USASP
US ASSOCIATION FOR THE STUDY OF PAIN

PUBLISHED BY
ELSEVIER

The Journal of Pain, Vol xxx, No. xxx (xxxx), xxxx: pp xxx–xxx
Available online at www.jpain.org and www.sciencedirect.com

Sex Differences in the Serum Proteomic Profile During Acute Low Back Pain—A Preliminary Study of the Relationship to Future Low Back Pain

Luke C. Jenkins,[*,†] Wei-Ju Chang,[*,‡] Peter Humburg,[*,§] Valerie C. Wasinger,[¶,‖] Laura S. Stone,[**] Susan G. Dorsey,[††] Cynthia Renn,[††] Angela Starkweather,[‡‡] and Siobhan M. Schabrun[*,§§,¶¶]

[*]*Centre for Pain IMPACT, Neuroscience Research Australia (NeuRA), Randwick, New South Wales, Australia,* [†]*School of Health Sciences, Western Sydney University, Penrith, New South Wales, Australia,* [‡]*School of Health Sciences, College of Medicine, Health and Wellbeing, University of Newcastle, New South Wales, Australia,* [§]*Mark Wainwright Analytical Centre, University of New South Wales, Sydney, New South Wales, Australia,* [¶]*Bioanalytical Mass Spectrometry Facility, Mark Wainwright Analytical Centre, UNSW, Kensington, NSW, Australia,* [‖]*School of Medical Science, UNSW, Kensington, NSW, Australia,* [**]*Department of Anesthesiology, Faculty of Medicine, University of Minnesota, Minneapolis, Minnesota,* [††]*Department of Pain & Translational Symptom Science, University of Maryland Baltimore, Baltimore,* [‡‡]*Department of Biobehavioral Nursing Science, University of Florida College of Nursing, Gainesville, Florida,* [§§]*The Gray Centre for Mobility and Activity, Parkwood Institute, London, Ontario, Canada,* [¶¶]*School of Physical Therapy, University of Western Ontario, London, Ontario, Canada*

Abstract: **The molecular processes driving the transition from acute to chronic low back pain (LBP) remain poorly understood and are likely to be sexually dimorphic. This study aimed to explore sex differences in the serum proteomic profile of people experiencing an acute LBP episode and determine if serum protein concentrations were associated with three-month outcome. Serum samples were collected through venepuncture from 30 female and 29 male participants experiencing an acute LBP episode. Serum samples underwent trypsin digestion and fractionation using hydrophobic interaction chromatography and were then analysed using mass-spectrometry. Mass-spectrometry spectra were searched in the Swissprot database for protein identification. Sex differences in protein abundance changes were evident upon inspection of fold changes. Multivariable data analysis identified 21 serum proteins during the acute episode that correctly classified 93% of males and 23 serum proteins that correctly classified 90% of females with ongoing LBP at 3 months. Pathway analysis suggested the differentially expressed proteins during acute LBP were frequently involved in immune, inflammatory, complement, or coagulation responses. This data provides preliminary evidence that biological processes during an acute LBP episode may contribute to the resolution, or persistence, of LBP symptoms at 3 months, however, these processes differ between males and females.**

Perspective: ***Differential expression of serum proteins was observed between male and female participants during an acute LBP episode. This preliminary work provides a foundation for future research targeting distinct immune system processes in males and females that may interfere with the transition from acute to chronic LBP.***

Key words: *Low back pain, Proteins, Mass spectrometry, Immune, Sex differences*

Received August 17, 2023; Received in revised form November 7, 2023; Accepted November 10, 2023
Supplementary data accompanying this article are available online at www.jpain.org and www.sciencedirect.com.
Address reprint requests to Siobhan M. Schabrun, School of Physical Therapy, University of Western Ontario, London, Ontario, Canada.
E-mail: s.schabrun@uwo.ca

1526-5900/$36.00

https://doi.org/10.1016/j.jpain.2023.11.009

Low back pain (LBP) is the most common musculoskeletal pain condition with an estimated one-month prevalence of approximately 23%.[1,2] Worldwide, LBP is the leading cause of years lived with disability,[3] resulting in considerable economic burden[4] that is projected to substantially increase.[5] Specific causes of LBP such as vertebral fracture, axial spondyloarthritis, malignancy and infection are rare, with estimates suggesting serious pathology is present in less than 1% of individuals presenting to primary care.[6] For most individuals with LBP, the specific cause of their symptoms is unknown and the condition is diagnosed as "non-specific" LBP.[7] Sex differences in non-specific LBP prevalence and severity have been explored, with most data suggesting a female predominance.[8]

Socioeconomic, occupational, and psychological risk factors for the development of chronic (symptoms lasting 3 months or more) LBP have been extensively studied,[9–11] however, these factors explain, at best, a moderate proportion of the variance in LBP outcome.[12–14] This has led to the exploration of the biological mechanisms underpinning LBP, focussing largely on the peripheral[15,16] and central nervous systems.[17–20]

It is increasingly accepted that LBP engages biological processes that extend beyond the nervous system.[21] For example, elevated serum levels of C-reactive protein during acute LBP is associated with better recovery at six months.[22] Further, substantial differences in the time course of transcriptomic changes have been demonstrated between people who recovered from their acute LBP episode and those with persistent symptoms at three-month follow-up.[23] When investigating pain biology researchers should consider sex differences.[24] For example, sex differences may exist in biological mechanisms linked to pain persistence such as cytokine expression,[25] neurotransmitter receptor activity,[26] cortical connectivity,[27] and diffuse noxious inhibitory control.[28] A lack of consideration for sexual dimorphism could hamper the development of more effective pain management options.[29,30]

The human 'proteome' refers to the total protein complement encoded by a given genome[31] and more studies are considering differential protein expression amongst clinical pain conditions. For example, in females with chronic widespread pain, upregulated proteins commonly involved in immune, inflammatory, and metabolic processes, have been linked to pain intensity, hypersensitivity, and psychological distress.[32] Other tissues and fluids including saliva, serum, urine, and cerebrospinal fluid have also been studied with proteomic methods, with differentially expressed proteins linked to neuropathic pain,[33] fibromyalgia,[34–36] rotator cuff related shoulder pain,[37] osteoarthritis[38] and chronic LBP.[39]

To our knowledge, no study has explored sex differences in the serum proteomic profile of individuals experiencing an acute episode of LBP or attempted to identify plausible serum proteomic biomarkers of poor LBP outcome. Therefore, the primary aim of this study was to investigate sex differences in the serum proteomic profile of individuals experiencing an acute episode of LBP and compare differences in the acute stage serum proteomic profile between males and females with, and without, ongoing LBP symptoms at three-month follow-up. The secondary aim was to investigate the relationship between male and female serum proteomic profiles in the acute stage of LBP and depression and anxiety, pressure pain sensitivity, and descending pain modulation.

Methods

Experimental Design

This study used a sub-sample of participants from the Understanding Persistent Pain Where it ResiDes (UPWaRD) longitudinal cohort who provided a serum sample at baseline assessment and were not consuming analgesic or anti-inflammatory medication prescribed by their physician for the treatment of pain at the time of sample collection (ACTRN12619000002189). Further, the sub-sample was purposively matched in age, sex, and recovery status (30 females [mean age 42 ± 15]; 29 males [mean age 38 ± 16]) to minimise the effect of potential confounding factors in order to address the primary study aim. Of the 59 participants included in this study, 15 male and 15 female participants were considered recovered from their LBP episode (average numerical rating scale [NRS] = .30 ± .47), while 14 male and 15 female participants were considered not recovered (average NRS = 4.0 ± 1.7) at three-month follow-up.

Sample sizes were not estimated for this study because of the explorative approach used to identify serum protein abundance with mass spectrometry. However, the LBP cohort described in this study (N = 59) is similarly sized to other human "omics" studies that have identified between-group differences.[32,40–43]

Participants

All other inclusion and exclusion criteria for this study were identical to those reported within the UPWaRD longitudinal cohort profile.[44] In brief, participants were recruited from local hospitals in South Eastern and South Western Sydney local health districts, New South Wales, Australia, primary care practitioners (eg, general practitioners and physiotherapists), newspaper/online advertisements, flyers, and social media sites such as Facebook. Ethical clearance was obtained from the institutional Human Research Ethics Committee (Western Sydney University: H10465 and Neuroscience Research Australia: SSA: 16/002). All procedures were conducted in accordance with the Declaration of the World Medical Association[45] and all participants provided written informed consent. This study is reported in line with the Sex and Gender Equity in Research guidelines[46] and the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[47]

Participants were recruited and assessed within 6 weeks of the onset of an acute LBP episode. LBP was defined as pain in the region of the lower back, superiorly bound by the thoracolumbar junction and inferiorly by the gluteal fold.[48] Participants remained eligible if they had pain referred beyond this region that was not associated with lumbosacral radiculopathy. Participants LBP episode must have been preceded by a period of at least one-month pain-free, the pain must

Table 1. Participant Characteristics at Baseline Compared Between Three-month Recovery Status

Characteristic	*Recovered (N = 30)*	*Non-recovered (N = 29)*	*P-value*
Age (years)	39.4 (16.7)	40.4 (15.1)	.81
BMI (kg/m^2)	24.4 (4.1)	25.0 (5.0)	.65
Medication use (yes, %)	17 (56.7)	11 (39.3)	.19
Previous LBP (yes, %)	23 (79.3)	20 (74.1)	.64
Comorbidity (yes, %)	9 (30.0)	7 (25.9)	.73
Pain duration (days)	18.6 (11.1)	15.1 (9.3)	.28
Baseline pain intensity (NRS)	3.8 (1.9)	5.0 (1.6)	.01
Depressive symptoms (DASS-21)#	9.4 (8.7)	15.3 (16.0)	.10
Pressure pain threshold (kPa)#	1005.2 (407.7)	894.9 (279.3)	.23
Conditioned pain modulation (kPa)	92.3 (178.5)	39.7 (168.1)	.26

NOTE. Continuous data described as mean and standard deviation. Categorical data described as number and percent. Variable means were compared between non-recovered and recovered low back pain participants using t-tests (continuous variable) or X^2 tests (categorical variables). # Welch's t-test performed.

have been present for at least 24 hours and participants must have reported pain of at least 2/10 (NRS, 0 = "no pain" and 10 = "worst pain imaginable") at any time during the 7 days preceding initial screening to be eligible for study inclusion.[48–51] Lumbosacral radiculopathy was defined as pain within a dermatomal-associated distribution, with or without neurological signs of spinal nerve involvement such as dermatome-associated sensory loss, impaired motor function, or attenuated reflexes. Individuals who presented with suspected serious spine pathology (eg, fracture, tumour, cauda equina syndrome), other major diseases/disorders (eg, schizophrenia, chronic renal disorder, multiple sclerosis), a history of spine surgery, or any other chronic pain conditions were excluded. Participants were asked to rate their "average" level of LBP over the previous 7 days using an 11-point (NRS: 0 ='no pain', 10 ='worst pain imaginable') on the day of baseline testing.[52] Characteristics of the participants included in this study are summarised in Table 1.

