 2 (MS2). The fragmented ions and precursor ions and their generated spectra containing information about m/z and intensity can then be compared to online databases and proteins can be identified [61].

When using LC-MS/MS, which is described briefly above, in MS1 (where the precursor ions are analyzed), a predefined m/z range is set (e.g., 300-1500). This set-up allows the MS instrument to analyze all peptides within this specific range. The fragmentation will then be performed on the ions with highest intensity. This forms a collection of data called data-dependent acquisition (DDA). Another set-up, data independent acquisition (DIA), is based on fragmenting all precursor ions within the specific m/z range window (i.e. not only choosing the ones with highest intensity) and works in a cycle-based manner and scans the entire mass range set in MS1 [62, 63].

The resulting m/z spectrum generate large data sets that need data processing to identify and quantify the peptides using existing protein databases (e.g., Uniprot). This can be achieved by using the software MaxQuant (for DDA analysis) or Spectronaut (for DIA analysis) [64, 65].

Multivariate data analysis and bioinformatics

Proteomics methods generate large data sets no matter whether they originate from 2-DE or LC-MS/MS analysis, which is difficult to process with traditional statistical methods such as Mann Whitney U or Student T-tests. These data sets often contain hundreds to thousands of variables (e.g., proteins) but few observations. Standard correlation methods have difficulties handling missing data and multicollinearity problems, so it assumes that variables are independent of one another. When investigating biological proteins with known interaction and functions, the “independence” between the proteins is challenged. To overcome this problem, advanced multivariate data analysis (MVDA) can be used, as MVDA can

deal with large data sets, missing values, and assumes and take advantage of exciting intercorrelations between investigated variables.

MVDA can roughly be divided into three groups: 1) supervision and overviewing of data using principal component analysis (PCA); 2) regression modelling using orthogonal partial least square (OPLS) analysis with two blocks of data: block one including a y-variable (e.g., clinical data from patients) and block two containing the x-variables (e.g., proteins); and 3) classification of discrimination between two or more groups (e.g., patients and controls) using orthogonal partial least square discriminant analysis (OPLS-DA) [66].

PCA, is an unsupervised method often used to discover systematic variance or structures in a data set. It displays how the observations (patients or controls) are either related or unrelated to one another and observed x-variables. It includes the Hotelling T2 and DmodX tests which are used to determine potential outliers. The OPLS modelling focus on x-variables that are related to a specific Y-variable e.g., a clinical variable to a specific group. The OPLS-DA modeling is a supervised method focusing on x-variables that can discriminate between two or more known groups. In the models, the x-variables are expressed as loadings. The higher the loading, the more important for the projection. Another variable that is often used is the variable important for projection or variable influence on projection (VIP). VIP describes the importance and relevance of each x-variables pooled over all dimensions and Y variables (patients and controls), which includes a set of variables that best explain Y. The higher the VIP-value, the more important regressor [66, 67]. A $VIP \geq 1$ is considered as a significant value and regarded as important for the model outcome. The loadings can also be scaled into a correlation coefficient, expressed as $p(\text{corr})$, which range from -1 to +1, the closer to 1 to stronger correlation. To validate a MVDA model, there are several parameters that needs to be taken into account especially when working with omics data [67]. First number of principal component (PC) and orthogonal components (OC) needs to be described. To validate the significance of each model, a cross-validated analysis of variance (CV-ANOVA) is used, where a P -value < 0.05 is considered significant. R^2 - *the goodness of model fit*, is a parameter that indicates how well the model explains the investigated data set (e.g., a R^2 of 0.80 explains 80% of the data). Q^2 – *goodness of prediction*, a cross-validated fraction of the data set that is used to measure the prediction of the model. Hence, how well the model can predict a new dataset. If R^2 is higher than Q^2 , it implies over-fitting, and the difference should not be greater than 0.2-0.3. [66, 67].

Bioinformatics

Classifying the biological function of proteins can be difficult and time consuming, and there are several databases available for this purpose. The UniProt database (www.uniprot.org) contains open source information about biological functions, processes, genes, taxonomy data, amino acid sequence, cross references etc. about proteins. Several other bioinformatic tools are available to analyze the

function of proteins and protein interaction networks such as Cytoscape, the Search Tool for Retrieval of Interacting Genes/Proteins (STRING), and Ingenuity Pathway Analysis (IPA) [68-70], which are widely used in proteomic studies.

Proteomic studies of CWP/FM

Proteomics can be used to study potential biomarkers in different body fluids or tissues such as blood, urine, feces, saliva, cerebrospinal fluid (CSF), muscles and mucosal biopsies. Although proteomics has great potential and is hypothesis generating, relatively few studies have explored the proteome of CWP/FM. In total, 17 published articles, have investigated different proteomes and correlations with clinical characteristics of CWP/FM (Table 1). The majority of these studies have been recently reviewed by Gerdle and Ghafouri [71]. All publications are relatively new and were published between 2009 and 2020. To date, four proteomes have been investigated using either 2-DE or LC-MS/MS techniques: blood [72-78], saliva [79-81], CSF [82-85], and muscle tissue [86-88] (Table 1). Of these publications, only seven (three of them are included in this thesis) focused on the plasma/serum proteome of CWP/FM. As noted, the most studies used the ACR 1990 criteria, but two serum proteome studies used the ACR 2010 criteria [77, 78]. Group differences comparing the proteome between CWP/FM and healthy controls dominate the proteome studies, but correlation of plasma proteome and clinical characteristics have been investigated in just a few [72-74, 77-79, 81]. Based on the plasma/serum proteomic studies by Ruggiero et al. [77], Wåhlén et al. (**paper I**) [76] and Ramirez-Tejero et al. [75], Gerdle and Ghafouri performed a protein network analysis to explore enriched protein pathways [71]. They found two clusters of proteins, one mainly involving proteins of cellular regulation of protein metabolic process and PTMs. The second cluster revealed proteins involved complement system. Overall, several of the investigated proteins in the network were involved in the immune system [71]. Han et al. recently investigated the serum proteome of FM compared to healthy controls [78]. They found similar results as Ramirez-Tejero et al. [75] with several proteins involved in inflammatory and coagulation pathways. In addition, inflammatory, metabolic, and immunity processes were also found in the most recent plasma proteomic study of FM compared to healthy controls (**paper III**) [72].

Table 1. Overview of proteomic studies of CWP/FM

Authors	Biofluid/ tissue	Number of CWP/FM and CON	Sex	ACR criteria	Proteomic method	Main variables analyzed
Blood						
Ruggiero et al. [77]	Serum	FM = 16 CON = 12	F	2010	2-DE	Group comparison
Han et al. [78]	Serum	FM = 20 CON = 20	F	2010	LC-MS/MS	Group comparison, clinical variables, heart rate variability
Wählén et al. [76] (paper I)	Plasma	CWP = 16 CON = 23	F	1990	2-DE	Group comparison
Ramirez-Tejero et al. [75]	Plasma	FM= 12 CON = 12	F	1990	LC-MS/MS	Group comparison
Wählén et al. [74] (paper II)	Plasma	CWP = 15 CON = 23	F	1990	2-DE	NRS, HADS total
Gerdle et al. [73]	Plasma	CWP = 15 CON = 23	F	1990	2-DE	PPT
Wählén et al. [72] (paper III)	Plasma	FM = 30 CON = 32	F	1990	2-DE	Group comparison, VAS, HADS total, PPT
Saliva						
Bazzichi et al. [81]	Whole saliva	FM = 22 CON = 26	F	1990	2-DE	Group comparison, clinical variables
Bazzichi et al. [80]	Whole saliva	FM = 40	F and M	1990	2-DE (pooled samples)	Comparison of Balneotherapy and mud bath intervention
Ciregia et al. [79]	Whole saliva	FM = 30 CON = 30	F and M	1990	2-DE (pooled samples)	Group comparison, clinical variables

CSF						
Olausson et al. [85]	CSF	CWP = 12 CON = 13	F	1990	2-DE	Group comparison
Lind et al. [82]	CSF	FM = 40 Two CON groups: Healthy CON = 11 Minor urology surgery CON = 28	FM = F CON = F and M	1990	Antibody suspen- sion bead arrays, Immunocapture - MS	Group comparison
Khoonsari et al. [83]	CSF	FM = 13 CON (other neurological diseases) = 8	F	1990	LC-MS/MS	Group comparison
Khoonsari et al. [84]	CSF	FM = 39 CON = 38	FM = F CON = F and M	1990	LC-MS/MS	Group comparison
Muscle						
Olausson et al. [88]	Dialysate from trapezius muscle	CWP = 18 CON = 22	F	1990	2-DE	Group comparison
Olausson et al. [87]	Trapezius muscle	CWP/FM = 18 CON = 19	F	1990	2-DE	Group comparison
Olausson et al. [86]	Trapezius muscle	CWP/FM = 18 CON = 19	F	1990	2-DE	NRS and PPT
F = Females, M = Males, CSF = Cerebrospinal fluid, FM = Fibromyalgia, CWP= Chronic widespread pain, CON = Controls, PPT = Pressure pain thresholds, VAS = Visual Analogue Scale, NRS = Numeric Rating Scale, 2-DE = Two-dimensional gel electrophoresis, LC-MS/MS = Liquid chromatography - mass spectrometry						

The plasma proteome

Common for all four papers included in this thesis is the plasma proteome. To get an overview of the composition of proteins present systemically and their overall function in the body, follows here a brief description of its components.

The blood consists of water, salts, ions, gases, lipids, proteins, metabolites, peptides and different types of cells such as erythrocytes, lymphocytes, and thrombocytes. Plasma is the liquid fraction of blood and the first plasma proteome was reported using 2-DE in 1977 by Anderson and Anderson [89]. Since then, both 2-DE and LC-MS/MS techniques have developed, and now high-throughput plasma proteome profiling are performed, which can identify thousands of proteins [90]. Plasma is routinely screened clinically e.g., during infection, hormone deficiency and drug monitoring, where known concentration intervals of specific proteins are established.

Plasma proteins can be divided into three major classes: 1) functional proteins that are highly abundant such as albumin, apolipoproteins, coagulation proteins and complement proteins; 2) tissue leakage proteins such as enzymes that have no assigned function in the circulation; and 3) signal proteins such as cytokines, chemokines and hormones. These three classes can be further divided into categories based on their function: proteins secreted by tissues (e.g., liver and intestines) and function in the circulation; immunoglobulins (antibodies); receptor ligands (peptides, hormones, cytokines, and chemokines); tissue leakage products (function is normal inside cells and is released during tissue damage e.g., troponins, creatine kinases); abnormal secretion proteins (tumor proteins); and foreign proteins (arising from infections as a results of viruses, bacteria, or parasites) [91].

Of interest in this thesis, are the proteins that have a function in the circulation. Albumin dominates the human plasma proteome followed by immunoglobulins, apolipoproteins, complement proteins, blood coagulation and fibrinolytic proteins. Several of these protein groups are known as acute-phase proteins which are increased or decreased during inflammation [92, 93].

As expected, no single method can cover the whole plasma proteome. The dynamic range of the plasma proteome is large, ranging from ten orders of magnitude [90, 91]. Hence, due to the high dynamic range of protein content, the plasma proteome presents not only analytical challenges but also great opportunities to investigate disease mechanisms and potential biomarkers suitable for use in the clinic.

AIMS

This thesis investigates the molecular mechanisms and potential biomarkers for chronic pain in women with CWP/FM. In addition, this thesis evaluates whether common pain characteristics such as pain intensity, sensitivity, and psychological distress in CWP/FM correlates with specific molecular protein profile in blood.

The general hypothesis is that there are differences in the plasma proteome in women with CWP/FM compared to healthy controls. Furthermore, the systemic protein changes are correlated with pain intensity, sensitivity, and psychological distress in CWP/FM.

The specific aims for each paper were:

- Paper I:** To analyze the protein pattern, and potential new biomarkers, in plasma samples from women with CWP compared to controls.
- Paper II:** To investigate the correlation between pain intensity, psychological distress, and plasma proteins among women with CWP compared to controls.
- Paper III:** To investigate the molecular pattern of plasma proteins and their correlation to pain intensity, sensitivity, and psychological distress in women with FM compared to controls.
- Paper IV:** To investigate the proteome of plasma in FM before and after a 15 weeks of resistance exercise intervention and to determine whether any of the clinical and exercises-related variables correlated with plasma proteins.

MATERIALS AND METHODS

Description of cohorts

Ethical approvals and consents

All participants in both cohorts were given written and oral information about the objectives, procedures, and sample collection and returned a signed written consent form for their participation. For cohort I, ethical approval was permitted by the regional ethical review board in Linköping, Sweden (Dnr: M10-08, M233-09, Dnr: 2010/164-32). For cohort 2, which is a part of a larger randomized controlled multicenter trial (Clinicaltrials.gov NCT01226784, 21 October 2010), was approved by the Ethical Review Board at Karolinska Institutet in Stockholm (Dnr: 2010/1121-31/3). All studies were conducted in accordance with the Helsinki Declaration and Good Clinical Practice. All participants were compensated economically for their participation in the studies.

Cohort I

Paper I and II, investigated a cohort consisting of women with CWP (the majority with a confirmed FM diagnosis) and healthy controls. The CWP/FM participants were recruited via the pain and rehabilitation Center in Linköping, Sweden or by advertisement and recruitment via an FM patient organization. The healthy controls were recruited via local newspaper advertisements. The recruitment process started in 2010 and ended in 2012. For primary screening of interested participants, a self-reported pain questionnaire assessing musculoskeletal symptoms conducted by the Nordic Ministry Council Questionnaire was used [94] in combination with a structured telephone interview to check for eligibility. Participants were then further referred for a clinical examination where they received a standardized and validated examination of the upper extremities [11] and confirmation of CWP/FM diagnosis using the ACR 1990 criteria. In the original study, the screening process resulted in recruitment of 19 CWP/FM and 24 control participants [95, 96], but due to difficulties retrieving blood samples or not enough sample for proteomic analysis, the final inclusion was 16 CWP/FM and 23 control participants (**paper I and II**).

