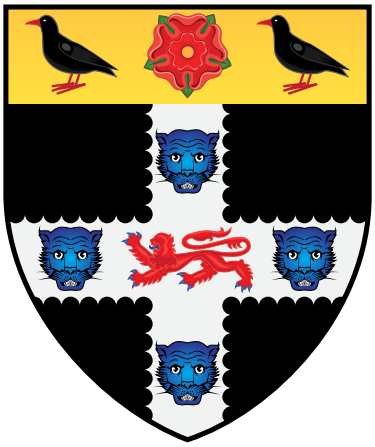
The Effect of Obesity Upon the Lumbar Spine



Dr Anand H Segar

Christ Church

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy in Musculoskeletal Sciences

Hilary Term 2015

Acknowledgements

The last three years in Oxford has been some of the best of my life. None of this would have been possible without the support and encouragement from so many. This has been a great start to a future career in academic orthopaedic surgery.

I am so lucky to have worked with my academic supervisors. I am indebted to Dr Jill Urban for all her support and guidance throughout my DPhil. I learnt a great deal from her analytical approach to scientific problems and her vast experience in this field. Thank you to Professor Jeremy Fairbank for his mentorship and for bringing a clinical perspective to my research. Also thank you to Associate Professor Andy Judge who helped me visualise and understand patterns in clinical data. Going forward this will be a very useful skill. A special thanks to Associate Professor Afsie Sabokbar who was instrumental in helping me secure my position in Oxford. We first met at the Botnar five years ago. This goes to show a DPhil is truly a marathon. I look forward to working with everyone on new projects in the future.

This would not have been possible without the generous support from all my financial sponsors. My funding came from a Commonwealth Scholarship awarded by the Commonwealth Scholarship Commission and the Department for Business, Innovation and Skills; the Oxford Nuffield Medical Fellowship awarded by the Nuffield Dominions Trust; a Foundation for Surgery Scholarship from the Royal Australasian College of Surgeons, and a research grant from the New Zealand Orthopaedic Association/Wishbone Trust. Thank you for supporting my research.

Thank you to my academic and clinical supporters from New Zealand. Professor John Windsor has been my academic mentor since I was as a second year medical student. I am truly grateful for his continuing encouragement and advice. A special thank you also to Mr Richard Nicol. Both Prof Windsor and Mr Nicol encouraged me to pursue a DPhil, helped me secure my funding and obtain my position at Oxford. I must acknowledge Mr Gary French, Mr Ali Bayan, and Mr Bill Farrington who also provided backing from the New Zealand orthopaedic community. Thank you all for believing in me and fostering my potential.

To my lab colleagues, Dr Olga Boubriak, Dr Jing Yu, Dr Ying Cui, Dr Heman Joshi, Dr Aasma Khan, and Dr Bob Lee, thank you for your patience and support, especially at the start of this DPhil when the scientific challenges seemed insurmountable. Thank you to the Oxford Musculoskeletal Biobank and the spine surgeons at the Nuffield Orthopaedic Centre, for their help in patient recruitment. I must also acknowledge Professor Sally Roberts, Dr Jude Meakin and Dr Sarah Turner from the Robert Jones and Agnes Hunt Orthopaedic Hospital for their help in sourcing plasma samples.

Thank you to Dr Holger Kramer for his help with mass spectrometry. To the Genodisc Consortium, particularly Professor Iain McCall, thank you for all your effort in recruiting the Genodisc population. I am privileged to have been part of your work.

I met so many amazingly talented and intelligent people while in Oxford and made lifelong friends. Thank you Dave, Mark, Pradeep, Geoff, and Phil for the conversations, support, intellectual challenges, curry nights and the long winter evenings spent in the pub. To my friends back in New Zealand, Janar, Jason, and my travelling companions Ashley and Cindy, thank you for your encouragement.

Finally, to my parents and sister. I am so grateful for your everlasting support and sacrifices. I hope to celebrate more success with you in the future. You are always there for me. I love you and I thank you wholeheartedly.

for my Mum and Dad.

Abstract

The Effect of Obesity Upon the Lumbar Spine

Thesis Submitted for the Degree of

Doctor of Philosophy in Musculoskeletal Sciences

Dr Anand H Segar

Christ Church

Hilary Term 2015

Word Count 417

Back pain is a massive global public health problem with multiple contributing factors including obesity. Obesity is thought to be linked to back pain through mechanical factors. However, obesity also causes a systemic low-grade inflammatory milieu. This would suggest a possible biochemical link between obesity, intervertebral disc degeneration, and back pain. Furthermore, the relationship between obesity and the clinical presentation of spine patients is unclear. This thesis aims to examine the effect of and relationship between obesity, the intervertebral discs, and back pain from biochemical, clinical, and epidemiological perspectives

In this thesis, an *in vitro* study assessed the effect of leptin, a fat-specific cytokine, upon the intervertebral disc. The bovine intervertebral disc was used as a model in a cell culture system. An *ex vivo* study examined leptin and pro-inflammatory cytokines produced by paraspinal adipose tissue taken during routine surgical procedures from spinal patients. Plasma taken from patients presenting with low back pain was analysed by mass spectrometry and multiplex immunoassay to identify possible protein biomarkers. At an epidemiological level, statistical modelling of the Genodisc pa-

tient population was conducted. This was a pan-European study of 2636 patients presenting to tertiary spinal units. Analyses were performed to examine relationships between obesity, quantified by body mass index (BMI), and pain, clinical diagnosis, and spinal degeneration identified on magnetic resonance imaging (MRI).

Leptin was shown to increase the production of and expression of degradative and pain-generating molecules by disc cells. A pro-inflammatory environment, especially IL-6, potentiated this response. Leptin and pro-inflammatory cytokines produced by paraspinal fat were unrelated to clinical symptoms. However, levels of the pro-inflammatory cytokines, TNF-α and IL-6, were raised in the plasma of patients with greater pain or those with spinal stenosis. Furthermore, clusterin and complement were identified, by mass spectrometry, as potential biomarkers for spine patients. Epidemiological analyses revealed that obesity was associated with greater back pain, although the magnitude of this association was small. Similarly, obesity was associated with a diagnosis of spinal stenosis. Finally, increased BMI was found to be an independent predictor of disc degeneration, spinal stenosis, and disc herniation on MRI.

In summary, this thesis has furthered the clinical understanding of lumbar spine pathology and back pain. It will provide clinicians with a better framework to assess spine patients. These results show that obesity is associated with lumbar spine degeneration and pain. Leptin could be a factor mediating this relationship. Further studies should concentrate on clarifying the mechanism of action of leptin upon the intervertebral disc and assessing the longitudinal effect of obesity upon the lumbar spine.

Contents

List of Figures

List of Tables

List of Abbreviations

18s rRNA	18s ribosomal subunit
2-ME	2-mercaptoethanol
A/A	antibiotic antimycotic solution
ADAMTS	A Disintegrin And Metalloproteinase with Thrombospondin Motifs
AF	annulus fibrosus
ANOVA	analysis of variance
APMA	amino-phenyl mercuric acetate
ATP	adenosine triphosphate
BMI	body mass index
BP	back pain
COPD	chronic obstructive pulmonary disease
CRP	C reactive protein
DMEM	Dulbecco's Modified Eagle's Medium
DMMB	1,9-dimethylmethylene blue
DNA	deoxyribonucleic acid
DS	degenerative spondylolisthesis
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
F12	Ham's F12 nutrient mixture
FBS	foetal bovine serum
GAG	glycosaminoglycan
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HPRT	hypoxanthine guanine phosphoribosyltransferase
IBS	irritable bowel syndrome
IFP	infrapatellar fat pad
Ig	immunoglobulin

IL	interleukin
iNOS	inducible nitric oxide synthase
IVD	intervertebral disc
LBP	low back pain
LC	liquid chromatography
LDH	lumbar disc herniation
LP	leg pain
LSS	lumbar spine stenosis
MAR	missing at random
MCAR	missing completely at random
MCE	Monte Carlo error
MI	multiple imputation
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MS	mass spectrometry
NF	normalisation factor
NG	normalisation gene
NP	nucleus pulposus
NSBP	non-specific back pain
OA	osteoarthritis
ODI	Oswestry Disability Index
OP	osteoporosis
PBS	phosphate buffered saline
PG	proteoglycan
PVDF	polyvinylidene difluoride
RA	rheumatoid arthritis
RNA	ribonucleic acid
RT-qPCR	real time quantitative polymerase chain reaction
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
T	Tween 20
TIMP	tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor-alpha
V	volt

v/v volume percent concentration
VAS visual analogue scale

w/v mass concentration

1
Introduction

Obesity and back pain are significant public health problems with substantial medical, personal, and financial burden globally. There are multiple contributing factors leading to back pain. Obesity is one factor which has not been fully studied for its effects on the lumbar spine. Specifically, the biochemical effects of obesity may contribute to pain and degeneration. Furthermore, the relationship between obesity and the clinical presentation of spine patients is unclear. This chapter is a focussed introduction consisting of background information, research hypotheses and thesis aims.

1.1 Low Back Pain

1.1.1 Low Back Pain

Low back pain (LBP) is the leading worldwide cause of years lived with disability (Figure 1.1).[1] It is defined as activity-limiting back pain, with or without pain referred into one or both lower limbs, that lasts for at least one day.[2] At any point in time, approximately 12% of the world's population suffers from LBP with a 1-month prevalence of up to 23%.[3] Given this, it is not surprising that LBP has an annual estimated total cost to the UK of £1.62 billion[4] and to the USA of US$34 billion,[5] with the financial lossed attributed to healthcare spending, personal costs and productivity losses.

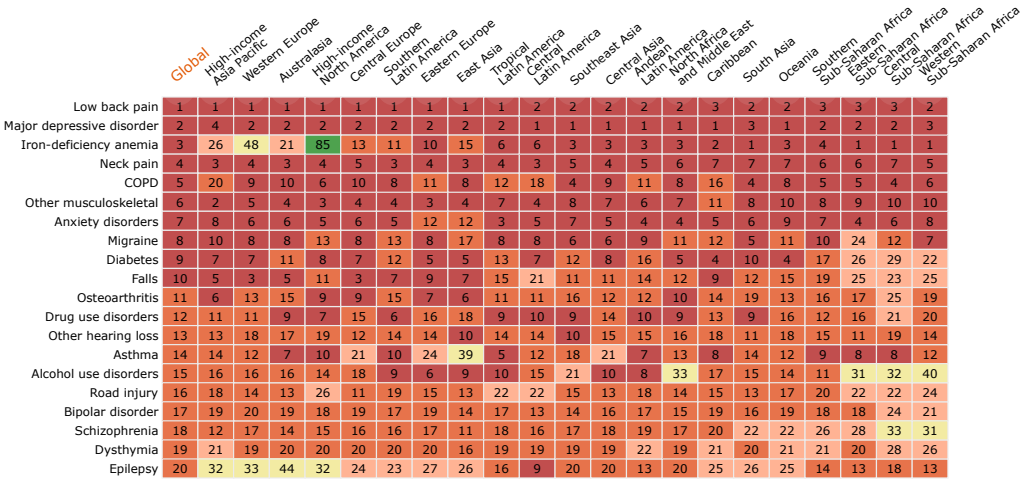


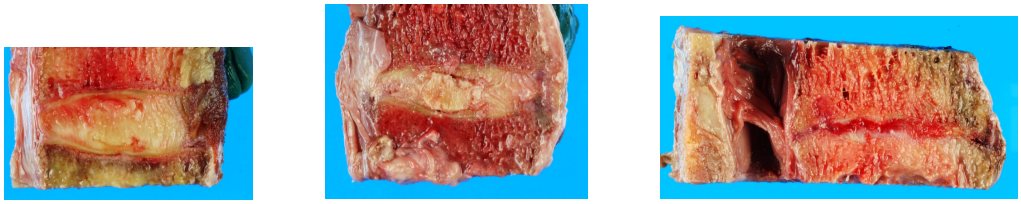
Figure 1.1: ***Worldwide leading cause of years lived with disability (YLDs).*** *Causes are ordered by global ranks with regional breakdown provided separately. Data obtained from the 2010 Global Burden of Diseases study and graphic sourced from the Institute of Health Metrics and Evaluation (http://ihmeuw.org/347z)*

1.1.2 Low Back Pain and Spinal Pathology

Degeneration of the lumbar spine has been associated with back and leg pain, however the relationship between pathology and symptoms is unclear. Many people with even severe disc degeneration or spinal stenosis can be asymptomatic. Nevertheless, the only current interventions are aimed at correcting spinal pathologies. Hence, four of these pathologies are discussed below.

Intervertebral Disc Degeneration

The intervertebral disc (IVD) is the primary joint of the spine. Many authors have linked disc degeneration (Figure 1.2) to LBP,[6–9] however, this relationship is unclear. Asymptomatic patients can have disc degeneration[10–14] and certain patients with LBP may have no degeneration.[13] Multiple factors have been implicated in disc degeneration including genetic factors, altered disc nutrition, abnormal mechanical load and local inflammation.[15,16] In reality, it is unlikely that any single factor is a lead culprit; rather it is a combination of factors. However, given the lack of a clear consensus on the causes of disc degeneration it is important to look for further pathological factors. The IVD will be discussed in greater detail in section 1.2.



a) Normal b) Moderate c) Severe

Figure 1.2: ***Spectrum of disc degeneration*** *Gross anatomical specimens of human disc showing spectrum of intervertebral disc degeneration. a) Normal disc b) Moderate degeneration and c) Severe degeneration (courtesy of Prof Sally Roberts and Dr Jude Meakin)*

Lumbar Disc Herniation

Anatomically, lumbar disc herniation (LDH) is a focal displacement of the nucleus pulposus beyond its normal confines. It is often symptomatic and associated with a radiculopathy. It has a lifetime prevalence of 1-2% (Figure 1.3a).[17] Disc herniation most commonly affects younger patients of

working age. They present with radiating or radicular leg pain termed sciatica, in combination with varying degrees of back pain.[18] Herniated discs usually have an element of pre-existing degeneration, and the herniation occurs when these discs are exposed to excessive mechanical forces.[15] Recently authors have also proposed end plate junction failure or sudden unexpected loading with subsequent annular rupture as mechanisms leading to LDH.[19,20]

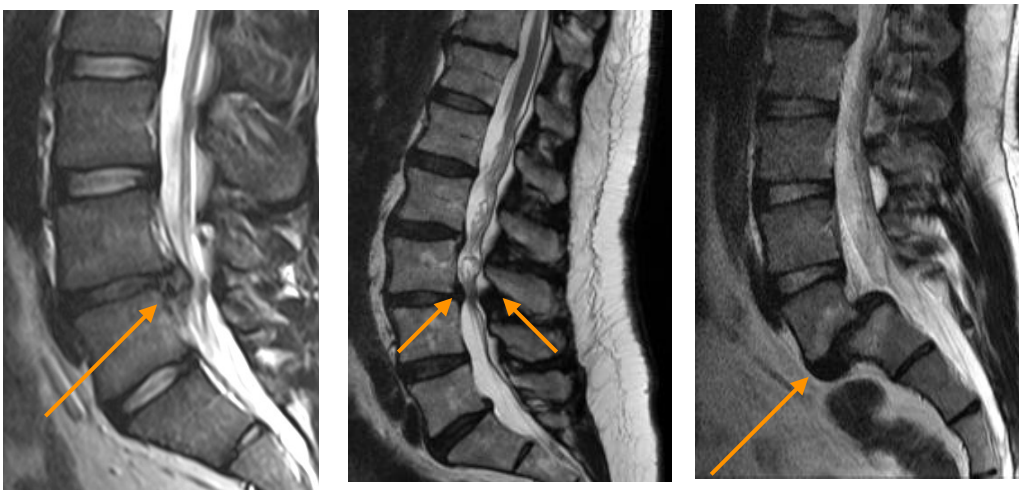
The pain associated with LDH is thought to arise from compressive pressure on the nerve root and local inflammatory changes. The herniated IVD can produce matrix metalloproteinases (MMPs), NO, interleukin (IL)-6 and prostaglandin E2.[21,22] Systemic inflammation has also been associated with sciatic pain and monoclonal anti-inflammatory molecules have shown promising results for the acute treatment of this pain.[23–26]

Lumbar Spine Stenosis

Lumbar spine stenosis (LSS) is defined as osteoligamentous narrowing of the vertebral canal with compression of the dural sac (Figure 1.3b).[27] It tends to affect an older population with neurogenic claudication typified by decreased walking distance, buttock pain, and intermittent sciatica.[28] LSS is a chronic problem with a large proportion of patients presenting with symptoms that have lasted longer than a year.[29] Like disc degeneration, a proportion of patients with anatomic LSS are asymptomatic.[28] Little is understood about the drivers of pain in symptomatic LSS but it is agreed symptomatic patients experience considerable functional limitation.[30]

Degenerative Spondylolsithesis

Degenerative spondylolisthesis (DS) is characterised by segmental spinal instability, usually at the level of L4-L5 and is seen in an older cohort of patients (Figure 1.3c).[29,31] There is a greater female predisposition with altered endocrine function and generalised ligamentous laxity attributed to this relationship.[31,32] Anatomically, facet orientation and arthritis has been implicated as another contributing factor. The pain associated with DS is primarily back pain due to instability, with associated neurogenic claudication and radicular leg pain as a result of nerve root compression.[31] It is not known if the degradative or pain processes in DS are the same as those for the other spine



a) Herniation b) Stenosis c) Spondylolisthesis

Figure 1.3: ***MRI showing different lumbar spine conditions*** *a) lumbar disc herniation b) spinal stenosis c) degenerative spondylolisthesis (courtesy of the Genodisc Study Group)*

conditions described above.

Non-Specific Back Pain

In addition to the above factors, there are a group of patients with no obvious cause for their back pain. Non-specific back pain (NSBP) can affect all age groups but imposes the greatest limitation upon adolescents and those of working age.[33] The Global Burden of Diseases study has shown this is a worldwide epidemic that afflicts people of all ethnicities and socioeconomic backgrounds (Figure 1.1).[1] These patients present a difficult problem as they have a poorly defined clinical phenotype[34] and the underlying pathology is unclear.[33] This has led for urgent calls to understand better the predictors and clinical course of NSBP.[2,3]

1.2 Intervertebral Disc

The IVD is the primary joint of the lumbar spine and allows for normal spinal biomechanics.[15] It is made up of two structurally and cellularly heterogeneous components, the nucleus pulposus (NP) and annulus fibrosus (AF) (Figure 1.4). The cells of the IVD primarily function to maintain the matrix. However, in human discs cell density is low and turnover of matrix macromolecules is slow. This

poses a problem of matrix preservation in the setting of degeneration.[35]

1.2.1 Nucleus Pulposus

The nucleus pulposus (NP) is the central gelatinous component of the IVD. It consists of chondrocyte-like cells producing a matrix similar to cartilage.[36] The two major components of this matrix are proteoglycans and collagen.

Proteoglycan (PG) accounts for 80% of the dry weight of young discs. However, in ageing and degeneration, PG content falls.[37,38] Aggrecan is the largest contributor to the PG network and it maintains disc hydration under load via its osmotic properties.[38,39] In a hydrated environment, aggrecan imbibes fluid through the Gibbs-Donnan swelling pressure developed by the negatively charged glycosaminoglycans (GAGs). A positive tissue pressure is created by the non-compliant network, consisting mainly of collagen II fibrils, and by external loads imposed by muscle activity and body weight. A fall in this swelling pressure is a sign of disc degeneration.[35]

As well as these major components, the matrix contains a rich complex of minor collagens, other proteins such as fibronection and thrombospondin, small proteoglycans such as lumican, decorin and biglycan, cytokines, growth factors and also proteases and their inhibitors.[15]

1.2.2 Annulus Fibrosus

The AF forms the outer part of the IVD and is arranged in 15-25 concentric lamellae. It consists mainly of collagen bundles running obliquely from one vertebral body to the next.[40] This organisation of collagen provides tensile strength and anchors the disc to the bone. Interspersed between the lamellae is a network containing elastic fibres and other proteins which maintains the integrity of the collagen network.[41] The lamellae are most densely packed in the outer region. Aggrecan concentration rises and collagen content falls towards the centre. Along with these changes, the cells of the outer annulus consist of fibroblastic spindle type cells which progress centrally to more rounded chondrocyte-like cells as seen in the NP.[42]

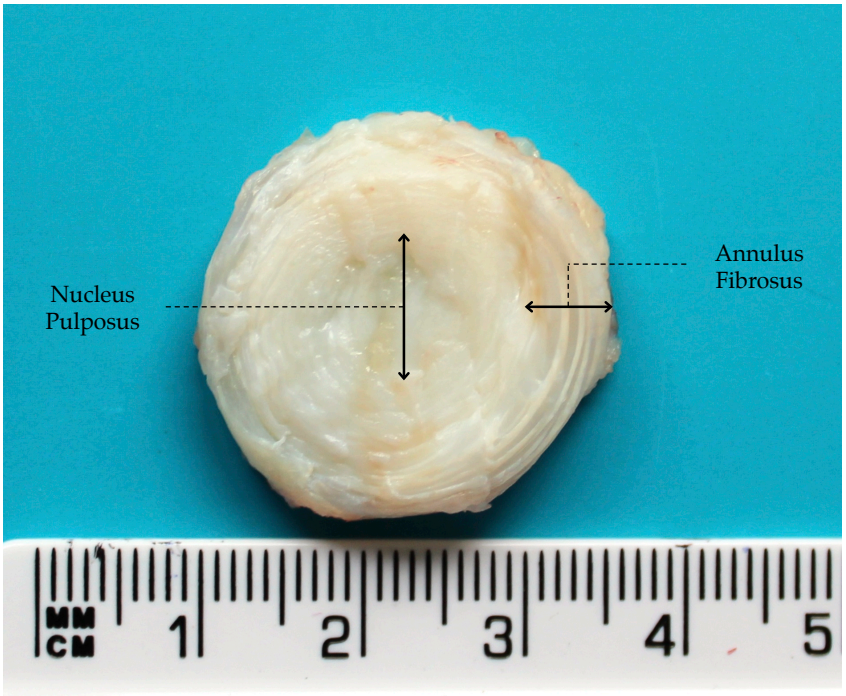


Figure 1.4: ***Photograph of the bovine intervertebral disc*** *showing the central nucleus pulpous and peripheral annulus fibrosus*

1.2.3 End Plate

The cartilaginous end plate is a layer of hyaline cartilage which lies immediately cranial and caudal to the IVD.[43] Resembling articular cartilage, the end plate is normally avascular (the adjacent bone is, and devoid of sensory innervation.[15] A thin layer of calcified cartilage joins the cartilaginous end plate to the vertebral body. End plate properties are critically important for IVD nutrition as the endplate is the main pathway for diffusion of nutrients and metabolites into the disc. It tends to calcify in ageing and pathology,[44] thus restricting passage of nutrients into the nucleus and inner annulus.[45]

The IVD is also surrounded by adipose tissue which is discussed further in section 1.5

1.2.4 Nutrition of the IVD

The disc is the largest avascular tissue in the body and receives nutrition by diffusion from blood vessels near the AF or through the end plate.[46,47] The NP is especially reliant on end plate diffusion given the distance from the peripheral AF blood supply. This diffusion pathway can be affected by

end plate calcification[48] or cartilage damage,[49] as observed in ageing.