Health and Medication Usage

Full details of data collected from participants within the UPWaRD LBP cohort are described in the cohort profile.[44] All participants self-reported general health characteristics including age (years), weight (kg), and height (m). Body mass index was then calculated (BMI: kg/m^2). Participants self-selected comorbid health conditions other than LBP from a list including "other" and reported all medications they were currently using (both prescribed and "over-the-counter"). Participants reported if they had experienced LBP at any time in the past (yes/no) and provided the date they believed their current pain episode had begun which was used to calculate pain duration. Average pain intensity over the week preceding baseline testing was reported by all participants using an 11-point NRS.

Sample Preparation

Peripheral venous blood was drawn into serum tubes (BD, SST II Advance) through venepuncture of the median cubital vein at baseline assessment. The sample was clotted (30 minutes, room temperature) then separated by centrifugation (2,500 rpm, 15 minutes). Samples were pipetted into 50 μL aliquots and immediately stored at –80 °C. After thawing, de-identified sera from all 59 participants were prepared by digesting 3 μL of serum (57 μg ul-1 ± 7 μg) in 50 μL of 50 mM AMBIC, 2 M urea, 10 mM DTT at pH 8 using trypsin at 25 °C for 16 hours in a 1:100 enzyme to protein ratio. Digestion was halted by acidification. Serum peptides were fractionated using hydrophobic interaction chromatography according to the manufacturers protocol (PolyLC Inc, MD) with the additional parameter of decreasing solvent releasing increasingly hydrophilic peptides. Five fractions with decreasing solvent were prepared (unbound fraction, 25%,50%,70%, and 100% sequential solvent extraction) with peptides released by 70% acetonitrile in 15 mM ammonium acetate fraction being evaluated further.

Mass Spectrometry of Samples

Digested and fractionated peptides were reconstituted in 5 μL .1% formic acid and separated by nano-LC using an Ultimate 3,000 high-performance liquid chromatography (HPLC) and autosampler (Dionex, Amsterdam, Netherlands). The sample, (.6 μL from 5 μL), was loaded onto a micro C18 pre-column (300 μm × 5 mm, Dionex) with H_2O:CH3CN (98:2, .1% trifluoroacetic acid) at 10 μL min^{-1}. After washing, the pre-column was switched (Valco 10 port valve, Dionex) into line with a fritless nanocolumn (75 μm id × 12 cm) containing reverse phase C18 media (1.9 μm, 120 °A, Dr. Maisch GmbH HPLC). Peptides were eluted using a linear gradient of H2O:CH3CN (98:2, .1% formic acid) to H_2O:CH3CN (64:36, .1% formic acid) at 250 nl min^{-1} over 90 minutes. The QExactive (Thermo Electron, Bremen, Germany) mass spectrometer (MS) was run in Data-Dependent Acquisition mode as previously described in ref.[53]

Protein Identification Relative Quantitation

Protein dataset-peak lists were generated from raw files using Mascot Daemon v2.5.1 (Matrix Science, London, UK, www.matrixscience.com). All MS/MS spectra were searched against Swissprot (downloaded February 2018; 556,568 sequences for protein identification with the following criteria: 1) species, *Human*; 2) allowed 1 missed cleavage; 3) variable modifications,

Oxidation (M), phosphorylation (S, T, Y); 4) peptide tolerance, ± 5 ppm; 5) Fragment tolerance, ± .05 Da; 6) peptide charge +2 and +3; and 7) enzyme specificity, semi-tryptic. A decoy database search was also performed. Only proteins identified from the Swissprot database, controlled by the Benjamini-Hochberg procedure for multiple comparisons, with 2 or more unique peptides were included in further analysis.

Three-month Low Back Pain Outcome

Participants completed the NRS at 3 months, providing an average pain intensity score for the week preceding the follow-up assessment. At 3 months follow-up participants who reported an NRS score ≥2 on average over the previous week were considered to have developed chronic or recurrent LBP (ie, not recovered) whereas participants who reported an NRS score of 0 or 1 were considered recovered. This threshold value of ≥2 on the NRS was chosen as it reflects cut-offs used in previous LBP prognostic research.[54–56]

Depressive Symptoms

Participants completed the 21-item depression, anxiety, and stress subscale (DASS-21[57]) on the day of baseline testing. The questionnaire evaluates symptoms of depression, anxiety and tension-stress, consisting of 21 items with responses quantified on a four-point Likert scale ranging from 0 ("not at all") to 4 ("applied to me very much, or most of the time"). A total score between 0 and 63 was calculated, where higher scores indicate higher levels of depression, anxiety, and/or stress.[58,59]

Pressure Pain Sensitivity and Descending Pain Modulation

On the day of baseline testing all LBP participants in the UPWaRD cohort completed numerous laboratory measures.[44] Two measures of pain sensitivity and their association with serum protein expression during acute LBP were explored in this study based on previous research identifying an association between these factors in a cohort of females experiencing chronic widespread pain.[32]

Pressure pain thresholds (PPTs) were assessed using a handheld pressure algometer (Somedic, Hörby, Sweden, probe size 1 cm^2) at the site of worst LBP (side of greatest pain on palpation). Pressure was applied at a rate of 40 kg Pascals per second (kPa/s) and participants used a hand-held trigger to indicate when the sensation of pressure first changed to one of pain. Three measures were obtained and averaged for analysis.

Descending pain modulation was assessed using an established conditioned pain modulation (CPM) paradigm.[60] First, heat pain threshold (HPT) was measured (Thermal Sensory Analyzer, TSA-2001, Q-Sense-CPM, Medoc Ltd, Ramat Yishai, Israel) using a 30 × 30 mm Peltier-based thermode placed on the skin of the ventral aspect of the forearm opposite the side of the worst pain. The temperature started at 32 °C and increased at a rate of .5 °C/second. Participants were instructed to push a button when the sensation of heat first changed to one of pain. Three measures were obtained and used as the average HPT. Next, 3 PPTs were measured at the site of LBP before the application of heat pain (test stimulus 1). Heat pain 1 °C greater than the participants HPT, was then applied to the ventral forearm opposite the side of LBP via the thermode, 10 cm distal to the medial epicondyle, and maintained for the duration of the test (conditioning stimulus). Three consecutive PPTs were re-measured 30 seconds post-heat application (test stimulus 2). Participants were instructed to rate their pain on an NRS (0–100) at 0 second, 30 seconds and immediately following the final PPT measurement. Pain scores were maintained between 50 and 80 during testing. The conditioning stimulus was adjusted by 1 °C as required to achieve a pain score within this range. The CPM response was calculated as test stimulus 2 minus test stimulus 1. A positive value indicates a normal CPM response. This CPM paradigm has shown good intra-session reliability.[61]

Statistical Analysis

Descriptive Statistics

Baseline data describing the cohort were compared between recovered and non-recovered, or male and female participants using chi squared (categorical variables) or independent t-tests (continuous variables). Homogeneity of variance was assessed using Levene's test and for variables that did not meet the equal variance assumption, a Welch's t-test was performed.

Protein Identification

Scaffold Software (version 4.8.7, Proteome Software Inc, Portland, OR) was used to compare the shotgun proteomic results using spectral counting. Peptide identifications were accepted if they could be established at greater than 95% probability using the Scaffold delta-mass correction. Expression changes across samples were measured via spectral count, normalised by total ion count with missing values kept at zero and recoded as not detected (ND) during further analysis.[18,19] Proteins which were present in < 50% of recovered or non-recovered groups were excluded from further functional analysis.

Multivariable Data Analysis

Orthogonal partial least squares (OPLS) analysis was performed using SIMCA software version 17 (Umetrix AB, Umea, Sweden). A discriminant analysis (OPLS-DA) was used to explore the longitudinal relationship between serum proteins (*X*-variables) and three-month LBP outcome (dichotomous outcome). OPLS regression was used to explore the longitudinal relationship between serum proteins (*X*-variables) and average pain intensity at three-month follow-up, or the cross-sectional relationship with baseline PPTs, baseline CPM, or baseline DASS-21 scores (continuous outcomes; *Y*-variables). The

procedures involved in multivariable data analysis (MVDA) using OPLS regression and reporting results from OPLS models have been described in detail elsewhere.[62–65]

In brief, OPLS is a form of supervised multivariable data analysis, suitable for variable (biomarker) selection.[66] OPLS models reduce the dimensionality of many variables into latent variables of interest. The latent variables are termed principal components, reflecting the coordinates of the original observation following a reduction in dimensionality. Variance that is unrelated to the hypothesis is reduced into latent variables termed orthogonal components.[66,67]

The first step of the analysis undertaken in this study was a principal component analysis. This was performed to identify outliers. Any participants deemed critical outliers based on Hotelling's T^2, T^2Crit (.99%), were excluded from further analysis.

OPLS was used for the regression analysis in this study,[68] and all variables were mean-centred and scaled for unified variance (UV-scaling). All variables with a low minimum/maximum ratio or high skewness were log-transformed prior to fitting the OPLS models. The variable influence on projection (VIP) measure indicates the relevance of each independent variable pooled overall dimensions and the group of variables that best explain the dependent variable. A VIP ≥ 1.0 combined with jack-knifed 95% confidence intervals (CI) in the regression coefficients plot not including zero were considered significant for this study. Coefficients were used to note the direction of the relationship (positive or negative). In the first step of the MVDA, of the 216 total identified proteins, 70 were present in at least 50% of both the recovered and non-recovered participant serum samples and were entered into the OPLS models. After the removal of any outliers, proteins with a VIP value ≥1.0 with jack-knifed CIs in the coefficients plot not including zero were included in the next step of the analyses.[69,70] To determine the relative importance of these significant proteins, a separate regression was carried out only including these significant proteins as the independent variables. Scaled loadings are presented for each significant independent variable. This is the loading scaled as a correlation coefficient, thus standardising the range from −1.0 to +1.0.[66]

Multivariable model fit: R^2 describes the goodness of fit explained by a principal component, whilst Q^2 describes the goodness of prediction—the amount of total variation of the variables that can be predicted by a principal component using cross-validation (CV) methods.[68] R^2 should not be considerably higher than Q^2. It is generally accepted that a difference of .2 to .3 between R^2 and Q^2 suggests an overfitted model.[66] Cross-validated analysis of variance (CV-ANOVA) was used to validate the models (seven-fold cross-validation). The *P*-value obtained from the CV-ANOVA is used to represent statistical significance. Finally, the score plot shows the observations (ie, recovered or non-recovered LBP), and by combining the score plot and loading plot, important variables can be linked to specific proteins or within group separations.

Results of this study are reported in line with recommendations for multivariable modelling suggested by Wheelock and Wheelock (ie, R^2, Q^2, number of principal/orthogonal components, CV-ANOVA, and *P*-value).[66]

Pathway Analysis

This study also uses bioinformatics analyses to visualise and contextualise interactions between the significant proteins identified in the multivariable data analysis. Protein network analysis was conducted using the online Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database Version 11.0.[71] Protein accession numbers obtained from the UniProt database for significant proteins in the MVDA models were entered in the STRING search engine (multiple proteins) using the following parameters: Organism = Homo sapiens, Network type = full STRING network, required interaction score = medium confidence (.400), and false discovery rate (FDR) stringency = ≤.01.