Cohort II

Paper III and IV investigated a cohort consisting of women with FM and healthy controls. These two studies are part of a large multicenter RCT study (Clinicaltrials.gov NCT01226784, October 21, 2010), with the main goal to investigate the effects of resistance exercise and relaxation therapy in FM. **Paper III and IV**,

included a subgroup of these participants. Recruitment of study participants in the original RCT started in 2010 and ended in 2013, and involved three centers in Sweden, located in Stockholm, Gothenburg, and Linköping. A total of 402 women with FM showed an interest in participating in the study. In the primary screening process, which consisted of telephone interviews, these women were assessed for eligibility using the inclusion and exclusion criteria before proceeding to medical examinations. A total of 177 women were eligible for the study and were further examined by an experienced physician to verify the ACR 1990 criteria. Of these 177 women, 130 with FM fulfilled the inclusion criteria for the study [37, 97]. The healthy controls were recruited via local newspaper advertisements in respective city and 182 women showed their interest in participating in the study. After telephone screening, 150 participants were eligible for medical examination and 137 of these were included as controls [98].

For the FM group, randomization was performed at each center to one of two intervention groups: 67 FM performing resistance exercise and 63 FM performing relaxation therapy. For the control group, 32 were randomized to perform resistance exercise and 105 were used as baseline controls [98]. In **paper III**, 32 plasma samples from FM and 30 from controls were available for baseline gel-based proteomic analysis. In **paper IV**, LC-MS/MS-based proteomics of 40 plasma samples from FM and 25 controls at baseline were available for analysis. After 15 weeks of resistance exercise, 21 plasma samples from FM and 24 plasma samples from controls were available from both time points for LC-MS/MS based proteomics.

Resistance exercise intervention program

The resistance exercise program focused on large muscle groups located in the lower extremities, trunk, and arms, and has been reported in a previous publication [37]. The intervention was person-centered, and each participant had a personalized meeting with a physiotherapist before start of the intervention to go through and practice each exercise assignment. The program initially started with low loads to avoid possible side effects. An estimation of one maximum repetition (1RM) was performed, which is the maximum number of repetitions until perceived exhaustion. The program started at a baseline of 40% of 1RM for 15-20 repetitions for 1-2 sets. After 3-4 weeks, an evaluation was made to increase loads or continue with the same loads. If deemed suitable, the loads were increased to 60% of 1RM for 10-12 repetitions for 1-2 sets. At week 6-8, 80% of 1RM for 5-7 repetitions for 1-2 sets was performed.

The resistance program consisted of ten minutes warm-up (on stationary bike or cross trainer), followed by 50 minutes of diverse exercise assignments. Between each set, there was one minute recovery period. The exercises were performed on either weight machines or free weights for leg-press, knee-extension, knee-flexion exercise, and biceps curl and hand grip strength, respectively. Heel raises and core stability was performed with body weight. Each exercise session ended

with ten minutes of stretching exercise. After five weeks of training, explosive strength was added, and after eight weeks rapid heel raises, and explosive knee-extensions were introduced into the training protocol.

Comparison of cohorts

These two cohorts have several features in common. The ACR 1990 criteria for diagnosing CWP/FM were used and all participants were women between 20 and 65 years old. To get an overview of the clinical picture of these two cohorts, background data were collected concerning age (20-65), sex (only females have been included for both CWP/FM and controls), height, weight, BMI, pain duration, and number of tender points. In addition, several Swedish validated questionnaires were included in both cohorts, that included questions about self-reported pain intensity (NRS or VAS), psychological distress (HADS total), pain catastrophizing (PCS), and Quality of life (QoL instrument, only **paper I** and **II**), or Fibromyalgia Impact Questionnaire (FIQ, **only paper III** and **IV**). In both cohorts, PPT was measured on different body sites. In **paper I** and **II**, a mean value of both left and right side of the trapezius muscle was used (results published in additional paper and not included in thesis [73]). In **paper III** and **IV**, PPT measurements were performed over eight of the 18 tender points, and a mean value of all PPT sites are presented and used in statistical analysis. Table 2 provides a comparison of the background data and shared clinical characteristics of the two cohorts and individual papers. The following section describes briefly the included self-reported instruments used.

Table 2. Comparison of background and shared clinical characteristics in cohort I (paper I and II) and cohort II (paper III and IV)

Variables	Cohort I				Cohort II			
	Paper I		Paper II		Paper III		Paper IV	
	CWP (n = 16)	CON (n = 23)	CWP (n = 15)	CON (n = 23)	FM (n = 30)	CON (n = 31)	FM (n = 40)	CON (n = 25)
	Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD	
Age (years)	47.4 $\pm$ 11.3	41.0 $\pm$ 10.2	49.2 $\pm$ 8.9	41.0 $\pm$ 10.2	52.2 $\pm$ 8.9	54.4 $\pm$ 8.1	50.2 $\pm$ 9.8	47.4 $\pm$ 12.6
BMI (kg/m ²)	25.8 $\pm$ 4.9	24.0 $\pm$ 2.8	26.0 $\pm$ 5.0	24.0 $\pm$ 2.8	27.1 $\pm$ 5.0	24.6 $\pm$ 4.4	26.9 $\pm$ 4.4	25.1 $\pm$ 4.8
Pain/FM duration (years)	12.5 $\pm$ 8.3	-	12.9 $\pm$ 8.4	-	13.0 $\pm$ 8.4	-	11.4 $\pm$ 7.9	-
Tender points (number)	-	-	-	-	16.0 $\pm$ 1.0	-	15.6 $\pm$ 2.1	-
Pain intensity (VAS)	-	-	-	-	50.2 $\pm$ 20.9	2.5 $\pm$ 6.7	52.1 $\pm$ 20.9	0.2 $\pm$ 0.9
Pain intensity whole body (NRS)	4.6 $\pm$ 2.3	0.0 $\pm$ 0.0	4.9 $\pm$ 2.0	0.0 $\pm$ 0.0	-	-	-	-
PPT mean measured sites (kPa)	251 $\pm$ 127	529 $\pm$ 90	247 $\pm$ 130	529 $\pm$ 90	184 $\pm$ 71	339 $\pm$ 105	154 $\pm$ 66	355 $\pm$ 106
FIQ total	-	-	-	-	60 $\pm$ 14	6.0 $\pm$ 7.8	60.3 $\pm$ 15.6	6.0 $\pm$ 6.3
HADS total (psychological distress)	13.3 $\pm$ 5.9	3.3 $\pm$ 2.8	14.0 $\pm$ 5.3	3.3 $\pm$ 2.8	14.1 $\pm$ 7.5	4.3 $\pm$ 4.3	15.9 $\pm$ 8.5	4.6 $\pm$ 4.6
PCS	12.5 $\pm$ 6.9	6.7 $\pm$ 6.4	13.0 $\pm$ 7.5	6.7 $\pm$ 6.4	19.8 $\pm$ 11.5	6.2 $\pm$ 10.7	19.6 $\pm$ 11.8	7.1 $\pm$ 9.6
QoL	86.6 $\pm$ 13.6	93.1 $\pm$ 9.7	82.5 $\pm$ 13.1	93.1 $\pm$ 9.7	-	-	-	-
Diagnosed with FM (n)	14	-	13	-	30	-	40	-

FM = Fibromyalgia, CWP = Chronic widespread pain, CON = Controls, BMI = Body mass index, VAS = Visual Analogue Scale, NRS = Numeric Rating Scale, PPT = Pressure pain thresholds, FIQ = Fibromyalgia Impact Questionnaire, HADS total = the Hospital Anxiety and Depression Scale total score, PCS = Pain Catastrophizing Scale, QoL = Quality of Life.

Included instruments to assess clinical characteristics in CWP/FM

Throughout **paper I-IV**, several self-reported instruments have been used. A summary of these results are presented in Table 2. For statistical results, see respective paper. In **paper I**, self-reported instruments were used to describe the background and the clinical picture of the cohort. In **paper II**, regression models of pain intensity (NRS) and psychological distress (HADS total) with plasma proteins were performed. In **paper III**, regression models of pain intensity (VAS global), pain sensitivity (PPT), psychological distress (HADS total), and plasma proteins were performed. In **paper IV**, the focus was on exercise in FM, and post exercise regression models of clinical variables, exercise-related variables and plasma proteins were analyzed.

The Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a self-reported instrument, consisting of 14 items, divided into two sub-scales, one focusing on questions concerning symptoms of anxiety (HADS-A), and one scale on depressive symptoms (HADS-D). Each scale consists of seven items graded on a four-point Likert scale with the range of 0-3, where 0 equals “absent symptom” and 3 equals “severe or persistent presence of symptom” [99, 100]. Each subscale ranges from 0-21 and they are summed to reflect symptoms of anxiety and depression - the higher value, the more anxiety or depressive symptoms. The cut-off values for classifying each subscale of anxiety and depression according to the HADS instrument are 0-7 (non-cases), 8-10 (doubtful cases), and 11-21 (cases) [100]. In both cohorts, the Swedish validated instrument for HADS was used [99]. In **paper II, III**, and **IV** the sum of both subscales has been used and are reported as HADS total (ranging 0-42) [101]. This is also defined as *psychological distress* in **paper II, III** and **IV**. The cut-off values for psychological distress in the three papers are <14 (normal), mild (15-20), and moderate (>21).

Pain intensity

Pain intensity is one of the fundamental traits in CWP/FM. One way to measure this aspect is by the different scales Numeric Rating Scale (NRS) or Visual Analogue Scale (VAS). Both scales are used for self-reported pain intensity. NRS consists of a 11-grade scale ranging 0-10 (0 = no pain and 10 = worst possible pain) [102]. The VAS scale consists of a 100 mm line (0 = no pain and 100 = worst possible pain) [103]. In **paper II, III** and **IV** whole body pain intensity is reported using either the NRS or VAS scale. Cut-off points for these pain intensity scales were used in **paper II** and **III**: mild (0-3/0-44 NRS/VAS), moderate (4-6/45-74, NRS/VAS), and severe pain intensity (7-10/75-100 NRS/VAS) [103, 104].

Pain sensitivity

In CWP/FM, generalized pain is one of the main clinical facets and increased pain sensitivity (i.e. lowered thresholds for e.g. mechanical pressure) is often present [73, 105, 106]. Clinically, pain sensitivity is assessed by either manual palpation the tender points or is semi-objectively measured on specific tender points or other muscles by using an electronic pressure algometer [107]. During the measurements of pressure pain thresholds (PPT), a mechanical stimulus is introduced through a probe (area 1 cm²), which is held vertically to the skin and pressed with a constant pressure of 50 kPa/s. This will induce a sensation of pressure. As soon the sensation of pressure is experienced as a painful stimulus, the participant terminates the procedure by pushing a stop button. If the participant does not push the button when the maximum pressure is reached (600–700 kPa), the examiner terminates the procedure. In either case, the examiner removes the algometer once the procedure is terminated. In **paper III** and **IV**, algometry was performed on eight of the 18 tender points defined by ACR 1990 criteria at the following sites: bilaterally over the supraspinatus muscle (at origin above the scapula spine near the medial border), the lateral epicondyle (2-cm distal to the epicondyles), over the gluteus maximus (in upper outer quadrants of buttocks in anterior fold of muscle), and inside of the knee (at the medial fat pad proximal to the joint line). To get an overall view of pain sensitivity, the mean value from all eight sites was used in all analysis, and PPT values < 200 kPa were regarded as very low pain sensitivity.

Fibromyalgia Impact Questionnaire

Fibromyalgia Impact Questionnaire (FIQ) is a self-reported instrument used to assess FM-related symptoms and severity. The FIQ consists of ten subscales, including questions about symptoms related to performing large muscle tasks, well-being, ability to perform work, pain intensity, tiredness in the morning, stiffness, anxiety, and depression [108]. The Swedish validated version has been used in **paper III** and **IV**. The total score for each question ranges from 0-100, and the mean from all ten subscales are summed in a FIQ total score - the higher score, the more FM-related symptoms, indicating lower health status [109].

Quality of Life instrument

The Quality of Life (QoL) instrument is a self-reported questionnaire consisting of 16 items regarding questions concerning health, relationships, material comforts, learning, socializing, and independence. The Swedish validated version has been used in **paper I** and **II**. The total score is summed from each item, ranging from 16-112 - a higher score indicates higher QoL and satisfaction [110, 111].

Pain Catastrophizing Scale

The Pain Catastrophizing Scale (PCS) is a self-reported instrument consisting of 13 items divided into three subscales that measures rumination, helplessness and magnification [112, 113]. In **paper I, II, and IV**, the total score of each subscale has been summed, ranging from 0-52 - a higher value indicates worse catastrophizing thoughts.

The Multidimensional Fatigue Inventory

Fatigue is one of common symptoms in CWP/FM and is assessed using the Multidimensional Fatigue Inventory (MFI) instrument. The MFI represents different dimensions of fatigue, and consists of 20 items covering general, physical, and mental fatigue and reduced motivation and activity [114, 115]. Each subscale ranges from 4-20. In **paper IV**, each dimension is reported - a higher score indicates more fatigue.

Chronic Pain Acceptance Questionnaire

Chronic Pain Acceptance Questionnaire (CPAQ) measures acceptance of pain and consists of 20 items divided into two subscales covering questions about activity engagement and willingness to experience pain [116]. In **paper IV**, the total CPAQ score is used, which is the sum of each item ranging from 0-6. The total CPAQ score ranges from 0-120 - a higher score indicates a higher degree of pain acceptance.

Proteomics

All four of the included papers in this thesis used proteomics to explore the plasma proteome in women with CWP/FM and healthy controls. Below is a description of the proteomic flow from the sample preparation to identified proteins used in **paper I-III** (2-DE) and **paper IV** (LC-MS/MS).

Sample collection

In all four papers, venous blood samples were collected in EDTA tubes and then centrifuged in either 1000 g for 15 mins (**paper I and II**) or 1500 g for 30 mins at room temperature (**paper III and IV**). After centrifugation, the plasma fraction was collected, aliquoted, and stored at a minimum of -70 °C until analysis.

2-DE

In **paper I-III**, 2-DE was used to study the plasma proteome of women with CWP/FM and healthy controls and related clinical characteristics.

Sample preparation

Before sample preparation, all samples were blinded. Albumin and immunoglobulin G (IgG) were depleted from each individual plasma sample using the ProteoPrep Immunoaffinity Albumin and IgG depletion kit (Sigma-Aldrich Co, St Louis, MO, USA) by adding 40 µl of crude plasma to the spin column following the instructions from the manufacturer's protocol. Protein concentration was then measured after depletion using Bradford, and the depleted sample was then desalted using PD-10 column (GE Healthcare, Little Chalfont, UK). Before lyophilization, to control for protein loss, the protein concentration was measured once again with Bradford. The lyophilized proteins were dissolved in 350 µl sample solution buffer containing 8M urea, 2% 3-((3-Cholamidopropyl)dimethylammonio)-1-propanesulfonate hydrate (CHAPS) (w/v), 0.3% dithiothreitol (DTT), 0.5% pharmalyte 3-10 (v/v) and bromophenol blue (BFB). Protein concentration was measured in the dissolved proteins in sample solution buffer, to assure 100 µg of total protein, using 2-D Quant kit (GE Healthcare) according to manufacturer's instructions.