Impaired nutritional inflow is compounded by the inability to remove metabolites from the disc. As the IVD is relatively avascular, glucose metabolism follows glycolytic pathways with lactate, a acidic molecule the main byproduct. As lactate builds up, the local pH of the IVD decreases resulting in decreased cell activity,[50] impaired matrix synthesis,[51,52] pain,[53] apoptosis,[54] and disc degeneration.[15]

1.3 Intervertebral Disc Degeneration

In disc degeneration (Figure 1.2), the matrix breaks down and loses integrity, the nucleus desiccates and cracks, and osteophytes form. Disc height decreases and there is vascular and neural ingrowth.[15] Disc degeneration is graded morphologically according to these matrix changes with Grade 1 being normal and Grade 4 or 5 very degenerate.[55]

1.3.1 Biochemistry of Disc Degeneration

IVD matrix turnover is a normal part of growth and adaptation. Matrix macromolecules are synthesised by disc cells. Matrix breakdown is mediated by two groups of proteases, MMPs and A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS), which are in turn regulated by tissue inhibitors of metalloproteinasess (TIMPs). Disc cells can also produce these proteases and inhibitors. An imbalance between matrix production and destruction underlies disc degeneration. In degenerate discs, the activity of MMPs and ADAMTS increases and expression of TIMP decreases.[56] Degenerate discs also produce pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), which potentiate degeneration by upregulating MMPs and ADAMTS.[57,58] Other proteases such as lysosomal cathepsins are also involved in disc degeneration .[59]

Group	MMP	Main Substrate
Collagenases	MMP-1, -8, -13	Collagens I, II, and III
Gelatinases	MMP-2, -9	Gelatin (degraded form of collagen) and collagen
Stromelysins	MMP-3, -10	Collagen IV and fibronectin
Matrilysins	MMP-7, -26	Fibronectin, aggrecan and laminin

Table 1.1: ***MMPs organised by substrate specificity***[60,63]

Matrix Metalloproteinases (MMP)

MMPs are a group of calcium-dependent, zinc-containing tissue proteases.[60] Mammalian MMPs share a conserved catalytic and auto-inhibitory pro-peptide domain. Cleavage of this pro-peptide is required to confer proteolytic activity.[61,62] MMPs can be broadly grouped by their primary substrate (Table 1.1).[60,63] Within the IVD, MMP-1, -2, -3, -9 and -13 amongst others have been found to be produced by degenerate disc cells and thus increased in degeneration.[63–65]

A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS)

ADAMTS are a group of 19 secreted proteases which unlike the MMPs are released in an active form.[59,66] Aggrecan, the primary component of the NP, is the main substrate for ADAMTS-1, -4, -5, -9 and -15. These five aggrecanases have all been implicated in disc degeneration[35,56,59,65] but the most active are ADAMTS-4 and -5, also known as aggrecanase-1 and -2.[35,67]

Tissue Inhibitor of Metalloproteinase (TIMP)

The four TIMPs (1-4) are the main regulators of MMP and ADAMTS activity.[62] Hence, tissue destruction is further controlled by the relative amounts of MMPs/ADAMTS to TIMPs. There is broad overlap in inhibition of MMPs with TIMPs-1 and -2 displaying slightly greater inhibition potential.[59] TIMP-3 is a potent inhibitor of the aggrecanases, ADAMTS-4 and -5.[59,66,68,69] In disc degeneration , there is a downregulation of TIMP gene expression and protein production.[63,65]

Pro-Inflammatory Cytokines

A variety of pro-inflammatory mediators have been implicated in disc degeneration and LBP. Tumor necrosis factor-alpha (TNF-α), IL-1α, IL-1β, IL-6 and IL-17 are all produced by degenerate AF and NP cells. These cytokines are involved in shifting the disc to a degradative phenotype by upregulating MMP and ADAMTS.[16,70] Both IL-1β and TNF-α increase the expression of MMP-3, -13 and ADAMTS-4 while decreasing the production of aggrecan, collagen-1 and -2.[57,58,71] These cytokines can also upregulate pain generating molecules such as substance P,[72] influence IVD cell differentiation,[70] and recruit inflammatory cells by promoting chemokine release by disc cells.[73] IL-6 is also secreted by disc cells. It acts by directly promoting degeneration and pain or by increasing local IL-1β and TNF-α.[16] Molecules such as TNF-α-stimulated gene-6 (TSG6) and its binding protein, inter-α-inhibitor (IαI) are found in the disc and may act to regulate the inflammatory cascade.[74] Finally, exogenous administered anti-inflammatory molecules are gaining increasing exposure as a useful treatment adjunct for patients with disc degeneration and LBP.[23–25,75]

1.3.2 Causes of Disc Degeneration

Although it has long been known that intervertebral discs degenerate earlier than any other tissue in the body, the exact causes are still not known. Age is the biggest risk factor. Environmental factors such as driving, occupation and heavy labour are not as significant as previously thought.[76] Twin studies have indicated a strong genetic link[77] and it was hoped that genetics would provide clarity to the complexity of spine phenotypes. However, despite many studies, no genes with strong effects have been identified.[78]

Obesity plays a significant role in multiple disorders, including degenerative musculoskeletal conditions. It appears to be a significant risk factor for LBP[79,80] and disc degeneration[81–85] but mechanisms which lead to these problems are poorly understood.[79,85] Altered biomechanics could be one mechanism which contributes to the development of the disorder.[86] However, it has been shown that limb fat mass, which would not alter spine biomechanics, is independently associated with LBP.[79] This raises the possibility of a biochemical link which is influenced by the chronic low-grade inflammatory state seen in the obese population.[87]

1.4 Obesity

1.4.1 Epidemiology of Obesity

Obesity is another public health epidemic that has been described as one the most important contributors to worldwide disease burden.[88] Increased body weight affects 33% of the global population[89] (Figure 1.5) and has been estimated to account up to 2.8% of a country's total healthcare expenditure.[90] This proportion will inevitably increase with the overweight and obese population reaching 3.3 billion in 2030 with a large contribution expected from developing countries.[89]

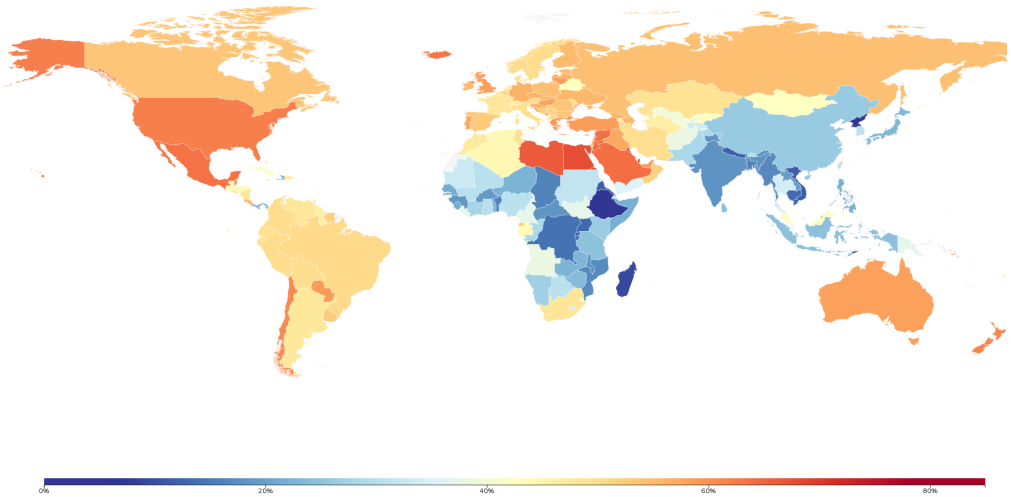


Figure 1.5: ***Worldwide prevalence of overweight and obesity*** *for BMI≥25kg/m^2 in adults of both genders over 20 years. Data obtained from the 2010 Global Burden of Diseases study and graphic sourced from the Institute of Health Metrics and Evaluation (http://vizhub.healthdata.org/obesity/)*

1.4.2 Obesity, Degeneration, and Pain

Until recently, the link between obesity and spine problems was thought to be mechanical.[91–93] However, this hypothesis has been recently challenged by recent systematic reviews showing no

relationship between LBP and occupational mechanical loading such as awkward postures,[94] standing,[95] pushing/pulling,[96] bending/twisting,[97] lifting,[98] or carrying.[99]

Obesity is known to cause a systemic low grade inflammatory milieu with growing evidence for a biochemical link between obesity, degeneration of musculoskeletal tissues and pain.[87] Obesity is associated with an elevated C reactive protein (CRP), a common and sensitive marker of inflammation.[100] In other disease processes, it is clear that obesity has a humoral influence. Perivascular adipose tissue, which is increased in obesity, has a direct link with the pathogenesis of atherosclerosis.[101] These same biochemical effects have been implicated in angiogenesis, an important step in tumour metastasis,[102] encephalomyelitis,[103] inflammatory bowel disease,[104] and most importantly osteoarthritis (OA).[105] These biochemical effects are not limited to inflammation and degeneration. Obesity can also influence and increase pain in fibromyalgia,[106] migraines,[107,108] and depression.[109,110]

1.5 Adipokines: A Biochemical Link?

1.5.1 Background

Adipose tissue or fat was once thought to be benign, serving as a source of energy. However, current understanding views fat as a metabolic and endocrine organ. It is now recognised that adipose tissue produces cytokines, termed adipokines. These include the specific fat cytokines; leptin, adiponectin, resistin and visfatin, as well as other more recognisable cytokines; IL-1, IL-6 and TNF-α.[22,105,111]

1.5.2 Leptin

The genetic control of body weight was noted in 1950 by Ingalls *et al.*[112] It was not until 1994, that the obese (*ob*) gene and its product, leptin, a 16kDa pleiotropic cytokine was discovered.[113] This finding heralded a new era in the understanding of obesity and its related disorders.

Leptin is primarily produced by adipocytes but is also produced by multiple other body tissues

such as the placenta,[114] stomach,[115] cartilage,[116] bone,[117] muscle,[118] and importantly the IVD.[119,120] In metabolic homeostasis, leptin provides an appetite suppression signal via the hypothalamus. Simplistically, a larger fat mass and hence more leptin, indicates adequate nutrition and thus further energy intake is not required. A state of central or hypothalamic leptin resistance is present in obesity and the negative feedback of adequate nutrition, provided by leptin is lost.[121] In a compensatory response, circulating leptin levels parallel body fat with greater levels seen in obesity.[122] Leptin levels are also influenced by other factors such as pro-inflammatory cytokines,[105] oestrogen,[105] psychological stress,[123] and corticosteroid intake.[124]

It has been suggested that leptin is the primary biochemical mediator of the inflammatory, degradative, and pain-related effects obesity described above. Looking to cartilage as an example, leptin is reported to increase the synthesis of pro-inflammatory cytokines, pain generators and destructive mediators in knee osteoarthritis.[125,126] Here, the sources of leptin are multiple and include distant fat stores, the infrapatellar fat pad,[127–129] osteoarthritic cartilage,[130] and bony osteophytes.[130] In chondrocytes, leptin has been shown to increase the synthesis of:

i pro-inflammatory cytokines: IL-1β,[127] IL-6 and IL-8,[125]

ii pain generators: prostaglandin E2 (PGE-2) and cyclooxygenase-2 (COX-2)[125] and

iii destructive mediators: nitric oxide (NO),[125] MMP-1,[126,131] MMP-3,[126] MMP-9[127] and MMP-13[126,127,131] by cells of the synovial joint.

Moreover, leptin potentiates the matrix-degrading activity of pro-inflammatory cytokines.[125,131] Taken together, adipose tissue and obesity appear to mediate OA progression.

It is hypothesised that a similar relationship could exist with respect to spinal degeneration and pain. The cells of the IVD produce both leptin and its receptor.[119,120] Leptin alters pain sensitisation in a lumbar nerve root compression model.[132] Although a relationship between adipokines and spinal degeneration and pain has been hypothesised, no clinical study has directly investigated any such role.[79,82,133] Leptin can reach the IVD via systemic circulation, via release by adjacent retroperitoneal adipose deposits and via production by the disc cells itself.

1.6 Summary

The pathways leading to degeneration and pain are unclear. Clinically, spinal degeneration may cause but is not always associated with back pain. There is also another group of patients with no clear cause for their pain. Given this uncertain relationship, further investigation into other, non-anatomical, predictors of LBP is required. There are risk factors common to both disc degeneration and LBP. Obesity is one of these.[9,80,134–136]

1.7 Thesis Hypotheses

The hypothesis behind this thesis is that obesity is associated with back pain and disc degeneration with biochemical factors playing an important role. Specific hypotheses are that:

1. Leptin, an adipokine, can induce inflammation and degeneration in the IVD. This effect is potentiated in a pro-inflammatory environment.
2. Paraspinal fat is a local source of adipokines and pro-inflammatory cytokines with levels correlating to patient-reported symptoms.
3. On a population level, increased BMI is associated with the experience of greater back and leg pain.
4. Obesity is an independent predictor of certain diagnostic patterns in the lumbar spine.
5. Obesity is associated with greater degeneration of the lumbar spine. Degeneration is quantified as disc degeneration, disc herniation, and lumbar stenosis.

1.8 Thesis Aims and Objectives

The main objective of this work is to understand better the relationship between obesity, lumbar spine degeneration and low back pain. To address these hypotheses, this thesis aims to:

1. Identify the response of both IVD cell types to leptin in isolation and within a pro-inflammatory context.
2. Investigate whether fat taken from symptomatic patients is related to clinical presentation and symptoms.
3. Identify plasma proteins which may be novel predictors of back pain or disc degeneration.
4. Establish associations between obesity and back pain, clinical diagnosis and spinal degeneration in a large heterogeneous patient population.

1.9 Thesis Structure

This thesis is a biochemical, clinical and epidemiological approach to answer the question of how obesity influences spinal degeneration and pain.

The first experimental chapter (chapter 3) investigates the role of leptin, the main adipokine, in intervertebral disc degeneration. This *in vitro* work attempts to identify a biochemical relationship between obesity and disc degeneration, in a cell culture model. This is followed by a clinical investigation of plasma and local adipose tissue adipokine levels. Pro-inflammatory mediators and the relationship to pain and clinical presentation are also analysed (chapter 4).

The next three chapters (chapters 6, 7 , and 8) are an epidemiological approach to define the relationship between obesity and back/leg pain; clinical diagnosis; and MRI defined spinal degeneration. A final summary is presented in chapter 9 with ideas for future investigations and studies.

Key Points

- Back pain and obesity are significant public health problems with considerable disease burdens.
- The causes of low back pain and disc degeneration are multifactorial. There has been an urgent call in the literature to understand both of these in greater detail.
- Obesity is a factor which has been relatively ignored in the lumbar spine.
- It is hypothesised that obesity is an important mediator of disc degeneration, back pain, and the clinical presentation of spine patients.

2

General Materials and Methods

This chapter describes the laboratory methods used in chapter 3 and chapter 4. Methods relating to clinical study design are included in chapter 4 and a description of the Genodisc population along with the statistical methods are detailed in chapter 5.

2.1 *In Vitro* Study

This section relates to the *in vitro* experimentation described in chapter 3.

2.1.1 Materials

All chemicals and reagents used are listed in Appendix A. Buffers are described in detail in Appendix B. Bovine caudal tails were sourced from 18-24 month cattle from a local abattoir (Mutch-Meats Ltd, Oxfordshire, UK). Tails were dissected and the IVDs isolated within four hours of slaughter.

2.1.2 Methods

Disc Cell Isolation and Culture

Intervertebral Disc Dissection Prior to dissection, tails were washed in sodium hypochlorite solution at a concentration of 10000ppm for 1 hour, followed by a brief wash with distilled water. All dissection was carried out under aseptic conditions in a laminar flow hood.

Only the proximal six IVDs were dissected and used for all experiments. Firstly, the disc was dissected completely from the surrounding fat and muscle. This was followed by separation of the cranial portion of the IVD from the cartilaginous end plate which allowed the disc to be visualised end-on as shown in Figure 2.1. The NP was identified centrally and separated from the inner annulus. The AF studied consisted only of the outer 3mm of the IVD. The remainder of the IVD was discarded. The outer AF was used due to the variable cell population and morphology of the inner annulus.[137]

Disc Cell Isolation Cell isolation was achieved by enzymatic digestion with the individual components of the IVD digested separately. Tissue explants were suspended in a solution of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with antibiotic–antimycotic solution (2% volume percent concentration (v/v)). The tissue was then incubated for 4 hours at 37 $^{\circ}$C with protease

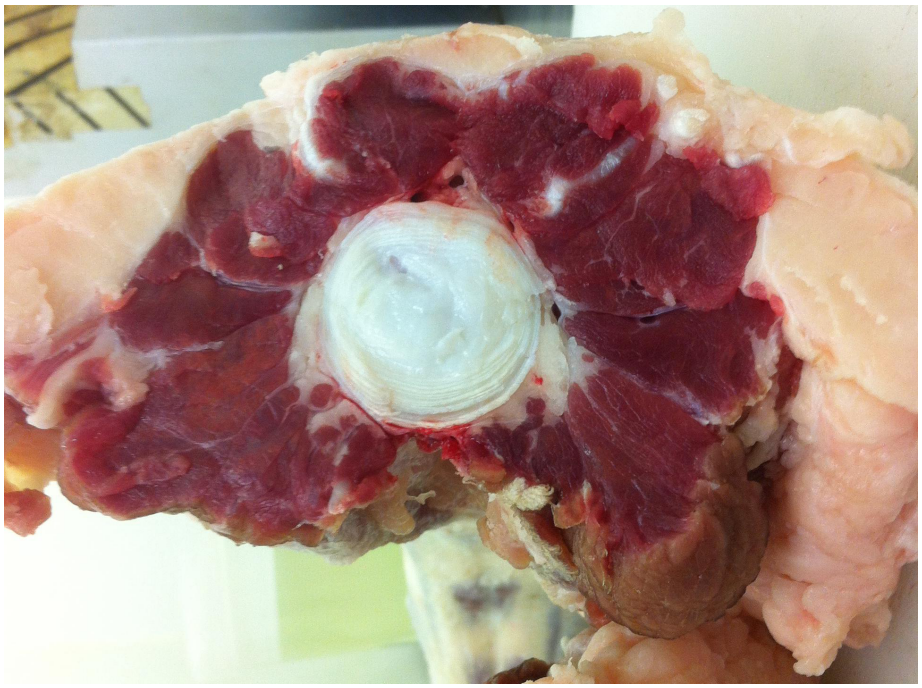


Figure 2.1: ***Photograph of the bovine intervertebral disc with surrounding soft tissues after separation from the cranial end plate.***

type XIV at a concentration of 0.5mg/ml for the NP and 1.5mg/ml for the AF. This was followed by incubation with collagenase type I at a the same concentrations for a further 18 hours.

The digests were then filtered through a 50µm mesh, washed three times in DMEM by repeated centrifugation at 2500g. The cell pellet was then resuspended in 5ml of DMEM. A 50µL aliquot of the suspension was mixed 1:3 with trypan blue and counted on a haemocytometer to quantify cell numbers and viability. The suspension was used immediately for culture if cell viability was greater than 95%.

Disc Cell Encapsulation To provide a more physiological environment, the cells were encapsulated in alginate beads. This technique was initially described, by Gou *et al.* as a method for chondrocyte culture as it maintains the spherical phenotype of a chondrocyte.[138] This technique has been shown to have similar benefits in IVD culture.[137]

In a modification of the protocol by Hauselmann *et al.* the cells were suspended in a solution of 1.2% alginate at a concentration of 4 million cells/ml.[139] This suspension was extruded through a 21G needle into 102mM $CaCl_2$ where insoluble calcium alginate beads are formed, encapsulating

the cells. Excess $CaCl_2$ was removed by washing the beads thrice with phosphate buffered saline (PBS). This procedure resulted in spherical alginate beads each of 15µl volume, containing 40,000 cells per bead.

Disc Cell and Explant Culture Unless otherwise stated, the initial culture medium used was DMEM (low glucose, pyruvate, HEPES) at 380mOsm supplemented with antibiotic antimycotic solution (A/A) (2% v/v) and foetal bovine serum (FBS) (10% v/v). Beads were cultured at a density of 1 million cells per ml of media. All culture was carried out at 37 °C, 100% humidity in 21% O_2 and 1% CO_2.

End of culture Upon completion of the experiment, alginate beads were dissolved by the addition of citrate buffer (Appendix A) and centrifuged for 5 minutes at 2500rpm. The supernatant was removed and digested with 2µl of papain at 60 °C for 24 hours. The cells were washed carefully with PBS to remove any carryover citrate buffer and the ribonucleic acid (RNA) was extracted as described below.

Outcome Measures

Lactate Assay

Owing to the low in-vivo oxygen pressure, both cell types of the IVD utilise anaerobic glycolysis as the primary adenosine triphosphate (ATP) generating pathway, even in the presences of oxygen. Lactate is the major end product of this reaction and as such such provides a good representation of the metabolic activity of both the NP and AF cells.[140,141]

The assay was performed using a commercially available kit. This enzymatic reaction is based on the principle that lactic acid is converted to pyruvate and subsequently hydrogen peroxide by lactate oxidase. The hydrogen peroxide produced allows horseradish peroxidase to catalyse the oxidative condensation of chromogen precursors to a coloured dye that is read at 540nm on a microplate spectrophotometer. Lactate dissolved in DMEM was used as standards. Results were normalised to cell number.

Glycosaminoglycan Assay

Sulphated GAGs are a major component of the IVD matrix and were measured as a marker of the synthetic function of the cells.

This assay utilised is a modification of the technique described by Farndale and relies on the colour change associated with the 1,9-dimethylmethylene blue (DMMB) dye at pH 6.8.[142] This reaction was measured on a microplate spectrophotometer at 525nm. Chondroitin sulphate dissolved in the papain buffer was used as a standard.

This analysis comes with two caveats. Firstly, this reaction is unstable as the GAG-DMMB complexes precipitate rapidly leading to a decrease in absorbance.[142] Hence, this reaction was read within the first minute of initiation. Secondly, the linear range for this assay is narrow and the supernatant frequently requires dilution. Furthermore, alginate interferes with the GAG assay but this was overcome by lowering the pH to 3.0.[143] Results were normalised to cell number when cultured in alginate beads.

Matrix Metalloproteinase Quantification

The activity and relative concentrations of MMPs were measured in the culture supernatant at various time points.

Protein Assay Soluble protein was quantified in the supernatant using the DC Protein Assay, This is a modification of the calorimetric Lowry Folin-phenol assay[144] with the advantage of rapid colour development and temporal stability. Colour is developed in two stages. First, the reaction between protein and copper sulphate occurs in an alkali environment. The copper-protein complex subsequently reduces the Folin reagent producing molybdenum/tungsten blue which exhibits maximum absorbance at 750nm.[144] Serially diluted bovine serum albumin was used as standards. Quantifying protein ensured equal loading in each well in the subsequent gel analysis.

Gelatin Zymography Zymography is an electrophoretic techniques that allows for substrate dependent identification of specific MMPs at the picogram level.[60] Specifically, gelatin zymography identifies MMP-2 (gelatinase-A) and -9 (gelatinase-B) in both the latent and active forms.

The technique used was a modification of that described by Hu *et al.*[145] Briefly, 10µg of protein from each sample was separated in 10% gelatin polyacrylamide gel under denaturing non-reducing conditions. Sodium dodecyl sulfate (SDS) in the sample and running buffer denatured and thus deactivated the MMPs. To allow for inter-gel comparison, 10ηg of recombinant human MMP-2 was used as a positive control. The gel was run at a constant 125volts (Vs) for 75 minutes. Kaleidoscope pre-stained standards was used as a molecular weight marker.

This was followed by two washes with 2.5% v/v Triton X-100 to remove the SDS, reactivating both the pro-MMPs (without cleavage) and active MMPs.[60] The gels were subsequently incubated in the zymogram renaturing buffer for 16 hours at 30 °C under gentle agitation. This allowed the MMPs time to digest the gelatin in the gel. After incubation, the gels were stained with 10ml of SimplyBlue SafeStain, a colloidal Coomassie G-250 stain, and destained with 100ml distilled water.

The proteolytic enzymes are detectible as clear bands against a blue background. Bands were quantified using densitometry on ImageJ. All results were normalised to the positive control, a 10ηg band of MMP-2.

Western Blotting Western blotting allowed for visualisation of MMP-1, -3 and -13. The protocol used was based on that provided by the manufacturer. *(Amersham ECL Western Blotting Detection Reagent Instructions 28-9829-42)*

To improve sensitivity, 100µg of protein was concentrated using StrataClean resin, a solid phase silica-based resin. The samples were then mixed with 10µl of 2x Laemmli sample buffer with 2-mercaptoethanol (2-ME) and boiled at 95 °C for 10 minutes. The samples were separated using a 12% Mini-PROTEAN TGX Gel at a constant 200Vs for 35 minutes. Recombinant human MMP-1, -3 and -13 western blot standards were used as positive control and Kaleidoscope prestained standards as a molecular weight marker.

Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot Gel Transfer Device and iBlot Regular PVDF Transfer Stack. After protein transfer, the membrane was the blocked with 5% mass concentration (w/v) ECL blocking solution in 0.1% v/v PBS-Tween 20 (T) for 1 hour at room temperature. This was followed by incubation with diluted, bovine reactive,

primary anti-MMP-1 , -3 and -13 antibodies in PBS-T at 4 °C overnight. Antibiody dilutions used were 1:1000, 1:1000 and 1:5000 respectively. The membrane was rinsed three times with PBS-T for 10 minutes at room temperature followed by 1 hour incubation in diluted (1:5000) donkey anti-rabbit IgG ECL antibody.