Results

Study Population

In line with our study methodology, this cohort was matched in age, sex and three-month LBP outcome. Rates of self-reported medication use were comparable between males (N = 13, 44.8%) and females (N = 15, 51.7%) at baseline. Baseline data describing the characteristics of recovered or non-recovered participants is presented in Table 1. The types of medication used by participants are listed in Supplemental File 1: Table S1. At baseline assessment, 8 (27.6%) females and 8 (28.6%) males reported at least one comorbid health condition (Table 2). The types of comorbidities are listed in Supplemental File 1: Table S2.

Protein Identification

A total of 216 serum proteins were identified confidently (2 peptide/protein identification and significance set at $P_{FDR} < .05$) (Supplemental File 1: Table S3). Of these identified proteins, 70 were present in at least 50% of both the recovered and non-recovered participant serum samples and were therefore eligible for multivariable data analysis. Visual inspection of the fold changes reported in Supplemental File 1: Table S3 highlights differences in protein abundance level between sex and three-month recovery status, thus male and female participants were first analysed separately.

Multivariable Data Analysis

Serum Protein Abundance Levels During Acute Low Back Pain are Associated With Three-month Low Back Pain Outcome

The remaining 70 proteins were first compared between male participants who had recovered or not recovered at the three-month follow-up using OPLS-DA.

Table 2. Participant Characteristics at Baseline Compared Between Sex

Characteristic	*Female (N = 30)*	*Male (N = 29)*	*P-value*
Age (years)	41.6 (15.3)	38.1 (16.4)	.40
BMI (kg/m^2)	23.3 (4.4)	26.1 (4.3)	.02
Medication use (yes, %)	15 (51.7)	13 (44.8)	.60
Previous LBP (yes, %)	19 (67.8)	24 (85.7)	.11
Comorbidity (yes, %)	8 (27.6)	8 (28.6)	1.00
Pain duration (days)	16.8 (11.0)	17.3 (9.8)	.88
Baseline pain intensity (NRS)	4.3 (1.8)	4.5 (1.9)	.78
Depressive symptoms (DASS-21)	14.1 (15.5)	10.5 (9.8)	.31
Pressure pain threshold (kPa)	787.5 (250.7)	1120.1 (364.9)	< .001
Conditioned pain modulation (kPa)	82.7 (122.2)	48.0 (217.2)	.46

NOTE. Continuous data described as mean and standard deviation. Categorical data described as number and percent. Variable means were compared between males and female participants using t-tests (continuous variable) or X^2 tests (categorical variable).

No critical outliers were present upon inspection of Hotelling's T^2. Twenty-one proteins had a VIP > 1.0 with jack-knife CI not crossing zero (Table 3). The model including 21 proteins was reduced in dimensionality to one principal component that was statistically significant (R^2 = .67, Q^2 = .62, CV-ANOVA: $P \leq .001$), (Fig 1). Serum protein concentrations of Albumin, Coagulation factor XII, Vitronectin, Afamin, Vitamin D-binding protein, Complement factor B, Apolipoprotein LI, and Complement component C8 gamma chain were upregulated in non-recovered males, whilst all other proteins identified as significant in males were downregulated. The final, OPLS-DA model had a cross-validated classification accuracy of 93% in male participants. The loading plot in Fig 2 displays the significant serum proteins identified in males.

All 70 proteins were then analysed in a separate OPLS-DA model for female participants. Again, no critical outliers were present. After inspection of VIP scores, 23 proteins were considered significant amongst females and retained in the model (Table 4). The model predicting three-month LBP outcome in females with 23

Table 3. Significant Serum Proteins in the OPLS-DA Model of Male Participants Recovered or Non-recovered From Acute Low Back Pain at Three-month Follow-up

Protein name	*Accession number*	*VIP*	*p (corr)*	*B*	*NR (N = 14)*	*R (N = 15)*	*Fold change (NR vs R)*
Serum albumin (ALB)*	P02768	2.25	.32	-.09	8.12 (.11)	7.84 (.12)	↑ 1.04
Kininogen-1 (KNG1)*	P01042	1.86	-.78	.09	5.78 (.14)	6.15 (.14)	↓ .94
Fibrinogen alpha chain (FGA)*	P02671	1.86	-.84	.09	6.74 (.31)	7.55 (.21)	↓ .89
Coagulation factor XII (F12)	P00748	1.75	.26	-.07	4.83 (1.42)	1.32 (2.28)	↑ 3.66
Complement factor H (CFH)*	P08603	1.60	-.74	.05	6.12 (.21)	6.33 (.17)	↓ .97
Immunoglobulin lambda constant 2 (IGLC2)*	P0DOY2	1.59	-.68	.06	4.15 (2.28)	4.99 (2.03)	↓ .83
Gelsolin (GSN)	P06396	1.56	-.69	.05	4.72 (1.40)	5.19 (1.45)	↓ .91
Complement C4-A (C4A)	P0C0L4	1.55	-.69	.06	6.36 (.40)	6.84 (.17)	↓ .93
Clusterin (CLU)*	P10909	1.51	-.63	.08	6.07 (.12)	6.38 (.14)	↓ .95
Inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1)*	P19827	1.51	-.62	.06	6.20 (.36)	6.28 (1.75)	↓ .99
Vitronectin (VTN)	P04004	1.46	.63	-.05	5.57 (1.62)	5.34 (1.49)	↑ 1.04
Afamin (AFM)	P43652	1.46	.63	-.05	3.48 (2.29)	.98 (2.03)	↑ 3.55
Immunoglobulin J chain (IGJ)	P01591	1.41	-.65	.07	5.29 (.49)	6.16 (.28)	↓ .86
Vitamin D-binding protein (GC)	P02774	1.36	.59	-.05	5.76 (1.69)	5.07 (2.08)	↑ 1.14
Haptoglobin (HP)*	P00738	1.36	-.57	.06	6.41 (.40)	6.83 (.22)	↓ .94
Complement factor B (CFB)*	P00751	1.33	-.56	.04	5.89 (.29)	5.73 (1.60)	↑ 1.03
Apolipoprotein LI (APOL1)	O14791	1.30	-.57	.05	5.91 (.34)	5.82 (1.62)	↑ 1.02
Apolipoprotein A-I (APOA1)	P02647	1.25	-.51	.06	6.68 (.28)	7.04 (.25)	↓ .95
Plasma kallikrein (KLKB1)	P03952	1.05	-.38	.05	4.78 (1.41)	5.10 (1.43)	↓ .94
Complement component C8 gamma chain (C8G)	P07360	1.02	.43	-.05	4.77 (1.40)	2.07 (2.63)	↑ 2.30
Apolipoprotein E (APOE)	P02649	1.01	-.47	.01	6.01 (.32)	6.16 (.25)	↓ .98

Abbreviations: R, recovered; NR, non-recovered.
NOTE. Accession numbers are derived from the protein data base UniProt (www.uniprot.org). Variable influence on projections (VIP) indicates the importance of the covariable. p(corr) is the loading of each variable scaled as a correlation coefficient. B is the regression coefficient. Recovered participants were coded as 0, and non-recovered participants were coded as 1. Fold change is a univariable measure whereby a value greater than 1 indicates a protein was upregulated in the non-recovered group (↑ = upregulated, ↓ = downregulated). Please note that fold change does not necessarily correspond to p(corr): that is, a positive fold change, is not automatically equal to a positive p(corr). Protein abundance is reported as the log10 scaled mean ± standard deviation.
*Indicates the protein was considered significant in male and female groups.

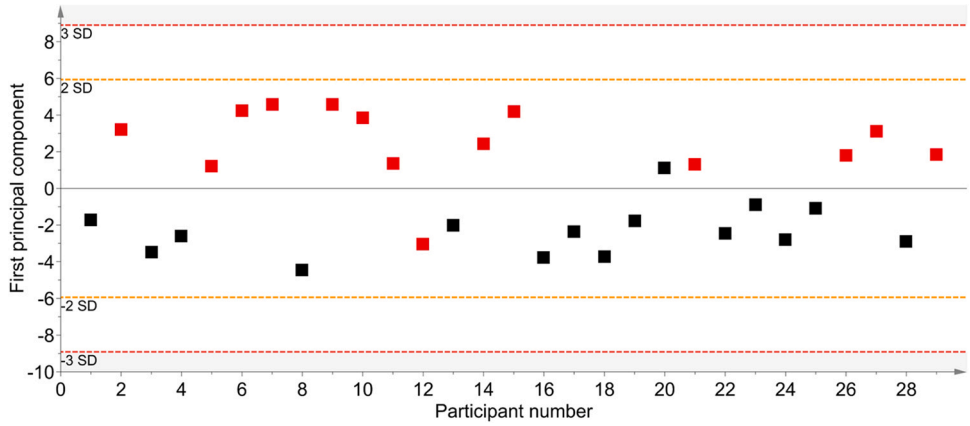

Figure 1. Score plot constructed with one principal component demonstrating separation between groups (R^2 = .67, Q^2 = .62, CV-ANOVA: $P \leq .001$). Black markers represent male participants who were recovered at three-month follow-up and red markers represent male participants that were considered not recovered at three-month follow-up (NRS ≥ 2).

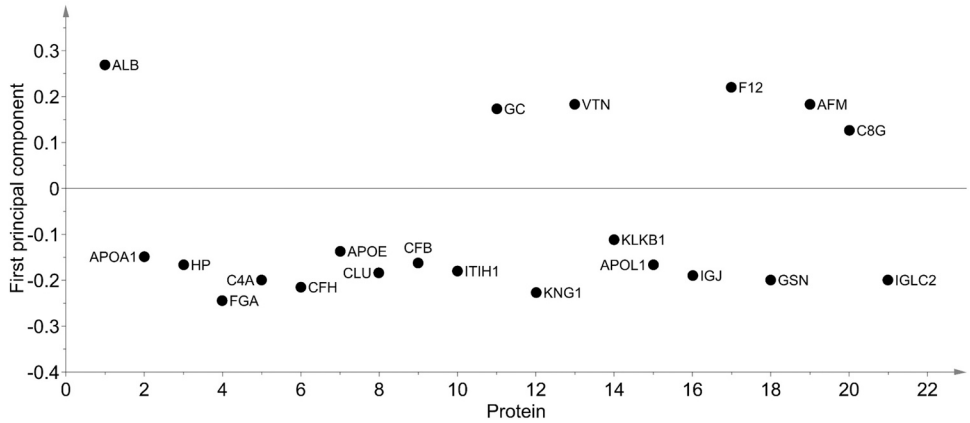

Figure 2. Loading plot displaying the raw loading values for significant proteins (VIP > 1.0) associated with three-month LBP recovery status in male participants. The protein abbreviations in the plot match the protein names in Table 3.

differentially expressed proteins was reduced in dimensionality to one principal component and one orthogonal component (R^2 = .65, Q^2 = .41, CV-ANOVA: P = .01), (Fig 3). Serum concentrations of Albumin, Kininogen-1, Fibrinogen alpha chain, Complement factor H, Immunoglobulin lambda constant 2, Clusterin, Haptoglobin, and Complement factor B were significantly downregulated in non-recovered males but upregulated in non-recovered females. Inter-alpha-trypsin inhibitor heavy chain H1 was significantly downregulated in both males and females. The loading plot in Fig 4 displays the significant serum proteins identified in females. The final, OPLS-DA model had a cross-validated classification accuracy of 90% in female participants.