First dimension

One hundred µg of total protein were applied to the 18-cm IPG strip, with the pH range 3-10 nonlinear (GE Healthcare) and run in the Ettan IPGphor 3 IEF system. By applying a voltage, the proteins migrate to their respective pI within their specific pH range. The IEF was performed during 38,000 Vhs overnight. When the electrophoresis was finished, each IPG strip was stored in -86 °C until the run in the second dimension.

Second dimension

Before molecular weight separation in the second dimension, six IPG strips were thawed and prepared simultaneously. First, the IPG strips were equilibrated for 15 mins in SDS solution containing 6 M urea, 4% SDS (w/v), 30.5% glycerol (w/v), 0.5 M trizma-HCl, BFB and 1% DTT to ensure and keep proteins denaturated and reduce disulfide bonds. Second, the IPG strips were equilibrated for 15 minutes in SDS solution including 4.5 % iodoacetamide (w/v) to alkylate the sulfhydryl groups. The second dimension was performed vertically on Ettan DALTsix Electrophoresis Unit (Amersham Pharmacia Biotech, Uppsala, Sweden). Pre-casted homogenous SDS gels containing 12.5% acrylamide (GE healthcare) were used. The IPG strips were mounted above the gels into the gel cassettes and casted in agarose solution to keep the IPGs in place. The electrophoresis was initially run at 80 V, 10 mA/gel for 1 h, followed by an increase to 600 V, 40 mA/gel, until the BFB line reached the bottom of the gel cassette. The run took approximately six hours. Immediately after the gel electrophoresis, each individual gel was put into a fixative solution containing 10% methanol (v/v), 7% acetic acid (v/v)), overnight. The gels were stained using the fluorescent dye SYPRO Ruby (Bio-Rad Laboratories,

Hercules, CA, USA) overnight and then rinsed with water to remove excess dye and prevent speckles. Visualization of the plasma proteome was done by scanning the gel with a charged coupled device camera (VersaDoc Imaging system 4,000 MP; Bio-Rad Laboratories) using UV-scanning illumination mode.

Spot detection and quantification

Scanned pictures of the gels were color inverted before loaded into the spot detection and quantification program PDQuest (Bio-Rad Laboratories). The initial spot detection wizard was used which automatically matches each spot on each gel. However, due to individual differences, manual review of each spot match was performed and corrected if necessary. For a spot to be valid, it had to be present in a minimum of 50% of one group. To account for individual differences in the gels and background, normalization was performed using the total quantity in valid spots. That is, the percentage of the optical density (OD) of the detected spots was determined by measuring the volume of each spot and dividing it by the total volume of all the detected spots of the same gel, multiplied by a scaling factor of 100. Thereby, a list of %OD values for all detected protein spots was generated and evaluated to detect differentially expressed proteins using MVDA.

Protein identification

Two MS techniques were used to identify significant plasma proteins: MALDI-TOF MS and the LC-MS/MS system LTQ Velos Orbitrap. Before MS analysis of each interested protein spot (which were significant from the MVDA analysis in respective paper), the spots were excised from the gel and de-stained from SYPRO Ruby dye using 50% ACN in 25 mM ammonium bicarbonate (AmBic) (v/v), dehydrated with 100% ACN, and dried in SpeedVac. Each protein gel piece was then subjected to proteolytic digestion using trypsin (Promega Biotech AB, Nacka, Sweden) at 37 °C overnight. After the digestion, peptides were extracted with 5% TFA/50% ACN for four hours, and then dried using SpeedVac. Dried peptides were stored at -20 °C until MS analysis was performed.

MALDI-TOF MS

Before MALDI-TOF MS, the peptides were reconstituted with 0.1% TFA. The reconstituted peptide solution was mixed in 1:1 ratio with the matrix solution 2,5-dihydroxybenzoic acid (DHB) dissolved in 70% ACN/ 0.3% TFA and 1 µl was spotted on a stainless target plate. An external peptide calibration standard (Bruker) was additionally spotted on the plate for external calibration. The MALDI-TOF MS (ultrafleXtreme, Bruker Daltonik GmbH, Bremen, Germany) was equipped with 337 nm N₂ laser, operated in positive reflector mode. The mass range was set between 300 and 3500 m/z. The instrument was calibrated using the external calibration standard with known peptides. Acquired MS spectra were pre-processed using flexAnalysis (Bruker) and internally calibrated against trypsin peaks

were performed. The 50-100 highest intensity peaks from each spectra was subjected to the search engine Protein Prospector MS-Fit (v. 5.14.4) using the Swissprot and NCBI databases. The search criteria were set as follows: *Homo sapiens*, mass tolerance 50 ppm, maximum miss cleavages by trypsin ≤ 1 , a minimum peptide required to match was two, fixed modifications were carbamidomethylating of cysteine, and variable modifications were oxidation of methionine, N-terminal glutamine to pyroglutamate, and N-terminus acetylation.

LC-MS/MS

In **paper II** and **III**, low abundant proteins were identified using a high-resolution MS LTQ Orbitrap Velos Pro (Thermo Scientific) coupled to an EASY-nLC system (Thermo Scientific). Proteins of interest followed the same de-staining and tryptic protocol as described above, with the exception that dried peptides were reconstituted in 0.1% FA to aid ionization, and six μ l were loaded onto the preanalytical C18 column (100 mm $\times$ 75 μ M, Agilent technologies). The mobile phase (buffer A) consisted of 0.1% FA (v/v) in MilliQ-H₂O and peptides were separated using a linear gradient increasing from 2 to 90% ACN including 0.1% FA (buffer B) for 30 minutes with a flow rate of 300 nL/min. The Orbitrap was operated in positive mode with a DDA approach. A mass range of 380–2000 m/z and a resolution of 30,000 was chosen for MS1 scan. The top 20 peaks from the full scan in MS1, were selected for fragmentation in MS2 scan. Peaks with +1 charge state were rejected, and the dynamic exclusion was set to 30 s.

The acquired raw files were analyzed with MaxQuant software (version 1.5.8.3) against the human Swissprot/UniProt database (downloaded in April 2017 and April 2018). The following parameters were used as search criteria: enzyme digestion with trypsin; two as the maximum number of missed cleavages; seven as minimum peptide length; 4,600 Da as maximum peptide mass; 4.5 ppm as parent ion mass tolerance; and 0.5 Da as fragment ion mass tolerance. Fixed modification was carbamidomethylation of cysteine, and variable modifications were oxidation of methionine and N-terminal acetylation. Protein false discovery rate (FDR) was set to < 1%, and a minimum of one unique peptide was needed to be considered identified.

Shotgun proteomics

In **paper IV**, the Q Exactive HF-X was used for plasma proteomics at baseline and after the 15 weeks resistance exercise intervention in women with FM and healthy controls. At baseline, 40 FM and 25 control samples were analyzed. Post exercise, only FM and controls performing the 15 weeks resistance exercise intervention and with plasma sample from both time points were analyzed, resulting in 21 FM and 24 controls.

Sample preparation

All sample preparations, protein de-salting, and protein digestion were performed on Agilent AssayMAP Bravo Platform (Agilent Technologies, Inc.). To check the sample preparation and instrument performance, a pool of all plasma samples including all participants were prepared and run simultaneously as a quality control sample (QC). Before sample preparation, plasma samples from each participant were diluted 1:10 with 100 mM AmBic, and ten μ l of each sample were transferred to a 96-well plate (Greiner G650201) and the proteins were denatured and digested as described in **paper IV**. The digested samples were de-salted using AssayMAP C18 cartridges (Agilent, PN: 5190-6532). The peptide concentration was determined using the Pierce Quantitative Colorimetric Peptide Assay (Thermo Fisher Scientific, Rockford, IL, USA) according to manufacturer's protocol. Each sample was spiked with synthetic iRT peptides (JPT Peptide Technologies), which are needed for calibration in DIA analysis and the use of downstream software Spectronaut.

Sample preparation to build library

Two pools were created, including ten random picked up plasma samples from FM at baseline and post exercise. These two pools of plasma samples and the QC sample were first depleted of the top 14 most abundant proteins using High Select™ Top14 Abundant Protein Depletion Mini Spin Columns (Thermo Fisher Scientific) as described by the manufacturer's protocol. The depleted samples were first subjected to protein denaturation and digestion as described above, then fractionated using Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions and as described in **paper IV**. All the fractions were dried by SpeedVac and re-suspended in 2% ACN/0.1% TFA and spiked with iRT peptides before LC-MS/MS analysis.

LC-MS/MS analysis

All individual plasma samples were analyzed on a Q Exactive HF-X MS coupled to Ultimate 3000 UHPLC system (Thermo Fischer Scientific). A two-column setup on the HPLC system was used and 1 μ g of peptides were loaded into an Acclaim Pep-Map 100 C18 precolumn (75 μ m x 2 cm, Thermo Scientific, Waltham, MA) and then separated on an EASY spray column (75 μ m x 50 cm, nanoViper, C18, 2 μ m, 100 Å) with the flow rate 300 nL/min. To elute the peptides, a 90 minute linear gradient from 4 to 38% of 80% ACN in 0.1% FA and 0.1% FA was used as described in **paper IV**.

DIA and DDA analysis

For the individual plasma samples, the Q-Exactive HF-X was operated in positive DIA mode with adopted instruments settings previously established by Bruderer

et al. [117]. The full MS1 survey scan performed in the Orbitrap detector ranged from 350 to 1205 m/z with a resolution of 120,000. In MS2, 26 segments with variable isolation windows were acquired with the resolution of 45,000.

For library generation, one non-depleted plasma sample, six depleted samples (QC sample, FM baseline, and FM post in duplicates), and eight fractions of three depleted samples (in total 24 fractions from QC sample, one FM baseline, and one FM post) were analyzed in positive DDA mode. The full MS1 scan was performed within the mass range 375-1500 m/z with a resolution set to 120,000 at m/z 200. Precursor ions were isolated with the isolation window of 1.2 m/z and fragmented by HCD with the normalized collision energy of 28. MS2 was detected in the Orbitrap with the resolution of 15,000.

Spectral library generation with Spectronaut

First, raw data from both the DIA and DDA analysis were loaded into Spectronaut Pulsar X (version 13, Biognosys) for the spectral library generation. The Uniprot Human FASTA file (downloaded 2019-12-03 entries 42410) was used as database that included isoforms and cysteine mutations. The default settings for Spectronaut were used: full trypsin specific cleavage; peptide length between 7 and 52 amino acids; maximum missed cleavage was set to two; and a maximum five variable modifications per peptide were allowed. Carbamidomethylation of cysteine residues was set as fixed modification and acetyl (Protein-N-term), oxidation (M), oxidation (P), and phospho (STY) as variable modifications. The FDR was set to 0.01. The library was filtered before data analysis. For a detail description see **paper IV**.

Data analysis of individual plasma samples

DIA data were analyzed using Spectronaut against the built spectral library. The data were extracted based on maximum intensity for both precursors and fragment ions. The default settings were applied for the peptide and protein identification and quantification. The area of MS2 level was used for quantitation. Peptide quantity was measured by the mean of 1–3 most intensive precursors, and protein quantity was calculated accordingly by the mean of 1–3 best peptides. For a detail description see **paper IV**.

Summary of the material and methods used in the studies

Table 3 provides an overview of the methods and statistical approaches used for each paper.

Table 3. Methodology comparison of paper I-IV.

Cohort I			Cohort II	
	Paper I	Paper II	Paper III	Paper IV
Number of CWP/FM	16	15	30	Baseline: 40, Post exercise: 21
Number of CON	23	23	32	Baseline: 25, Post exercise: 24
Plasma sample tube	EDTA	EDTA	EDTA	EDTA
Centrifugation	1000 g x 15 min	1000 g x 15 min	1500 g x 30 min	1500 g x 30 min
Proteome analysis	2-DE	2-DE	2-DE	LC-MS/MS
Sample preparation before analysis	Albumin/ IgG removal, PD-10 desalting columns	Albumin/ IgG removal, PD-10 desalting columns	Albumin/ IgG removal, PD-10 desalting columns	Grude plasma. Desalting: AssayMap C18 Cartridges. For library build: Top 14 abundant protein removal + desalting Amicon 0.5 ml 10 kDa cut-off, Fractionation
Amount of total protein/peptide used for analysis	100 µg	100 µg	100 µg	1 µg (peptides)
Staining	SYPRO Ruby	SYPRO Ruby	SYPRO Ruby	-
Quantification	PDQuest	PDQuest	PDQuest	Spectronaut
Number of proteins detected	414 (including proteoforms)	414 (including proteoforms)	381 (including proteoforms)	575 unique proteins
Number of proteins included in MVDA	325 (including proteoforms)	325 (including proteoforms)	381 (including proteoforms)	484 unique proteins
MVDA models	OPLS-DA	OPLS	OPLS-DA, OPLS	OPLS-DA, PCA, OPLS
Protein identification method	MALDI-TOF, (and map matching)	MALDI-TOF, LC-MS/MS (and map matching)	LC-MS/MS, (and map matching)	LC-MS/MS in DIA mode (samples) Samples for Library building LC-MS/MS in DDA and DIA mode
Protein digestion	Trypsin	Trypsin	Trypsin	Lys-C, Trypsin
Software/database for protein identification	Protein Prospector MS fit, Expsy database	Protein Prospector MS fit, MaxQuant, Expsy database	MaxQuant, Expsy database	Spectronaut
Protein validation	Vitamin D- binding protein/25-hydroxy vitamin D ₂ and D ₃ Methods used: Peggy Sue and HPLC	-	-	-

Statistics

Univariate statistics

All four papers used standard traditional univariate and bivariate statistics to calculate background, clinical variables, and exercise-related variables. The data were processed with either Mann Whitney U or Student's t-test after test for normality using Shapiro-Wilk test. For within comparison between variables at baseline and post exercise (**paper IV**), Wilcoxon signed-rank test or paired sample t-test was used. All statistical tests were performed in SPSS (version 23 or 25).

The R software (version 3.6.1) with the package Corrplot (version 0.84) was used to create Spearman's correlation matrices (supplementary Figure S2-S4 **paper III**). A *P*-value of ≤ 0.05 was regarded as significant for all tests.

Multivariate data analysis

Throughout all four papers, MVDA has been applied using PCA (**paper I-IV**), OPLS-DA (**paper I, III, and IV**), and OPLS (**paper II-IV**) modelling in SIMCA P+ (ver 13 and ver 15, Sartorius Stedim Biotech, Umeå, Sweden).