Amersham ECL Plus Western Blotting Detection reagent was pipetted on the membrane and incubated at room temperature for 1 hour. Chemiluminescence was detected on Amersham ECL Hyperfilm. Protein bands were quantified by densitometry on ImageJ and normalised to the relevant positive standard.

MMP Fluorometric Assay The SensoLyte 520 Generic MMP Assay Kit was used to quatify total MMPs produced by IVD cells exposed to experimental conditions. Like zymography, this is a substrate dependant reaction and uses a fluorescence resonance energy transfer peptide as a MMP substrate.[146] Both a fluorescent molecule, 5-FAM and a quencher, QXL520, is bound to this peptide. When intact, the fluorescence is suppressed by QXL520. Exposure to MMPs leads to cleavage of the peptide and liberation of fluorescence which is detected at 490nm excitation and 520nm emission wavelengths in relative fluorescence units. The assay is able to detect the presence of MMP-1, -2, -3, -7, -8, -9, -12, -13, and -14 down to nanogram concentrations. All MMPs were activated using amino-phenyl mercuric acetate (APMA) immediately prior to the measurement and the manufacturer's technical protocol was followed.

Quantification of Gene Expression

At the completion of the experiments, cells were isolated and gene expression quantified to compare relative differences between controls and treated conditions.

RNA Extraction and Purification RNA was isolated using the Qiagen RNeasy Mini Kit. Cells were lysed using the provided RLT buffer with 2-ME, inactivating RNases and releasing the RNA. Homogenisation, to reduce sample viscosity, was achieved with the QIAshredder. The remainder of the RNA extraction followed the followed the *Purification of Total RNA from Animal Cells Unsing Spin Technology Protocol* provided by Qiagen. In short, the homogenate was combined with

70% ethanol and applied to a silica membrane, to which RNA binds. Repeated washing and centrifugation with the provided buffers purifies the RNA, which is finally eluted in RNase/DNase free water.

RNA concentration and purity was assessed using a NanoDrop 1000 spectrophotometer. Samples were only used if the 260:280 ratio was greater than 1.75. Genomic deoxyribonucleic acid (DNA) was eliminated from the RNA by adding Precision DNase and heating to 55 °C. This DNAse enzyme has no activity against RNA.

Complementary DNA Synthesis Complementary DNA (cDNA) was synthesised from the RNA samples using the nanoScript reverse transcription premix (PrimerDesign RT-premix-48), which includes oligodT and random primers. This reaction accommodates up to 2µg of RNA and yields a volume of 10µl.

Normalisation Gene Selection The normalisation gene (NG), also known as the housekeeping gene or internal control gene, is an essential part of evaluating qPCR data. Strict normalisation of data is needed to control for a variety of factors, which include the total amount of template cDNA, the cell type, and primer efficiencies.[147] A NG used for one experimentation protocol may not be suitable for another.[148] Given this, expression profiling under the experimental condition of interest is required to select a suitable number of NGs to ensure valid results.[147]

Commonly used NGs for qPCR involving the bovine IVD are glyceraldehyde-3-phosphate dehydrogenase (GAPDH),[149–151] hypoxanthine guanine phosphoribosyltransferase (HPRT)[152] and the 18s ribosomal subunit (18s rRNA).[153,154] There are no published reports of a NG expression stability assessment for the bovine IVD. Using IVD cells and chondrocytes from other species as a guide, the 18s rRNA[155–158] and GAPDH[155,157] appear some of the least stably expressed NGs tested, although commonly used.

The genes listed in Table 2.1 were selected to examine expression stability in the system tested. A bovine primer for GAPDH was unavailable for this testing.

Samples from both NP and AF cells, treated with leptin, were used in this qPCR experiment

Symbol	Name	Accession Number	Function
EIF2B2	eukaryotic translation initiation factor 2B	NM_001015593.1	Protein synthesis
ACTB	β-actin	NM_173979.3	Cell structure and integrity
SDHA	succinate dehydrogenase complex A	NM_174178.2	Electron transport chain
PP1A	protein phosphatase 1, α isozyme	NM_001035316.2	Cell division, protein sythesis
HPRT1	hypoxanthine phosphoribosyltransferase 1	NM_001034035.2	Purine synthesis via the purine salvage pathway
18s	ribosomal protein S18	NM_001033614.2	18s ribosomal subunit

Table 2.1: ***Genes assessed for expression stability under experimental conditions described in subsubsection 2.1.2***

with the primers from the six genes in Table 2.1. Data analysis was conducted using the validated GeNorm algorithm designed by Vandesompele *et al.*[147] The algorithm relies on the principle that the expression ratio of two ideal NGs is constant in all samples. Any variation in this ratio would suggest that either one of the genes is variably expressed.

The *M* (Figure 2.2) value gives a measure of the average stability of a selected NG based on the pairwise variation of the selected NG with all the other NGs in the experiment. NGs with the lowest *M* value have the most stable expression. In this system, the two most stably expressed genes were EIF2B2 and ACTB (Figure 2.2). Interestingly, two commonly used NGs as cited in the literature, 18S and HPRT1, were the least stable in this experiment. If either of these NGs had been chosen, based on the literature or previous experience, interpretation of results may have been compromised.

The GeNorm algorithm also calculates a normalisation factor (NF) for each gene based on the geometric mean of its expression profile. The geometric mean is preferred to the arithmetic mean as it less susceptible to outliers.[147] To determine the ideal number of NGs to use, another pairwise variation calculation *V* is made and is defined as n/(n+1), where n is the number of genes assessed (Figure 2.3). It measures the effect of adding further NGs (n+1) on the NF obtained from n NGs. Additional NGs are added until there is no significant variation in the calculated NF. Vandesompele *et al.* defined a cut-off level of variation as 0.15, below which no further NGs are required.[147]

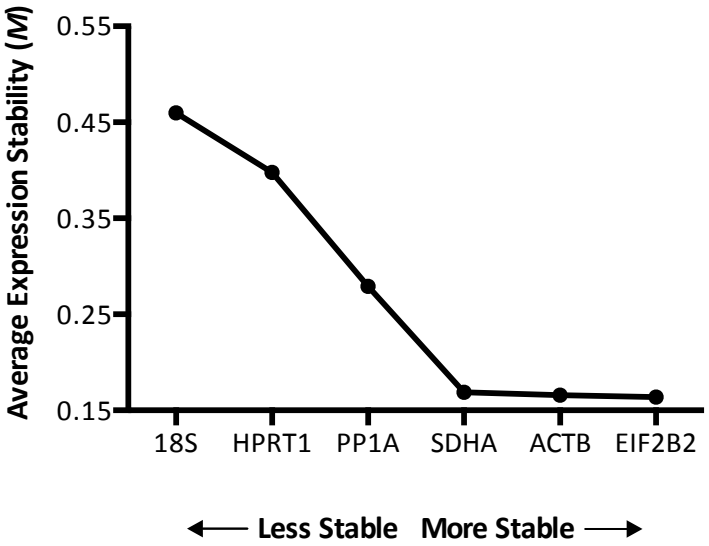


Figure 2.2: ***Ranking of the stability of six potential normalisation genes.*** *Expression stability (M) of normalisation genes in both cell types (NP and AF) under leptin stimulation. The lower the (M) value, the more stable the NG.*

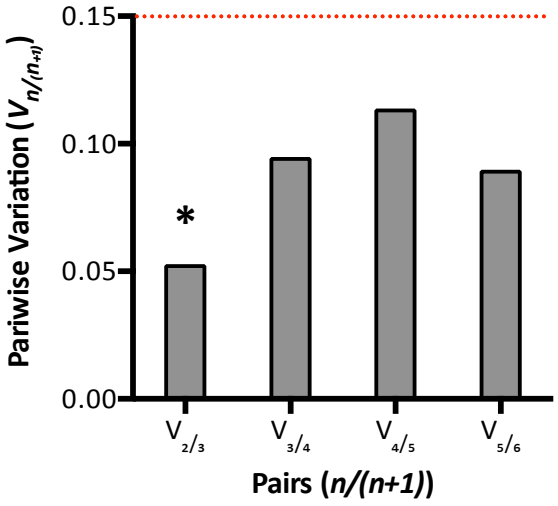


Figure 2.3: ***Pairwise variation (V) to determine the optimal number of control genes for an accurate normalisation factor ($V_{n/(n+1)}$).*** *The variation between two consecutive normalisation factors with n and n+1 genes. Red line represents the cut-off level of variation below which no further NGs are required. * represents optimal number of control genes for normalisation.*

It is generally accepted that more than one NG should be employed in qPCR experiments.[159] Figure 2.3 shows that with two NGs, the addition of a third (V2/3) has no significant effect on NF variation. Variation is increased further with the addition of more NGs. In this situation, two NGs provide the ideal normalisation strategy. Finally, given ACTB and EIF2B2 are the most stably expressed (lowest *M* value in Figure 2.2) these genes were chosen for qPCR normalisation.

Real-Time Quantitative PCR (RT-qPCR) Oligonucleotide primers, listed in Appendix C were designed and validated by PrimerDesign. All PCR reactions utilised the 2x Precision Mastermix with SYBR green dye according using the protocol supplied by PrimerDesign. Each reaction volume was 20µl that consisted of 12.5µg of cDNA and primers at a 300nM concentration.

The amplification protocol consisted of a hot start with 10 minutes at 95 °C followed by 40 cycles of 15 seconds denaturation at 95 °C and 60 seconds data collection at 60 °C. The reaction was read on the ABI Prism 7000 (Applied Biosystems). Melting curves were performed at the end of 40 cycles to ensure a single amplification product. All data were analysed using the $2^{-\Delta\Delta Ct}$ method with statistical analysis performed using Student's *t*-test as described by Schmittgen and Livak.[160]

Statistical Analysis

All data is presented as the mean ± standard error of the mean (SEM) of at least three biological replicates (n≥3). Statistical analysis was performed using GraphPad Prism version 6.0. Comparison across multiple groups was achieved using the two way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. A P value ≤ 0.05 was considered statistically significant.

2.2 *Ex Vivo* Study

The general methods relating to this observational study are detailed in the section below with specific study design methods detailed in chapter 4. At the time of surgery, paraspinal adipose tissue, serum and plasma was obtained from spinal patients and processed as described below.

2.2.1 Materials

The materials used for the analysis of biological samples obtained from patients, along with the suppliers, are listed in Appendix A.

2.2.2 Methods

Adipose Tissue Explant Culture

Adipose tissue was acquired at the commencement of surgery near the location of the skin incision. Approximately 500 to 1500mg was received from each patient. Each sample had a maximum warm ischemia time of 15 minutes prior to stabilisation at 4 $^{\circ}$C in DMEM-Ham's F12 nutrient mixture (F12).

The adipose tissue was processed according to the modified protocol described by Fain *et al.*[161] The explants were initially morcellised into to approximate 1-2mm^3 pieces. These were the resuspended in DMEM and centrifuged at 350rpm for 1 minute thus separating adipose tissue, which floated, from connective tissue and blood, which formed a pellet. The adipose tissue was removed, dried with sterile gauze and weighed into approximately 500mg fractions.

Fractions were then cultured for 48 hours in 2.5ml of DMEM per 500mg of tissue. The media was supplemented with 2% v/v A/A and DMEM-F12. Culture was performed in duplicate if the initial tissue volume allowed. Media was changed at 24 hours and this was analysed.

Serum and Plasma Preparation

Venous blood samples were obtained at the time of anaesthetic induction. As the patients were presenting for surgery, they had been fasting for at least eight hours. Serum was collected in a serum separation tube and allowed to clot for one hour. Plasma was collected with ethylenediaminetetraacetic acid (EDTA), an anticoagulant. Both samples were centrifuged at 5000rpm for 10 minutes, separated into aliquots and stored at -80 $^{\circ}$C until analysis as described below.

Single and Multiplex Immunoassay

Leptin, adiponectin and 10 pro-inflammatory cytokines in the adipose explant culture supernatant and plasma, were measured using the Meso Scale Discovery Assay System. Leptin and adiponectin were measured in singleplex, one protein target per well, where as the pro-inflammatory cytokines were measured in multiplex, 10 targets per well (Figure 2.4). The benefits of this system over a traditional enzyme-linked immunosorbent assay is the large dynamic range of detection and the ability to examine multiple proteins within a single well.

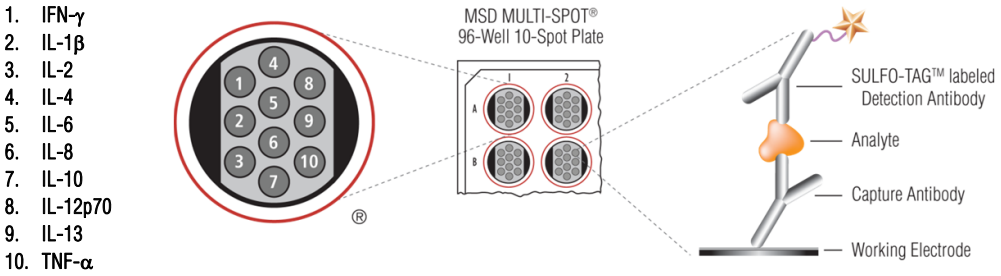


Figure 2.4: ***Schematic depicting the multiplex sandwich immunoassay principle.****(© MesoScale Diagnostics LLC)*

The assay is principally an electro-chemiluminescent sandwich immunoassay. Briefly, a capture antibody is pre-coated to an electrode within a 96-well plate. When sample is added, the protein of interest binds to the capture antibody. A chemiluminescent labelled detection antibody is then added, this also binds to the protein thus completing the 'sandwich'. A voltage is then passed through the plate causing the labelled antibody to emit light, the intensity of which correlates the quantity of protein (Figure 2.4).

Liquid Chromatography–Mass Spectrometry

Liquid chromatography (LC) with mass spectrometry (MS) is a powerful exploratory proteomic analytical tool which provides an unbiased assessment of the proteins present in a biological sample. In its broadest form MS can detect, identify, quantify proteins and their associated modifications by measuring the masses of peptides and using a protein sequence database to identify the proteins present.[162] MS is also very sensitive with a detection range into the femtomole range.[163] Plasma

samples from patients were analysed by LC-MS to identify novel proteins. The LC-MS workflow is described below.

Abundant Protein Depletion

Due to the large dynamic range of proteins in plasma, proteome analysis is complex with trace protein MS signal suppressed by highly abundant proteins.[164–167] It has been estimated that only a few abundant proteins, such as albumin and immunoglobulin (Ig)G make up 90% of total plasma proteins.[168] For example, the concentration of albumin, the most abundant plasma protein is approximately 10 orders of magnitude greater than that of "more interesting" proteins such as cytokines and chemokines (Figure 2.5).[165]

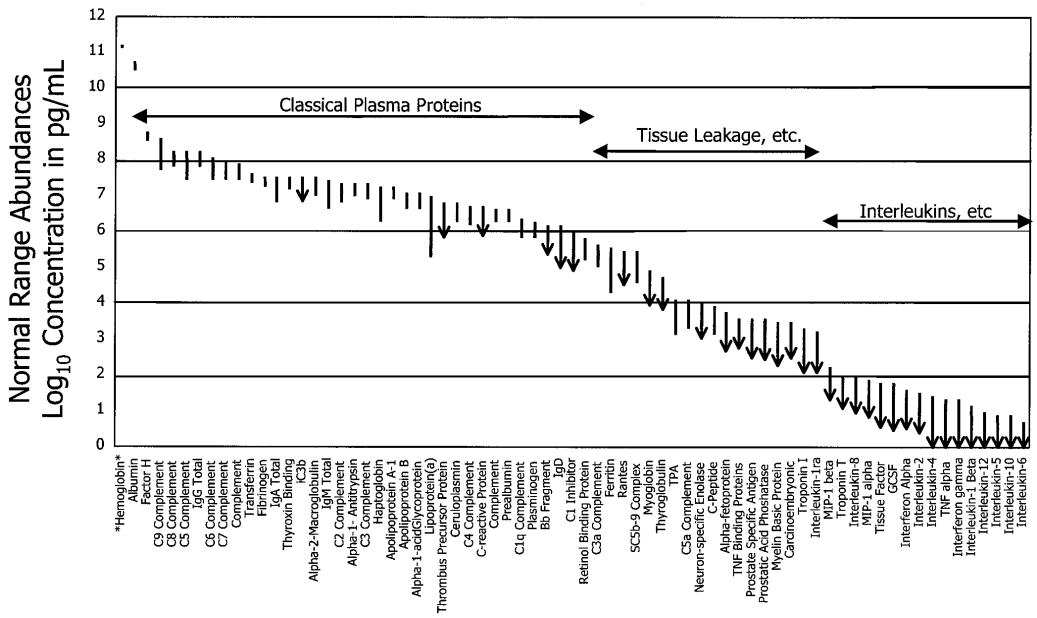


Figure 2.5: ***Graph showing the dynamic range of proteins in normal human plasma.*** *This research was originally published in Molecular & Cellular Pharamcology. Anderson & Andreson. The Human Plasma Proteome. Molecular & Cellular. 2002; 1:847-867.[165] © The American Society for Biochemistry and Molecular Biology."*

In an effort to overcome sampling issues, methods have been developed to deplete abundant proteins while retaining potential proteins of interest. In this study, two methods were trialled in an attempt to identify the optimal technique for improvement of protein discovery.

Immunoaffinity High Abundant Protein Depletion This immunoprecipitation technique utilises resin-immobilised anti-protein antibodies which bind to and subsequently deplete abundant pro-

teins. The Pierce Top 12 Abundant Protein Depletion spin columns removes greater than 95% of the 12 most abundant plasma proteins (albumin, Ig G, α1-acid glycoprotein, α1-antitrypsin, α2-macroglobulin, apolipoprotein A-I, apolipoprotein A-II, fibrinogen, haptoglobin, IgA, IgM, and transferrin) thus improving the number of ”interesting” proteins identifiable.

The procedure for abundant protein removal involves loading 10µl of plasma into a pre-loaded spin column. The columns are incubated at room temperature for 60 minutes with gentle mixing allowing the proteins time to bind to their corresponding antibodies. Subsequent centrifugation elutes the unbound proteins in a buffer (10mM PBS, 0.15M NaCl, 0.02% azide, pH 7.4) that is suitable for downstream LC-MS analysis. Bound proteins remain attached to the resin in the spin column which is then discarded. The proteins in eluate were then digested.

A diverse range of abundant proteins can be depleted using this technique. However, complete depletion will lead to a loss important protein complexes. Analysis of co-depletion proteins has shown that more than 80% of plasma proteins detected on MS are bound to albumin or IgG.[169,170]

Low Abundant Protein Enrichment using Solid Phase Combinatorial Hexapeptide Ligand Libraries This technique is based on the principle of affinity chromatography. Porous chromatographic beads (solid phase) are divided into equal batches and attached to hexapeptide/amino acid sequence. The same sequence is bound to each bead batch which then binds to a unique and reciprocal sequence of proteins in the plasma sample.[168] The ProteoMiner Protein Enrichment kit contains a library of 64 million unique peptide sequences. It is suggested that this vast library is likely to capture nearly any possible protein.[171] The Small-Capacity ProteoMiner spin columns were used in experimentation.

After equilibration, columns were washed thrice with PBS by centrifugation at 1000rpm for 60 seconds. At least 200µl of plasma was loaded into each column and incubated for two hours under gentle agitation allowing proteins to bind to the hexapeptides. Following this, the columns were centrifuged and the flow through discarded. Highly abundant proteins, such as albumin, quickly saturate their ligands (blue and yellow in Figure 2.6), remain in solution and are washed out. Conversely, low abundance proteins (green and grey in Figure 2.6) are concentrated on their beads. After three more washes with PBS, the bound proteins were eluted from the beads using the elu-

tion reagent (8 M urea with 2% CHAPS) and digested for mass spectometry.

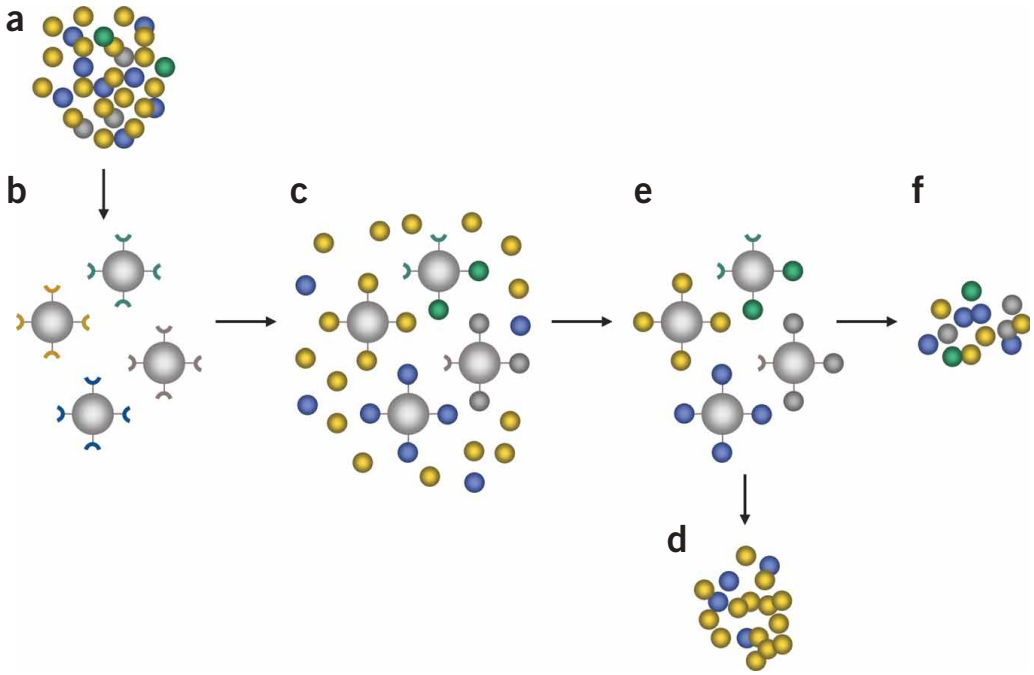


Figure 2.6: ***Schematic describing the protein enrichment process using combinatorial hexapeptide ligand libraries.*** *Various plasma proteins a) are incubated with hexapeptide ligand library b) until each bead binds its corresponding protein until saturation c). The unbound protein d) is washed and discarded while the bound fraction e) is eluted f) and used in downstream MS.(Copyright License Number 3458220406196 provided by Nature Publishing Group)*[172]

This technique effectively depletes highly abundant proteins while enriching trace proteins thus reducing the dynamic range of the sample and retaining protein complexes which may be lost during immunoaffinity depletion. A concern with hexapeptide ligand enrichment is that the relative protein concentrations may be altered thus making results difficult to quantify.[172] However, it has been found that the initial protein proportions remained unchanged for serum spiked with various concentrations of complex protein samples and subsequently processed with Proteominer, suggesting the MS results are still quantifiable.[168]

Protein Digestion

To generate peptides suitable for LC-MS analysis, the proteins obtained above were digested with trypsin either in-gel or in-solution.

In-Gel Digestion The proteins were run onto the top of a 12% Mini-Protean TGX Gel and stained with InstantBlue, a Coomassie based stain. The protein band was cut into 1-2mm^3 pieces and washed twice for a total of 18 hours in a wash solution (1ml, 50% methanol, 5% acetic acid). The supernatant was removed and the gel pieces dehydrated in acetonitrile under gentle agitation.

Disulphide bonds were reduced using 10mM dithiothreitol (DTT) followed by alkylation with 100mM iodoacetamide. Following this, gel pieces were washed by repeated dehydration, with acetonitrile, and rehydration with 100mM ammonium bicarbonate. The gel pieces were dehydrated, dried and rehydrated with freshly prepared trypsin solution (150µl, 10ηg/µl MS grade trypsin in 50mM ammonium bicarbonate). After rehydration, excess trypsin solution was removed and 200uL of 50mM ammonium bicarbonate was added to prevent dehydration. Digestion was carried out at 37 °C for 18 hours.

Peptides from the gel pieces were extracted sequentially using 50mM ammonium bicarbonate, followed by 50% acetonitrile with 5% formic acid and finally with 85% acetonitrile and 5% formic acid.