Protein Network Analysis of the Significant Serum Proteins Predicting Three-month LBP Outcome in Male and Female Participants

The 21 proteins associated with three-month LBP outcome in males were entered into the STRING database, and twenty were included in the network analysis. Immunoglobulin lambda constant 2 was not identified by the search engine. The enriched network comparing non-recovered males to recovered males consisted of 20 nodes and 103 edges (Fig 5). The average local clustering coefficient was .75 and the protein-protein interaction enrichment score was deemed statistically significant ($P \leq$ 1.0E-16).

Amongst females, the 23 significant proteins were entered into the STRING database and 20 were included in the network analysis. Ig alpha-1 chain C region, Immunoglobulin lambda constant 2, and Ig mu chain C region were not identified by the search engine. The enriched network comparing non-recovered females to recovered females consisted of 20 nodes and 127 edges. The average local clustering coefficient was .80 and the protein-protein interaction enrichment score was statistically significant ($P \leq$ 1.0E-16) (Fig 6).

Serum Protein Abundance Levels During Acute Low Back Pain are Associated With Three-month Pain Intensity

Using an OPLS model the longitudinal relationship between baseline serum protein expression and three-month pain intensity (continuous outcome: NRS) was explored in male participants. The 21 significant

Table 4. Significant Serum Proteins in the OPLS-DA Model of Female Participants Recovered or Non-recovered From Acute Low Back Pain at Three-month Follow-up

Protein name	Accession number	VIP	p (corr)	B	NR (N = 14)	R (N = 15)	Fold change (NR vs R)
Alpha-1-antitrypsin (SERPINA1)	P01009	1.67	-.57	.27	4.78 (1.97)	5.84 (.30)	↓ .82
Apolipoprotein D (APOD)	P05090	1.49	.55	-.18	3.44 (2.53)	2.25 (2.49)	↑ 1.53
Ig alpha-1 chain C region (IGHA1)	P01876	1.46	.61	-.22	6.74 (.43)	6.36 (.28)	↑ 1.06
Complement factor H (CFH)*	P08603	1.44	.48	-.02	6.18 (.21)	5.99 (.24)	↑ 1.03
Alpha-1B-glycoprotein (A1BG)	P04217	1.43	.38	-.06	5.97 (1.69)	5.85 (.48)	↑ 1.02
Beta-2-glycoprotein 1 (APOH)	P02749	1.41	-.19	.18	4.05 (2.10)	5.13 (.26)	↓ .79
Serum albumin (ALB)*	P02768	1.35	-.23	.00	7.97 (.27)	8.11 (.19)	↓ .98
Immunoglobulin lambda-1 light chain (IGL1)	P0DOX8	1.32	.47	-.08	3.61 (2.65)	3.58 (2.26)	↑ 1.01
Alpha-2-HS-glycoprotein (AHSG)	P02765	1.32	-.57	.13	6.47 (1.83)	7.06 (.56)	↓ .92
Kininogen-1 (KNG1)*	P01042	1.31	.48	-.08	5.92 (.24)	5.72 (.26)	↑ 1.03
Immunoglobulin lambda constant 2 (IGLC2)*	P0DOY2	1.29	.32	-.08	3.33 (2.82)	1.38 (2.38)	↑ 2.41
Complement factor B (CFB)*	P00751	1.26	.20	-.03	5.58 (1.58)	5.77 (.30)	↓ .97
Hemopexin (HPX)	P02790	1.25	.36	-.07	5.66 (1.59)	5.76 (.32)	↓ .98
Complement C1s subcomponent (C1S)	P09871	1.17	.43	-.10	5.26 (1.48)	5.05 (1.42)	↑ 1.04
Ig mu chain C region (IGHM)	P01871	1.15	.27	.01	5.35 (1.53)	5.56 (.33)	↓ .96
Antithrombin-III (SERPINC1)	P01008	1.14	.37	-.07	4.55 (1.88)	3.69 (2.32)	↑ 1.23
Complement C3 (C3)	P01024	1.10	.23	.04	6.65 (.34)	6.51 (.30)	↑ 1.02
Inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1)*	P19827	1.10	.17	.05	5.95 (1.68)	6.23 (.35)	↓ .96
Inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2)	P19823	1.05	.18	-.14	6.35 (.28)	6.20 (.23)	↑ 1.02
Transthyretin (TTR)	P02766	1.05	-.54	.10	3.03 (2.58)	4.20 (2.19)	↓ .72
Fibrinogen alpha chain (FGA)*	P02671	1.04	.31	.03	7.15 (.53)	6.76 (.53)	↑ 1.06
Clusterin (CLU)*	P10909	1.02	.28	.03	6.16 (.27)	6.12 (.18)	↑ 1.01
Haptoglobin (HP)*	P00738	1.01	.31	-.01	6.68 (.38)	6.57 (.31)	↑ 1.02

Abbreviations: R, recovered; NR, non-recovered.
NOTE. Accession numbers are derived from the protein database UniProt (www.uniprot.org). Variable influence on projections (VIP) indicates the importance of the covariable. p(corr) is the loading of each variable scaled as a correlation coefficient. B is the regression coefficient. Recovered participants were coded as 0, and non-recovered participants were coded as 1. Fold change is a univariable measure whereby a value greater than 1 indicates a protein was upregulated in the non-recovered group (↑ = upregulated, ↓ = downregulated). Please note that fold change does not necessarily correspond to p(corr): that is, a positive fold change, is not automatically equal to a positive p(corr). Protein abundance is reported as the log10 scaled mean ± standard deviation.
*Indicates the protein was considered significant in male and female groups.

proteins listed in Table 3 were entered into an OPLS model with continuous three-month pain intensity as the outcome of interest. All 21 serum proteins displayed a VIP value > 1.0 with jack-knifed CI not crossing zero. The model was reduced in dimensionality to 1 predictive component that explained 48% of the variation in males three-month pain intensity (R^2 = .48, Q^2 = .38, CV-ANOVA: P = .002).

The same process was repeated for female participants. Of the 23 proteins entered into the OPLS model (Table 4), 14 remained significant with a VIP value > 1.0 and jack-knife CI not crossing zero: Alpha-1-antitrypsin,

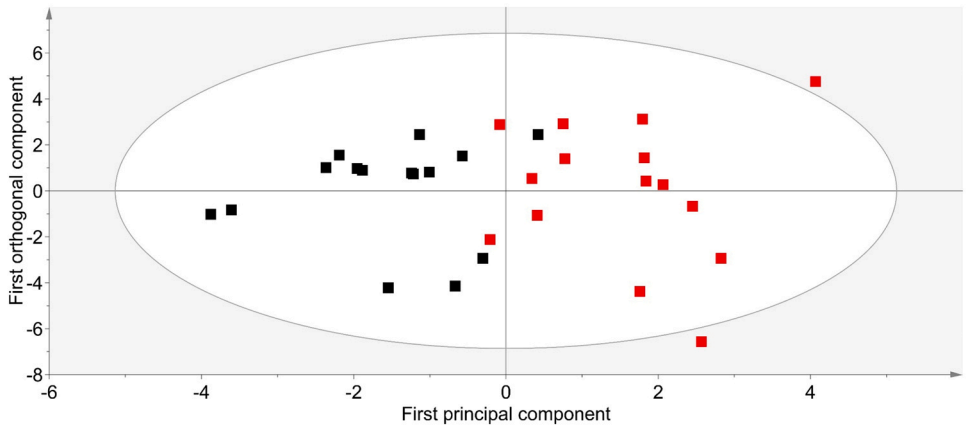

Figure 3. Score plot constructed with one principal component and one orthogonal component demonstrating separation between groups (R^2 = .65, Q^2 = .41, CV-ANOVA: P = .01) for female participants. Black markers represent female participants who were recovered at the three-month follow-up and red markers represent female participants that were considered not recovered at the three-month follow-up (NRS ≥ 2).

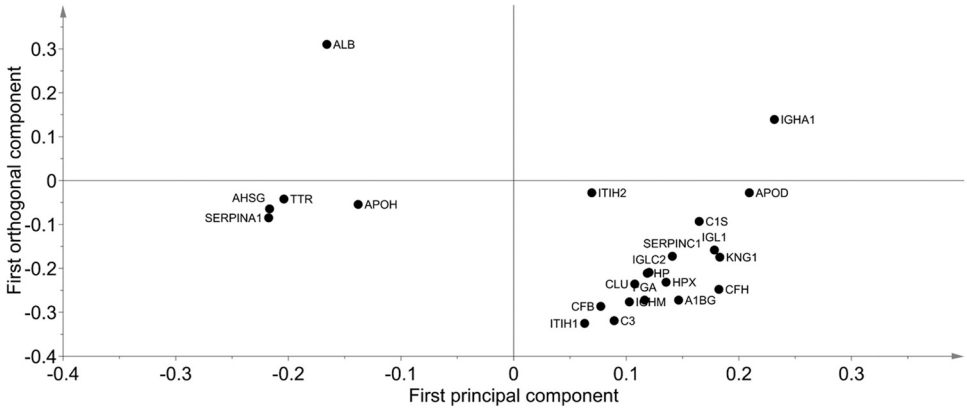

Figure 4. Loading plot displaying the raw loading values for significant proteins (VIP > 1.0) associated with three-month LBP recovery status in female participants. The protein abbreviations in the plot match the protein names in Table 4.

Apolipoprotein D, Complement factor H, Albumin, Immunoglobulin lambda-1 light chain, Alpha-2-HS-glycoprotein, Immunoglobulin lambda constant 2, Complement factor B, Hemopexin, Inter-alpha-trypsin inhibitor heavy chain H1, Fibrinogen alpha chain, Clusterin and Haptoglobin. The model including 14 proteins was reduced in dimensionality to one principal component and 1 orthogonal component (R^2 = .71, Q^2 = .52, CV-ANOVA: P = .001).

Serum Protein Abundance Levels and Depression, Anxiety and Stress During an Acute Episode of Low Back Pain

For male participants baseline DASS scores were not associated with serum protein abundance levels after exclusion of 2 missing data points and 2 critical outliers (1 principal component and 1 orthogonal component, R^2 = .69, Q^2 = –.39, CV-ANOVA: P = 1.00). In females, 2 missing data points and 1 critical outlier were excluded and the OPLS model again demonstrated no association between baseline serum protein abundance levels and depression, anxiety or stress (1 principal component and 1 orthogonal component; R^2 = .80, Q^2 = –.14, CV-ANOVA: P = 1.00).