PCA

For all four papers, PCA analysis was used to review the dataset and detect potential multiple outliers using DModX and Hotelling's T² as a complement. In this unsupervised model, all observations from the investigated cohort and all proteins were included as x-variables. Most of the time, no outliers were detected; however, if an observation was flagged as an outlier, a deeper review of the data was performed leading to either exclusion (as in **paper III** one control sample) or rejection (in **paper IV**, no obvious reason for exclusion).

Additionally, a PCA model was used in **paper IV** to compare the plasma proteome profile at baseline and post exercise in FM.

OPLS-DA

The supervised model OPLS-DA was used to compare and investigate whether any of the quantified proteins generated from either 2-DE (**paper I and III**) or LC-MS/MS (**paper IV**) analysis could discriminate the two groups of CWP/FM from the healthy controls. Before the analysis, all proteins that were valid for inclusion in the MVDA analysis were classified as x-variables, and the data were mean centered, UV scaled, and log transformed (if necessary). Each group was then assigned a number (0 = CON, CWP/FM = 1). The OPLS-DA analysis was performed in two steps. In the first step, the generated model included all x-variables. The proteins with a VIP ≥ 1 and 95% jack-knifed confidence interval were selected

and included in a new secondary regression model. The new second OPLS-DA model, including a reduced number of x-variables with new VIP values, were used in the results.

Throughout the papers, several variables are presented that describe the model statistics as suggested by Wheelock and Wheelock [67]: number of principle- and orthogonal components (PC, OC), R^2 , Q^2 , CV-ANOVA, $p(\text{corr})$, VIP, and VIPpred.

OPLS

OPLS modelling was used to investigate correlation structures between x-variables (e.g., proteins) and a pre-defined y-variable (e.g., clinical variables such as pain intensity, HADS total and FM duration) in **paper II**, **III**, and **IV**. These models were performed with the same procedure as described for OPLS-DA. In several of the OPLS models, a subgroup division was seen based on reported pain intensity (NRS or VAS), sensitivity (PPT), psychological distress (HADS total score), FM duration, and knee extension force.

Bioinformatics

In **paper I** and **II**, the assigned protein groups were based on the biological process defined by the Uniprot database (www.uniprot.org). In **paper III** and **IV**, the STRING (ver. 11)[68] was used to classify the biological process of significant proteins from respective MVDA model. In this analysis, the UniProt accession number from respective MVDA model were submitted to the search engine using multiple protein settings. This database automatically identified the submitted protein list of interest and organism. The following settings were used to investigate the enriched protein networks: maximum number of interaction was set to query (submitted) proteins only; interaction score was medium (**paper III**) and high (**paper IV**) confidence; and a FDR ≤ 0.05 was set for classification of all biological processes. In addition, the Venn diagrams and heatmap (**paper III**) were created in R with the packages VennDiagram (version 1.6.20) and heatmap.2 included in gplots (version 3.0.1.1).

Methodology considerations

There are several limitations with 2-DE. First, it is a laborious technique that requires a lot of sample preparation and inevitable result in loss of some proteins. Low abundant proteins can be difficult to detect, and the technique has difficulties separating very hydrophobic or membrane proteins, which could be very large (> 200 kDa) or very small (< 10 kDa). In general, proteins with extreme pI (very low < 3 or high > 10) and expression of proteins with the same MW or pI can be difficult to distinguish from one another [57]. The major advantage with 2-DE is that one can study whole proteins (not peptides), and the technique can easily

distinguish a protein's different proteoforms and potential PTMs. In addition, 2-DE has relatively high sensitivity and very good resolution [118, 119]. Although there might be issues with reproducibility, ours and other previous studies have found less than 20% (even as low as 5%) of variability between specific protein spots, and from replicates of gels from the same sample and between runs [120-122].

LC-MS/MS-based proteomics have several major advantages such as large sample size with a high throughput that can be run in a short amount of time. In addition, LC-MS/MS-based proteomic are based on peptide identification with highly sensitive and specific instruments that are constantly improving. The major disadvantage with LC-MS/MS-based proteomics is that it is based on peptides and not the full protein (as in 2-DE). In addition, like 2-DE, it also requires extensive sample preparation especially if fractionation is used, which results in more sources for protein loss. Furthermore, increasing acquisition time to separate full proteomes, column blockage, are some parameters that are challenging.

RESULTS AND DISCUSSION

All four papers included in this thesis used proteomics to investigate the plasma proteome profile. In addition, the papers analyzed pain characteristics such as pain intensity, sensitivity, psychological distress, and exercise. Since **paper I** and **II** are based on **cohort I** and **paper III** and **IV** are based on **cohort II**, these results will be presented and discussed together. To get an overview of the molecular pain profile in CWP/FM, new additional pathway analysis comparing the results from all four papers will be presented.

Cohort I (paper I and II)

The plasma proteomic data from cohort I, including women with CWP and healthy controls, have resulted in two publications included in this thesis [74, 76]. In **paper I**, the plasma proteome was analyzed with 2-DE and proteomic data were further analyzed using OPLS-DA modeling. In total, 22 proteins including several proteoforms were found significantly altered and able to discriminate between the CWP group and controls. The significant proteins were grouped into four different biological processes, immunity, metabolic, inflammatory, and iron ion hemostasis. Hence, the majority of proteins belonged to metabolic and immunity processes. The proteins with highest discriminating power (highest VIP values) were proteins mainly involved in the complement system and blood coagulation cascade such as complement C1r (subcomponent), complement factor I light chain, prothrombin, and plasminogen. Three proteoforms of vitamin D-binding protein were found upregulated in CWP compared to controls. This increase of vitamin D-binding protein was confirmed by additional analysis with an antibody-based method (Peggy Sue). Furthermore, HPLC analysis of the levels of 25-hydroxyvitamin D₂ and D₃ revealed increased levels in CWP compared to controls [76].

Pain intensity, and psychological distress were among those variables that were significantly higher in CWP compared to controls. Therefore, investigations were made to see if any of these clinical variables correlated with the analyzed plasma proteome profile in **paper II**.

In **paper II**, a total of seven OPLS models were made including the plasma proteomic data and the clinical variables pain intensity and psychological distress. Since there was a significant difference in age between the two groups, confounding effect of age and BMI were also investigated. The major findings of these seven OPLS models were the CWP model of pain intensity and psychological distress [74].

The results showed that a distinct proteome profile was related to respective clinical outcome in CWP. For pain intensity, 20 protein alterations were significant (including proteoforms). These proteins were involved in several biological processes such as immunity, inflammatory, iron ion hemostasis, metabolic and lipid metabolism. Hence, most of the proteins belonged to metabolic and immunity processes.

For psychological distress in CWP, 18 proteins (including proteoforms) were found significantly altered and belonged to the biological processes: immunity, inflammatory, iron ion hemostasis, metabolic and lipid metabolism. Hence, the majority of significant proteins belonged to immunity process. For the controls, the biological processes related to normal psychological distress, were related to a more metabolic profile.

Furthermore, as displayed in the OPLS models of pain intensity and psychological distress in CWP, a subgroup division within the CWP group was related to self-reported pain intensity and psychological distress score, classified as mild, moderate, and severe pain intensity, and normal and mild moderate psychological distress. Finally, there were specific proteins associated with increased pain intensity and moderate psychological distress.

To compare protein processes involved in **paper I** and **II**, to those seen in **paper III** and **IV**, new STRING models were analyzed (Figure 2). A highly significant enriched protein network (PPI enrichment P -value $< 1e-16$, local clustering coefficient of 0.72) was found when including the 22 proteins (including proteoforms) that were important discriminating proteins between CWP and controls. In total, 20 unique proteins were identified in the network and several biological processes were found (e.g., protein metabolic process, regulation of inflammatory response, immune response, and blood coagulation). Metabolic, immunity and to some extent inflammatory proteins dominated the network, which was also shown in the analysis of each protein based on the Uniprot database (Table 2 **paper I**).

Of the 16 significant proteins (including proteoforms) identified in the pain intensity model of CWP in **paper II**, STRING identified 11 unique proteins that were included in the interacted protein network (PPI enrichment P -value $< 1e-16$, local clustering coefficient of 0.82). Similar biological processes were identified as in the model for group differences (e.g., blood coagulation, immune system process, inflammatory response, and protein metabolic process). The identified enriched biological processes were in line with the processes identified in **paper II** (Table 2, **paper II**).

Of the 17 significant proteins (including proteoforms) identified in the psychological distress model of CWP in **paper II**, STRING identified 11 unique proteins in the significant enriched protein network (PPI enrichment P -value $< 1e-16$, local

clustering coefficient of 0.64). However, few protein interactions were seen and several biological processes were enriched and related to immune response, iron ion transport, and regulation of acute inflammatory response. For comparison of the three STRING models, see Figure 2.

Figure 2. Protein interaction networks of group differences chronic widespread pain (CWP) patients and controls (CON) (**paper I**), pain intensity, and psychological distress in CWP (**paper II**). Non-colored nodes were not included in any of the chosen biological process in the specific network. A1BG = Alpha-1B-glycoprotein; AHSB = Alpha-2-HS-glycoprotein; APOA1 = Apolipoprotein A-I; APOC2 = Apolipoprotein C-II; APOC3 = Apolipoprotein C-III; C1R = Complement C1r subcomponent; C1S = Complement C1s subcomponent; C3 = Complement C3; C7 = Complement component C7, CFB = Complement factor B; CFI = Complement factor I; CHI3L1 = Chitinase-3-like protein 1, CLU = Clusterin; CP = Ceruloplasmin; F13B = Coagulation factor XIII B chain; F2 = Prothrombin; FCN3 = Ficolin-3; FGA = Fibrinogen alpha chain; FGG = Fibrinogen gamma chain; GC = Vitamin D-binding protein; GSN = Gelsolin; HP = Haptoglobin; HPX = Hemopexin; KNG1 = Kininogen-1; LRG1 = Leucine rich alpha-2-glycoprotein 1; PLG = Plasminogen; SERPINA1 = Alpha-1-antitrypsin; SERPINF2 = Alpha-2-antiplasmin; TF = Serotransferrin; TTR = Transthyretin

As noted, there were many similar biological processes involved in all three models identified by the new STRING analysis. Hence, in the model of group differences, several processes were identified and most proteins belonged to immunity and metabolic processes. Metabolic profile and to some extent immunity proteins were dominant for pain intensity in CWP, whereas immunity process was more prevalent for psychological distress. Taken together, it is evident that these three biological processes are involved in this subset of CWP patients. Increased

pain intensity is often reported among CWP/FM patients [123] and is an important facet in pain perception. Furthermore, depression, which is part of psychological distress in this study has shown a strong relationship with pain intensity and physical functioning in CWP/FM [124]. Therefore, it is not surprising that similar proteins and processes are identified in each model and that these are seen when only comparing group difference.

Additionally, in **paper II**, a subgroup division among the CWP patients were seen in both the pain intensity model and psychological distress model. From a clinical point of view, these subgroups are interesting, as it might reflect different ongoing mechanisms related to the intensity and severity of each clinical outcome. Investigation of the plasma/serum profile of patients with major depressive disorder have found alteration of proteins involved in similar metabolic process (e.g., blood coagulation cascade) and immunity and inflammation (e.g., acute-phase response and complement activation) [125-127]. It is tempting to speculate that even though this CWP group was not clinically diagnosed with major depression or anxiety or had severe pain intensity, similar processes are found in severe cases of depression and this might affect protein changes systemically. This might help explain the alteration of immunity and metabolic processes seen in CWP. Therefore, these protein changes and involved processes in combination with a higher pain intensity and psychological burden might be viewed as potential risk factors. That is, knowledge from the plasma proteome and involved protein processes might be used to predict symptom severity and potential progression in CWP. CWP/FM is a complex pain condition and pain intensity, anxiety, and depression are just few of the common symptoms. Other factors that could affect clinical outcome are related to the concept of pain and psychological distress such as mood, fatigue, disability, and life quality.

Overall, the results from **paper I** and **II** indicate that important systemic differences exist between CWP and controls. Furthermore, the pain profile of CWP consists of plasma proteins associated with pain intensity and psychological distress involved in several biological processes of immunity and protein metabolic process, including proteins that exert their major function in the complement system and blood coagulation cascade.

Cohort II (paper III and IV)

In **paper III** and **IV**, the plasma proteome has been investigated in women with FM and healthy controls using both 2-DE and LC-MS/MS. In addition, the correlation analysis of clinical characteristics and the plasma protein profile have been investigated using MVDA modelling. The main purpose of **paper III** was to confirm the previous findings from **paper I** and **II**, but with an additional cohort. The same methodology was used (2-DE) and resulted in analysis of almost twice as large a cohort with respect to FM patients (Table 3).

In total, 18 unique proteins expressed as 35 proteoforms were found to be significant important for group separation between FM and controls. In the protein network analysis, these proteins belonged to similar protein processes as seen in **paper I**, which were immune response, inflammatory response, protein metabolic process and blood coagulation. The majority of proteins positively associated with FM were proteins mainly included in inflammatory and immune response processes [72]. In FM, 11 unique proteins expressed as 13 proteoforms were found significantly correlated with reported pain intensity. The enriched protein network showed that these proteins were highly interacted and involved in the same processes as previously found in pain intensity model of CWP in **paper II** (i.e., immune response, protein metabolic process, blood coagulation and inflammatory response). Most of proteins were involved in immunity and metabolic processes. A sub-group division among the FM group was also found based on reported pain intensity score, showing several proteins related to moderate and severe pain intensity.

As in **paper II**, correlation of psychological distress with the plasma protein profile was investigated and showed 17 unique proteins expressed as 26 proteoforms. These proteins were subjected to STRING pathways analysis and identified several biological processes such as blood coagulation/fibrin clot formation, immune response, acute-phase response, and vitamin transport. As noted, in the sub-group division, the proteins associated with moderate psychological distress mainly belonged to blood coagulation/ fibrin clot formation process.

This study, is the first study to investigate pressure pain thresholds (PPT), referred to as pain sensitivity, and PPT's correlation with specific plasma proteins. In total, 10 unique proteins expressed as 14 proteoforms were significantly correlated with PPT in FM. These proteins formed a small integrated network with most proteins belonging to inflammatory response, hence blood coagulation cascade and immunity processes were also present. Figure 3 displays the protein interaction networks for each MVDA model from **paper III** [72].

Figure 3. STRING proteins networks from paper III. Significant proteins of group differences in fibromyalgia (FM) and controls (CON), and pain intensity, sensitivity, and psychological distress in FM. Pictures were kindly reproduced with permission from [72].