In-Solution Digestion The proteins in suspension were dehydrated in a vacuum centrifuge followed by resuspension in 7.2M urea and 100mM ammonium bicarbonate. Reduction and alkylation was achieved with 45mM DTT and 100mM iodoacetamide respectively. The urea was then diluted to 1M with ultra-pure water and 10mM ammonium bicarbonate. Freshly prepared 10ηg/µl MS grade trypsin was added in a ration of 1:25 trypsin to protein. Digestion was carried out overnight at 37 °C. The reaction was the quenched with 10µl of glacial acetic acid. Peptides were extracted from solution using Isolute-PH solid phase extraction columns and eluted into 80% acetonitrile and 0.1% formic acid.

The final step of sample preparation was the same for both in-gel and in-solution digestion. The extracts were then concentrated in a vacuum centrifuge, resuspended in 20µl of 5% acetonitrile, 0.1% formic acid in an ultrasonic bath and transferred to a LC-MS vial.

LC-MS Equipment

Samples were analysed by Ultimate 3000 nano-HPLC system (Dionex, Sunnyvale, CA, USA) which

was coupled by an electrospray ionisation (ESI) interface to a 3D high capacity ion trap mass spectrometer (amaZon; Bruker Daltonics).

Database Searching and Data Analysis

Using the UniProt_SwissProt database with human restriction, searches were performed using the Mascot (version 2.4) algorithm,[173] a probability based scoring system. The following parameters were applied: 2+, 3+ and 4+ ions, peptide mass tolerance 0.3 Da, 13C = 2, fragment mass tolerance 0.6 Da, number of missed cleavages: two, instrument type: ESI-TRAP, fixed modifications: Carbamidomethylation (Cys), variable modifications: Oxidation (Met).

Operation of the LC-MS equipment and database searching was performed in conjunction with Dr Holger Kramer, OXION, Oxford University.

3

Leptin and the Intervertebral Disc: An *In Vitro* Study

This chapter investigates the hypothesis that fat-specific cytokines can induce and influence intervertebral disc degeneration. A bovine IVD model was exposed to varying concentrations of leptin, alone or in combination with other pro-inflammatory cytokines, TNF-α, IL-1β or IL-6. It was found that leptin in isolation can induce degeneration of the IVD. These effects are potentiated in a pro-inflammatory environment. Taken together, this suggests leptin could be an important mediator of disc degeneration and a biochemical link between obesity and disc degeneration.

3.1 Introduction

Adipokines are fat-specific cytokines which have deleterious effects on musculoskeletal tissues. Leptin and its receptor have been localised in the IVD but apart from this little about the possible role of leptin in the disc is known.[119,120] More is known about the effect of leptin on articular cartilage. Multiple authors have shown that leptin can upregulate degradative enzymes[126,131] and lead to local expression of pro-inflammatory cytokines in OA.[125] Given the similarities between cartilage and the IVD, it was thought leptin could mediate similar degradative processes in the IVD and prove to be a biochemical link between obesity and disc degeneration .

3.2 Hypothesis

That leptin, an adipokine, can induce inflammation and degeneration in the IVD. This effect is potentiated in a pro-inflammatory environment.

3.3 Experimental Plan

The experimental process is summarised in Figure 3.1. After isolation, described in chapter 2, cells were placed in media supplemented with 10% FBS for 24 hours, followed by media with no FBS for a further 24 hours, to allow the cells to equilibrate to the conditions. Leptin alone, at a concentration of 5, 10 or 25µg/ml, was then added to the cells in serum-free medium for 48 hours. The concentrations used are in keeping with those used on chondrocytes.[131] At the end of the experiment, the media and alginate were stored for later analysis while RNA was extracted from the cells. Following a similar protocol, cells were also exposed to the pro-inflammatory cytokine (TNF-α, IL-1β or IL-6) alone, at concentrations of 0.1, 1.0, 10 or 100ηg/ml, or in combination with leptin at 25µg/ml. This combination of cytokines and leptin aimed to mimic the pro-inflammatory environment described in disc degeneration[16] and herniation.[21] The concentration of 25µg/ml for leptin was chosen for the pro-inflammatory experiments as this showed the greatest effect in isolated culture.

Lactate production rates were measured as a marker of cell metabolism. GAG and MMP pro-

duction were quantified to assess the respective anabolic and catabolic functions of the AF and NP cells. Finally, real time quantitative polymerase chain reaction (RT-qPCR) was used to assess the changes in gene expression in response to changes in experimental conditions.

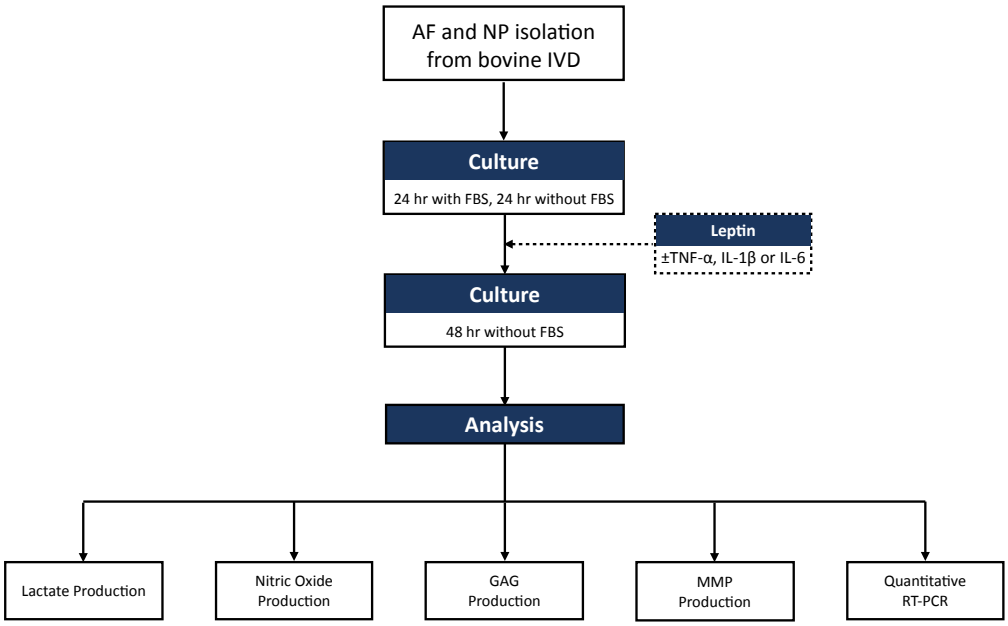


Figure 3.1: ***In Vitro Experimental Plan***

3.4 Results

3.4.1 Leptin In Isolation

Lactate Production Rate Leptin alone had minimal effect on cellular metabolism. In the AF, there was no discernible change in lactate production with increasing leptin concentration. The NP however showed a trend for a fall in metabolism with increase in leptin concentration, but this did not reach statistical significance (Figure 3.2A). Lactate production per cell was significantly greater under all conditions for NP than for AF cells.

Glycosaminoglycan Production Rate Figure 3.2B shows the GAG production by both cells types. With increasing leptin, there was a trend for a fall in rates of production but this was not significant. Compared to the control, AF and NP cells exposed to 25µg/ml of leptin produced 14% and 19% less

GAGs respectively.

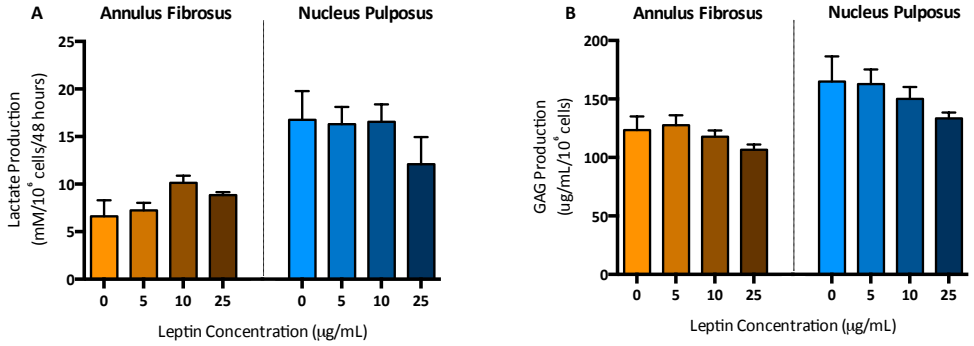


Figure 3.2: ***Lactate (A) and GAG (B) production by AF and NP cells upon exposure to 3 concentrations of leptin.****. Assays performed after 48 hours of culture and exposure to leptin. Data represented as mean ± SEM.*

Matrix Metalloproteinases at the Protein Level There was a clear dose-dependent increase in MMP-3 and MMP-9 production, by both AF and NP cells, with increases in leptin concentrations (Figure 3.3). MMP-1 and MMP-2 did not follow this pattern in the AF and these levels were not detectable in the NP. MMP-13 was not detectable for either cell type at any leptin concentration. Importantly, total MMP production, shown in Figure 3.7, was markedly increased with the addition of 25µg/ml of leptin by both AF and NP cells.

RT-qPCR As leptin at 25µg/ml had the greatest effect on cell metabolism and protein production, gene expression was quantified in both cell types exposed to this concentration. Figure 3.4A shows that for the AF, MMP-7, MMP-11 and TNF-α were all markedly increased relative to the control. For the NP, there was an increase in expression of the ADAMTS-4, ADAMTS-5, IL-6 and TNF-α. There were no changes in the expression of the anabolic genes, aggrecan and collagen, and the TIMPs.

3.4.2 Leptin within a Pro-Inflammatory Environment

Given the up-regulation of the pro-inflammatory cytokines, it was hypothesised that leptin and these cytokines may act synergistically thus, potentiating further degradation within an already degenerate environment. Three cytokines, TNF-α, IL-1β or IL-6, were individually added to the culture system described above along with leptin at 25µg/ml.

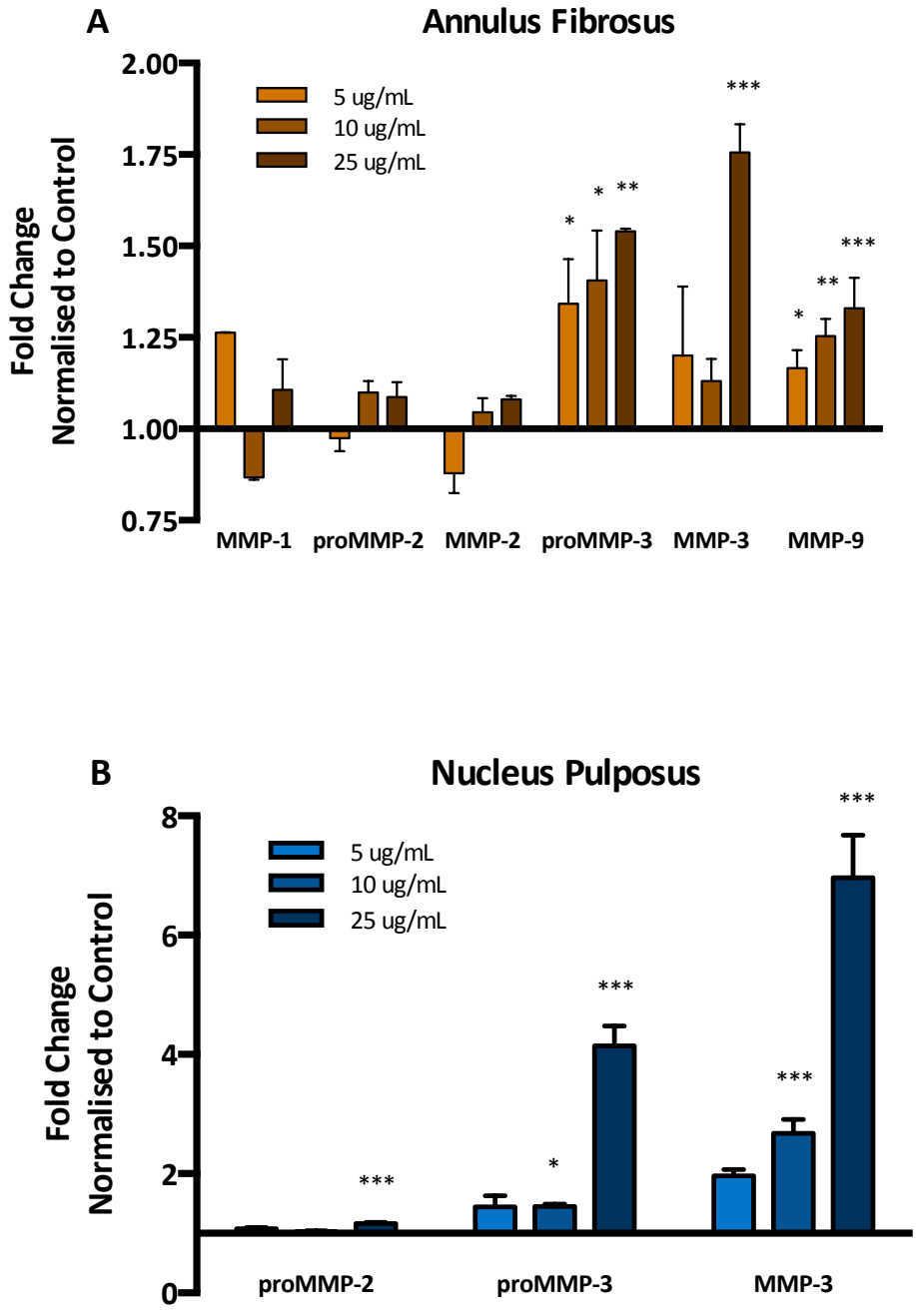


Figure 3.3: ***Matix metalloproteinase production by annulus fibrosus and nucleus pulposus cells****. MMP-2 and -9 measured by gelatin zymography and MMP-1 and-3 quantified by western blotting. At three concentrations of leptin (5, 10 and 25µg/ml). Intensity normalised and represented as fold change relative to control. MMP-13 was not detected from either NP or AF cells, MMP-1 and -9 not detected from NP cells. Data represented as mean ± SEM. *p<0.05 **<p<0.01 ***p<0.001*

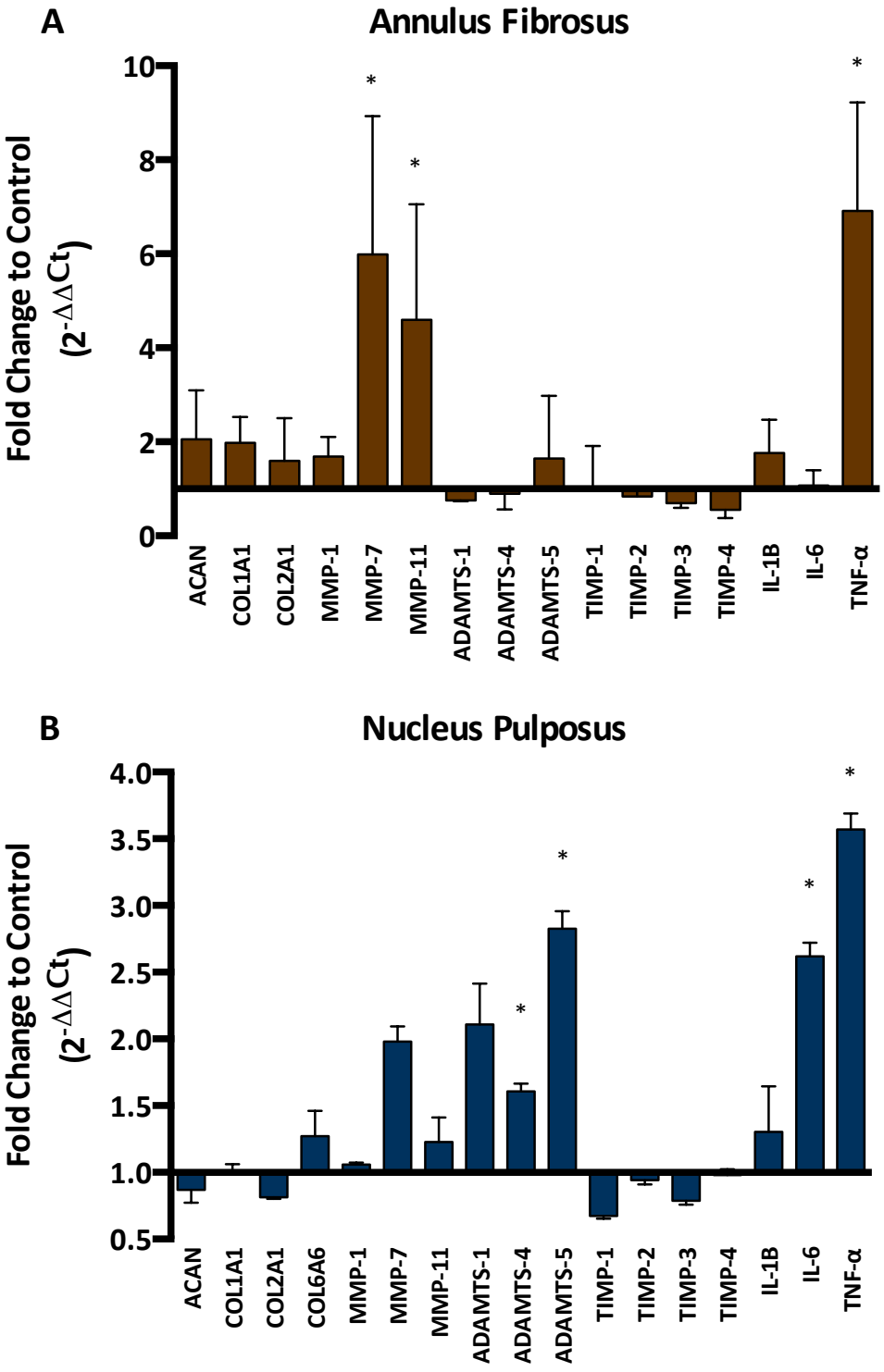


Figure 3.4: ***Gene expression by both annulus fibrosus and nucleus pulposus cells in response to leptin.*** *Normalised to ACTB and EIF2B2 and shown as fold change ($2^{-\Delta\Delta Ct}$) to control. Data represented as mean ± SEM of three independent experiments. *p<0.05*

Lactate Production Rates Figure 3.5A and B shows the combined effect of leptin and TNF-α. For the AF, there was a trend for increased energy requirement as TNF-α concentration increased with a small synergistic effect of leptin. Conversely, in the NP, TNF-α was the primary driver of lactate production with no effect of leptin evident. The combination of leptin and IL-1β had very little effect on lactate production in either cell type (Figure 3.5C and D) with the difference to the control driven mostly by IL-1β. Lactate production rates with IL-6 were increased by the addition of leptin at lower concentrations (0.1 and 1.0ηg/ml) in the AF.

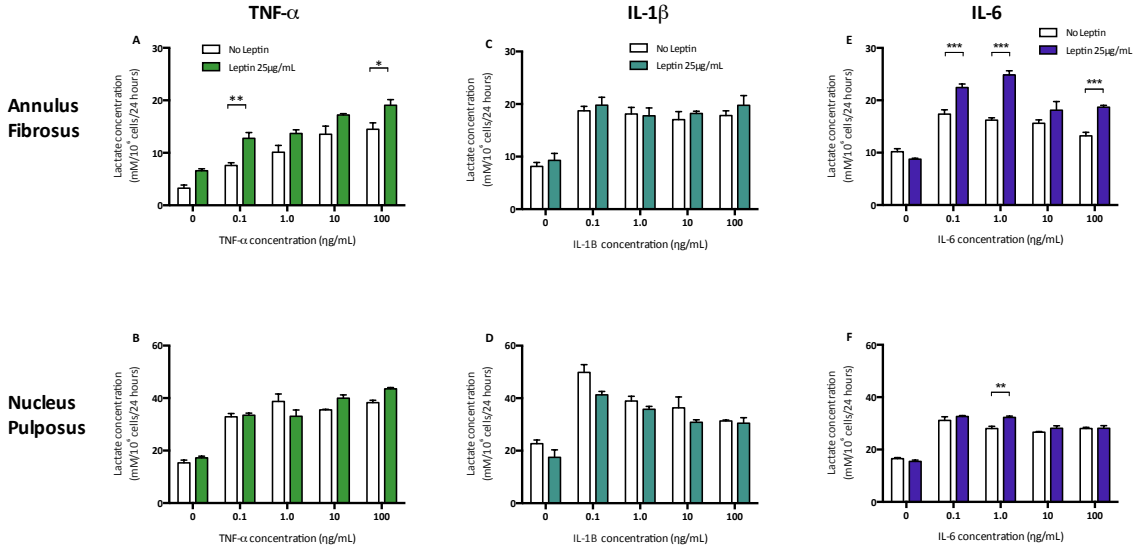


Figure 3.5: ***Lactate production by annulus fibrosus and nucleus pulposus cells in response to leptin and pro-inflammatory cytokines****. Four concentrations (0.1, 1.0, 10 and 100ηg/ml) of either TNF-α, IL-1ß or IL-6 with or without 25μg/ml of leptin. Data represented as mean ± SEM of three independent experiments. *p<0.05 **<p<0.01 ***p<0.001*

Nitric Oxide Production Rates Leptin alone significantly increased nitric oxide (NO) production rates in NP cells (Figure 3.6). There was a synergistic increase with both TNF-α and IL-6 which was more marked at the lower concentrations of TNF-α and at higher concentrations of IL-6. Similar to the results seen with lactate, IL-1β was the primary driver of NO production with most of the effect seen at lower concentrations. NO was not detected in AF cells.

MMP Production Figure 3.7A and B shows the total MMP production, as measured by fluorescent assay by both cell types in response to leptin alone, pro-inflammatory cytokines (0.1 or 100ηg/ml)

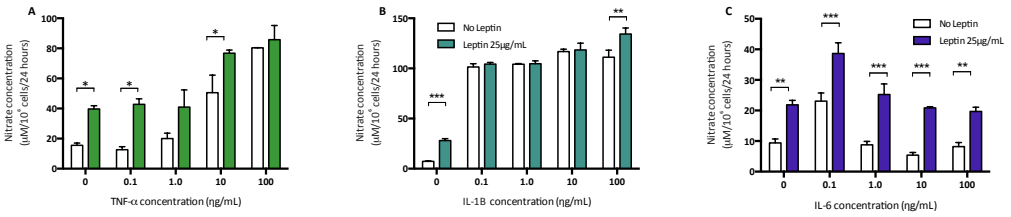


Figure 3.6: ***Nitrate production of nucleus pulposus cells in response to leptin and pro-inflammatory cytokines.***. *Four concentrations (0.1, 1.0, 10 and 100ηg/ml) of either TNF-α, IL-1β or IL-6 with or without 25μg/ml of leptin. Nitrate production was only detected by annulus fibrosus cells at a concentration of 100ηg/ml of TNF-α (not shown). Data represented as mean ± SEM of three independent experiments. *p<0.05 **<p<0.01 ***p<0.001*

alone or the combination of the two. For both the AF and NP, leptin alone increased the production of total MMPs significantly. Although the fold changes are larger than that seen in Figure 3.3, the assay used here is sensitive to a spectrum of MMPs and prior to quantification, pro-MMPs were activated.

For TNF-α and IL-1β, the majority of MMP production was driven by the pro-inflammatory cytokines and only marginal synergy with leptin was evident. IL-6 showed synergism with leptin in the NP with total MMP production increasing by 25-30% compared to leptin addition, at both IL-6 concentrations.

RT-qPCR As the greatest synergistic effect was seen with leptin and 0.1ηg/ml IL-6, RNA was isolated from cells exposed to this combination of factors and gene expression assayed by RT-qPCR. The only important differences in AF gene expression was with IL-6. In isolation, exposure to IL-6 led to downregualtion of IL-6 gene expression but with the addition of leptin there was a small increase in expression. There was a similar trend in the NP with a two fold increase in IL-6 expression after leptin addition (Figure 3.8B).

Furthermore, in the NP, there was a significant synergistic up-regulation in the expression of TNF-α, IL-6, and ADAMTS-4, by leptin. No change was seen with MMP-2 and -9 and there was a slight non-significant decrease in the expression of TIMP-3.