Serum Protein Abundance Levels and Pressure Pain Threshold During an Acute Episode of Low Back Pain

Using an OPLS model the cross-sectional association between serum protein abundance levels and PPTs during an acute episode of LBP was explored. In males, thirteen proteins were associated with baseline PPTs at

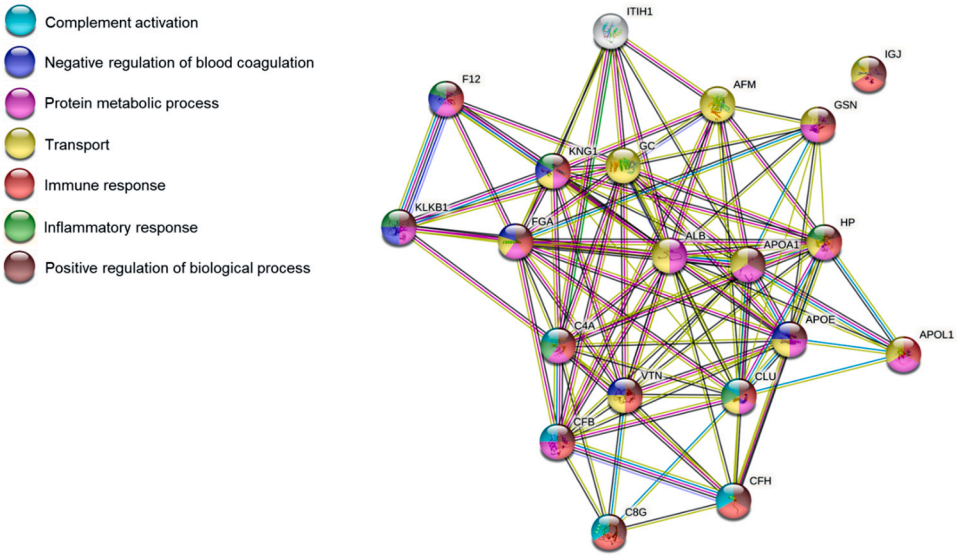

Figure 5. Pathways analysis of group differences between males considered recovered or non-recovered at three months. The functional protein network was derived from significant serum proteins. Immunoglobulin lambda constant 2 was not identified by the search engine. STRING version 11 was used to create the network analysis (https://string-db.org/). Abbreviations used for each protein are listed in Table 3. In the network, each protein is represented by a node (circles), coloured according to its biological processes (legend to the left of figure). The protein-protein interaction is represented by an edge visualised as a coloured line. Known interactions were from curated databases (turquoise) or experimentally determined (pink). Predicted interactions (coloured line) were identified from gene neighbourhoods (green), gene fusion (red), and gene co-occurrence (dark blue), and other interactions were identified using text mining (yellow), co-expression (black), and protein homology (purple).

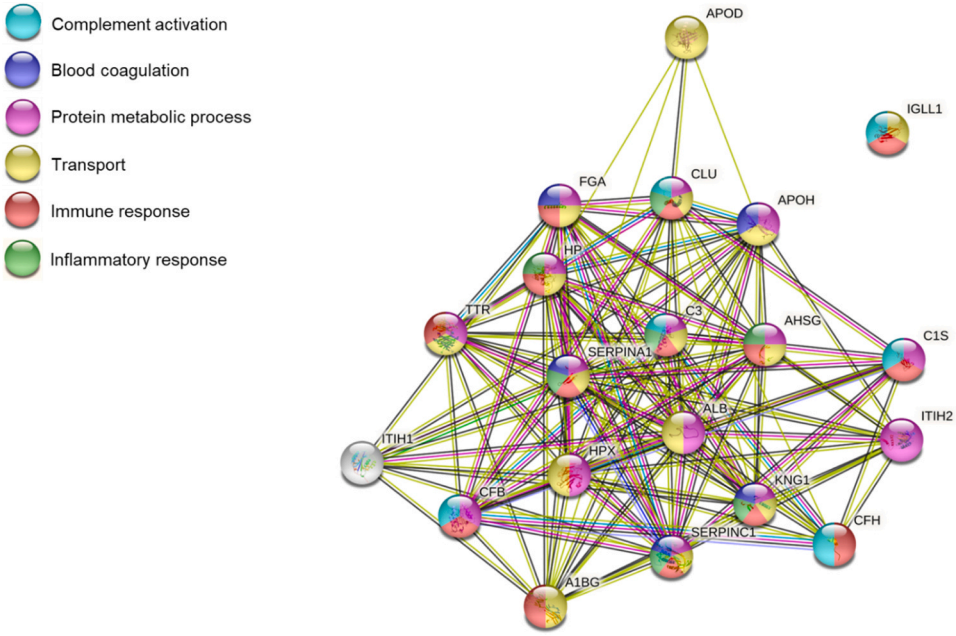

Figure 6. Pathways analysis of group differences between females considered recovered or non-recovered at three months. The functional protein network was derived from significant serum proteins. Ig alpha-1 chain C region, Immunoglobulin lambda constant 2, and Ig mu chain C region were not identified by the search engine. STRING version 11 was used to create the network analysis (https://string-db.org/). Abbreviations are listed in Table 4. In the network, each protein is represented by a node (circles), coloured according to its biological processes (legend to the left of figure). The protein-protein interaction is represented by an edge visualised as a coloured line. Known interactions were from curated databases (turquoise) or experimentally determined (pink). Predicted interactions (coloured line) were identified from gene neighbourhoods (green), gene fusion (red), and gene co-occurrence (dark blue), and other interactions were identified using text mining (yellow), co-expression (black), and protein homology (purple).

the site of LBP (Table 5). The multivariable model was reduced in dimensionality to 1 principal and 1 orthogonal component (R^2 = .55, Q^2 = .33, CV-ANOVA: P = .04). The score plot (Fig 7) and loading plot (Fig 8) suggest that four proteins: Serum amyloid A-4 protein, Vitronectin, Serum albumin and Prothrombin, were associated with lower PPTs. The thirteen significant proteins were entered into the STRING database. The enriched network consisted of 13 nodes and 42 edges. The average local clustering coefficient was .71 and the protein-protein interaction enrichment score was statistically significant ($P \leq$ 1.0E-16) (Fig 9). Amongst female participants there was no evidence to suggest serum protein abundance levels were associated with PPTs during an acute LBP episode (1 principal component and 1 orthogonal component; R^2 = .11, Q^2 = .01, CV-ANOVA: P = .88).

Serum Protein Abundance Levels and Descending Pain Modulation During an Acute Episode of Low Back Pain

Serum protein abundance levels were not associated with the CPM response at baseline in males after exclusion of 2 participants with missing CPM data at baseline and the exclusion of 2 participants considered critical outliers (1 principal component, R^2 = .50, Q^2 = .19, CV-ANOVA: P = 1.00). Similarly, baseline CPM response was not associated with serum protein abundance in females after the removal of one participant with missing CPM baseline data and one participant deemed a critical outlier in the OPLS model (1 principal component, R^2 = .59, Q^2 = .13, CV-ANOVA: P = .17).

Sensitivity Analysis

A sensitivity analysis was conducted to further understand sex differences in this data. First, the OPLS-DA model fitted in male participants including 21 significant proteins was tested on the female dataset. The significant proteins identified in male participants classified LBP outcome correctly in only 37% of females, which is less than chance (1 principal component and 1 orthogonal component; R^2 = .68, Q^2 = .62, CV-ANOVA: $P \leq$ .001).

Next, an OPLS-DA model was developed for all recovered participants with sex entered as the dichotomous outcome of interest. In the recovered participants, 25 proteins were considered significant predictors of sex (Supplemental File 1: Table S4). The OPLS-DA model including these significant proteins was reduced in dimensionality to 1 principal component (R^2 = .66, Q^2 = .60, CV-ANOVA: P = < .001). The model correctly classified sex in 87% of the recovered participants. The same process was repeated in non-recovered participants and 14 significant proteins were identified, 9 of which were also considered significant in the model predicting the sex of recovered participants (Immunoglobulin J chain, Fibrinogen alpha chain, Kininogen-1, Serum albumin, Clusterin, Vitronectin, Complement factor H, Apolipoprotein A-I, Inter-alpha-trypsin inhibitor heavy chain H1) (Supplemental File 1,

Table 5. Serum Proteins Significantly Associated With Baseline Pressure Pain Thresholds at the Site of Low Back Pain in Male Participants

Protein name	Accession number	VIP	p (corr)	B
Cystatin-C (CST3)	P01034	1.79	.55	.21
Serum amyloid A-4 protein (SAA4)	P35542	1.68	-.63	-.26
Alpha-1B-glycoprotein (A1BG)	P04217	1.67	.67	.18
Complement component C9 (C9)	P02748	1.51	.62	.24
Kininogen-1 (KNG1)	P01042	1.49	.30	.15
Apolipoprotein A-I (APOA1)	P02647	1.40	.37	.16
Fibrinogen alpha chain (FGA)	P02671	1.17	.26	.03
Immunoglobulin J chain (IGJ)	P01591	1.13	.21	.10
Gelsolin (GSN)	P06396	1.11	.41	-.05
Vitronectin (VTN)	P04004	1.09	-.14	-.06
Complement component C8 alpha chain (C8A)	P07357	1.05	.28	.22
Serum albumin (ALB)	P02768	1.05	-.15	.09
Prothrombin (F2)	P00734	1.04	-.31	.09

NOTE. Accession numbers are derived from the protein database UniProt (www.uniprot.org). Variable influence on projections (VIP) indicates the importance of the covariable. p(corr) is the loading of each variable scaled as a correlation coefficient. B is the regression coefficient. The pressure pain threshold (*Y*-variable) remained a continuous variable for the OPLS analysis.

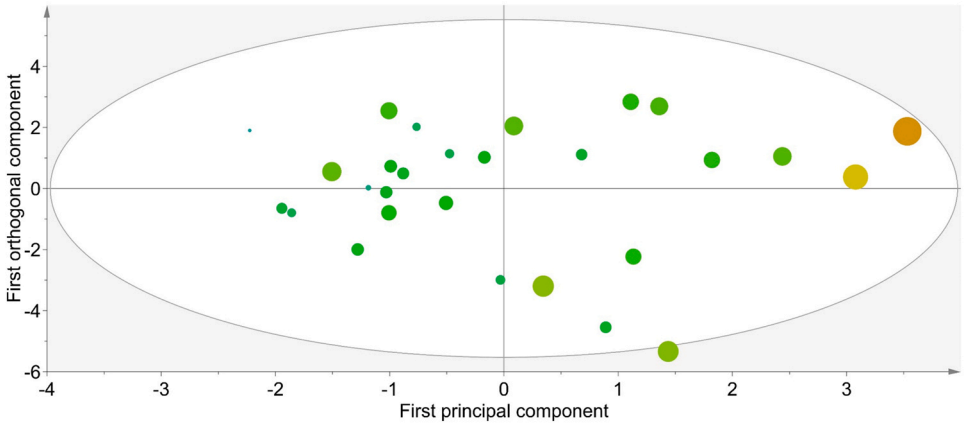

Figure 7. Score plot constructed with one principal component and one orthogonal component separating males based on their pressure pain threshold at the site of low back pain (R^2 = .55, Q^2 = .33, CV-ANOVA: *P* = .04). Each circle represents a participant and the larger circles with warmer colours represent higher pressure pain thresholds at the site of low back pain.

Table S5). The OPLS-DA model including these significant proteins correctly classified sex in 69% of non-recovered participants (1 principal component; R^2 = .26, Q^2 = .20, CV-ANOVA: *P* = .06).

Discussion

This preliminary study explored sex differences in the serum proteomic profile of individuals experiencing an acute episode of LBP. Our data provide evidence to suggest sex-specific differences in the expression of proteins, measured from human serum, contribute to recovery or ongoing pain at three-month follow-up.