A1BG = Alpha1B-glycoprotein; A2M = Alpha-2-macroglobulin; AGT = Angiotensinogen; AHSG = Alpha-2-HSglycoprotein; APOA1 = Apolipoprotein A-I; APOC3 = Apolipoprotein C-III; APOH = Beta-2-glycoprotein 1; C1R = Complement C1r subcomponent; C3 = Complement C3; C4B = Complement C4-B; C4BPA = C4b-binding protein alpha chain; CFI = Complement factor I; CLU = Clusterin; CP = Ceruloplasmin; FCN3 = Ficolin-3; FGA = Fibrinogen alpha chain; FGB = Fibrinogen beta chain; GC = Vitamin D-binding protein; GSN = Gelsolin; HP = Haptoglobin; HPX = Hemopexin; IGG = Immunoglobulin J chain; KNG1 = Kininogen-1; PLG = Plasminogen; SAA4 = Serum amyloid A-4 protein; SERPINA1 = Alpha-1-antitrypsin; SERPINC1 = Antithrombin-III; SERPINF2 = Alpha-2-antiplasmin; TF = Serotransferrin.

In **paper IV**, the plasma proteome was analyzed using shotgun proteomics before start of intervention, referred to as baseline, and 15 weeks after the resistance exercise intervention in FM and healthy controls. At baseline, 40 women with FM and 25 controls were included in the analysis. The proteomic analysis following OPLS-DA modelling resulted in 19 proteins that were significantly altered and important for group separation between FM and controls. These proteins belonged to different biological processes related to stress, regulation of mRNA stability, and three processes of immunity - i.e., defense response to gram-positive bacterium, opsonization, and complement activation (lectin pathway). After 15 weeks of resistant exercise, 28 proteins were able to discriminate between FM and controls. These proteins were involved in regulation of immune system process, muscle structure development, and response to stress.

Exercise in FM and physical functioning and proteins

Of the significant proteins from both baseline and post exercise found to be useful for discriminating between FM and controls, seven proteins showed a significant alteration in the FM group (**Figure 3, paper IV**): myosin regulatory light polypeptide 9, zinc finger homeobox protein 3, histone H4, histone H2B type -K, and myelin basic protein were significantly reduced, and afadin- and alpha-actinin-binding protein and prolow-density lipoprotein receptor-related protein 1 were significantly increased. To further evaluate whether any clinical characteristics significantly correlated with the plasma proteome post exercise in FM, additional OPLS analysis was made. PPT was the only clinical characteristic that was found significantly correlated to the plasma proteome post exercise in FM. Hence, the significant proteins were involved in several biological processes, most of the proteins belonged to the immunity and metabolic process (**Figure 4, paper IV**). Additionally, the significant proteins were all associated with very low PPT scores in FM.

Additionally, an interesting subgroup division was found in the PCA comparing the total plasma proteome with the FM group at baseline and post exercise (**score plot Figure 5, paper IV**). After closer investigation, evident differences were found in both clinical and exercise-related variables among the two subgroups (e.g., a significant difference in FM duration and tender points at baseline were found). Hence, the group with shorter FM duration (subgroup 1) showed correlation with plasma proteins involved in stress, blood coagulation and immune response, and the group with longer FM duration (subgroup 2) showed correlations with proteins involved in stress and several immunity processes and inflammation (**Figure 7 C, paper IV**). In addition, the subgroup with longer FM duration significantly improved their physical capacity.

Post exercises, in the individual protein graphs (**Figure 3, paper IV**), a cluster of eight FM participants showed a higher expression of three of the proteins compared to rest of the FM group (histone H4, histone H2B type 1-K, and myelin basic protein). After closer investigation, these participants belonged to subgroup 1 having shorter FM duration, which had no significant improvement in the exercise-related variables, and they showed the opposite protein alteration compared to FM group with longer FM duration.

As a group, FM improved their general and mental fatigue, pain catastrophizing, and psychological distress post exercise. Furthermore, significantly higher pain acceptance and reduction in pain intensity was noted. No significant improvement in PPT was seen. FM significantly improved their physical capacity in the quadricep muscle on the left side, handgrip force (both sides), and elbow strength right side (**Table 2, paper IV**). Investigating the clinical and exercise-related variables alone, the results show that exercise clearly had beneficial effects on this FM group concerning experienced symptoms, which is of high importance for

these patients. If exercise can reduce pain intensity, fatigue and psychological burden, and improve muscle strength and force, that is evidence enough that exercise is a great tool to be used for reducing symptoms in FM.

In the two subgroups of FM, the post exercise-related differences could be due to different pre-conditions such as subgroup 2 had fewer tender points as these differences might reflect that this subgroup is more susceptible to perform exercise since they do not have as many painful sites that could hinder them from performing the exercise intervention. Furthermore, with a lower muscle strength to start with, there might be more room for muscle strength improvements after performing this type of resistance exercise for 15 weeks. It also seems that the alteration of specific proteins is affected by exercise, although further investigations are needed to evaluate if these changes are the effect of resistance exercise or due to other more clinical changes in FM. Nevertheless, the plasma proteome at baseline might be able to distinguish two subsets of FM that seem to improve their physical function more after exercise. Therefore, this subset of patients might be more prone to gain from exercise intervention than others. If there is a positive effect overall on discovered proteins, then these processes need to be investigated in future studies.

The plasma proteome of CWP/FM

It is evident that there are several proteins that can discriminate CWP/FM patients from controls as shown in **paper I, III, and IV**. To summarize these changes, a protein network was analyzed including all significant proteins from respective paper investigating group differences. As shown in Figure 4, a large network with two major clusters was found significantly enriched (PPI enrichment P -value $< 1.0e-16$, avg. local clustering coefficient: 0.60). In the whole network, protein of metabolic process and immunity proteins were present. Additionally, cluster I proteins were highly interacted and involved in blood coagulation, whereas cluster II proteins were involved in complement activation. For both clusters, several proteins were also related to inflammatory response.

Figure 4. Comparison of the plasma proteome from paper I, III and IV. Cluster I include proteins of blood coagulation process and cluster II include proteins of complement activation.

A1BG = Alpha-1B-glycoprotein; A2M = Alpha-2-macroglobulin; AHSB = Alpha-2-HS-glycoprotein; APOA1 = Apolipoprotein A-I; APOC3 = Apolipoprotein C-III; APOH = Beta-2-glycoprotein 1; C1R = Complement C1r subcomponent; C1S = Complement C1s subcomponent, C3 = Complement C3; C4B = Complement C4-B; CFB = Complement factor B; CFI = Complement factor I; COLEC11 = Collectin-11; CP = Ceruloplasmin; CRP = C-reactive protein; DPF3 = Zinc finger protein DPF3; F13B = Coagulation factor XIII B chain; F2 = Prothrombin; FCN3 = Ficolin-3; FGA = Fibrinogen alpha chain; FGB = Fibrinogen beta chain; FGG = Fibrinogen gamma chain; GC = Vitamin D-binding protein; GSN = Gelsolin; HIST1H2AB = Histone H2A type 1-B/E; HIST1H2BK = Histone H2B type 1-K; HIST1H4E = Histone H4; HP = Haptoglobin; KHSRP = Far upstream element-binding protein 2; KNG1 = Kininogen-1; MBL2 = Mannose-binding protein C; MBP = Myelin basic protein; MYLK = Myosin light chain kinase (smooth muscle); NPC1L1 = Niemann-Pick C1-like protein 1; PLG = Plasminogen; PRPF40B = Pre-mRNA-processing factor 40 homolog B; RRP1 = Ribosomal RNA processing protein 1 homolog; SERPINA1 = Alpha-1-antitrypsin; SERPINF2 = Alpha-2-antiplasmin; TF = Serotransferrin; TTR = Transthyretin; UBB = Polyubiquitin-B; YWHAZ = 14-3-3 protein zeta/delta; ZFHX3 = Zinc finger homeobox protein 3.

Paper I, III, and IV show similar results as the other reported studies that have investigated the plasma and serum proteome of CWP/FM [75, 77, 78]. The first paper was published by Ruggerio et al. [77]. In their preliminary 2-DE serum proteome study from FM and healthy controls, they found only alpha-1-antitrypsin, transthyretin, and retinol binding protein 4 that were significantly increased, suggesting involvement of oxidative stress process in FM [77]. Ramirez-Tejero et al. [75], in a LC-MS/MS based plasma proteome study of FM and healthy controls, found 33 proteins that were significantly different in FM and controls, where most of the proteins were upregulated and involved in several enriched pathways. The most important pathways were acute-phase response signaling, Liver-X Receptor/Retinoid-X Receptor activation, Farnesoid-X Receptor/Retinoid-X Receptor activation, and coagulation and complement systems, suggesting an overall involvement of inflammation in FM [75]. In the most recent study, Han et al. [78] investigating the LC-MS/MS based serum proteome of FM and controls, found 22 significantly altered proteins that were mainly involved in blood coagulation, immune response, and extracellular matrix–receptor interactions [78]. Overall, these six studies have found similar altered pathways related to immunity, blood coagulation with more specific proteins involved in the complement system, acute-phase proteins, and metabolic processes. Hence, they all suggest that there is an inflammatory profile involved in the pathophysiology behind CWP/FM [72, 75-78]. Irrespective of differences in specific proteins in these studies, they have several pathways in common. Together with the results from this thesis, it seems that CWP/FM might have a dysregulation effect on the immune system tightly linked to several proteins in both the complement system and blood coagulation cascade. What is important to remember is that all these studies are highly exploratory and contain few observations. Nonetheless, these results warrant further investigations into the proposed biological pathways in CWP/FM.

Clinical characteristics in CWP/FM and correlation to plasma proteins

As noted in Table 2, both these cohorts do not suffer from severe pain intensity or have a clinical diagnosis of anxiety or depression. In **paper II and III**, plasma proteins were found significantly correlated to reported pain intensity. Although, the same proteins were not found significantly altered in the two groups, similar processes were detected. Common for both pain intensity and psychological distress are the presence of immune proteins. Metabolic proteins were present through the whole network of pain intensity. Meanwhile, for psychological distress, inflammatory and acute-phase response processes were found enriched, among others.

So far, few studies have managed to find correlation of clinical variables and protein markers in plasma/serum in CWP/FM. Ruggerio et al. found no significant correlations between the investigated serum proteome and pain intensity, pain

duration or FIQ [77]. Han et al. found positive correlation with Ig heavy chain V-I region HG3 and pain intensity in FM, and negative correlation of keratin, type II cytoskeletal 80 was found for depression (Becker's Depression Inventory) [78]. Hence, several immunoglobulins were found correlated in both **paper II** and **III** in the OPLS models of pain intensity in CWP/FM. Other attempts have failed in finding significant correlation between clinical characteristics and the saliva proteome in FM [79, 81]. Furthermore, the trapezius muscle proteome of cohort I has previously been investigated by Olausson et al. [86]. They found 12 proteins classified as metabolic, contractile, inflammatory, and stress proteins that were significantly correlated with pain intensity in CWP. Overall, it seems that there are several proteins associated with clinical characteristics in CWP/FM and several processes specific to each clinical outcome.

Another important factor that needs to be addressed is the fact that in cohort II increased BMI was found in FM compared to controls. Several of the noted biological processes identified in all models are involved in the immune system and to some extent classified as inflammatory or even acute-phase proteins. Increased BMI is often present among CWP/FM, and several proteomic studies have shown that increased weight and obesity can influence circulating immunity proteins especially those belonging to the complement system [128, 129]. Other proteomic studies have shown that obesity and inflammation are associated [130], and low-grade inflammation is present in obesity, which is sustained after heavy weight loss [131]. Therefore, the influence of BMI could have affected the proteomic profile seen in these studies. Hence, the FM participants were not classified as obese in cohort II. However, BMI might play a part in the complex etiology of CWP/FM and therefore could be an underlying cause and response to the changes seen in the proteome profile in the two cohorts. This needs to be addressed in larger studies investigating CWP/FM with obesity compared to normal weighted CWP/FM participants.

Medications can influence the proteomic profile as well as the clinical variables investigated in these studies. The reality is that several of the CWP/FM participants are on long-term medications and were probably unable to refrain from analgesics before blood sampling. Ongoing medical treatment probably influenced several of the clinical variables such as pain intensity, psychological distress and the PPT measurements, which would most likely have been higher. However, it would not be ethical to completely remove ongoing medical regimes. Moreover, such restrictions would have given a false picture of how CWP/FM patients live their daily lives.

CONCLUSION

CWP/FM is a complex pain condition where little is known about the molecular mechanisms contributing to its pathophysiology. The clinical significance of finding biomarkers for CWP/FM are not only beneficial for diagnosis, but also for future mechanism-based treatments/rehabilitations. To date, there are no established biomarkers for CWP/FM.

In this thesis, the pain profile of CWP/FM, which includes the plasma proteome and clinical characteristics, were analyzed using proteomics, multivariate modeling, and bioinformatics. This thesis illustrates the potential use of these methods to identify systemic protein changes associated with CWP/FM and potential mechanisms involved in this complex pain condition. The results indicate that there are prominent systemic changes related to immunity, inflammation, and metabolic processes, in women with CWP/FM. Furthermore, in CWP/FM the results show that there are specific protein profiles involved in processes of immunity, metabolic and immunity, and inflammatory processes related to pain intensity, psychological distress, and pain sensitivity, respectively.

Exercise is recommended as the first line of treatment for CWP/FM. The plasma proteome was investigated before and after a 15 weeks resistance exercise intervention in FM. Both at baseline and post exercise in FM and controls, prominent protein alterations were found that were involved in immunity, stress, mRNA stability, and muscle structure development processes. Exercise seems to influence the clinical characteristics and circulating proteins in FM. Furthermore, specific plasma proteome profile related to grade of chronification, pain sensitivity and improved muscle force of the quadriceps muscle was also found.

To summarize, the results from this thesis suggest that in CWP/FM there might be a dysregulation in the biological processes involved in the immune system and metabolic processes, which are tightly linked to several proteins in the complement system and blood coagulation cascade. These results have shed lights on ongoing mechanisms involved in the pathophysiology of chronic pain.

FUTURE PERSPECTIVES

The painomics research field is still in an early stage, especially regarding CWP/FM. To date, the molecular pain profile of CWP/FM is poorly understood, and only few proteomic studies have been conducted. The molecular function of the discovered proteins in this thesis might already be known, but how they act and function in the pathophysiology of CWP/FM needs further investigation. The results have shown that there are different proteoforms due to the post translational modifications (PTM) in plasma that are altered in CWP/FM. The function of the proteoforms are not known. Therefore, the PTM has not been investigated in this thesis, an investigation that will require large-scale proteoformics studies. By using a back-translational approach, experimental animal models can be used to target specific proteins of interest (e.g., of immunity processes) and to evaluate its specific involvement in chronic pain.