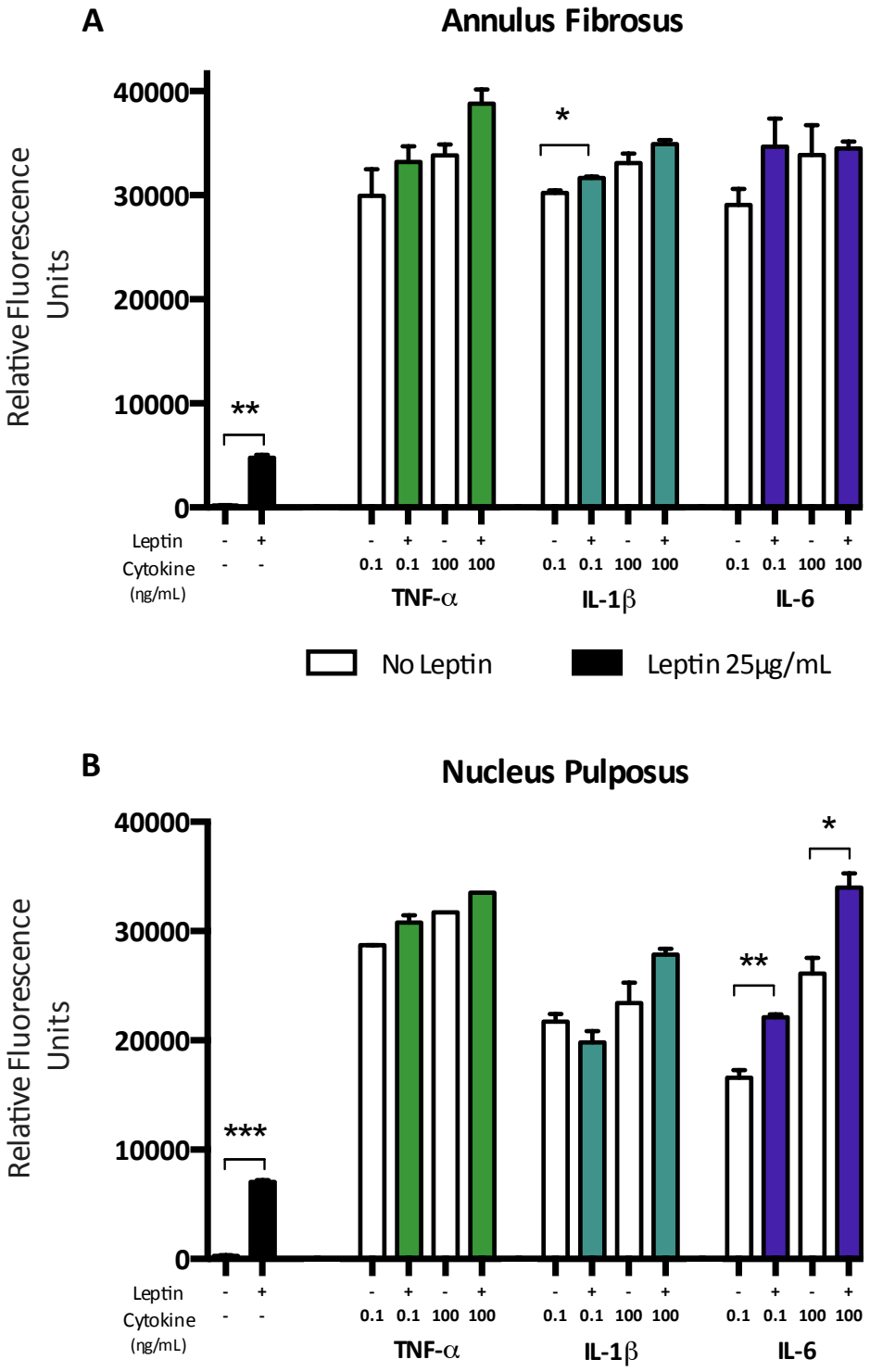


Figure 3.7: ***Total MMP production by annulus fibrosus and nucleus pulposus cells in response to leptin and pro-inflammatory cytokines.*** *Two concentrations (0.1 and 100ηg/ml) of either TNF-α, IL-1β or IL-6 with or without 25µg/ml of leptin. Assay detects the cumulative activity of MMP-1, 2, 3, 7, 8, 9, 12, 13, and 14. Data represented as mean ± SEM of two independent experiments. *p<0.05 **<p<0.01 ***p<0.001*

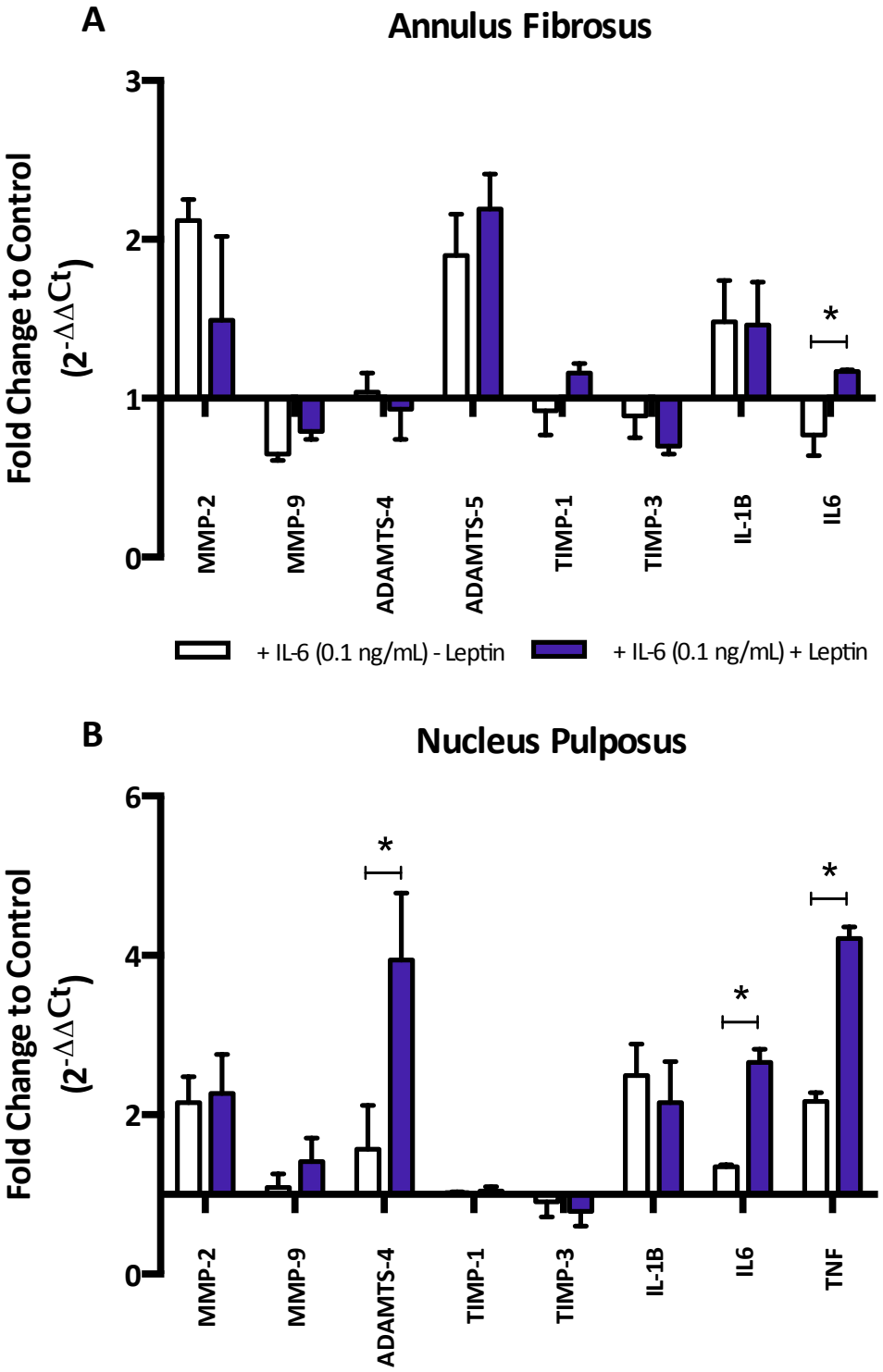


Figure 3.8: ***Gene expression changes in both annulus fibrosus and nucleus pulposus cells in response to leptin and 0.1ηg/ml of IL-6****. Normalised to ACTB and shown as fold change ($2^{-\Delta\Delta Ct}$) to control. Data represented as mean ± SEM of three independent experiments. *p<0.05*

3.5 Discussion

Leptin is the prototypical adipokine; a group of cytokines primarily but not exclusively produced by adipocytes.[87] Initially discovered in 1994 from adipose tissue, leptin is now known to be produced by multiple tissues including cartilage and intervertebral disc.[105,119,120] There is a growing body of both biological and clinical evidence implicating leptin in the pathogenesis of musculoskeletal degeneration.[87,131]

The results presented above suggest that leptin alone is able to induce a degenerative and inflammatory cascade within the IVD (Figures 3.3 and 3.4) by upregulating production of some but not all MMPs, nitric oxide and also by increasing expression of inflammatory cytokines. Furthermore, in a simulated pro-inflammatory environment, particularly in the presence of IL-6 and TNF-α, the effects of leptin are magnified (Figures 3.6, 3.7, and 3.8) with possibly deleterious consequences.

There are few studies looking at the effects of adipokines upon the intervertebral disc. The presence of leptin and its receptor in NP cells was first described by Zhao.[119] Using human herniated disc samples, the authors showed that leptin was associated with cell clusters and proliferating fibrocartilaginous areas, common markers of degeneration.[174] This was supported by finding that leptin stimulated the proliferation of rat NP cells *in vitro*; given that disc cell proliferation leads to cell cluster formation, leptin could be involved in formation of the clusters seen in degenerate discs. Clinically, Zhao *et al.* also showed a moderate correlation between increase in age and a larger proportion of both leptin and leptin-receptor positive cells.[119]

Using similar tissue samples, Li *et al.*[175] confirmed these findings and showed this process was stimulated by the upregulation of cyclins, important controllers of cell-cycle progression which are also involved in TGF-β induced NP mitogenesis.[176] Leptin has been shown to be pathologically mitogenic in other tissues such as vascular smooth muscle,[177] an important contributor to coronary artery disease as well as in multiple tumours including colorectal, endometrial, breast and prostate.[178]

AF cells also express the leptin receptor and produce leptin.[120,179] Gruber *et al.* showed that leptin was produced in discs throughout the spectrum of degeneration, viz. from healthy discs to

those severely degenerated.[120] These leptin-immunopositive cells were more common in normal compared to degenerated discs but similar quantities of leptin was produced by all discs. This discrepancy is possibly due to age-related changes which are present in the stained tissue specimens in which leptin-immunopositive cells were identified. Leptin production was measured in cells that were expanded in culture, possibly there losing any differences in phenotype. Gruber *et al.* also found leptin localised adjacent to AF cells and was contained by the pericellular encapsulating matrix. This capsule has previously been shown to be primarily collagen-6, which is upregulated by leptin (Figure 3.4).[180] However, firm conclusions from these experiments are limited by the small sample size examined.

Leptin has also been shown to have effects on disc matrix. Aggrecan is the major proteoglycan of the disc matrix and critical for maintaining disc morphology and hydration.[15] It is degraded primarily by ADAMTS, specifically ADAMTS-4 and -5.[35,67] The disc matrix is dynamic and some degradation is seen as part of normal remodelling. However, as shown by Li *et al.*[181] and this study (Figure 3.2 and Figure 3.4), leptin can tip the balance towards a greater degradation by downregulating aggrecan while increasing the expression of important ADAMTS.[59]

Collagen, specifically collagen-1 and -2, is the other important macromolecule of the intervertebral disc with breakdown controlled mostly by MMPs.[63–65] To date, no authors have investigated the role of leptin upon collagen matrix turnover in the disc. This study showed that MMPs, particularly the collagenases (MMP-1), gelatinases (MMP-2 and -9) and stromelysin (MMP-3) are upregulated by leptin at the protein and gene level (Figure 3.4). *In vivo*, these proteases have the potential to degrade the disc matrix and lead to degeneration. These findings are similar to the effect that leptin has upon chondrocytes where various authors have shown an increase production of MMP-1,[126,131] -3,[126] -9[127] and -13.[126,127,131]

Pro-inflammatory cytokines are also important mediators of disc degeneration. IL-1β, -6 and TNF-α are all produced by the IVD, increased in degeneration and in isolation increase the production of degradative mediators shifting the disc to a more catabolic phenotype.[16] This study has shown that in the presence of leptin, both the AF and NP express greater levels of IL-6 and TNF-α but not IL-1β. This in itself could be devastating for the disc. When cultured together, the potentiating effect of leptin has downstream consequences with increased production of lactate, NO

and MMPs. Vuolteenaho *et al.* described a similar phenomenon in osteoarthritic cartilage with leptin increasing the production of IL-6, -8 and NO.[125] Furthermore, increased leptin production has been localised to parts of the IVD which exhibit increased IL-6 and TNF-α levels, suggesting a degenerative link.[179]

Given the relative avascular nature of the IVD, the cells are specialised at anaerobic metabolism, converting glucose to ATP with lactate the primary by-product. Lactate is produced as lactic acid, an acidic molecule and with pH one of the primary regulators of disc cell activity, increases in lactate marks an increase in acidity with potentially deleterious consequences upon these cells.[50] Glycolysis falls with increase in acidity, resulting in lower ATP production. Furthermore, lower disc pH leads to reduced matrix synthesis,[51,52] fall in production of TIMPs[52] and ultimately cell death.[54] Lactic acid also activates acid sensing ion channels which are implicated in the pathogenesis of pain.[53] Pain fibre activation is most important for the outer AF which is the only innervated region in normal discs. In isolation, leptin had no significant effect on lactate production, however, in combination with the pro-inflammatory mediators lactate was greatly increased.

Nitric oxide, as quantified by nitrate and nitrite, has also been implicated in spine-related pain,[182] disc herniation,[21] and reduced disc matrix synthesis.[183] This study showed that leptin alone and in combination with inflammatory mediators resulted in greater rates of NO production than in controls. Within the IVD, previous authors have shown that pro-inflammatory cytokines are critical to the regulation of inducible nitric oxide synthase (iNOS), the main pathway for NO synthesis in disc degeneration.[16,184] Leptin could increase NO production by direct upregulation of iNOS, as it does in chondrocytes,[185,186] or indirectly via pro-inflammatory mechanisms.

To the disc, the most devastating synergy would be between leptin and IL-6. A combination of leptin and this cytokine increased cellular metabolism, NO, MMPs, and IL-6 itself in disc cells (Figures 3.7, 3.5, 3.6, and 3.8). IL-6 is a cytokine which has both pro- and anti-inflammatory actions.[187] Within the disc, IL-6 has been associated with pain and degeneration.[16] Both IL-6 and leptin signal through a class I cytokine receptor and certain authors believe that it is through this homology that leptin mediates its cytokine like effects.[16,188] These results could be seen indirectly to support such a hypothesis especially given the effects of leptin and IL-6 upon MMP production (Figure 3.7). However, trying to dissect the exact signalling pathways of leptin is complex with mul-

tiple pathways implicated including mitogen-activated protein kinases,[125,175,181,189] protein kinase B,[175,190,191] protein kinase C,[125] nuclear factor kappa B,[125,191] JAK-STAT,[175,189] cyclins,[175] NO,[175] and phosphoinositide 3-kinase.[190]

Additionally, leptin and IL-6 have been co-localised within degenerated IVDs.[179] Leptin can also upregulate IL-6 directly via the IL-6 specific receptor as has been shown in synovial fibroblasts,[190] B-cells,[189] and microglia.[191] On the other hand, IL-6 can control leptin release centrally.[192] Clinically, both leptin and IL-6 have been implicated in hip osteoarthritis, and the effect of leptin on OA is modulated by IL-6.[193]

An important consideration of an *in vitro* study is the extent to which physiological conditions are reproduced. In this study, three concentrations of leptin (5, 10 and 25µg/ml) were investigated with the greatest effect seen at 25µg/ml. Given the relative paucity of data regarding the IVD and leptin, these concentrations were drawn from the cartilage literature.[125,131] Although, circulating leptin is found at higher levels in obesity and in women, it is usually in the range of 10 to 50ŋg/ml, an order of magnitude less than used experimentally.[87,122,128,194–196] Importantly, Hui *et al.* showed that despite using a higher concentration of leptin (25µg/ml) comparable levels of MMP production were obtained from using pathological intra-articular fat conditioned media, suggesting that cartilage may be exposed to local leptin concentrations at much greater levels than those found in serum. Indeed, other authors have shown that synovial leptin, produced mostly by local fat, is greater than serum leptin.[128] This topic, with respect to spinal adipose tissue is explored in chapter 4.

IVD cells, like chondrocytes, can produce leptin[119,120,175] and thus could increase local leptin concentrations. It is hypothesised that this local leptin could act in a paracrine or autocrine fashion exerting actions upon nearby cells. Supporting this, Gruber *et al.* showed leptin to be encapsulated in a matrix surrounding AF cells,[120] possibly explaining why higher concentrations of leptin are required to stimulate effects in IVD cells. Zhao *et al.* also found that NP cells from older discs produced greater concentrations of leptin,[119] which is in keeping with the clinical findings shown in chapter 8. Unfortunately, neither author described the relative concentration of leptin produced by or found within the disc.

Others could argue that leptin has no influence on IVD cells *in vivo* and only when exposed to

supra-physiological concentrations is an effect seen. There are two counters to this argument. Firstly, as explained above, local leptin concentrations are likely to be greater than those seen in serum; whether the concentrations used in this and other studies are super-physiological is not known at present. Secondly, Li *et al.* have shown that human NP cells can respond to recombinant human leptin at a concentration of 10ηg/ml.[175,181,197] Recombinant bovine leptin was used in this study and inter-species differences could explain the discrepancy in concentrations required to produce an effect in this bovine model. Another explanation is that bovine model used is young healthy cells whereas the cells used by Li are from degenerate discs. It is possible that degenerate cells are more sensitive to leptin and thus adipokines are more deleterious in this situation.

Clinically, multiple authors have shown that leptin and other adipokines are mediators of musculoskeletal degeneration. Most recently, Fowler-Brian *et al.* found half of the effect of increased BMI upon knee OA could be explained by leptin, a strong indictment for deleterious biochemical effects of obesity.[198] This effect was greatest in women, in keeping with a study by Karvonen-Gutierrez *et al.* which showed a similar independent association.[199] Greater serum and synovial leptin has also been correlated with greater severity of knee and hip OA.[193,200,201] Other adipokines such as adiponectin,[202,203] resistin,[204] and visfatin[205] have all been associated with OA. Unfortunately, all but one of these studies are cross sectional and causation cannot be established. The working hypothesis is that obesity leads to greater leptin levels which leads to degeneration. However, it is possible that existing degeneration could lead to decreased mobility, subsequent obesity and thus greater leptin levels. The latter situation would lead to an erroneous association.

There are no large population studies investigating the relationship between leptin and back pain or disc degeneration. However, in smaller studies Urquhart *et al.* found greater fat mass was associated with increased back pain and reduced disc height and hypothesised biochemical factors as a mediator.[79,133] In chapter 4, the relationship between plasma and local leptin and back pain is investigated. A follow-up of the Genodisc population, as described in chapter 5 or using the UK Biobank population would provide large scale evidence for a relationship between adipokines and back pain or disc degeneration. The ideal study design would be a longitudinal patient cohort to confirm the potential causation shown in this study.

3.6 Conclusion

Taken together, these data show that both AF and NP cells can respond to leptin with increased production and expression of degradative and pain generating molecules. Crucially, leptin can initiate degenerative processes and within an inflammatory environment it can potentiate degeneration. This supports a causative link between increased leptin and degeneration. Further confirmatory clinical studies are required.

Key Points

- Leptin, the prototypical adipokine, can initiate and potentiate the production of molecules involved in intervertebral disc degeneration and back pain.
- The negative effects of a pro-inflammatory environment, especially IL-6, are potentiated by leptin.
- These results demonstrate a possible biochemical link between obesity and disc degeneration.

4

Paraspinal Fat, Plasma Cytokines and Back Pain: An *Ex Vivo* Study

Chapter 3 showed that adipokines can have deleterious effects upon the IVD in an *in vitro* model. Chapter 4 aims to validate these findings clinically by investigating the paraspinal fat and plasma as local and distant sources of adipokines. However, no correlation was found between symptoms and adipokine concentrations. Patient heterogeneity could explain the varied results. Plasma proteins, that had not been previously noted in spine patients were also identified using mass spectrometry. These may have a role in spine disease and could be mediators of degeneration and further confirmatory studies are required.

4.1 Introduction

Local and distant adipose tissue can act as sources of adipokines which can lead to degeneration and pain in spine patients. In OA, leptin from both the infrapatellar fat pad (IFP)[206,207] and distant sites have been shown to induce degeneration.[198] A similar situation could exist in the spine. Here, paraspinal fat is the main source of local adipokines and while global adipose tissue and thus obesity, is the primary driver of plasma leptin levels.

The diagnosis of LBP is unsatisfactory and conditions driving it are complex.[34] Biomarkers and novel plasma proteins provide a promising investigative avenue to identify potential mediators of spine related disorders and stratification into distinct phenotypes.[208] In an attempt to find markers for these patients, mass spectrometry was used to identify plasma proteins which may mediate spine related symptoms and degeneration.

4.2 Hypothesis

The first two aspects of this study were hypothesis driven and stated that:

1. Paraspinal fat is a local source of adipokines and pro-inflammatory cytokines, with levels correlating to patient reported symptoms.
2. Plasma adipokines and pro-inflammatory cytokines are elevated in patients when compared to controls.

The final aspect of the study was an exploratory analysis of plasma, performed to generate hypotheses and guide further investigation.

4.3 Methods

4.3.1 Participant Recruitment

Figure 4.1 is a schematic summarising participant recruitment and sample analysis.

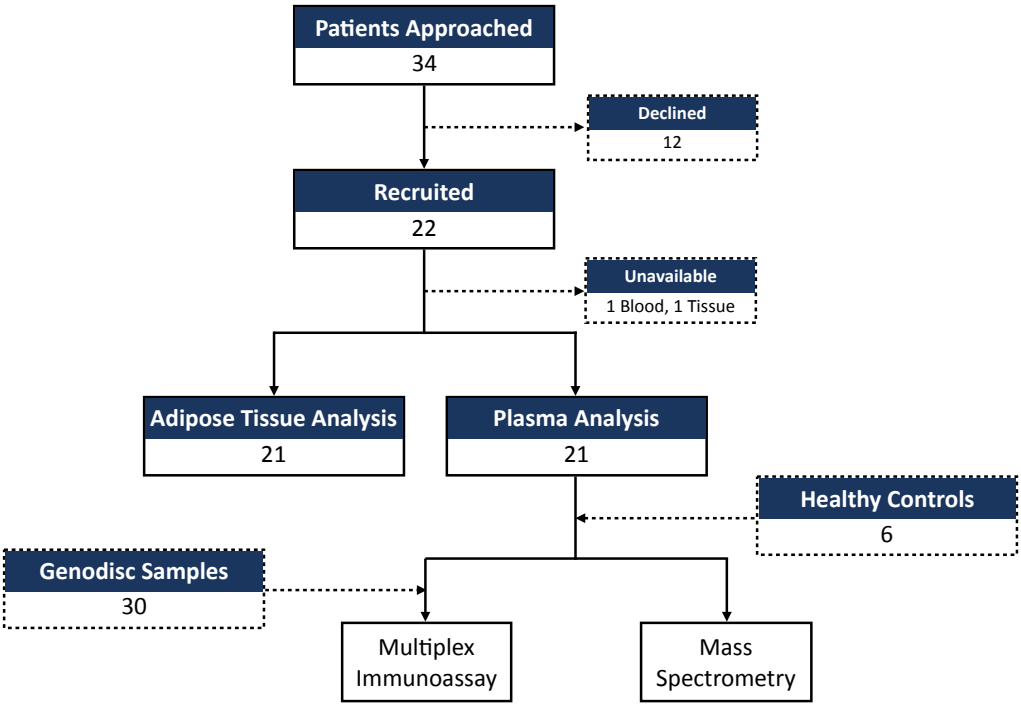


Figure 4.1: ***Flow diagram showing the patient recruitment process and patient samples for analysis.***

Participants were recruited from three areas.

Oxford Patients undergoing spine surgery at the Nuffield Orthopaedic Centre were invited to participate. Over a one year period, a total of 34 patients were approached with 22 recruited. After informed consent, patients completed the clinical assessment described below with blood collected at anaesthetic induction. Adipose tissue, adjacent to the paraspinal musculature, was isolated shortly after the the commencement of surgery thus minimising mechanical trauma. Blood and adipose tissue was processed and analysed as described in chapter 2.

Oswestry A subgroup of 30 patients were selected from the Oswestry Genodisc group (chapter 5). This group was chosen to match the diagnoses of those recruited from Oxford. As this was a post-hoc selection, only previously gathered data and plasma was available. Unfortunately, patients had not completed the Oswestry Disability Index (ODI) and paraspinal adipose tissue was not available.