Amongst male participants with ongoing LBP at 3 months, the serum concentration of proteins involved in immune processes such as Fibrinogen alpha chain and Immunoglobulin J Chain were lower during acute LBP than their recovered counterparts. The impact of immunity on pain has been considered since early observations that individuals with chronic pain exhibit symptoms that parallel typical systemic illnesses such as lethargy, depression and anxiety.[72,73] A growing body of evidence now highlights neuroimmune interaction as a plausible mechanism driving the transition from acute to chronic pain.[74]

Our data also identified several proteins linked to complement and coagulation systems (eg, Complement factor H, Complement factor B, Coagulation factor XII) expressed during acute LBP in both male and female participants. The complement system represents an intricate immune surveillance system that attempts to discriminate between healthy tissue, cellular debris, apoptotic and foreign intruders, before regulating response to maintain homoeostasis.[75] An imbalance between complement activation and regulation is associated with various immune, inflammatory, neurodegenerative, and age-related disease.[76] Interactions between complement and coagulation systems play a pivotal role in regulating inflammation.[77]

Significant proteins identified in the multivariable data analysis (ie, Kininogen, Fibrinogen alpha chain,

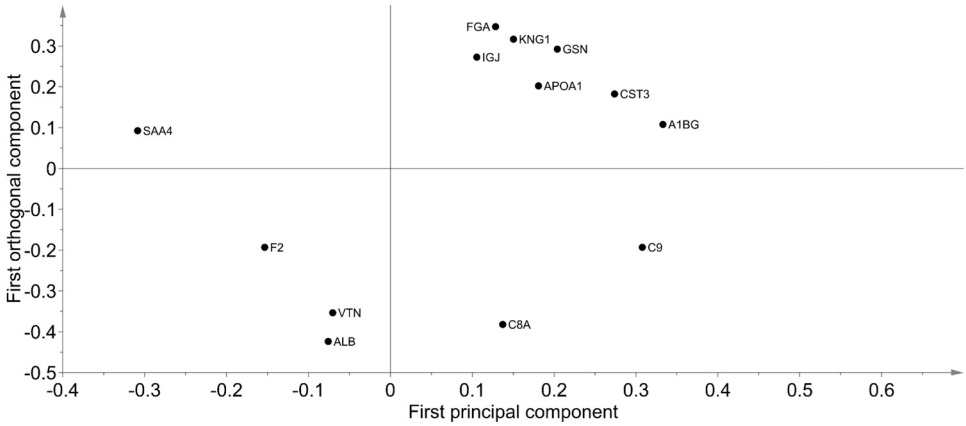

Figure 8. Loading plot displaying the raw loading values for significant proteins associated with baseline pressure pain threshold at the site of low back pain in males. Four of the proteins (SAA4, F2, VTN, ALB) were associated with greater sensitivity to pressure (ie, lower pressure pain threshold). The protein abbreviations in the plot match the protein names in Table 5.

Complement factor H, Immunoglobulin lambda constant 2, Clusterin, Inter-alpha-trypsin inhibitor heavy chain H1, Haptoglobin, Complement Factor B) and the derived functional protein networks (ie, immune, inflammatory, complement, coagulation, transport, and metabolic) suggest female participants express proteins involved in similar biological processes as males during acute LBP. However, our data suggest that for nearly all significant proteins identified in this study, proteins downregulated during acute LBP in non-recovered males were upregulated in non-recovered females. For example, Fibrinogen alpha chain and Immunoglobulin lambda constant 2 were upregulated during acute LBP in females not recovered at three-month follow-up, as were inflammatory proteins such as Haptoglobin and Kininogen. A similar proteomic profile has been associated with chronic widespread pain and fibromyalgia in females.[32,63,78] Specifically, high levels of haptoglobin and fibrinogen have been proposed as plausible biomarkers of fibromyalgia,[78] and several proteins involved in an acute phase response, the coagulation cascade, and the complement system are upregulated in females with fibromyalgia compared to pain-free controls.[32]

Our sensitivity analysis suggests protein expression differs in response to an acute LBP episode dependent on sex. When proteins predicting three-month LBP outcome in male participants were used to predict outcome in females, cross-validated classification accuracy was only 37%, much less than chance. If there was no difference between sex in protein expression during

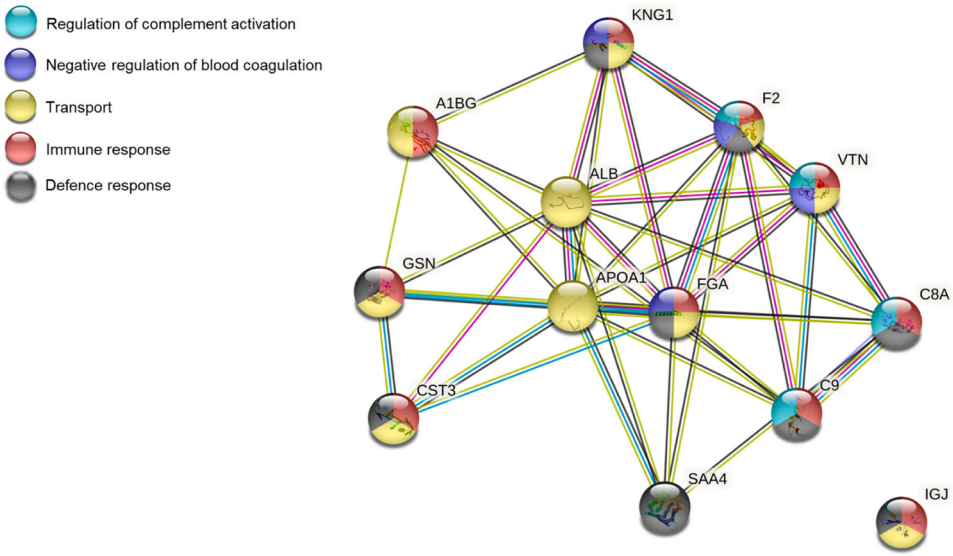

Figure 9. Pathways analysis of group proteins significantly associated with baseline pressure pain thresholds at the site of low back pain in male participants The functional protein network was derived from significant serum proteins. STRING version 11 was used to create the network analysis (https://string-db.org/). Abbreviations are listed in Table 5. In the network, each protein is represented by a node (circles), coloured according to its biological processes (legend to the left of figure). The protein-protein interaction is represented by an edge visualised as a coloured line. Known interactions were from curated databases (turquoise) or experimentally determined (pink). Predicted interactions (coloured line) were identified from gene neighbourhoods (green), gene fusion (red) and gene co-occurrence (dark blue), and other interactions were identified using text mining (yellow), co-expression (black), and protein homology (purple).

acute LBP, classification accuracy should have been similar when predicting outcome in the opposite sex with the same significant proteins. We also explored the magnitude of sex differences in the study data by attempting to predict sex in a multivariable model based on serum protein abundance levels. Significant proteins classified sex correctly in 87% of the recovered participants. Conversely, sex was correctly classified in 69% of the non-recovered participants. Taken together, this sensitivity analysis provides further evidence to suggest sex differences in protein expression occur during acute LBP. Further, these sex differences within our data appear to be more prominent in participants who recovered from their acute LBP episode.

Immune system-related sex differences may explain these observations. For example, Sorge and colleagues determined that after spinal nerve injury in mice of both sexes, the application of glial inhibitors reversed allodynia in male, but not in female mice.[79] The authors speculate that female mice preferentially adopt an adaptive immune cell response to spinal nerve injury, whilst male mice are less able to mediate hypersensitivity through T-Cell infiltration.[79] Sex-dependent differences in proteins extracted from the sciatic nerve of male and female mice following a chronic constriction injury have also been identified. In the injured mice, 44 proteins were differentially expressed between sex.[80] Furthermore, females typically demonstrate a more robust adaptive immune system then males,[81] females have greater cellular and humoral immune response to vaccination or infection,[82] and females have higher amounts of CD4+ and CD8+ T-cells then males.[83]

Based on our data, we speculate, that males who recovered from an acute LBP episode could more effectively upregulate an immune driven inflammatory response during LBP. This hypothesis is also supported by the work of Vacca and colleagues,[80] identifying higher accumulations in males than in females of several proteins associated with inflammation. In our study sample, this inflammatory response appears to be tightly controlled through regulation and interaction of the complement and coagulation systems, aiming to achieve homoeostasis and subsequent recovery from injury to cell membranes and intracellular structures, possibly in the context of LBP a result of overload, overstretch, compression or anoxia. Conversely, due to sex-specific immune differences, females may have a predisposition towards a heightened immune response to injury and therefore to achieve the level of homoeostasis needed for recovery, females must down-regulate their immune response. Females who are unable to achieve the level of molecular plasticity necessary to dampen this response are more likely to experience ongoing LBP at three-month follow-up. These findings may highlight the potential need for distinct strategies targeting immune system processes to prevent the transition from acute to chronic LBP in males versus females.[23,79]

Our study found no evidence to support a relationship between serum protein abundance levels and baseline depression, anxiety, and stress during acute LBP. Similarly, there was no evidence to suggest serum protein abundance levels were associated with an impaired CPM response. This lack of evidence for an association between serum protein abundance, psychological status and descending pain modulation could be explained by the relatively low levels of psychological distress in this cohort, and no obvious impairment in descending pain modulation. Amongst males, 13 proteins were associated with baseline PPTs, however, no significant association between serum protein abundance levels and baseline PPTs was identified for females. The observed association between the serum proteomic profile and PPT in males, but not females, may again provide further evidence of sex differences in pain phenotype, warranting further, longitudinal investigation in larger cohorts.

Enhanced nervous system sensitivity[48,84–86] and psychological distress[87,88] occur more frequently once LBP has already persisted. Interestingly, some psychosocial factors are thought to disrupt the balance of the immune system[89,90] and are associated with increased systemic inflammation.[22,91–94] This relationship between psychological factors and biological mediators/moderators of systemic inflammation is considered reciprocal.[95] The data presented here suggests that plasticity occurring in the human serum proteome during acute LBP is not clearly associated with psychological status, descending pain modulation or pain sensitivity to pressure. However, it remains possible that differential protein expression during acute pain triggers a cycle of altered immune system responsiveness in some people, driving alterations in systemic inflammation, ongoing pain and associated psychological distress.[95,96] Emerging evidence has suggested that after a primary immune challenge, microglia may undergo epigenetic modifications and upregulated transcriptional activity, resulting in an enhanced response to future immune challenges, termed immune priming.[97–100]

This study has several limitations. First, as we were unable to analyse three-month serum samples in this study, we cannot determine whether alterations in serum protein during an acute LBP episode were sustained at the three-month follow-up. Second, the time-of-day blood was collected was not standardised. Evidence suggests proteins involved in inflammatory processes may have diurnal variation and this is an important consideration for future work.[101] Third, despite the intentional selection of a sub-sample of participants with minimal comorbidity/medication usage some potential confounding still exists (eg, the use of ibuprofen or inhaled bronchodilator/corticosteroid). Whilst medication usage and comorbid health conditions could have impacted some participant's proteomic profile, complete exclusion of these participants would affect the generalisability of results. Fourth, the mass spectrometry methodology applied in this study is inherently limited in its depth of coverage and is not optimised to detect low-abundance proteins. For example, Afamin was significantly upregulated in non-recovered males. Whilst this could reflect a state of systemic inflammation and metabolic dysfunction

relevant to recovery from LBP,[102] the serum concentration of this protein amongst males could also be a consequence of its high abundance.[103] Finally, care should be taken interpreting these results due to the study sample size. We attempted to minimise the effect of this limitation using multivariable data analysis reported in line with known recommendations.[66] However, the risk of overfitting statistical models with large 'omics' datasets remains.[67] Considering the generally accepted rule that a difference of .2 to .3 between R^2 and Q^2 indicates model overfitting, the statistical models fitted in this study, particularly those modelled with female participants should be considered preliminary in nature until these findings are replicated in larger samples.