The CWP/FM included in both cohorts did not have severe pain. Therefore, it will be highly interesting to investigate the plasma proteome profile in severe cases to see if the proteome profile differs compared to CWP/FM patients with moderate pain. Additionally, a higher BMI was seen in the FM patients in two of the studies. Future studies should include CWP/FM with high and normal BMI to investigate if the proteome profile differs and if the discovered processes are affected by this factor. This knowledge could contribute to insights into mechanisms related to higher BMI, psychological distress, and pain intensity, as well as potential effects of the immunity/inflammatory profile seen in this group. It will also add value about potential subgroups of CWP/FM and if there are different ongoing mechanisms involved. Hence, maybe we should start to consider these clinical factors as part of the diagnosis, and not just part of the clinical presentation as symptoms of CWP/FM. In fact, we should stop treat them as confounding factors and investigate their role in CWP/FM.

There are other research areas within the omics field of chronic pain that are still in developing such as metabolomics and transcriptomics. Disease prediction might be possible if data from these fields and the clinical characteristics are combined using advance bioinformatics such as machine learning. Furthermore, adding a multi-omics approach that includes the analysis of different biofluids, might take us one step closer to finding out the complete molecular pain profile for CWP/FM in different levels. By applying advanced bioinformatics to the large-scale data from multi-omics, specific molecular pain “signatures” can be predicted. With a high precision for the prediction, these objective models can be used to predict clinical diagnosis, early onset, subgroups of CWP/FM, evaluate

mechanism-based treatments/rehabilitation, and lead to new drug developments. Before the potential identified biomarkers in this thesis can be used for CWP/FM, the specificity needs to be clinically assessed.

This type of biomarker research has great potential in increasing knowledge about mechanisms involved in CWP/FM and can hereby open for better clinical understanding and management of this and other chronic pain states. The clinical value of collecting a blood sample and measuring stable pain mechanism markers in combination with evaluation of anamnesis and clinical examination would in the future help clinicians and patients receive a faster and more precise diagnosis and ultimately better treatment strategies.

REFERENCES

1. *Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy.* Pain Suppl, 1986. **3**: p. S1-226.
2. Raja S.N., Carr D.B., Cohen M., Finnerup N.B., Flor H., Gibson S., Keefe F.J., Mogil J.S., Ringkamp M., Sluka K.A., Song X.J., Stevens B., Sullivan M.D., Tutelman P.R., Ushida T., and Vader K., *The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises.* Pain, 2020. **Articles in Press**.
3. Treede R.D., Rief W., Barke A., Aziz Q., Bennett M.I., Benoliel R., Cohen M., Evers S., Finnerup N.B., First M.B., Giamberardino M.A., Kaasa S., Korwisi B., Kosek E., Lavand'homme P., Nicholas M., Perrot S., Scholz J., Schug S., Smith B.H., Svensson P., Vlaeyen J.W.S., and Wang S.J., *Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11).* Pain, 2019. **160**(1): p. 19-27.
4. Mills S.E.E., Nicolson K.P., and Smith B.H., *Chronic pain: a review of its epidemiology and associated factors in population-based studies.* Br J Anaesth, 2019. **123**(2): p. e273-e283.
5. Breivik H., Collett B., Ventafridda V., Cohen R., and Gallacher D., *Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment.* Eur J Pain, 2006. **10**(4): p. 287-333.
6. Cimmino M.A., Ferrone C., and Cutolo M., *Epidemiology of chronic musculoskeletal pain.* Best Pract Res Clin Rheumatol, 2011. **25**(2): p. 173-83.
7. Wolfe F., Smythe H.A., Yunus M.B., Bennett R.M., Bombardier C., Goldenberg D.L., Tugwell P., Campbell S.M., Abeles M., Clark P., and et al., *The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee.* Arthritis Rheum, 1990. **33**(2): p. 160-72.
8. Wolfe F., Clauw D.J., Fitzcharles M.A., Goldenberg D.L., Katz R.S., Mease P., Russell A.S., Russell I.J., Winfield J.B., and Yunus M.B., *The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity.* Arthritis Care Res (Hoboken), 2010. **62**(5): p. 600-10.
9. Wolfe F., Clauw D.J., Fitzcharles M.A., Goldenberg D.L., Hauser W., Katz R.S., Mease P., Russell A.S., Russell I.J., and Winfield J.B., *Fibromyalgia criteria and severity scales for clinical and epidemiological studies: a*

- modification of the ACR Preliminary Diagnostic Criteria for Fibromyalgia. J Rheumatol*, 2011. **38**(6): p. 1113-22.
10. Wolfe F., Clauw D.J., Fitzcharles M.A., Goldenberg D.L., Hauser W., Katz R.L., Mease P.J., Russell A.S., Russell I.J., and Walitt B., *2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. Semin Arthritis Rheum*, 2016. **46**(3): p. 319-329.
 11. Ohlsson K., Attewell R.G., Johnsson B., Ahlm A., and Skerfving S., *An assessment of neck and upper extremity disorders by questionnaire and clinical examination. Ergonomics*, 1994. **37**(5): p. 891-7.
 12. Dansie E.J. and Turk D.C., *Assessment of patients with chronic pain. BJA: British Journal of Anaesthesia*, 2013. **111**(1): p. 19-25.
 13. Kosek E., Cohen M., Baron R., Gebhart G.F., Mico J.A., Rice A.S., Rief W., and Sluka A.K., *Do we need a third mechanistic descriptor for chronic pain states? Pain*, 2016. **157**(7): p. 1382-6.
 14. Gerdle B., Bäckryd E., Novo M., Roeck-Hansen E., Rothman M., Stålnacke B.-M., Westergren H., and Rivano-Fischer M., *Smärtanalys : diagnos, smärtmekanismer, psykologisk och social bedömning*. 2020, Lund: Studentlitteratur.
 15. Mansfield K.E., Sim J., Jordan J.L., and Jordan K.P., *A systematic review and meta-analysis of the prevalence of chronic widespread pain in the general population. Pain*, 2016. **157**(1): p. 55-64.
 16. Gran J.T., *The epidemiology of chronic generalized musculoskeletal pain. Best Pract Res Clin Rheumatol*, 2003. **17**(4): p. 547-61.
 17. Jones G.T., Atzeni F., Beasley M., Fluss E., Sarzi-Puttini P., and Macfarlane G.J., *The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. Arthritis Rheumatol*, 2015. **67**(2): p. 568-75.
 18. Queiroz L.P., *Worldwide epidemiology of fibromyalgia. Curr Pain Headache Rep*, 2013. **17**(8): p. 356.
 19. Senna M.K., Ahmad H.S., and Fathi W., *Depression in obese patients with primary fibromyalgia: the mediating role of poor sleep and eating disorder features. Clin Rheumatol*, 2013. **32**(3): p. 369-75.
 20. Jaremo P., Arman M., Gerdle B., Larsson B., and Gottberg K., *Illness beliefs among patients with chronic widespread pain - associations with self-reported health status, anxiety and depressive symptoms and impact of pain. BMC Psychol*, 2017. **5**(1): p. 24.
 21. Staud R., *Peripheral pain mechanisms in chronic widespread pain. Best Pract Res Clin Rheumatol*, 2011. **25**(2): p. 155-64.
 22. Okifuji A., Bradshaw D.H., and Olson C., *Evaluating obesity in fibromyalgia: neuroendocrine biomarkers, symptoms, and functions. Clin Rheumatol*, 2009. **28**(4): p. 475-8.

23. Creed F., *A review of the incidence and risk factors for fibromyalgia and chronic widespread pain in population-based studies*. Pain, 2020. **161**(6): p. 1169-1176.
24. Wolfe F., Walitt B., Perrot S., Rasker J.J., and Hauser W., *Fibromyalgia diagnosis and biased assessment: Sex, prevalence and bias*. PLoS One, 2018. **13**(9): p. e0203755.
25. Weir P.T., Harlan G.A., Nkoy F.L., Jones S.S., Hegmann K.T., Gren L.H., and Lyon J.L., *The incidence of fibromyalgia and its associated comorbidities: a population-based retrospective cohort study based on International Classification of Diseases, 9th Revision codes*. J Clin Rheumatol, 2006. **12**(3): p. 124-8.
26. Hauser W., Perrot S., Sommer C., Shir Y., and Fitzcharles M.A., *Diagnostic confounders of chronic widespread pain: not always fibromyalgia*. Pain Rep, 2017. **2**(3): p. e598.
27. Coster L., Kendall S., Gerdle B., Henriksson C., Henriksson K.G., and Bengtsson A., *Chronic widespread musculoskeletal pain - a comparison of those who meet criteria for fibromyalgia and those who do not*. Eur J Pain, 2008. **12**(5): p. 600-10.
28. Staud R., *Chronic widespread pain and fibromyalgia: two sides of the same coin?* Curr Rheumatol Rep, 2009. **11**(6): p. 433-6.
29. Macfarlane G.J., Kronisch C., Dean L.E., Atzeni F., Häuser W., Fluß E., Choy E., Kosek E., Amris K., Branco J., Dincer F., Leino-Arjas P., Longley K., McCarthy G.M., Makri S., Perrot S., Sarzi-Puttini P., Taylor A., and Jones G.T., *EULAR revised recommendations for the management of fibromyalgia*. Annals of the rheumatic diseases, 2017. **76**(2): p. 318-328.
30. Mccloughlin M.J., Colbert L.H., Stegner A.J., and Cook D.B., *Are Women with Fibromyalgia Less Physically Active than Healthy Women?* Medicine & Science in Sports & Exercise, 2011. **43**(5): p. 905-912.
31. Mannerkorpi K., Burckhardt C.S., and Bjelle A., *Physical performance characteristics of women with fibromyalgia*. Arthritis care and research : the official journal of the Arthritis Health Professions Association, 1994. **7**(3): p. 123-129.
32. Henriksen M., Lund H., Christensen R., Jespersen A., Dreyer L., Bennett R.M., Danneskiold-Samsøe B., and Bliddal H., *Relationships between the fibromyalgia impact questionnaire, tender point count, and muscle strength in female patients with fibromyalgia: a cohort study*. Arthritis and rheumatism, 2009. **61**(6): p. 732-739.
33. Nijs J., Roussel N., Van Oosterwijck J., De Kooning M., Ickmans K., Struyf F., Meeus M., and Lundberg M., *Fear of movement and avoidance behaviour toward physical activity in chronic-fatigue syndrome and fibromyalgia: state of the art and implications for clinical practice*. Clinical rheumatology, 2013. **32**(8): p. 1121-1129.

34. Turk D.C., Robinson J.P., and Burwinkle T., *Prevalence of fear of pain and activity in patients with fibromyalgia syndrome*. The journal of pain : official journal of the American Pain Society, 2004. **5**(9): p. 483-490.
35. Assumpção A., Matsutani L.A., Yuan S.L., Santo A.S., Sauer J., Mango P., and Marques A.P., *Muscle stretching exercises and resistance training in fibromyalgia: which is better? A three-arm randomized controlled trial*. European journal of physical and rehabilitation medicine, 2018. **54**(5): p. 663-670.
36. Busch A.J., Webber S.C., Richards R.S., Bidonde J., Schachter C.L., Schafer L.A., Danyliw A., Sawant A., Dal Bello-Haas V., Rader T., and Overend T.J., *Resistance exercise training for fibromyalgia*. The Cochrane database of systematic reviews, 2013. **2013**(12): p. CD010884-CD010884.
37. Larsson A., Palstam A., Lofgren M., Ernberg M., Bjersing J., Bileviciute-Ljungar I., Gerdle B., Kosek E., and Mannerkorpi K., *Resistance exercise improves muscle strength, health status and pain intensity in fibromyalgia-a randomized controlled trial*. Arthritis Res Ther, 2015. **17**: p. 161.
38. Ericsson A., Palstam A., Larsson A., Lofgren M., Bileviciute-Ljungar I., Bjersing J., Gerdle B., Kosek E., and Mannerkorpi K., *Resistance exercise improves physical fatigue in women with fibromyalgia: a randomized controlled trial*. Arthritis Res Ther, 2016. **18**: p. 176.
39. Bidonde J., Busch A.J., Schachter C.L., Overend T.J., Kim S.Y., Góes S.M., Boden C., and Foulds H.J., *Aerobic exercise training for adults with fibromyalgia*. The Cochrane database of systematic reviews, 2017. **6**(6): p. CD012700-CD012700.
40. Valkeinen H., Häkkinen A., Hannonen P., Häkkinen K., and Alén M., *Acute heavy-resistance exercise-induced pain and neuromuscular fatigue in elderly women with fibromyalgia and in healthy controls: effects of strength training*. Arthritis and rheumatism, 2006. **54**(4): p. 1334-1339.
41. Khalsa P.S., *Biomechanics of musculoskeletal pain: dynamics of the neuromatrix*. J Electromyogr Kinesiol, 2004. **14**(1): p. 109-20.
42. Woolf C.J. and Salter M.W., *Neuronal plasticity: increasing the gain in pain*. Science, 2000. **288**(5472): p. 1765-9.
43. Staud R. and Smitherman M.L., *Peripheral and central sensitization in fibromyalgia: Pathogenetic role*. Current Pain and Headache Reports, 2002. **6**(4): p. 259-266.
44. May A., *Neuroimaging: visualising the brain in pain*. Neurol Sci, 2007. **28 Suppl 2**: p. S101-7.
45. Woolf C.J., *Central sensitization: implications for the diagnosis and treatment of pain*. Pain, 2011. **152**(3 Suppl): p. S2-S15.
46. Fillingim R.B., *Individual differences in pain: understanding the mosaic that makes pain personal*. Pain, 2017. **158 Suppl 1**: p. S11-s18.
47. Group N.D.W., *Biomarkers and surrogate endpoints: preferred definitions and conceptual framework*. Clin Pharmacol Ther, 2001. **69**(3): p. 89-95.