Controls Healthy participants with no history of spinal problems completed the an assessment as described below and plasma was collected for analysis.

4.3.2 Clinical Assessment

At the time of recruitment, Oxford and control participants, were assessed clinically according to a short questionnaire detailed in Appendix D. Baseline demographics including age, gender, smoking history, and occupation were collected. Medical co-morbidities and current medications were obtained from direct questioning or the clinical records.

Anthropometric measurements included height (cm), weight (kg), and waist circumference (cm). Waist circumference was measured at the level of the iliac crest as recommended by the American Heart Association.[209] Body mass index (BMI) was calculated using the standard formula of weight, in kg, divided by height, in m, squared. Disease specific questions included the specific diagnosis, duration of symptoms, and previous surgery.

4.3.3 Oswestry Disability Index

The ODI is a patient reported spine specific questionnaire. Developed by Fairbank *et al.* in 1980, the ODI is one of the most widely used and validated questionnaires to assess spine related disability and functional restriction.[210,211]

It is a 10-item questionnaire where participants are asked to rate their functional limitation in everyday life. Each item has six options with normal function scored as 0, complete limitation as 5, and the intervening statements scored on rank. The maximum possible score is 100. The mean ODI in a normal population is 10; it is 27 in patients with idiopathic back pain and 48 in those with spinal metastases.[212] Permission for use of the ODI was granted by the Mapi Research Trust (academic agreement 1479) and ODI version 2.1a was used for all patients (Appendix E).

Patients and controls from Oxford completed this questionnaire. An alternative to the ODI is the Roland-Morris (RM) questionnaire. Both the ODI and RM questionnaire have similar sensitivity and are are widely used. In this study, the ODI was chosen given the experience our research group has with this tool.[213,214]

Visual Analogue Scale Patients from Oswestry completed the Genodisc questionnaire as described in chapter 5 and pain was scored on a visual analogue scale (VAS) from 0 to 10.

Correlation between ODI and VAS To analyse the Oxford and Oswestry data together, an attempt was made to categorise symptoms into mild, moderate and severe groups. The VAS and ODI have strong positive correlation which has been shown by multiple authors.[215–218] These scores were were stratified into mild, moderate or severe as described in Table 4.1.

Category	ODI Score	VAS Score
Mild	<40	<4
Moderate	40-60	4-7
Severe	>60	>7

Table 4.1: ***Categorisation of spine symptom scores***

4.3.4 Ethical Approval

Recruitment of patients was conducted through the Oxford Musculoskeletal Biobank which is part of Oxford BioResource. Ethical approval was granted under the Oxford REC C 09/H0606/11 and Human Tissue Act Licence 12217. Ethical approval for Genodisc and control participants was granted under UK REC 09/H0501/95 GENODISC Study.

4.3.5 Protein Depletion Method Selection

As described in chapter 2, two methods, high abundance depletion and low abundance enrichment, were trialed to ensure the optimal method was used for MS. Figure 4.2 shows the large protein band, corresponding to albumin, is decreased after ProteoMiner low abundance enrichment and nearly absent after the Pierce high abundance immunodepletion. One plasma sample was passed through a workflow described in Figure 4.3. From this, it was clear that the high abundance immunodepletion followed by an in-solution digestion yielded the greatest number of protein hits. Thus this method was chosen to process the remaining samples.

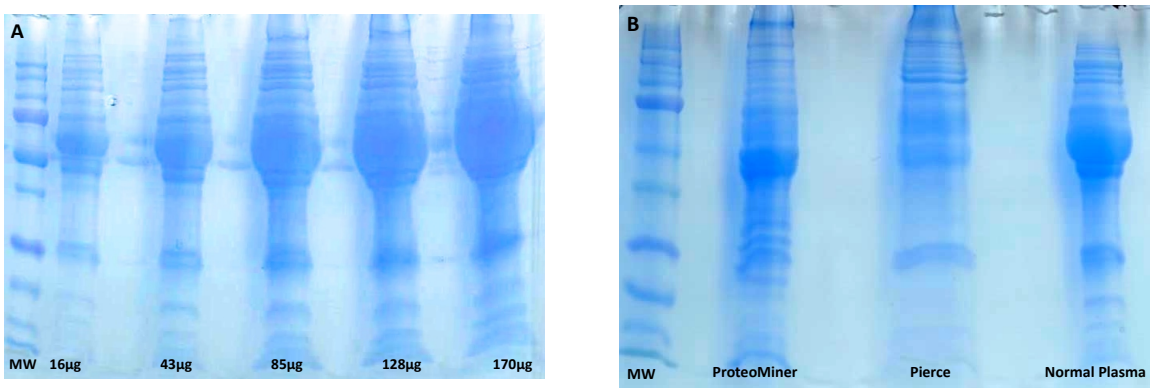


Figure 4.2: ***Photograph of gel showing plasma dilution and depletion.*** *A) Serial dilutions of whole plasma proteins. Note the largest band corresponds to the molecular weight of albumin. B) Two abundant plasma protein depletion techniques compared to whole plasma. Each lane was loaded with with 38.75µg of protein. Note the large band corresponding to albumin is not present in the ProteoMiner and Pierce lanes. MW is the molecular weight marker.*

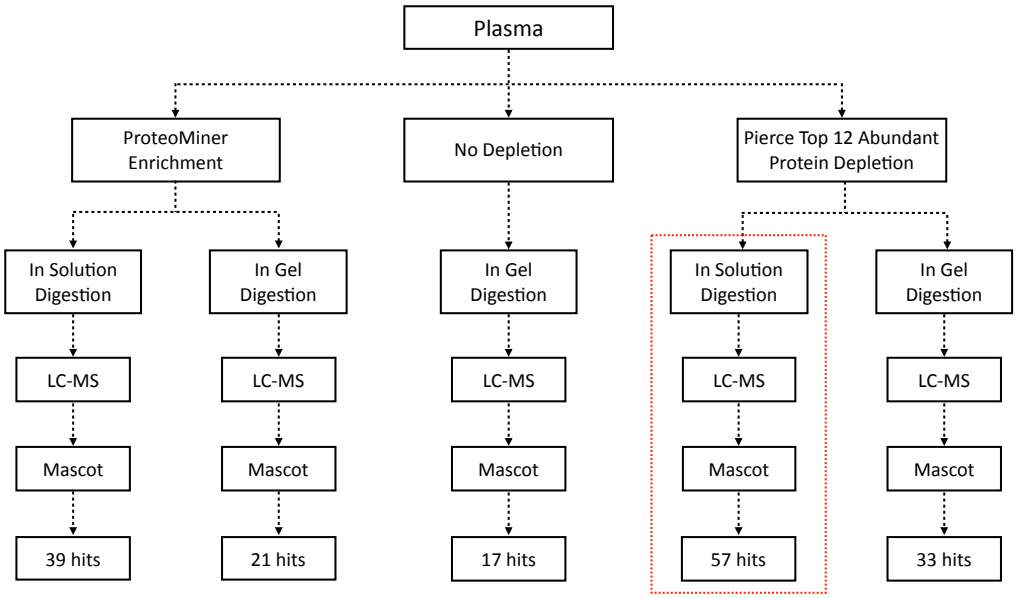


Figure 4.3: ***Flow diagram showing mass spectrometry method development protocol.*** *Red box represents optimal method.*

4.4 Results

4.4.1 Patient Demographics

Table 4.2 describes the patients and controls. In total, there were 20 recruited participants from Oxford, 30 selected participants from the Oswestry Genodisc patients, and six controls with no back pain. Mean age and BMI across all three groups was similar. Patients from the Oxford group were considerably disabled with a mean ODI of 50 in contrast to a mean score of 3 for controls.

	Oxford	Oswestry	Controls
Number	22	30	6
Age (years)	51.6 (14)	52.2 (14)	52.7 (16.9)
Women n (%)	8 (40%)	14 (47%)	4 (67%)
BMI (kg/m^2)	28.6 (5.6)	29.2 (6.5)	26.0 (6.5)
Waist Circumference (cm)	99 (14)	-	-
ODI	49.7 (13.5)	-	2.7 (6.5)
Back Pain Score	-	6.0 (3.1)	-
Duration of Symptoms (months)	13.5 (9)	22.9 (32.1)	-
Smoking Status			
Non-Smoker	9 (45%)	11 (37%)	5 (83%)
Previous Smoker	8 (40%)	8 (27%)	0
Current Smoker	3 (15%)	11 (37%)	1 (17%)
Work Type			
Sedentary	3 (15%)	2 (7%)	-
Light	4 (20%)	6 (20%)	6 (100%)
Medium	6 (30%)	10 (33%)	-
Heavy	7 (35%)	12 (40%)	-
Clinical Diagnosis			
Disc Herniation	14 (70%)	22 (73%)	-
Spine Stenosis	5 (25%)	9 (30%)	-
Spondylolisthesis	1 (5%)	3 (10%)	-
Non-specific Back Pain	0 (0%)	5 (17%)	-

Table 4.2: ***Patient Demographics*** *summarising Oxford, Oswestry and controls.*

4.4.2 Paraspinal Adipose Tissue Analysis

Table 4.3 describes the mean amount of cytokines (pg or ηg per gram of tissue) produced by paraspinal adipose tissue after 24 hours of culture in HAMS-F12 supplemented media. Leptin, TNF-α, IL-1β, -6, -8 and interferon-γ all showed showed a trend of decreasing concentration with increase in disability, however none reached statistical significance. No trend was seen for adiponectin, IL-2, -4, -10, -12 and -13.

	ODI<40	ODI 40-60	ODI >60
Number	8	7	5
Decreasing Trend			
Leptin	20.7 ± 32.0	12.7 ± 11.0	6.90 ± 5.80
TNF-α	4.51 ± 3.14	1.99 ± 2.00	1.02 ± 0.44
IL-1β	25.3 ± 47.6	3.77 ± 6.03	4.62 ± 9.95
IL-6	32.8 ± 43.5	3.56 ± 5.85	0.93 ± 1.09
IL-8 (ηg)	33.9 ± 56.9	33.3 ± 39.5	4.18 ± 3.66
Interferon-γ	6.28 ± 2.45	4.53 ± 2.76	4.07 ± 2.50
No Trend			
Adiponectin (ηg)	583 ± 313	678 ± 339	611 ± 402
IL-2	1.67 ± 0.35	0.97 ± 0.76	1.36 ± 0.94
IL-4	0.39 ± 0.65	0.09 ± 0.1	0.16 ± 0.10
IL-10	8.91 ± 13.5	10.0 ± 10.8	6.69 ± 12.9
IL-12	0.92 ± 0.62	0.75 ± 0.51	0.96 ± 0.19
IL-13	7.12 ± 7.82	6.45 ± 6.46	7.75 ± 7.54

Table 4.3: ***Cytokine production by paraspinal fat samples*** *All values are pg per gram of adipose tissue (unless otherwise stated) and is after 24 hours of culture in HAMS F12 supplemented media. Values are expressed as mean with standard deviation. Patient sample is the 20 patients from Oxford region only.*

Figure 4.4 shows leptin and adiponectin production in relation to disability/ODI. No trend line was significantly different from zero. There was an outlier with one patient displaying very high leptin production compared to the remainder. This was not a sampling error as other cytokine results from this patient were not outliers. Upon checking this patient's data, there was nothing to suggest a reason for this result.

In an attempt to ascertain if obesity had a role in cytokine production, patients were split into

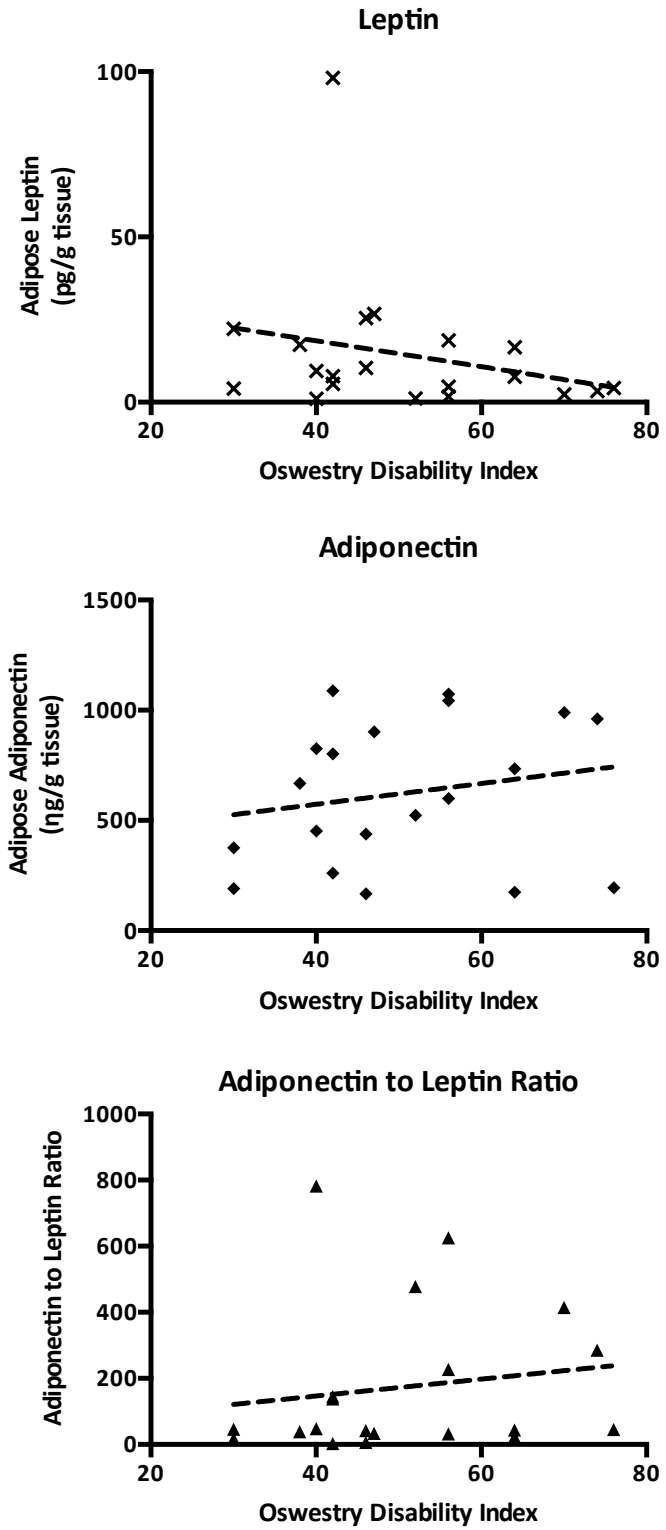


Figure 4.4: ***Paraspinal adipose tissue production of leptin and adiponectin as a function of Oswestry Disability Index.*** *Data derived from paraspinal fat of 20 patients in the Oxford group. Leptin quantified in pg per gram of tissue and adiponectin is ηg per gram of tissue after 24h hours of culture in HAMS-F12 supplemented media. Regression line was not statistically different from zero.*

two groups with a BMI cut off set at 28kg/m^2. Figure 4.5 illustrates the results for TNF-α and IL-8. No relationship for any cytokine was significant.

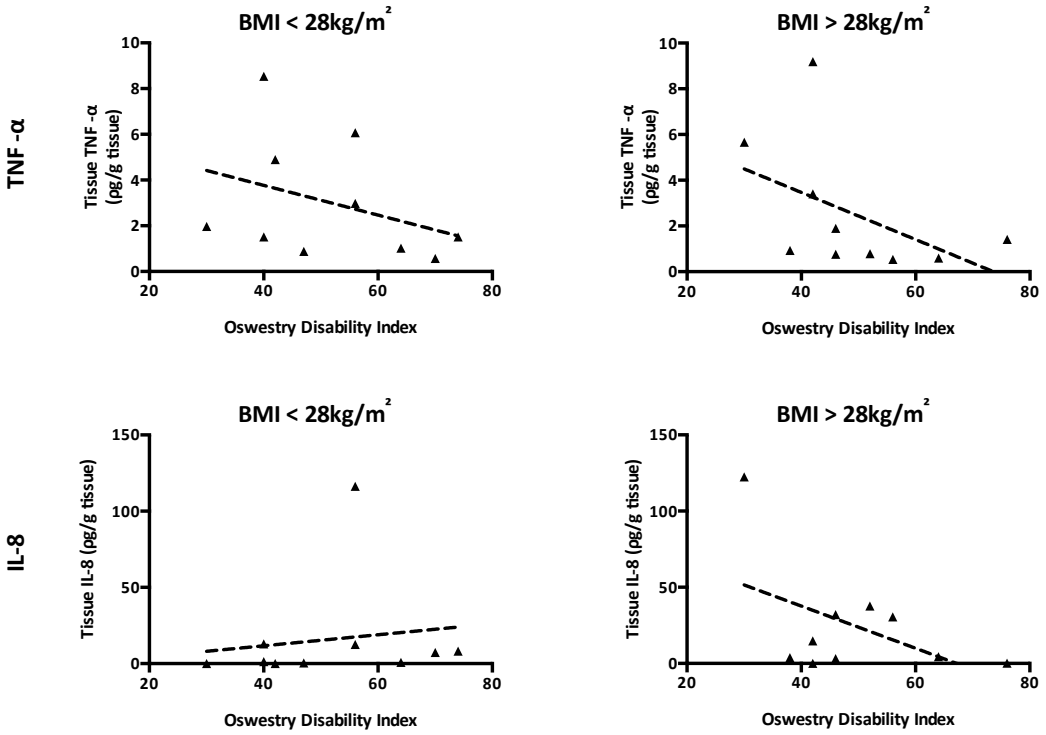


Figure 4.5: ***Paraspinal adipose tissue production of TNF-α and IL-8 by Oswestry Disability Index in low and high BMI categories.*** *Both TNF-α and IL-8 are quantified in pg per gram of tissue after 24 hours of culture in HAMS-F12 supplemented media. Regression line was not statistically different from zero.*

4.4.3 Plasma Cytokine Analysis

Table 4.4 describes the concentrations of various plasma cytokines in patients, both Oxford and Oswestry, compared to controls. Leptin, TNF-α, IL -6 and leptin showed a trend higher for higher concentrations in patients when compared to controls. These results are displayed graphically in Figure 4.6. TNF-α was statistically greater in all patients compared to controls. IL-6 showed a similar trend but this did not reach statistical significance. TNF-α and IL -6 were also raised in patients with LSS but not LDH (Figure 4.7). There was no correlation between adipose and plasma cytokine values.

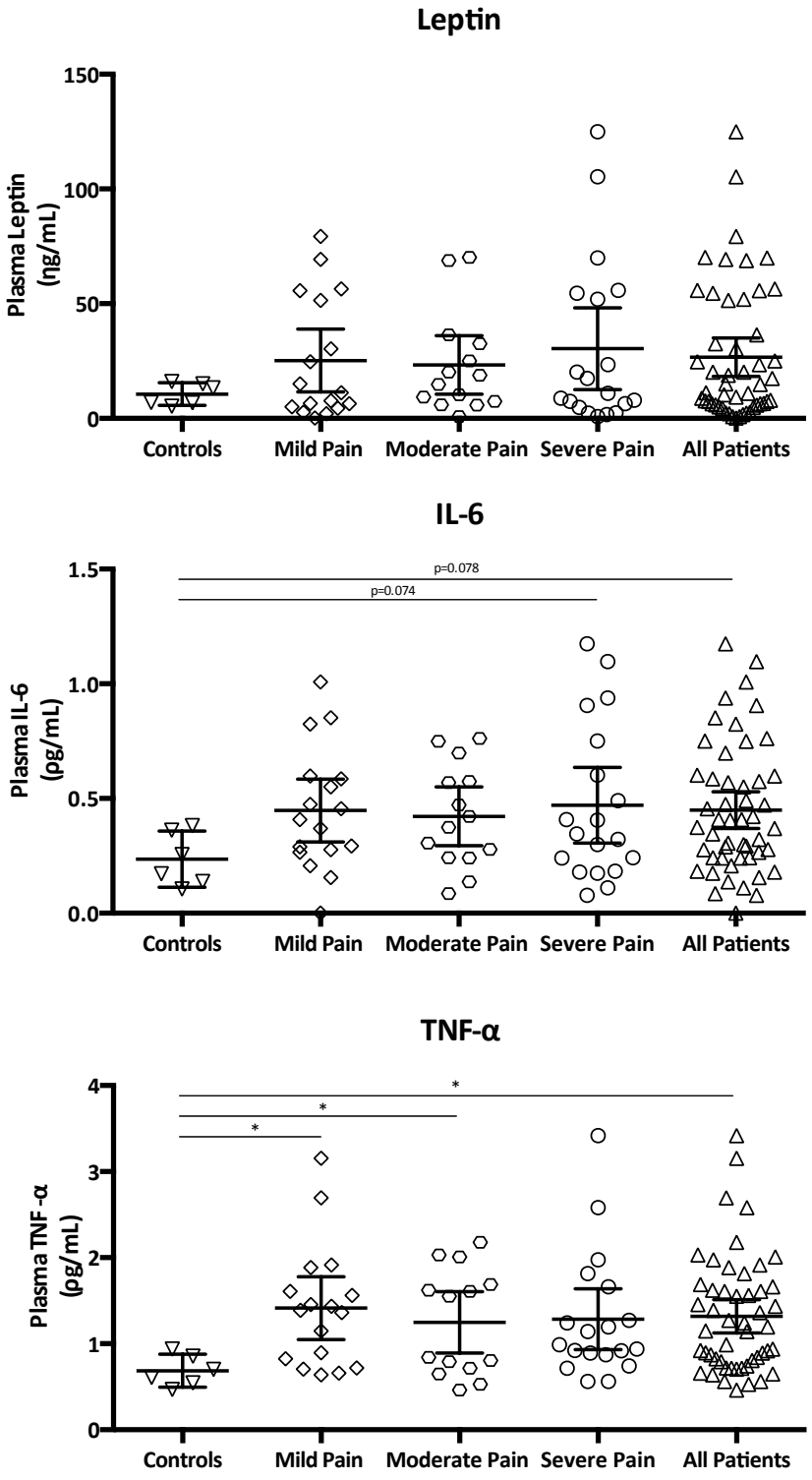


Figure 4.6: ***Plasma leptin, IL-6 and TNF-α by pain severity.*** *Leptin measured in ηg/ml, IL-6 and TNF-α quantified in pg/ml in patients from Oxford and Oswestry compared to controls.* p<0.05*

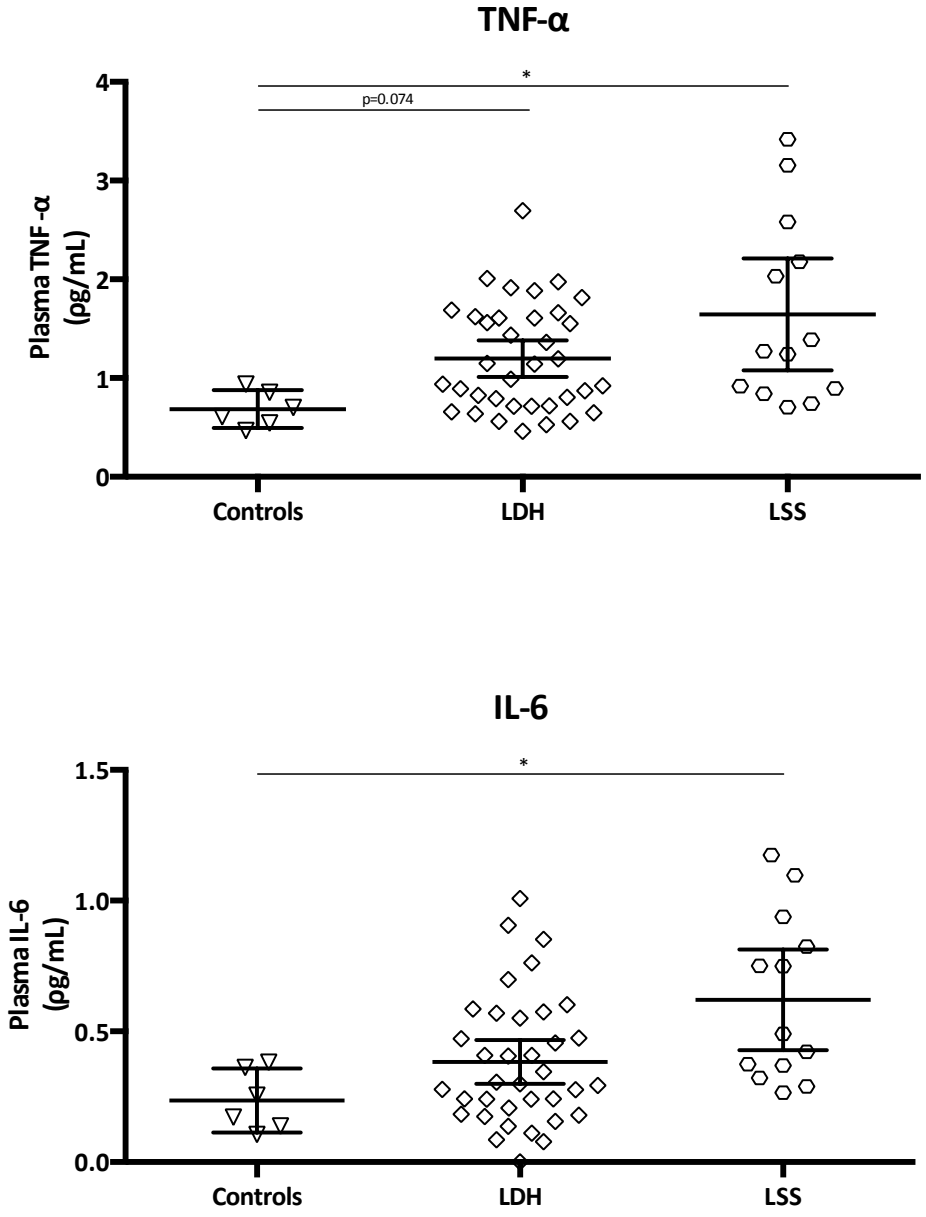


Figure 4.7: ***Plasma TNF-α and IL-6 concentrations within each diagnostic category.*** *Plasma TNF-α and IL-6 quantified in pg/ml in patients from Oxford and Oswestry compared to controls. LDH is lumbar disc herniation and LSS is lumbar spine stenosis. * p<0.05*