Conclusions

This study explored sex differences in the serum proteomic profile of individuals experiencing an acute episode of LBP and identified several proteins related to immune, inflammatory, complement, coagulation, transport and metabolic processes associated with three-month LBP outcome. Differential expression of serum proteins was observed between male and female participants during an acute LBP episode. This preliminary work provides a foundation for future research in larger and independent cohorts targeting distinct immune system processes in males and females that may interfere with the transition from acute to chronic LBP.

Disclosures

This work was supported by Grant 1059116 from the National Health and Medical Research Council (NHMRC) of Australia. Funding has been applied to achieve the objectives and outcomes described in this protocol and analysis plan. Funding has been spent only on direct research costs as described within the NHRMC funding agreement. SMS receives salary support from the National Health and Medical Research Council of Australia (1105040).

The authors have no potential conflicts of interest to declare.

Author contributions

L.C.J., V.C.W., S.M.S.: Conceptualization. **L.C.J., W.J.C, V.C.W.:** Methodology. **L.C.J., V.C.W.:** Software. **L.C.J., V.C.W.:** Validation. **L.C.J., V.C.W, P.H.:** Formal analysis. **V.C.W, S.M.S.:** Resources. **L.C.J., W.J.C, V.C.W, S.M.S.:** Data curation. **L.C.J.:** Writing – original draft preparation. **L.C.J., W.J.C, P.H, V.C.W, L.S.S, S.G.D, C.R, A.S, S.M.S.:** Writing – review & editing. **L.C.J., W.J.C, P.H, V.C.W, L.S.S, S.G.D, C.R, A.S, S.M.S.:** Visualization. **V.C.W, L.S.S, S.G.D, C.R, A.S, S.M.S.:** Supervision. **L.C.J., W.J.C, V.C.W, S.M.S.:** Project administration. **S.M.S.:** Funding acquisition.

Data availability statement

The mass spectrometry proteomics data have been deposited to the Dryad Repository. Pre-publication sharing link: doi: 10.5061/dryad.1rn8pk0xm.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpain.2023.11.009.

References

1. Hoy D, Bain C, Williams G, *et al.* A systematic review of the global prevalence of low back pain. Arthritis Rheum 64(6):2028-2037, 2012. https://doi.org/10.1002/art.34347

2. Manchikanti L, Singh V, Falco FJ, Benyamin RM, Hirsch JA: Epidemiology of low back pain in adults. Neuromodulation 17:3-10, 2014.

3. Vos T, Abajobir AA, Abate KH, *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390(10100):1211-1259, 2017.

4. Dagenais S, Caro J, Haldeman S: A systematic review of low back pain cost of illness studies in the United States and internationally. Spine J 8(1):8-20, 2008.

5. Ferreira ML, de Luca K, Haile LM, *et al.* Global, regional, and national burden of low back pain, 1990–2020, its attributable risk factors, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 5(6):316-329, 2023.

6. Henschke N, Maher CG, Refshauge KM, *et al.* Prevalence of and screening for serious spinal pathology in patients presenting to primary care settings with acute low back pain. Arthritis Rheum 60(10):3072-3080, 2009.

7. Maher C, Underwood M, Buchbinder R: Non-specific low back pain. Lancet 389(10070):736-747, 2017.

8. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley IIIJL: Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain 10(5):447-485, 2009.

9. Foster NE, Thomas E, Bishop A, Dunn KM, Main CJ: Distinctiveness of psychological obstacles to recovery in low back pain patients in primary care. Pain 148(3):398-406, 2010.

10. Lagersted-Olsen J, Bay H, Jørgensen MB, Holtermann A, Søgaard K: Low back pain patterns over one year among 842 workers in the DPhacto study and predictors for chronicity based on repetitive measurements. BMC Musculoskel Disord 17(1):1-11, 2016.

11. Manek NJ, MacGregor A: Epidemiology of back disorders: prevalence, risk factors, and prognosis. Curr Opin Rheumatol 17(2):134-140, 2005.

12. Linton JS: A review of psychological risk factors in back and neck pain. Spine 25(9):1148-1156, 2000. https://doi.org/10.1097/00007632-200005010-00017

13. Pincus T, Burton AK, Vogel S, Field AP: A systematic review of psychological factors as predictors of chronicity/disability in prospective cohorts of low back pain. Spine 27(5):109-120, 2002.

14. Pincus T, Vogel S, Burton AK, Santos R, Field AP: Fear avoidance and prognosis in back pain: a systematic review and synthesis of current evidence. Arthritis Rheum 54(12):3999-4010, 2006.

15. Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain 152(3):S2-S15, 2011.

16. Roussel NA, Nijs J, Meeus M, Mylius V, Fayt C, Oostendorp R: Central sensitization and altered central pain processing in chronic low back pain: fact or myth? Clin J Pain 29(7):625-638, 2013.

17. Wand BM, Parkitny L, O'Connell NE, *et al.* Cortical changes in chronic low back pain: current state of the art and implications for clinical practice. Manual Ther 16(1):15-20, 2011.

18. Apkarian AV, Hashmi JA, Baliki MN: Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. Pain 152(3 Suppl):S49-S64, 2011. https://doi.org/10.1016/j.pain.2010.11.010

19. Baliki MN, Petre B, Torbey S, *et al.* Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci 15(8):1117-1119, 2012. https://doi.org/10.1038/nn.3153

20. Jenkins LC, Chang W-J, Buscemi V, *et al.* Cortical function and sensorimotor plasticity are prognostic factors associated with future low back pain after an acute episode: the Understanding persistent Pain Where it ResiDes prospective cohort study. Pain 164(1):14-26, 2022. https://doi.org/10.1097/j.pain.0000000000002684

21. Hodges PW, Barbe MF, Loggia ML, Nijs J, Stone LS: Diverse role of biological plasticity in low back pain and its impact on sensorimotor control of the spine. J Orthopaed Sports Phys Ther 49(6):389-401, 2019.

22. Klyne D, Barbe M, Hoorn W, Hodges P: ISSLS PRIZE IN CLINICAL SCIENCE 2018: longitudinal analysis of inflammatory, psychological, and sleep-related factors following an acute low back pain episode—the good, the bad, and the ugly. Eur Spine J 27(4):763-777, 2018. https://doi.org/10.1007/s00586-018-5490-7

23. Parisien M, Lima LV, Dagostino C, *et al.* Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci Transl Med 14(644):eabj9954, 2022.

24. Mogil JS: Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci 13(12):859-866, 2012.

25. Torres-Chávez KE, Fischer L, Teixeira JM, *et al.* Sexual dimorphism on cytokines expression in the temporomandibular joint: the role of gonadal steroid hormones. Inflammation 34:487-498, 2011.

26. Dong X-D, Mann MK, Kumar U, *et al.* Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. Neuroscience 146(2):822-832, 2007.

27. Linnman C, Beucke J-C, Jensen KB, Gollub RL, Kong J: Sex similarities and differences in pain-related periaqueductal gray connectivity. Pain 153(2):444-454, 2012.

28. Popescu A, LeResche L, Truelove EL, Drangsholt MT: Gender differences in pain modulation by diffuse noxious inhibitory controls: a systematic review. Pain 150(2):309-318, 2010.

29. Olson EM, Akintola T, Phillipsc J, *et al.* Effects of sex on placebo effects in chronic pain participants: a cross-sectional study. Pain 162(2):531, 2021.

30. Keogh E, McCracken LM, Eccleston C: Do men and women differ in their response to interdisciplinary chronic pain management? Pain 114(1–2):37-46, 2005.

31. Wasinger VC, Cordwell SJ, Cerpa-Poljak A, *et al.* Progress with gene-product mapping of the Mollicutes: mycoplasma genitalium. Electrophoresis 16(1):1090-1094, 1995.

32. Wåhlén K, Ernberg M, Kosek E, Mannerkorpi K, Gerdle B, Ghafouri B: Significant correlation between plasma proteome profile and pain intensity, sensitivity, and psychological distress in women with fibromyalgia. Sci Rep 10(1):1-19, 2020.

33. Lind A-L, Just D, Mikus M, *et al.* CSF levels of apolipoprotein C1 and autotaxin found to associate with neuropathic pain and fibromyalgia. J Pain Res 12:2875-2889, 2019. https://doi.org/10.2147/JPR.S215348

34. Furer V, Hazan E, Mor A, *et al.* Elevated levels of eotaxin-2 in serum of fibromyalgia patients. Pain Res Manag 2018:1-4, 2018. https://doi.org/10.1155/2018/7257681

35. Ciregia F, Giacomelli C, Giusti L, *et al.* Putative salivary biomarkers useful to differentiate patients with fibromyalgia. J Proteom 190:44-54, 2019.

36. Ruggiero V, Era B, Cacace E, *et al.* A preliminary study on serum proteomics in fibromyalgia syndrome. Clin Chem Lab Med 52(9):207-210, 2014.

37. Sejersen MHJ, Frost P, Hansen TB, Deutch SR, Svendsen SW: Proteomics perspectives in rotator cuff research: a systematic review of gene expression and protein composition in human tendinopathy. PLoS One 10(4):e0119974, 2015.

38. Balakrishnan L, Bhattacharjee M, Ahmad S, *et al.* Differential proteomic analysis of synovial fluid from rheumatoid arthritis and osteoarthritis patients. Clin Proteom 11(1):1-14, 2014.

39. Lim TK, Anderson KM, Hari P, *et al.* Evidence for a role of nerve injury in painful intervertebral disc degeneration: a cross-sectional proteomic analysis of human cerebrospinal fluid. J Pain 18(10):1253-1269, 2017.

40. Dorsey SG, Renn CL, Griffioen M, *et al.* Whole blood transcriptomic profiles can differentiate vulnerability to chronic low back pain. PLoS One 14(5):e0216539, 2019.

41. Theken KN, Hersh EV, Lahens NF, *et al.* Variability in the analgesic response to ibuprofen is associated with cyclooxygenase activation in inflammatory pain. Clin Pharmacol Therap 106(3):632-641, 2019.

42. Olausson P, Ghafouri B, Ghafouri N, 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 9:345-356, 2016.

43. Boisson M, Borderie D, Henrotin Y, *et al.* Serum biomarkers in people with chronic low back pain and Modic 1 changes: a case-control study. Sci Rep 9(1):1-5, 2019.