48. Niederberger E. and Geisslinger G., *Proteomics in neuropathic pain research*. Anesthesiology, 2008. **108**(2): p. 314-23.
49. Graves P.R. and Haystead T.A., *Molecular biologist's guide to proteomics*. Microbiol Mol Biol Rev, 2002. **66**(1): p. 39-63; table of contents.
50. O'Farrell P.H., *High resolution two-dimensional electrophoresis of proteins*. J Biol Chem, 1975. **250**(10): p. 4007-21.
51. Klose J., *Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals*. Humangenetik, 1975. **26**(3): p. 231-43.
52. Friedman D.B., Hoving S., and Westermeier R., *Isoelectric focusing and two-dimensional gel electrophoresis*. Methods in enzymology, 2009. **463**: p. 515-540.
53. Gorg A., Drews O., Luck C., Weiland F., and Weiss W., *2-DE with IPGs*. Electrophoresis, 2009. **30 Suppl 1**: p. S122-32.
54. Gorg A., Obermaier C., Boguth G., Harder A., Scheibe B., Wildgruber R., and Weiss W., *The current state of two-dimensional electrophoresis with immobilized pH gradients*. Electrophoresis, 2000. **21**(6): p. 1037-53.
55. Westermeier R. and Gorg A., *Two-dimensional electrophoresis in proteomics*. Methods Biochem Anal, 2011. **54**: p. 411-39.
56. N. B.K., Birte S., F. L.M., H. S.T., Alla B., Gary S., Annie W., and F. P.W., *An improved formulation of SYPRO Ruby protein gel stain: Comparison with the original formulation and with a ruthenium II tris (bathophenanthroline disulfonate) formulation*. PROTEOMICS, 2002. **2**(5): p. 486-498.
57. Meleady P., *Two-Dimensional Gel Electrophoresis and 2D-DIGE*. Methods in molecular biology (Clifton, N.J.), 2018. **1664**: p. 3-14.
58. Goetz M.M., Torres-Madronero M.C., Rothlisberger S., and Delgado-Trejos E., *Preprocessing of 2-Dimensional Gel Electrophoresis Images Applied to Proteomic Analysis: A Review*. Genomics Proteomics Bioinformatics, 2018. **16**(1): p. 63-72.
59. Hortin G.L., *The MALDI-TOF mass spectrometric view of the plasma proteome and peptidome*. Clin Chem, 2006. **52**(7): p. 1223-37.
60. Wilm M., *Principles of electrospray ionization*. Mol Cell Proteomics, 2011. **10**(7): p. M111.009407.
61. Cottrell J.S., *Protein identification using MS/MS data*. J Proteomics, 2011. **74**(10): p. 1842-51.
62. Bilbao A., Varesio E., Luban J., Strambio-De-Castillia C., Hopfgartner G., Müller M., and Lisacek F., *Processing strategies and software solutions for data-independent acquisition in mass spectrometry*. Proteomics, 2015. **15**(5-6): p. 964-80.
63. Wood J.N., Gomez-Varela D., and Schmidt M., *The Proteomics and Metabolomics of Pain—Opportunities for Systems Medicine*. 2018, Oxford University Press.

64. Cox J. and Mann M., *MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification*. Nat Biotechnol, 2008. **26**(12): p. 1367-72.
65. Gomez-Varela D., Barry A.M., and Schmidt M., *Proteome-based systems biology in chronic pain*. Journal of Proteomics, 2019. **190**: p. 1-11.
66. Eriksson L., Byrne T., Johansson E., Trygg J., and Vikström C., *Multi-and megavariate data analysis: basic principles and applications*. 3rd revised ed. 2013: Umetrics Academy, MKS Umetrics AB, Malmö, Sweden.
67. Wheelock A.M. and Wheelock C.E., *Trials and tribulations of 'omics data analysis: assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine*. Mol Biosyst, 2013. **9**(11): p. 2589-96.
68. Szklarczyk D., Gable A.L., Lyon D., Junge A., Wyder S., Huerta-Cepas J., Simonovic M., Doncheva N.T., Morris J.H., Bork P., Jensen L.J., and Mering C.V., *STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets*. Nucleic Acids Res, 2019. **47**(D1): p. D607-D613.
69. Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B., and Ideker T., *Cytoscape: a software environment for integrated models of biomolecular interaction networks*. Genome Res, 2003. **13**(11): p. 2498-504.
70. Krämer A., Green J., Pollard J., Jr., and Tugendreich S., *Causal analysis approaches in Ingenuity Pathway Analysis*. Bioinformatics, 2014. **30**(4): p. 523-30.
71. Gerdle B. and Ghafouri B., *Proteomic studies of common chronic pain conditions - a systematic review and associated network analyses*. Expert Review of Proteomics, 2020: p. 1-23.
72. Wåhlén K., Ernberg M., Kosek E., Mannerkorpi K., Gerdle B., and Ghafouri B., *Significant correlation between plasma proteome profile and pain intensity, sensitivity, and psychological distress in women with fibromyalgia*. Scientific Reports, 2020. **10**(1): p. 12508.
73. Gerdle B., Wåhlén K., and Ghafouri B., *Plasma protein patterns are strongly correlated with pressure pain thresholds in women with chronic widespread pain and in healthy controls-an exploratory case-control study*. Medicine (Baltimore), 2020. **99**(22): p. e20497.
74. Wåhlén K., Ghafouri B., Ghafouri N., and Gerdle B., *Plasma Protein Pattern Correlates With Pain Intensity and Psychological Distress in Women With Chronic Widespread Pain*. Front Psychol, 2018. **9**(2400): p. 2400.
75. Ramirez-Tejero J.A., Martinez-Lara E., Rus A., Camacho M.V., Del Moral M.L., and Siles E., *Insight into the biological pathways underlying fibromyalgia by a proteomic approach*. J Proteomics, 2018. **186**: p. 47-55.
76. Wåhlén K., Olausson P., Carlsson A., Ghafouri N., Gerdle B., and Ghafouri B., *Systemic alterations in plasma proteins from women with chronic widespread pain compared to healthy controls: a proteomic study*. J Pain Res, 2017. **10**: p. 797-809.

77. Ruggiero V., Era B., Cacace E., Molin L., Corda M., Fais A., and Utzeri S., *A preliminary study on serum proteomics in fibromyalgia syndrome*. Clin Chem Lab Med, 2014. **52**(9): p. e207-10.
78. Han C.L., Sheng Y.C., Wang S.Y., Chen Y.H., and Kang J.H., *Serum proteome profiles revealed dysregulated proteins and mechanisms associated with fibromyalgia syndrome in women*. Sci Rep, 2020. **10**(1): p. 12347.
79. Ciregia F., Giacomelli C., Giusti L., Boldrini C., Piga I., Pepe P., Consensi A., Gori S., Lucacchini A., Mazzoni M.R., and Bazzichi L., *Putative salivary biomarkers useful to differentiate patients with fibromyalgia*. J Proteomics, 2019. **190**: p. 44-54.
80. Bazzichi L., Da Valle Y., Rossi A., Giacomelli C., Sernissi F., Giannaccini G., Betti L., Ciregia F., Giusti L., Scarpellini P., Dell'Osso L., Marazziti D., Bombardieri S., and Lucacchini A., *A multidisciplinary approach to study the effects of balneotherapy and mud-bath therapy treatments on fibromyalgia*. Clinical and experimental rheumatology, 2013. **31**(6 Suppl 79): p. S111-S120.
81. Bazzichi L., Ciregia F., Giusti L., Baldini C., Giannaccini G., Giacomelli C., Sernissi F., Bombardieri S., and Lucacchini A., *Detection of potential markers of primary fibromyalgia syndrome in human saliva*. Proteomics Clin Appl, 2009. **3**(11): p. 1296-304.
82. Lind A.L., Just D., Mikus M., Fredolini C., Ioannou M., Gerdle B., Ghafouri B., Bäckryd E., Tanum L., Gordh T., and Månberg A., *CSF levels of apolipoprotein C1 and autotaxin found to associate with neuropathic pain and fibromyalgia*. J Pain Res, 2019. **12**: p. 2875-2889.
83. Khoonsari P.E., Ossipova E., Lengqvist J., Svensson C.I., Kosek E., Kadetoff D., Jakobsson P.J., Kultima K., and Lampa J., *The human CSF pain proteome*. J Proteomics, 2019. **190**: p. 67-76.
84. Khoonsari P.E., Musunri S., Herman S., Svensson C.I., Tanum L., Gordh T., and Kultima K., *Systematic analysis of the cerebrospinal fluid proteome of fibromyalgia patients*. J Proteomics, 2019. **190**: p. 35-43.
85. Olausson P., Ghafouri B., Backryd E., and Gerdle B., *Clear differences in cerebrospinal fluid proteome between women with chronic widespread pain and healthy women - a multivariate explorative cross-sectional study*. J Pain Res, 2017. **10**: p. 575-590.
86. Olausson P., Ghafouri B., Ghafouri N., and Gerdle B., *Specific proteins of the trapezius muscle correlate with pain intensity and sensitivity - an explorative multivariate proteomic study of the trapezius muscle in women with chronic widespread pain*. J Pain Res, 2016. **9**: p. 345-56.
87. Olausson P., Gerdle B., Ghafouri N., Sjöström D., Blixt E., and Ghafouri B., *Protein alterations in women with chronic widespread pain--An explorative proteomic study of the trapezius muscle*. Sci Rep, 2015. **5**: p. 11894.
88. Olausson P., Gerdle B., Ghafouri N., Larsson B., and Ghafouri B., *Identification of proteins from interstitium of trapezius muscle in women*

- with chronic myalgia using microdialysis in combination with proteomics.* PLoS One, 2012. **7**(12): p. e52560.
89. Anderson L. and Anderson N.G., *High resolution two-dimensional electrophoresis of human plasma proteins.* Proceedings of the National Academy of Sciences of the United States of America, 1977. **74**(12): p. 5421-5425.
 90. Geyer P.E., Holdt L.M., Teupser D., and Mann M., *Revisiting biomarker discovery by plasma proteomics.* Mol Syst Biol, 2017. **13**(9): p. 942.
 91. Anderson N.L. and Anderson N.G., *The human plasma proteome: history, character, and diagnostic prospects.* Mol Cell Proteomics, 2002. **1**(11): p. 845-67.
 92. Jain S., Gautam V., and Naseem S., *Acute-phase proteins: As diagnostic tool.* J Pharm Bioallied Sci, 2011. **3**(1): p. 118-27.
 93. Schrödl W., Büchler R., Wendler S., Reinhold P., Muckova P., Reindl J., and Rhode H., *Acute phase proteins as promising biomarkers: Perspectives and limitations for human and veterinary medicine.* PROTEOMICS – Clinical Applications, 2016. **10**(11): p. 1077-1092.
 94. Kuorinka I., Jonsson B., Kilbom A., Vinterberg H., Biering-Sorensen F., Andersson G., and Jorgensen K., *Standardised Nordic questionnaires for the analysis of musculoskeletal symptoms.* Appl Ergon, 1987. **18**(3): p. 233-7.
 95. Gerdle B., Larsson B., Forsberg F., Ghafouri N., Karlsson L., Stensson N., and Ghafouri B., *Chronic widespread pain: increased glutamate and lactate concentrations in the trapezius muscle and plasma.* Clin J Pain, 2014. **30**(5): p. 409-20.
 96. Ghafouri N., Ghafouri B., Larsson B., Stensson N., Fowler C.J., and Gerdle B., *Palmitoylethanolamide and stearoylethanolamide levels in the interstitium of the trapezius muscle of women with chronic widespread pain and chronic neck-shoulder pain correlate with pain intensity and sensitivity.* Pain, 2013. **154**(9): p. 1649-58.
 97. Palstam A., Larsson A., Bjersing J., Lofgren M., Ernberg M., Bileviciute-Ljungar I., Ghafouri B., Sjors A., Larsson B., Gerdle B., Kosek E., and Mannerkorpi K., *Perceived exertion at work in women with fibromyalgia: explanatory factors and comparison with healthy women.* J Rehabil Med, 2014. **46**(8): p. 773-80.
 98. Gerdle B., Ernberg M., Mannerkorpi K., Larsson B., Kosek E., Christidis N., and Ghafouri B., *Increased Interstitial Concentrations of Glutamate and Pyruvate in Vastus Lateralis of Women with Fibromyalgia Syndrome Are Normalized after an Exercise Intervention - A Case-Control Study.* PLoS One, 2016. **11**(10): p. e0162010.
 99. Lisspers J., Nygren A., and Soderman E., *Hospital Anxiety and Depression Scale (HAD): some psychometric data for a Swedish sample.* Acta Psychiatr Scand, 1997. **96**(4): p. 281-6.

100. Zigmond A.S. and Snaith R.P., *The hospital anxiety and depression scale*. Acta Psychiatr Scand, 1983. **67**(6): p. 361-70.
101. LoMartire R., Ang B.O., Gerdle B., and Vixner L., *Psychometric properties of Short Form-36 Health Survey, EuroQol 5-dimensions, and Hospital Anxiety and Depression Scale in patients with chronic pain*. Pain, 2020. **161**(1): p. 83-95.
102. Ferreira-Valente M.A., Pais-Ribeiro J.L., and Jensen M.P., *Validity of four pain intensity rating scales*. Pain, 2011. **152**(10): p. 2399-404.
103. Jensen M.P., Chen C., and Brugger A.M., *Interpretation of visual analog scale ratings and change scores: a reanalysis of two clinical trials of postoperative pain*. J Pain, 2003. **4**(7): p. 407-14.
104. Boonstra A.M., Schiphorst Preuper H.R., Balk G.A., and Stewart R.E., *Cut-off points for mild, moderate, and severe pain on the visual analogue scale for pain in patients with chronic musculoskeletal pain*. Pain, 2014. **155**(12): p. 2545-50.
105. Giesecke T., Williams D.A., Harris R.E., Cupps T.R., Tian X., Tian T.X., Gracely R.H., and Clauw D.J., *Subgrouping of fibromyalgia patients on the basis of pressure-pain thresholds and psychological factors*. Arthritis Rheum, 2003. **48**(10): p. 2916-22.
106. Gerhardt A., Eich W., Janke S., Leisner S., Treede R.D., and Tesarz J., *Chronic Widespread Back Pain is Distinct From Chronic Local Back Pain: Evidence From Quantitative Sensory Testing, Pain Drawings, and Psychometrics*. Clin J Pain, 2016. **32**(7): p. 568-79.
107. Petzke F., Clauw D.J., Ambrose K., Khine A., and Gracely R.H., *Increased pain sensitivity in fibromyalgia: effects of stimulus type and mode of presentation*. Pain, 2003. **105**(3): p. 403-13.
108. Bennett R., *The Fibromyalgia Impact Questionnaire (FIQ): a review of its development, current version, operating characteristics and uses*. Clin Exp Rheumatol, 2005. **23**(5 Suppl 39): p. S154-62.
109. Hedin P.J., Hamne M., Burckhardt C.S., and Engstrom-Laurent A., *The Fibromyalgia Impact Questionnaire, a Swedish translation of a new tool for evaluation of the fibromyalgia patient*. Scand J Rheumatol, 1995. **24**(2): p. 69-75.
110. Liedberg G.M., Burckhardt C.S., and Henriksson C.M., *Validity and reliability testing of the Quality of Life Scale, Swedish version in women with fibromyalgia -- statistical analyses*. Scand J Caring Sci, 2005. **19**(1): p. 64-70.
111. Burckhardt C.S. and Anderson K.L., *The Quality of Life Scale (QOLS): reliability, validity, and utilization*. Health Qual Life Outcomes, 2003. **1**: p. 60.
112. Leung L., *Pain catastrophizing: an updated review*. Indian journal of psychological medicine, 2012. **34**(3): p. 204-217.