	Controls	Mild	Moderate	Severe	All
Number	6	17	14	19	50
			Controls Lower		
Leptin (ηg)	10.6 ± 4.8	25.2 ± 26.7	23.3 ± 22.1	30.4 ± 36.9	26.6 ± 29.5
Adiponectin (µg)	14.0 ± 10.0	20.8 ± 10.6	18.9 ± 11.2	16.9 ± 14.2	18.7 ± 12.1
TNF-α	0.69 ± 0.18	1.41 ± 0.71	1.25 ± 0.62	1.28 ± 0.73	1.32 ± 0.68
IL-6	0.24 ± 0.12	0.45 ± 0.27	0.42 ± 0.22	0.47 ± 0.34	0.45 ± 0.28
IL-8	1.90 ± 0.76	4.32 ± 4.37	4.75 ± 4.82	4.05 ± 4.22	4.33 ± 4.36
			No Trend		
Interferon-γ	3.27 ± 3.27	3.19 ± 4.22	3.2 ± 4.19	8.77 ± 25.1	5.89 ± 15.6
IL-2	0.00 ± 0	0.15 ± 0.25	0.00 ± 0.01	0.02 ± 0.03	0.20 ± 0.64
IL-10	0.08 ± 0.06	0.24 ± 0.30	0.16 ± 0.14	0.19 ± 0.15	0.20 ± 0.21
IL-12	0.10 ± 0.07	0.14 ± 0.11	0.16 ± 0.1	0.12 ± 0.06	0.14 ± 0.09
IL-13	0.22 ± 0.4	0.68 ± 0.68	0.36 ± 0.68	0.30 ± 0.56	0.45 ± 0.73

Table 4.4: ***Plasma cytokine concentrations*** *All values are per pg/ml unless otherwise stated and expressed as mean with standard deviation. Oxford and Oswestry patient samples combined and grouped by severity of symptoms. IL-1ϐ and IL-4 not detected.*

4.4.4 Plasma Mass Spectrometry Analysis

Figure 4.8 graphically shows the protein hits for patients and controls after LC-MS with each square representing unique protein (Uniprot ID). The light blue boxes represent proteins common to both groups which were excluded from further analysis.

In general, patients had a greater number of total hits compared to controls. Table 4.5 represents those proteins found commonly in patients but not in controls. Apolipoprotein B-100, clusterin, corticosteroid- binding globulin, complement-C9 and -C4b were all present in patients but not in controls.

To improve the power of analysis, mass spectra for controls, patients with LSS and LDH were pooled, into these three groups and reanalysed. Again, the complement proteins (C4b, C5, C6, C7, C9 and factor H) and clusterin were present in both patient groups but not controls.

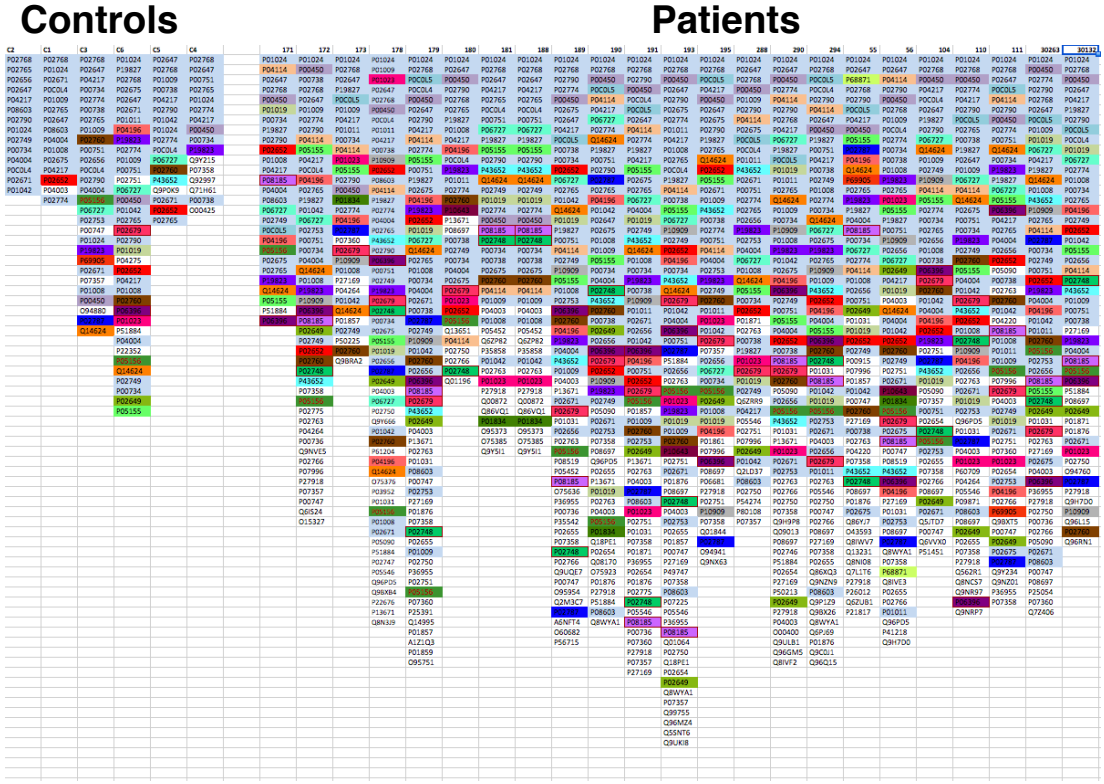


Figure 4.8: ***Schematic of different mass spectrometry protein hits.*** *Controls and patients seperated. Light blue represents highly scored proteins common to both groups. Other colours represent different proteins.*

Protein	Controls	Patients
Apolipoprotein B-100	0	23
Clusterin	0	16
Complement component C9	0	16
Complement C4-B	0	15
Corticosteroid-binding globulin	0	15
Plasma protease C1 inhibitor	1	22
Angiotensinogen	1	20
Histidine-rich glycoprotein	1	20
Afamin	1	19
Gelsolin	1	17
Fibrinogen gamma chain	1	17
Apolipoprotein E	1	16
Lumican	1	6
Inter-alpha-trypsin inhibitor heavy chain H4	2	21
Inter-alpha-trypsin inhibitor heavy chain H2	3	20

Table 4.5: ***Mass spectrometry protein hits*** *Table shows the number of controls and patients the protein was found in. Data only displayed for proteins which were less prevalent in controls.*

4.5 Discussion

Both systemic as well as local adipose tissue has been shown to be inflammatory.[219,220] Paraspinal fat was studied to assess if local adipokines and cytokines are present at levels to mimic the results seen in chapter 3. There is no literature into the role of paraspinal fat in spinal disability or degeneration. However, adipose tissue in healthy and obese patients has been studied extensively.[161,221–223] Due to the different analytic techniques, culture duration and normalisation method used it is difficult to directly compare the cytokine production but, the relative quantities of cytokines appear similar to the literature for obese subjects.[161]

In other areas of the body, local fat can be independently inflammatory in a situation analogous to the IFP in osteoarthritis[220] or pericardial fat in coronary atherogenesis.[224] Multiple authors have described the deleterious effects of the IFP, which takes on an inflammatory phenotype in OA.[206] Distel *et al.* showed that the IFP was a important source of IL-6 and it was produced in greater amounts than other fat. It is hypothesised that in this situation, the local fat is involved in paracrine

inflammation.[225] These findings were supported by Klein-Wieringa *et al.* and Ushiyama *et al.* who independently showed elevated TNF-α and IL-6 from the IFP.[206,207]

Although not directly correlated with plasma levels, synovial leptin is generally higher and synovial adiponectin lower than the plasma levels in normal patients.[128,226] Elevated synovial leptin has been associated with OA in general,[129] increased OA severity[129,200,227] and greater pain in OA.[228] Additionally, microarray analysis of the IFP showed a 10-fold increase in leptin gene expression in end stage OA.[229] Adiponectin is less studied but early results suggest it is also elevated in OA along with visfatin.[206] Ghandhi *et al.* suggested the adiponectin to leptin ratio was inversely correlated to pain.[230] However, this ratio was unremarkable in the results presented above (Figure 4.4).

Paraspinal fat is not the only source of local cytokines and adipokines. As the field of adipokines have developed there has been concurrent advancement in understanding myokines; cytokines produced by muscle. The most studied myokine, IL-6, has been described by some as an adipomyokine given its role in adipose-muscle cross talk.[231,232] Furthermore, in animal models of disc injury, the paraspinal muscles produce TNF-α and IL-1β.[233] Epidural fat, that is adipose tissue surround the spinal cord, is another local adipose source. However, this is a difficult tissue to source due to its close association with vertebral venous plexus. Genevay *et al.* were able to isolate periradicular fat, surrounding the nerve root, in patients with LDH. They found that this adipose tissue produced greater quantities of TNF-α, IL-1β, -6, and -8 compared to controls.[22] Finally, the disc itself can produce adipokines as discussed in chapter 3.

Adipose tissue consists primarily of adipoctyes, which is the cell type responsible for the majority of leptin production as well as a large number of other cytokines.[161] The other cellular components include pre-adipocytes, fibroblasts, endothelial cells and macrophages.[219] Adipose-localised macrophages have been shown to produce considerable quantities of TNF-α, IL-6, -8, iNOS, and inflammatory cell chemoattractants.[234,235] Furthermore, the numbers of macrophages, in the subcutaneous fat, have been positively correlated to obesity.[234,235] A similar trend has not been shown in the IFP.[206] In this study, the cellular constituents were not separated, thus cytokine production is a combination of both adipose tissue and macrophages.

This pilot study on the inflammatory nature of paraspinal fat is informative even though the

hypothesised relationship between the cytokine production from paraspinal adipose tissue and spinal disability was not evident. Possible relationships might have been masked by limitations in the experimental design. Firstly, the study consists of a small sample of heterogeneous patients. As a result, the findings were strongly influenced by outliers. Second, there were no controls because of ethical constraints. Ideally both an internal control, fat from the same patient sourced from a distant site and an external control, paraspinal fat from asymptomatic healthy volunteers are required. To compensate for this, the patients were categorised by ODI which reduced study power. Lastly, sampling difficulty meant paraspinal fat was substituted for other adipose deposits, epidural or retroperitoneal, which are in closer apposition to the disc. The differences, if any, between these deposits should be further investigated.

These limitations were less important during the plasma analysis given the larger sample size and availability of some controls. The most striking finding of these results was the relationship between plasma cytokines, spine related symptoms, and clinical diagnosis. Both TNF-α and IL-6 were higher in patients with LSS (Figure 4.7). Only TNF-α was elevated in those with greater disability although IL-6 and leptin showed a trend towards significance (Figure 4.6).

Wang *et al.* has described the relationship of TNF-α with back pain. In two separate cohorts, the authors showed back pain patients were more likely to be TNF-α positive and had significantly greater TNF-α levels than healthy controls.[236,237] Furthermore, Strumer *et al.* found that high sensitivity C reactive protein, a marker of subclinical systemic inflammation, was associated with greater pain in patients with acute LDH.[238] Furthermore, several authors have shown that systemic and local anti-TNF agents are useful in the treatment of the pain and disability associated with acute sciatica.[23–25,75] Importantly, it has been shown that TNF-α blockade depresses central pain pathways before any local articular anti-inflammatory effect.[26] These findings in combination to those shown above would point to a global inflammatory milieu in addition to local inflammation.

Plasma MS analysis revealed novel proteins, such as clusterin and complement and this could be a focus of further investigation. Clusterin, also known as apolipoprotein-J, is a ubiquitous protein involved in apoptosis and implicated in multiple diseases including neurodegeneration and OA.[239] Synovial, serum, and cartilage clusterin is raised in early OA[240,241] and within the disc, expression is increased in the painful degenerative AF.[242]

Complements are a group of proteins involved in innate immunity. C4b and C9, found in patients but not controls, are important proteases involved in the formation of the terminal membrane attack complex (MAC). When formed, the MAC inserts into the target cell wall thus leading to disruption and cellular death.[243] Complement dysregulation is present in OA cartilage and is also involved in the pathogenesis of this disease. Greater MACs lead to increased chondrocyte expression of MMPs, ADAMTSs, and inflammatory cytokines; cell lysis, and ultimately necrosis.[244] There are no studies investigating the role of complement and the IVD.

Apolipoprotein-B100, a lipid transporter, has shown to be increased by starvation.[245] In this study, the patients were starved preoperatively whereas the controls were not and this difference could explain why apolipoprotein-B100 was found more commonly in patients.

The plasma analysis is limited in two aspects. In grouping patients by severity, an assumption was made that the ODI correlates with visual analogue scale (VAS) and patients can be broadly grouped by symptoms. The literature suggest this assumption is correct but the cut-offs chosen may have misclassified patients. Furthermore, the current study only provides a snapshot of symptoms and cytokines at a single time point in the disease process. The best study design would be longitudinal patient cohort with analysis at multiple time points.

4.6 Conclusion

This study investigated both systemic and local sources of adipokines and cytokines. Results suggest that systemic adipokines could be mediators of pain and degeneration. Both TNF-α and IL-6 are associated with a spinal stenosis and more severe spine related disability. Although the adipose samples did not reveal any significant results, trends were apparent and further investigation is required. Finally, clusterin and complement are proteins targets which require more understanding in spinal patients.

Key Points

- Paraspinal fat was not related to disability or symptoms however a trend was apparent. A larger sample size or comparison to control sample may reveal important differences.

- TNF-α and IL-6 were raised in patients with spinal stenosis and severe disability. This has not been previously reported.

- Complement and clusterin are plasma proteins that may be implicated in back pain or spinal degeneration. Further investigation is required.

5

Genodisc Population and General Statistical Methods

In this chapter, the Genodisc study is introduced and the population described. The study design and process of data acquisition is explained along with a description of the statistical methods used in chapters 6, 7, and 8.

5.1 Introduction

The Genodisc Study was a pan-European observational study with the aims of developing a greater understanding of spine-related conditions and of defining specific clinical phenotypes.[34] Although I was not involved in the study design, patient recruitment or data collection these will be described for completeness. My involvement was with data cleaning, formulation of research questions, analysis and synthesis.

5.2 Genodisc Study Methods

5.2.1 Recruitment

All patients presenting to six tertiary spine centres in four countries were invited to participate. Over a period of five years (2008 to 2013), 2636 patients were enrolled into the study (Table 5.1). Data were captured in three broad areas:

- participant survey which included patient reported demographics, comorbidities and pain measures,
- clinician reported diagnoses, and
- quantitative and qualitative MRI findings.

Country	City	Spine Unit	Patients
United Kingdom	Oxford	Nuffield Orthopaedic Centre, Oxford University Hospitals NHS Trust	395
	Oswestry	The Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust	247
	Kettering	East Midland Spine, The Woodland Hospital	259
Hungary	Budapest	National Center for Spinal Disorders, Buda Health Center	1351
Italy	Milan	IRCCS Istituto Ortopedico Galeazzi	364
Slovenia	Ljubljaja	MD Medicina	20

Table 5.1: ***Genodisc recruitment centres***

5.2.2 Participant Survey

Along with general demographics, patients were asked to complete a short questionnaire which was organised in three domains.

Spine Related Pain

Information on both back and leg pain was obtained. Back pain (BP) was defined as pain the lower back. In an attempt to differentiate radicular leg pain from other causes of leg pain (LP), such as hip osteoarthritis or spinal stenosis, participants were specifically asked to score leg pain which went below their knee. Data on duration of pain, number of recent pain episodes and indicators of disability such as walking distance, absence from work and pain interfering with work were also obtained.

Medical, family and social history

Using yes-no questions, participants were questioned on their medical comorbidities and previous spine surgery. Specific medical conditions asked about were: OA, osteoporosis (OP), rheumatoid arthritis (RA), fibromyalgia, asthma, chronic obstructive pulmonary disease (COPD), emphysema, migraine, angina, high blood pressure, peripheral vascular disease, irritable bowel syndrome (IBS), upper gastrointestinal disease (ulcer, hernia, reflux), depression, anxiety or panic disorders, diabetes and cancer.

Other important questions included receipt of a disability payment, smoking history, occupation intensity and a family history of the same condition.

Zung Self Rating Depression Scale

As an assessment of mood, participants completed the Zung self-rating depression scale (Zung SDS). This is a 20-item questionnaire with each item scored on a four point Likert scale.[246] The result ranges between 20 to 80 and a score greater than 50 is considered depression.[246] The Zung SDS has been found to be an appropriate measure of depression in BP patients.[247]

5.2.3 Clinical Data

Using all clinically available information (including magnetic resonance imaging (MRI) data), the treating surgeon completed a diagnostic form categorising participants into either LDH, LSS, DS, scoliosis or NSBP.

5.2.4 MRI Acquisition and Scoring

MRIs were obtained as part a participant's normal clinical care and the imaging protocol followed standard practice at the recruitment site. All MRIs consisted of sagittal and axial images with both T1- and T2-weighting. These were anonymised and read by a single experienced musculoskeletal radiologist. Six spinal levels from T12-L1 to L5-S1 were assessed systematically with attention to the intervertebral disc, vertebral end-plates (upper and lower), vertebral canal, facet joints and nerve roots with associated foramina. Specific details on MRI scoring are discussed in chapter 8.

5.2.5 Ethical Approval

The Genodisc Study was conducted in compliance with the Helsinki Declaration. The study was approved by local Research Ethics Committees in each recruiting country; ethical approval number in the UK was UK REC 09/H0501/95 GENODISC Study.

5.3 General Statistical Methods

All statistical analysis was conducted using STATA 13 (Stata Statistical Software: Release 13. College Station, TX: StataCorp LP)

5.3.1 Database Cleaning

As data were gathered across six different sites, a small number of erroneous or incorrect entries were to be expected. Variables were tabulated and Italy used '999' and '9999' as missing whereas

Hungary and the UK centres used '0'. Furthermore, for the comorbidity questions, the study protocol specified yes as '-1' and no as '0'. However, the data from Hungary had a majority of patients with blank or missing values. Given the large proportion of potential missing values and the otherwise precise data from Hungary, a decision was made to consider missing values as no and '-1' as yes. To avoid further confusion, all yes-no binary variables were recoded to a consistent scheme of '1' for yes '0' for no and '.' for missing. Finally, extreme and implausible values, such as height of 100cm were considered incorrect and replaced as missing.

5.3.2 Multiple Imputation

Owing to the cumulative effects of missing data in the questionnaires, the sample size for certain analyses was reduced considerably. Performing a complete case analysis can lead to important bias, loss of precision and power.[248] This is especially important when data is missing at random (MAR) as opposed to missing completely at random (MCAR). The potential bias can be overcome by using multiple imputation (MI) which allows for uncertainty about missing data by creating several plausible imputed datasets and combining the results appropriately according to Rubin's rules.[249] The imputed values are sampled from their predictive distribution based on the observed data while introducing an appropriate amount of variability into the results.

Before conducting MI, it is important to understand the patterns of missing data. Although there is no formal testing, attempts can be made to understand a pattern in more detail. Logit models were used to assess if certain variables predict missingness of other variables. In cases of MCAR, there are no variables in the dataset that predict missingness and a complete case analysis, although with a reduced sample size, would not result in biased estimates.[248] However, if the data is MAR, a complete case analysis can lead to important bias which can be overcome by using MI.

Imputation was performed using multiple imputation by chained equations. Both the predictor variables and the outcome were included in the multiple imputation process. The outcome was included as it carries information about missing values of the predictors and it is an accepted methodological process.[248,249] Observed and imputed summary statistics were compared and found to be similar.

Convergence was checked after a single test imputation with 100 iterations. Trace plots of the imputed mean and standard deviations for certain variables were found to converge after approximately 20-25 imputations. Convergence was also checked after the completion of 50 imputations and was found to be acceptable. As multiple imputation is a simulation procedure, Monte Carlo error (MCE) was considered. MCE is defined the standard deviation of the results across repeated runs of the same imputation procedure with the same data and should tend to 0 as the number of imputations increase.[249] Further MI methods are described with the related analysis plan.

5.3.3 Linear Regression Models and Diagnostics

Outcome variables in univariate and multivariate linear regression models are continuous and were found to be normally distributed for the analyses presented.

Regression diagnostics were performed for all multivariate models. Linearity was tested using fractional polynomials and by categorising the exposure variable. Residuals were graphically assessed for normality using kernel density, quantile and normal probability plot. Quantile plots are sensitive to deviations from normality at the tails of distribution whereas standardised normal plots are useful for deviations near the centre.[250] Residual homoscedasticity or homogeneity of residual variance was also assessed visually with residual versus fitted plots and tested with both White's and Breusch-Pagan tests. Both test the null hypothesis that variance of the residuals is homogenous and a small P value suggests heteroscedasticity or unequal residual variance.[250]

Multicollinearity was assessed by computing the variance inflation factor, which was less than 2 for all predictors. Model specification error was tested using STATA's linktest and ovtest. Finally, the presence of influential observations was reviewed by looking for outlying residuals, assessing leverages and Cook's D.[250]

5.3.4 Logistic Regression Models and Diagnostics

The outcome variables for logistic regression models are binary. Diagnostics performed included checking for linearity and specification error as described above.

5.4 The Genodisc Population

The Genodisc database contained information on 2636 patients. Table 5.2 describes the Genodisc population along with the number of patients with missing items. BMI and age were normally distributed with a mean of 27.2kg/$m^2$and 50.9 years respectively. Across all participants, the mean back and leg pain scores were 6.2 and 6.7 respectively, which is not surprising as patients were recruited from tertiary spine clinics. The most common comorbidities were hypertension (27%) followed by migraines (15%), and osteoarthrits (11%). Intensity of work was equally distributed among patients. Furthermore, 46% of patients were non-smokers with the remainder equally distributed between current and previous smokers.