44. Jenkins LC, Chang WJ, Buscemi V, *et al.* The Understanding persistent Pain Where it ResiDes study of low back pain cohort profile. N Z J Physiother 51:199-216, 2023. https://doi.org/10.15619/nzjp.v51i3.375z

45. Declaration of Helsinki: Ethical principles for medical research involving human subjects. WHO drug information. 2000;14(3):160.

46. Heidari S, Babor TF, De Castro P, Tort S, Curno M: Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. Res Integrity Peer Rev 1(1):2. https://doi.org/10.1186/s41073-016-0007-6, 2016.

47. Vandenbroucke JP, Elm Ev, Altman DG, *et al.* Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Ann Intern Med 147(8):W-163-W-194, 2007.

48. Müller M, Curatolo M, Limacher A, *et al.* Predicting transition from acute to chronic low back pain with quantitative sensory tests—a prospective cohort study in the primary care setting. Eur J Pain 23(5):894-907, 2019. https://doi.org/10.1002/ejp.1356

49. De Vet CWH, Heymans WM, Dunn MK, *et al.* Episodes of low back pain: a proposal for uniform definitions to be used in research. Spine 27(21):2409-2416, 2002. https://doi.org/10.1097/01.BRS.0000030307.34002.BE

50. Williams CM, Maher CG, Latimer J, *et al.* Efficacy of paracetamol for acute low-back pain: a double-blind, randomised controlled trial. Lancet 384(9954):1586-1596, 2014. https://doi.org/10.1016/S0140-6736(14)60805-9

51. Stanton T, Henschke N, Maher CG, Refshauge K, Latimer J, McAuley J: After an episode of acute low back pain, recurrence is unpredictable and not as common as previously thought. Spine 33(26):2923-2928, 2008. https://doi.org/10.1097/BRS.0b013e31818a3167

52. Cleeland C.S., Ryan K. The brief pain inventory. *Pain Research Group*; 1991.

53. Wasinger VC, Curnoe D, Boel C, Machin N, Goh HM: The molecular floodgates of stress-induced senescence reveal translation, signalling and protein activity central to the post-mortem proteome. Int J Mol Sci 21(17):6422, 2020.

54. Hill JC, Dunn KM, Lewis M, *et al.* A primary care back pain screening tool: identifying patient subgroups for initial treatment. Arthritis Care Res 59(5):632-641, 2008.

55. Hush JM, Refshauge K, Sullivan G, De Souza L, Maher CG, McAuley JH: Recovery: what does this mean to patients with low back pain? Arthritis Care Res 61(1):124-131, 2009.

56. Traeger AC, Henschke N, Hübscher M, *et al.* Estimating the risk of chronic pain: development and validation of a prognostic model (PICKUP) for patients with acute low back pain. PLoS Med 13(5):e1002019, 2016.

57. Lovibond SH, Lovibond PF: Manual for the Depression Anxiety Stress Scales. Psychology Foundation of Australia; 1996

58. Antony MM, Bieling PJ, Cox BJ, Enns MW, Swinson RP: Psychometric properties of the 42-item and 21-item versions of the depression anxiety stress scales in clinical groups and a community sample. Psycho Assess 10(2):176-181, 1998. https://doi.org/10.1037/1040-3590.10.2.176

59. Parkitny L, McAuley JH, Walton D, *et al.* Rasch analysis supports the use of the depression, anxiety, and stress scales to measure mood in groups but not in individuals with chronic low back pain. J Clin Epidemiol 65(2):189-198, 2012.

60. Klyne DM, Schmid AB, Moseley GL, Sterling M, Hodges PW: Effect of types and anatomic arrangement of painful stimuli on conditioned pain modulation. J Pain 16(2):176-185, 2015. https://doi.org/10.1016/j.jpain.2014.11.005

61. Lewis GN, Luke H, Rice DA, Rome K, McNair PJ: Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. Pain Res Manag 17(2):98-102, 2012.

62. Eriksson L., Johansson E., Kettaneh-Wold N., Trygg J., Wikström C., Wold SJUUA. Multi-and Megavariate Data analysis; part I and II; 2006;

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

64. Wold S, Sjöström M, Eriksson L: PLS-regression: a basic tool of chemometrics. Chemom Intell Lab Syst 58(2):109-130, 2001.

65. Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J: OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. J Chemom 20(8–10):341-351, 2006.

66. Wheelock ÅM, Wheelock CE: Trials and tribulations of 'omics data analysis: assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine. Mol BioSyst 9(11):2589-2596, 2013.

67. Diez D, Wheelock ÅM, Goto S, *et al.* The use of network analyses for elucidating mechanisms in cardiovascular disease. Mol BioSyst 6(2):289-304, 2010.

68. Eriksson L, Andersson PL, Johansson E, Tysklind M: Megavariate analysis of environmental QSAR data. Part II–investigating very complex problem formulations using hierarchical, non-linear and batch-wise extensions of PCA and PLS. Mol Divers 10(2):187-205, 2006. https://doi.org/10.1007/s11030-006-9026-4

69. Tukey J: Bias and confidence in not quite large samples. J Ann Math Statist 29:614, 1958.

70. Quenouille M: Problems in plane sampling. Ann Math Stat 20(3):355-375, 1949. https://doi.org/10.1214/aoms/1177729989

71. Szklarczyk D, Gable AL, Lyon D, *et al.* STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 47(D1):D607-D613, 2019. https://doi.org/10.1093/nar/gky1131

72. Maier SF, Wiertelak EP, Martin D, Watkins LR: Interleukin-1 mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin. Brain Res 623(2):321-324, 1993.

73. Ferreira S, Lorenzetti B, Bristow A, Poole S: Interleukin-1β as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature 334(6184):698-700, 1988.

74. Grace PM, Hutchinson MR, Maier SF, Watkins LR: Pathological pain and the neuroimmune interface. Nat Rev Immunol 14(4):217-231, 2014.

75. Ricklin D, Hajishengallis G, Yang K, Lambris JD: Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11(9):785-797, 2010.

76. Pettigrew HD, Teuber SS, Gershwin ME: Clinical significance of complement deficiencies. Ann NY Acad Sci 1173(1):108-123, 2009.

77. Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD: Interactions between coagulation and complement—their role in inflammation. Semin Immunopathol 34(1):151-165, 2012.

78. Ramírez-Tejero JA, Martínez-Lara E, Rus A, Camacho MV, Del Moral ML, Siles E: Insight into the biological pathways underlying fibromyalgia by a proteomic approach. J Proteom 186:47-55, 2018.

79. Sorge RE, Mapplebeck J, Rosen S, *et al.* Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci 18(8):1081-1083, 2015.

80. Vacca V, Marinelli S, Pieroni L, Urbani A, Luvisetto S, Pavone F: Higher pain perception and lack of recovery from neuropathic pain in females: a behavioural, immunohistochemical, and proteomic investigation on sex-related differences in mice. Pain 155(2):388-402, 2014.

81. Rosen S, Ham B, Mogil JS: Sex differences in neuroimmunity and pain. J Neurosci Res 95(1–2):500-508, 2017.

82. Fish EN: The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8(9):737-744, 2008.

83. Amadori A, Zamarchi R, De Silvestro G, *et al.* Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med 1(12):1279-1283, 1995. https://doi.org/10.1038/nm1295-1279

84. Leresche L, Turner JA, Saunders K, Shortreed SM, Von Korff M: Psychophysical tests as predictors of back pain chronicity in primary care. J Pain 14(12):1663-1670, 2013. https://doi.org/10.1016/j.jpain.2013.08.008

85. Marcuzzi A, Dean CM, Wrigley PJ, Chakiath RJ, Hush JM: Prognostic value of quantitative sensory testing in low back pain: a systematic review of the literature. J Pain Res 9:599, 2016.

86. O'Neill S, Kjær P, Graven-Nielsen T, Manniche C, Arendt-Nielsen L: Low pressure pain thresholds are associated with, but does not predispose for, low back pain. Eur Spine J 20(12):2120-2125, 2011. https://doi.org/10.1007/s00586-011-1796-4

87. Campbell P, Bishop A, Dunn KM, Main CJ, Thomas E, Foster NE: Conceptual overlap of psychological constructs in low back pain. Pain 154(9):1783-1791, 2013.

88. Nicholas MK, Costa DS, Blanchard M, Tardif H, Asghari A, Blyth FM: Normative data for common pain measures in chronic pain clinic populations: closing a gap for clinicians and researchers. Pain 160(5):1156-1165, 2019.

89. Dowlati Y, Herrmann N, Swardfager W, *et al.* A meta-analysis of cytokines in major depression. Biol Psychiatry 67(5):446-457, 2010.

90. Segerstrom SC, Miller GE: Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull 130(4):601-630, 2004. https://doi.org/10.1037/0033-2909.130.4.601

91. Felger JC, Lotrich FE: Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience 246:199-229, 2013.

92. Edwards RR, Kronfli T, Haythornthwaite JA, Smith MT, McGuire L, Page GG: Association of catastrophizing with interleukin-6 responses to acute pain. Pain 140(1):135-144, 2008.

93. Kubera M, Obuchowicz E, Goehler L, Brzeszcz J, Maes M: In animal models, psychosocial stress-induced (neuro) inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. Prog Neuro-Psychopharmacol Biol Psychiatry 35(3):744-759, 2011.

94. Slavich GM, Irwin MR: From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. Psychol Bull 140(3):774-815, 2014. https://doi.org/10.1037/a0035302

95. Klyne DM, Barbe MF, James G, Hodges PW: Does the interaction between local and systemic inflammation provide a link from psychology and lifestyle to tissue health in musculoskeletal conditions? Int J Mol Sci 22(14):7299, 2021. https://doi.org/10.3390/ijms22147299

96. Kosek E, Cohen M, Baron R, *et al.* Do we need a third mechanistic descriptor for chronic pain states? Pain 157(7):1382-1386, 2016.

97. Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A: Physiology of microglia. Physiol Rev 91(2):461-553, 2011.

98. Loram LC, Taylor FR, Strand KA, *et al.* Prior exposure to glucocorticoids potentiates lipopolysaccharide induced mechanical allodynia and spinal neuroinflammation. Brain Behav Immunity 25(7):1408-1415, 2011.

99. Hains LE, Loram LC, Taylor FR, *et al.* Prior laparotomy or corticosterone potentiates lipopolysaccharide-induced fever and sickness behaviors. J Neuroimmunol 239(1–2):53-60, 2011.

100. Loram LC, Grace PM, Strand KA, *et al.* Prior exposure to repeated morphine potentiates mechanical allodynia induced by peripheral inflammation and neuropathy. Brain Behav Immunity 26(8):1256-1264, 2012.

101. Jasim H, Carlsson A, Gerdle B, Ernberg M, Ghafouri B: Diurnal variation of inflammatory plasma proteins involved in pain. Pain Rep 4(5):e776, 2019. https://doi.org/10.1097/PR9.0000000000000776

102. Furman D, Campisi J, Verdin E, *et al.* Chronic inflammation in the etiology of disease across the life span. Nat Med 25(12):1822-1832, 2019.

103. Lichenstein HS, Lyons DE, Wurfel MM, *et al.* Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding protein gene family. J Biol Chem 269(27):18149-18154, 1994.