113. Sullivan M.J.L., Bishop S.R., and Pivik J., *The Pain Catastrophizing Scale: Development and validation*. Psychological Assessment, 1995. **7**(4): p. 524-532.
114. Smets E.M., Garssen B., Bonke B., and De Haes J.C., *The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue*. J Psychosom Res, 1995. **39**(3): p. 315-25.
115. Ericsson A. and Mannerkorpi K., *Assessment of fatigue in patients with fibromyalgia and chronic widespread pain. Reliability and validity of the Swedish version of the MFI-20*. Disabil Rehabil, 2007. **29**(22): p. 1665-70.
116. McCracken L.M., Vowles K.E., and Eccleston C., *Acceptance of chronic pain: component analysis and a revised assessment method*. Pain, 2004. **107**(1-2): p. 159-66.
117. Bruderer R., Bernhardt O.M., Gandhi T., Xuan Y., Sondermann J., Schmidt M., Gomez-Varela D., and Reiter L., *Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results*. Mol Cell Proteomics, 2017. **16**(12): p. 2296-2309.
118. Rabilloud T., *Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains*. Proteomics, 2002. **2**(1): p. 3-10.
119. Celis J.E., Ratz, G., Basse, B., Lauridsen, J.B., Celis, A., Jensen, N.A. and Gromov, P. (1997), in *Cell Biology: A Laboratory Handbook (Celis, J.E., Carter, N., Hunter, T., Shotton, D., Simons, K. and Small, J.V., Eds.)*, Vol. IV, pp. 375–386, Academic Press, San Diego, CA.
120. Ghafouri B., Ståhlbom B., Tagesson C., and Lindahl M., *Newly identified proteins in human nasal lavage fluid from non-smokers and smokers using two-dimensional gel electrophoresis and peptide mass fingerprinting*. PROTEOMICS, 2002. **2**(1): p. 112-120.
121. Wåhlén K., Fornander L., Olausson P., Ydreborg K., Flodin U., Graff P., Lindahl M., and Ghafouri B., *Protein profiles of nasal lavage fluid from individuals with work-related upper airway symptoms associated with moldy and damp buildings*. Indoor Air, 2016. **26**(5): p. 743-54.
122. Bech-Serra J.-J., Borthwick A., Colomé N., ProteoRed C., Albar J.-P., Wells M., Sánchez del Pino M., and Canals F., *A multi-laboratory study assessing reproducibility of a 2D-DIGE differential proteomic experiment*. Journal of biomolecular techniques : JBT, 2009. **20**(5): p. 293-296.
123. Farrar J.T., Young J.P., Jr., LaMoreaux L., Werth J.L., and Poole R.M., *Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale*. Pain, 2001. **94**(2): p. 149-58.
124. Steiner J.L., Bigatti S.M., Slaven J.E., and Ang D.C., *The Complex Relationship between Pain Intensity and Physical Functioning in Fibromyalgia: The Mediating Role of Depression*. Journal of applied biobehavioral research, 2017. **22**(4): p. e12079.

125. Gui S.-W., Liu Y.-Y., Zhong X.-G., Liu X., Zheng P., Pu J.-C., Zhou J., Chen J.-J., Zhao L.-B., Liu L.-X., Xu G., and Xie P., *Plasma disturbance of phospholipid metabolism in major depressive disorder by integration of proteomics and metabolomics*. *Neuropsychiatric disease and treatment*, 2018. **14**: p. 1451-1461.
126. Lee J., Joo E.J., Lim H.J., Park J.M., Lee K.Y., Park A., Seok A., Lee H., and Kang H.G., *Proteomic analysis of serum from patients with major depressive disorder to compare their depressive and remission statuses*. *Psychiatry Investig*, 2015. **12**(2): p. 249-59.
127. Ruland T., Chan M.K., Stocki P., Grosse L., Rothermundt M., Cooper J.D., Arolt V., and Bahn S., *Molecular serum signature of treatment resistant depression*. *Psychopharmacology (Berl)*, 2016. **233**(15-16): p. 3051-9.
128. Sahebkhitiari N., Saraswat M., Joenvaara S., Jokinen R., Lovric A., Kaye S., Mardinoglu A., Rissanen A., Kaprio J., Renkonen R., and Pietilainen K.H., *Plasma Proteomics Analysis Reveals Dysregulation of Complement Proteins and Inflammation in Acquired Obesity-A Study on Rare BMI-Discordant Monozygotic Twin Pairs*. *Proteomics Clin Appl*, 2019. **13**(4): p. e1800173.
129. Oberbach A., Bluher M., Wirth H., Till H., Kovacs P., Kullnick Y., Schlichting N., Tamm J.M., Rolle-Kampczyk U., Murugaiyan J., Binder H., Dietrich A., and von Bergen M., *Combined proteomic and metabolomic profiling of serum reveals association of the complement system with obesity and identifies novel markers of body fat mass changes*. *J Proteome Res*, 2011. **10**(10): p. 4769-88.
130. Cominetti O., Núñez Galindo A., Corthésy J., Valsesia A., Irincheeva I., Kussmann M., Saris W.H.M., Astrup A., McPherson R., Harper M.-E., Dent R., Hager J., and Dayon L., *Obesity shows preserved plasma proteome in large independent clinical cohorts*. *Scientific reports*, 2018. **8**(1): p. 16981-16981.
131. Bruderer R., Muntel J., Müller S., Bernhardt O.M., Gandhi T., Cominetti O., Macron C., Carayol J., Rinner O., Astrup A., Saris W.H.M., Hager J., Valsesia A., Dayon L., and Reiter L., *Analysis of 1508 Plasma Samples by Capillary-Flow Data-Independent Acquisition Profiles Proteomics of Weight Loss and Maintenance*. *Mol Cell Proteomics*, 2019. **18**(6): p. 1242-1254.

ACKNOWLEDGEMENTS

What a PhD journey this has been. Who would have thought that a girl who loves Hello Kitty and is always dressed in pink would do a PhD... I have always known I would engage in research, but not to what extent. I'm so proud of my-self.

There are numerous people I would like to express my gratitude to which have helped me before and during this process, both related to my PhD studies and the "normal" life outside the PhD bubble.

First and foremost, I would like to thank **all women with CWP/FM and healthy controls** who participated in the studies. Without you, these studies would not have been possible!

My main supervisor, **Professor Bijar Ghafouri**. Words cannot express how grateful I am for this opportunity and that you have taken me under your wing as a PhD student. Not only have you opened the door to the world of proteomics, you have also showed me that hard work pays off. You have really prepared me for an upcoming bright research career within the field of painomics and you have showed me that research can be fun, inspiring, rewarding and sometimes really challenging.

پاش ١٦٠٠ کوی قالا
تو بوویا برادره کی زور نئزیک و هه میسه له بیرمه هو هامو شته خوشانه که کردومانا. قات له بیرم ناچئ هم سه فیره

Without you I would not have come this far.

My co-supervisor, **Professor Björn Gerdle**. Your supervision and feedback during this process has been greatly appreciated. Thank you for believing in me and challenging the medical biologist in me to think more as a clinician. I will try to bring all your advice forward in my research career, and hopefully I have taught you some tricks, too...

My co-supervisor, **Associate professor Mika Gustafsson**. You came into this PhD process halfway through. Although our collaboration took a different path than we anticipated from the beginning, you and your group have introduced me to bioinformatics which I am thankful for. I look forward to our upcoming projects.

I would also like to thank all my **co-authors** for valuable input and fruitful discussions on the papers: Professor **Malin Ernberg**, Professor **Eva Kosek**, Professor **Kaisa Mannerkorpi**, Associate professor **Nazdar Ghafouri**, Post doc **Hong Yan**, Associate professor **Charlotte Welinder**, PhD **Patrik Olausson** and M. Sc. **Anders Carlsson**.

Anders Kjellgren, the director of Pain and Rehabilitation Center (SORC), Region Östergötland, Linköping. Thank you for the opportunity to do a PhD and for all the support along the way. I would also like to thank **all my colleagues** at SORC for cheering on me during this journey.

Eva-Britt Lind – Our retired research nurse. Thank you so much for all the help regarding recruitment of all the participants in these studies. You were a great teacher when showing me how to draw blood samples and performing microdialysis insertions. I have come far since the first time and this knowledge is of great value for my ongoing and future research carrier.

This thesis would not have been possible without the members of the **PAINOMICS® laboratory**. I am glad and proud to be part of this team. **Anders Carlsson** – our research engineer. What would I do without you? Thank you for all your help in the lab and for being a great team player. Your nerdy features and passion for beer and ice cream never stop to intrigue me. **Niclas Stensson**, thank you for supporting me during this process and for being the best post doc mate. We sure need to practice our sailing theory skills for next time... **Mica Jönsson**, our newest member, I'm glad we have another female in the crew. Good luck with your upcoming PhD journey and hopefully I can guide you through this process and make it as fun as mine has been. **Patrik Olausson**, you are the whole reason why I started this PhD journey. I think of you every time I open SIMCA (which is a lot), and I miss you deeply. Cancer took you away, but no one can take away our memories. I will cherish them forever. #fuckcancer

To **all employees** at floor 14 in "Växthuset" LiU. Thank you for all the good fika times and lunch company. It has been a great place to have my office at and I look forward to the day when you all will come dressed in pink.

Stefan Ljunggren, Liam Ward, Helen Karlsson, Maria Assenhøj - What many great memories we have from all late calibration nights, epic Fridays, Medoc Marathon (I still cannot believe I won 😊) and not to forget all the skiing trips. It has been great fun and you all have really made this time enjoyable and unforgettable.

Cynthia Veenstra - Thank you so much for proofing my manuscript and for tips during the thesis writing. You are one of a few who understands the importance of a good manicure and who loves brussel sprouts as much as I do.

Johanna Huoman - WE DID IT! Who would have thought that we would defend our thesis within two days of time...Thank you for all the support (especially during the past weeks) and friendship during these years.

Elisabeth Paul - I'm so glad I met you and found a great friend in. Your support has been "von unschätzbarem Wert" during this process, and soon it's your turn 😊.

Anik Islam – Thank you for all fun memories during this PhD journey, and for being such a generous and loyal friend.

Maite Bedmar Casado – We started this journey together and ends it together. I wish you all the best for your post doc, and I can't believe it took me four years to understand jajajajajaja...

Daniel Sjögren – Thank you for being my friend and for welcoming me into your lovely family. I always have so much fun with you. After this I might have time to engage in a putting lesson or two so we can win that golf competition...

Josephine, Louise, Emma, Johanna, Anna C, Christine, Anneli – Even though we don't meet so often, it is always great when we get together, and when we do, it is as if time has stood still. I'm so grateful and glad to have you in my life and it's been lovely to follow you building your own families.

Emelie Blixt and Therese Ljunggren – I do not know what I would do without you! You really know when I need a break. If it's just going to Ullared, hang out with Emil and Ellen, picking mushrooms, doing cross country skiing 90 km in "Öppet Spår" in -27 °C, go shopping or just have dinner – it is always so much fun and I love and value our friendship.

Family Omar – Mohammad, Shenga, Shermi and Shiwana. I love your Kurdish traditions and food. I always feel so welcome in your home. It's been great fun to get to know you all and I look forward to dance "korvdansen" soon...

Not to forget, **Hello Kitty** and all her friends. You always make me happy. I'm longing for when I can go back to Japan and visit you again in Puroland.

Family Karlsson/Wintler – Ewa and Björn – Thank you for welcoming me into your family more than 10 years ago. You are amazingly kind and always look after us "kids". Thank you so much for supporting me before and during this PhD journey. When corona is over I hope we can play golf together again. **Anna-Maria and Jing** - it's going to be a lot of doctors in the family...I look forward to your PhD defenses 😊

My family – you are my biggest supporters and I love you deeply! Without you I would be nothing. My twin brother **Peter** – You are a true fighter! I wish I had the same passion for training and skills for making amazing desserts as you do. My dad **Jan** – Thank you for your endless support and love. Soon I will get the "murkla" 😊. Btw now you two can finally call me a doctor (however not the doctor you sometimes think I am 😊). **Catrin** – thank you for being in our lives and for supporting me during all these years.

Mom – I have missed you every day since you sadly left us 21 years ago. I hope you are having a great time sitting on your pink fluffy cloud watching down on us. I long for the day until we meet again. But that must wait because first I want to become a professor...

Karl-Johan – My love, my mushroom and my "rauk". This PhD journey would have never happened if we wouldn't have met on Skytte-C in 2009. Thank you for your patience, for taking care of me, for your endless support and for loving me for who I am. I really look forward the most important journey called LIFE with you, and to start our new house adventure. I love you ∞

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-170794>

FACULTY OF MEDICINE AND HEALTH SCIENCES

Linköping University Medical Dissertations No. 1757, 2020
Department of Health, Medicine and Caring Sciences

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se

Karin Wåhlén

PhD at PAINOMICS® laboratory
M.Sc. in Medical Biology at LiU 2013
Contact: karin.wahlen@liu.se

Publication list

Wåhlén, K. et al. Sci Rep 10, 12508 (2020)
Gerdle, B. et al. Medicine 99, e20497 (2020)
Tobieson, L. et al. Proteome Sci 18: p. 7 (2020)
Peolsson, A. et al. BMC Musc Disord 20, 51 (2019)
Wåhlén, K. et al. Front Psycho 9, 2400 (2018)
Sørensen, L.B. et al. Sci Rep 8, 3034 (2018)
Wåhlén, K. et al. J Pain Res 10, 797-809 (2017)
Wåhlén, K. et al. Indoor Air 26, 743-754 (2016)
Shimada, A. et al. Eur J Pain 20, 1502-1512 (2016)
Fornander, L. et al. PloS One 8, e83089 (2013)