Table 5.2: ***Entire Genodisc Population with missing data***[1]

Characteristic		Missing (n)
Age (years), mean (SD)	50.9 (14.6)	111
Women	1349 (54%)	127
BMI (kg/m^2), mean (SD)	27.2 (4.8)	169
Pain Score (units), mean (SD)		
Back	6.2 (2.9)	211
Leg	6.7 (2.9)	217
Duration of Symptoms (months), median [IQR]		
Back	10 [4-24]	432
Leg	8 [3-20]	436
Zung Depression Score, mean (SD)	39.8 (9.0)	942
Sport per Week (episodes), median [IQR]	0 [0-2]	133
Disability Benefit	296 (12%)	183
Family History	747 (30%)	172
Previous Surgery	715 (28%)	69
Smoking Status		264
Non-Smoker	1079 (46%)	
Previous Smoker	622 (26%)	
Current Smoker	671 (28%)	
Work Type		234

Continued

Characteristic		Missing (n)
Sedentary	684 (29%)	
Light	605 (25%)	
Medium	563 (23%)	
Heavy	550 (23%)	
Clinical Diagnosis		
Disc Herniation	1413 (56%)	90
Spine Stenosis	968 (39%)	161
Spondylolisthesis	400 (16%)	186
Non-specific Back Pain	359 (15%	198
Comorbidities		0
Hypertension	715 (27%)	
Type 2 Diabetes Mellitus	214 (8%)	
Rheumatoid Arthritis	233 (9%)	
Osteoarthritis	298 (11%)	
Osteoporosis	180 (7%)	
Fibromyalgia	21 (1%)	
Migraine	381 (15%)	
Irritable Bowel Syndrome	162 (6%)	
Anxiety	233 (9%)	
Cancer	82 (3%)	

KEY POINTS

- Genodisc was a pan-European observational study which recruited 2,636 patients.
- Data were collected on patient characteristics, pain symptoms, clinical diagnosis and MRI features.
- Multiple imputation was used to overcome bias associated with missing data.

6

Obesity and Spine Related Pain

This chapter reports the association between obesity and back or leg pain as a symptom. Pain is important to both patients, indeed this is usually their primary concern, and also to clinicians. This analysis showed that increased BMI was associated with greater back and leg pain, although the magnitude of this association was small. The association of pain with other predictors is also described.

6.1 Introduction

The correlation between pain and pathological change in the spine is not always clear[30] and further investigation is required into other, non-pathological, predictors such as obesity.[2] From population-based studies, it has been established that BMI increases the odds of low back and leg pain.[80,134] However, these studies consider pain as a binary outcome and provide little information for the effect of obesity upon the severity of pain. Furthermore, there is limited information as to the relationship between BMI and pain in the patient population seen in a tertiary care setting. It is important to understand the contributors to back and leg pain in this population, as surgeons, rheumatologists and physiotherapists are presented with these people daily.

6.2 Analysis Plan

6.2.1 Hypothesis

The hypothesis for this analysis is that obesity, as quantified by increased BMI, will be an independent predictor of back and leg pain in spinal patients.

6.2.2 Outcome

Back and leg pain were considered separately. Pain was assessed on a 11-point scale, ranging from 0, meaning no pain to 10 being the worst pain imaginable. Both BP and LP were normally distributed and considered continuous for this analysis.

6.2.3 Exposure

BMI was the exposure of interest and considered continuous.

6.2.4 Confounders

Given the comprehensive nature of the dataset, adjustment for a large number of possible confounders was possible. A confounder must have an independent relationship with both the exposure (BMI) and the outcome (pain) but not be on the direct causal pathway between the exposure and outcome. For example, migraines,[109,251] fibromyalgia,[252] anxiety[251,253] and IBS[254,255] are all independently related to both back pain and obesity and are unlikely to lie on the causal pathway thus these are valid confounders. Other confounders included in the model were age, gender, Zung SDS, sport, disability benefit, family history, smoking status, work intensity, comorbidities (T2DM, hypertension, RA, OA, OP), and the clinical diagnoses (LDH, LSS, DS and NSBP).

6.2.5 Missing Data

Missing data, primarily from the individual categories of the Zung Depression scale resulted in a considerably smaller complete case analysis. Complete cases were 51% (n= 1346) and 50% (n=1325) for back pain and leg pain models respectively. Unlike the SF-36, there is no accepted method of accounting for missingness within the Zung SDS and if an individual question was missing, the questionnaire was considered to be invalid.

Logit models were created to assess if missingness can be predicted by other variables. An example from this dataset was work category missingness was predicted by RA and OA. A hypothesis could be that patients suffering form RA and OA are less likely to work and as such failed to answer this question. As missing values can be predicted by other variables, data was assumed to be MAR as opposed to MCAR and MI was performed. MI has also been shown as a valid method of managing missing data in the Zung SDS.[256]

Two separate imputations were carried out, one for each multivariate model of BP and LP. Composite variables, where two or more individual variables are combined, such as BMI and the Zung SDS were imputed as the individual variables (height and weight) and combined to give the final variable (BMI). Continuous variables, such as height, weight, age, were normally distributed and imputed using linear regression. Back pain score, leg pain score, work type and the individual

components of the Zung score were imputed as ordered categorical variables. Smoking status, a nominal categorical variable, was imputed using multinomial logistic regression. Finally, the binary variables (gender, disability award, family history, previous surgery, clinical diagnoses and comorbidities) were imputed using logistic regression.

A total of 50 imputed datasets were created. Prior to the recording of an imputed dataset, 25 iterations were conducted resulting in a total of 1500 iterations for the entire imputation procedure. Convergence and MCE were both acceptable. Observed and imputed summary statistics as well as crude univariate regression, before and after imputation, were found to be similar.

6.2.6 Statistical Technique

Univariate and multivariate linear regression models, as described in chapter 5, were used to model the relationship between BMI and pain scores. All coefficients for BMI and age relate to an increase of 5kg/m^2or 10 years respectively.

6.3 Results

6.3.1 Description within BMI Categories

Patient Distribution and Characteristics

The largest group of participants were overweight (38%) followed by those with normal BMI (31%), obese (10%), morbidly obese (6%) and underweight (1.4%) with 6.4% of patients missing a BMI (Table 6.1). Mean ages of the overweight (52 years), obese (54 years) and morbidly obese (54 years) groups were similar. Patients of a normal BMI were slightly younger (47 years) and those underweight were younger still, approximately 10 years younger than the overweight groups (44 years). There was a female predominance at the extremes of BMI with 87% in the underweight group and 59% in the morbidly obese.

Table 6.1: ***Genodisc Population described in BMI Categories***[1]

	Underweight (<18.5 kg/m^2)	Normal (18.5-25 kg/m^2)	Overweight (25-30 kg/m^2)	Obese (30-35 kg/m^2)	Morbid Obese (>35 kg/m^2)	Missing
Number of patients	37	804	992	474	160	169
Age (years), mean (SD)	43.7 (16.4)	47.1 (15.4)	52.1 (13.8)	54.0 (13.1)	53.7 (12.5)	50.9 (18.5)
Female Gender, n (%)	32 (87%)	496 (62%)	454 (46%)	232 (49%)	95 (59%)	40 (69%)
Pain Score (VAS units)						
Back, mean (SD)	5.4 (2.9)	6.0 (2.9)	6.2 (2.9)	6.2 (2.8)	7.2 (2.6)	6.3 (3.0)
Leg, mean (SD)	6.3 (3.5)	6.4 (3.1)	6.6 (2.9)	7.0 (2.7)	7.7 (2.1)	6.5 (3.0)
Symptoms Duration (months)						
Back, median [IQR]	11.0 [5,45]	9.0 [3,24]	9.0 [4,24]	11.5 [5,24]	13.0 [4,36]	10.0 [4,21]
Leg, median [IQR]	7.0 [4,24]	7.0 [3,18]	7.0 [3,18]	10.0 [4,24]	12.0 [4,26]	9.5 [4,18]
Zung Depression Score	42.1 (13.0)	39.6 (8.8)	39.1 (8.9)	40.5 (8.6)	42.3 [8.5]	42.7 [10.0]
Sport per week (episodes), median [IQR]	0 [0,2]	0 [0,2]	0 [0,2]	0 [0,1]	0 [0,0]	0 [0,0]
Disability Award, n (%)	2 (6%)	65 (8%)	126 (13%)	58 (13%)	40 (26%)	5 (11%)
Family History, n (%)	11 (31%)	245 (31%)	297 (31%)	140 (30%)	43 (28%)	11 (22%)
Previous Surgery, n (%)	7 (19%)	218 (28%)	282 (29%)	143 (30%)	53 (33%)	13 (11%)
Smoking Status, n (%)						
Non-Smoker	15 (40%)	336 (44%)	414 (44%)	212 (48%)	70 (47%)	32 (70%)
Previous Smoker	8 (22%)	163 (22%)	262 (28%)	125 (28%)	54 (36%)	10 (22%)
Current Smoker	14 (38%)	260 (34%)	264 (28%)	103 (24%)	26 (17%)	4 (8%)

Continued

	Underweight	Normal	Overweight	Obese	Morbid Obese	Missing
Work Type, n (%)						
Sedentary	11 (31%)	218 (28%)	269 (29%)	131 (29%)	46 (30%)	9 (20%)
Light	9 (26%)	225 (29%)	221 (23%)	98 (22%)	39 (25%)	13 (29%)
Medium	9 (26%)	202 (26%)	207 (22%)	105 (23%)	31 (20%)	9 (20%)
Heavy	6 (17%)	127 (16%)	244 (26%)	120 (26%)	39 (25%)	14 (31%)
Clinical Diagnosis, n (%)						
Disc Herniation	21 (58%)	482 (60%)	561 (57%)	252 (54%)	79 (49%)	56 (47%)
Spine Stenosis	7 (19%)	233 (30%)	397 (42%)	217 (47%)	82 (54%)	47 (42%)
Spondylolisthesis	3 (8%)	117 (15%)	116 (18%)	79 (18%)	27 (18%)	6 (14%)
Non-specific Back Pain	7 (19%)	102 (13%)	141 (15%)	62 (14%)	24 (16%)	23 (21%)
Comorbidities, n (%)						
Hypertension	4 (11%)	120 (15%)	274 (28%)	216 (46%)	86 (54%)	15 (9%)
Type 2 Diabetes Mellitus	0 (0%)	31 (4%)	73 (7%)	71 (15%)	34 (21%)	5 (3%)
Rheumatoid Arthritis	2 (5%)	50 (6%)	92 (9%)	64 (14%)	22 (14%)	3 (2%)
Osteoarthritis	2 (5%)	79 (10%)	123 (12%)	62 (13%)	27 (17%)	5 (3%)
Osteoporosis	5 (14%)	76 (9%)	63 (6%)	26 (5%)	9 (6%)	1 (1%)
Fibromyalgia	0 (0%)	11 (1%)	3 (0%)	4 (1%)	2 (1%)	1 (1%)
Migraine	7 (19%)	134 (17%)	134 (14%)	69 (15%)	26 (16%)	11 (7%)
Irritable Bowel Syndrome	2 (5%)	60 (7%)	61 (6%)	24 (5%)	11 (7%)	4 (2%)
Anxiety	7 (19%)	78 (10%)	73 (7%)	50 (11%)	18 (11%)	7 (4%)
Cancer	1 (3%)	25 (3%)	31 (3%)	17 (4%)	8 (5%)	0 (0%)

[1]Data are n (%), mean (standard deviation, SD) or median [interquartile range,IQR]

Duration of Pain Underweight and morbidly obese patients presented after a longer duration of both back and leg pain (Figure 6.1). For all patients, the median (interquartile range [IQR]) duration of back and leg pain symptoms was 10 (4-24) months and 8 (3-20) months respectively (Table 5.2).

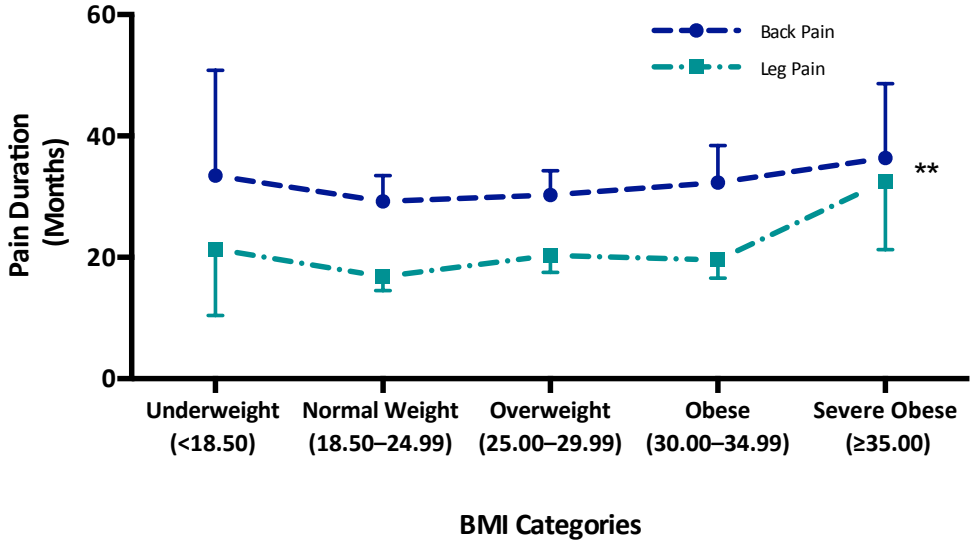


Figure 6.1: ***Mean duration of pain (months) experienced by patients categorised by BMI.*** *Markers represent mean and 95% confidence intervals. **p<0.01 when compared to normal BMI*

Sporting Activity There was a trend for lower sporting activity with both higher pain (Figure 6.2) and greater BMI (Figure 6.3).

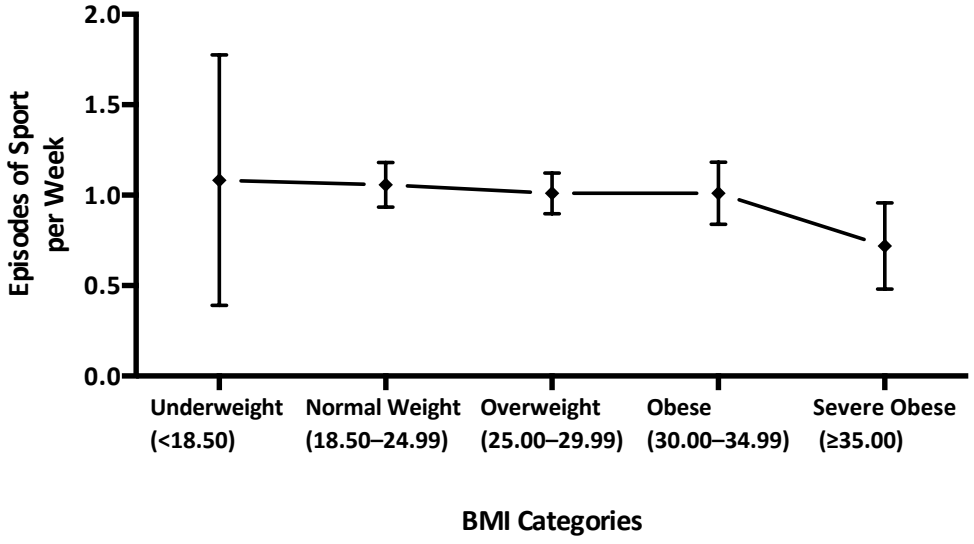


Figure 6.2: ***Sport participation by BMI categories.*** *Markers represent 95% confidence intervals.*

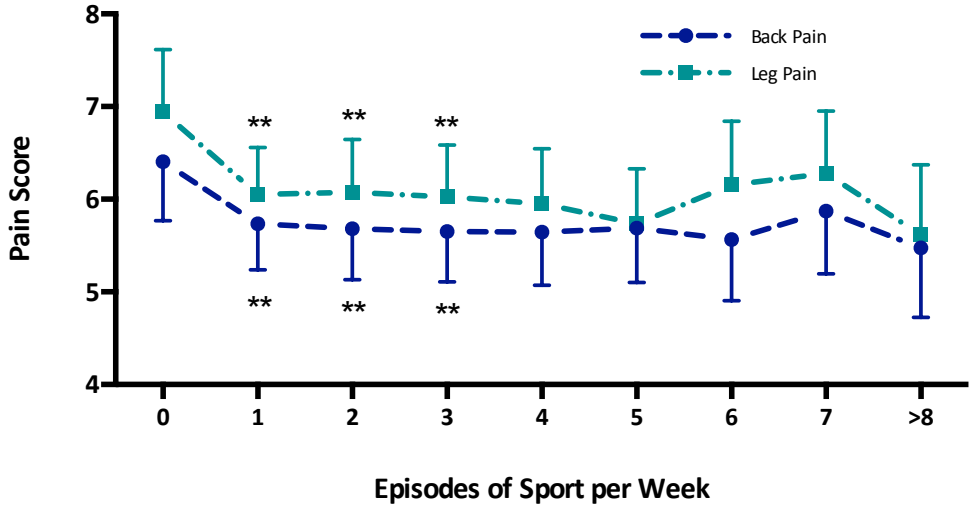


Figure 6.3: ***Mean pain scores for patients who are categorised by participation in sport.*** *Markers represent mean and 95% confidence intervals. **p<0.01 when compared no sport.*

Comorbidities The patients in the higher BMI groups had a greater prevalence of hypertension, diabetes, RA, and OA. This correlation was most striking for hypertension and diabetes with 54% and 21% of morbidly obese patients suffering from these diagnoses compared to 15% and 4% of those with a normal BMI. Osteoporosis (14%) and anxiety (19%) were most common in patients who were underweight. Depression was marginally greater in the underweight and morbidly obese group but the difference (2 points on the Zung SDS) is unlikely to be clinically relevant. No obvious differences were seen for fibromyalgia, migraines, IBS and cancer (Table 6.1).

Smoking Smoking status was similar in the normal, overweight and obese groups. Current smokers were most common in the underweight group (38%) and least in the morbidly obese group (17%). Furthermore, the latter group also had the largest percentage of previous smokers (36%) (Table 6.1).

Occupation There was an equal distribution of occupational intensity across all four intensity categories and BMI groups. However, a disability award or benefit was most common in the morbidly obese group (26%) with the proportion steadily lower in the lower BMI groups (Table 6.1).

6.3.2 Pain Scores

With higher BMI there was an increase in both unadjusted BP and LP scores (Table 6.1). Figure 6.4 shows this relationship persisted after adjustment for multiple confounders

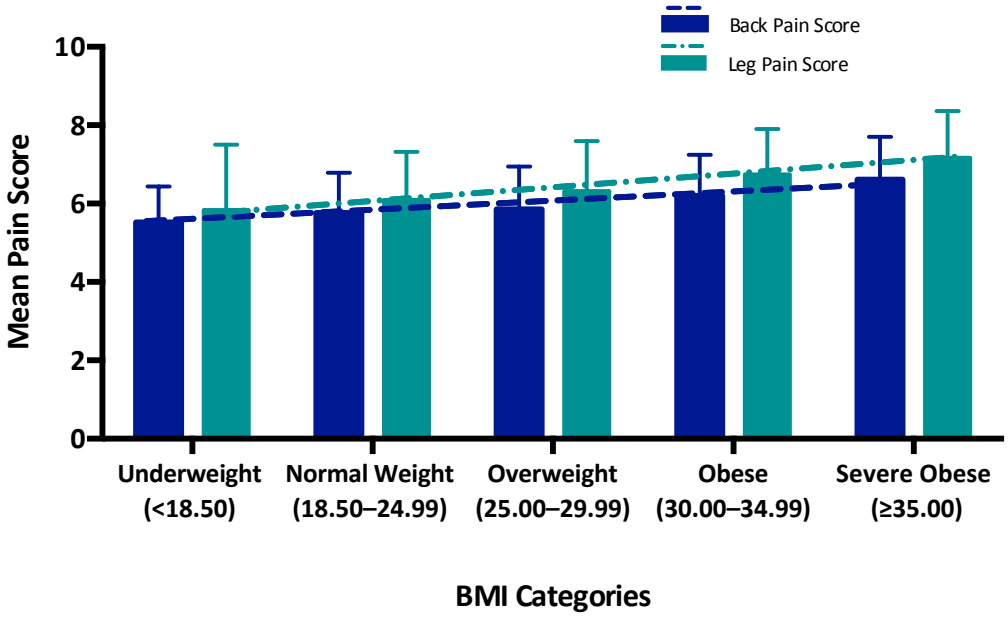


Figure 6.4: ***Adjusted (from multivariate model 2) mean back and leg pain score for each BMI category.*** *Bars show mean and 95% confidence interval.*

Back Pain Score

Table 6.2 summarises the main result of the univariate and multivariate linear regression models. Multivariate adjusted model 1 (hypertension and diabetes included) for BP shows that BMI was associated with greater but non-significant BP score (0.10 units [95% CI -0.02, 0.22]). However in the univariate model, this association was significant. To investigate where this relationship was lost confounders were sequentially removed. When hypertension and diabetes were removed (back pain multivariate model 2), the effect of BMI upon BP remained significant at 0.15 units (95% CI 0.04,0.27) with minimal change to the coefficients of the other confounders (Figures 6.5 and 6.6).

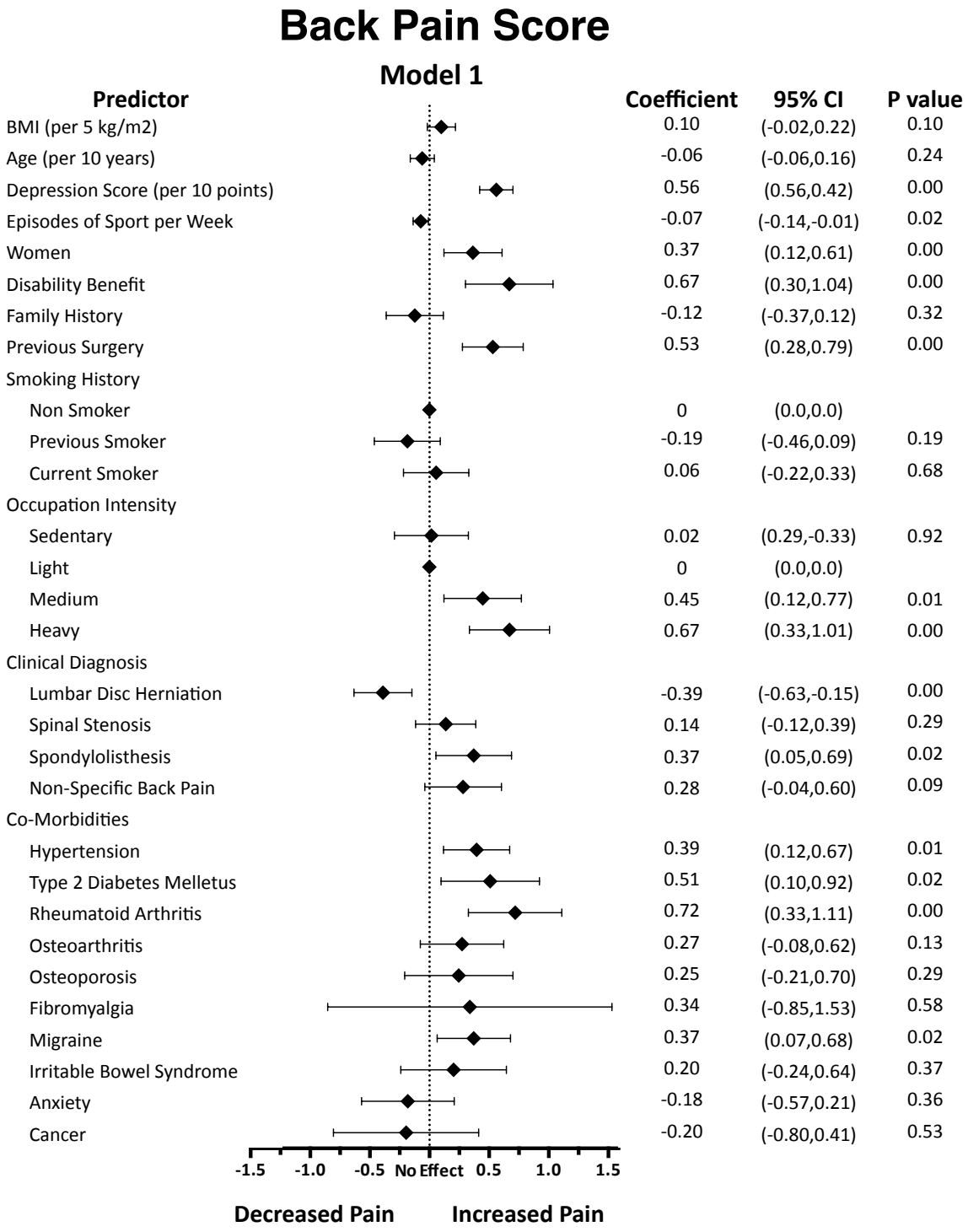


Figure 6.5: ***Confidence interval plot showing regression coefficients of back pain multivariate model 1 (hypertension and diabetes included)*** *Models fitted to 50 multiple imputation datasets (n=2636). Coefficient is the regression coefficient. A positive coefficient represents higher levels pain and a negative coefficient represents lower levels pain. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

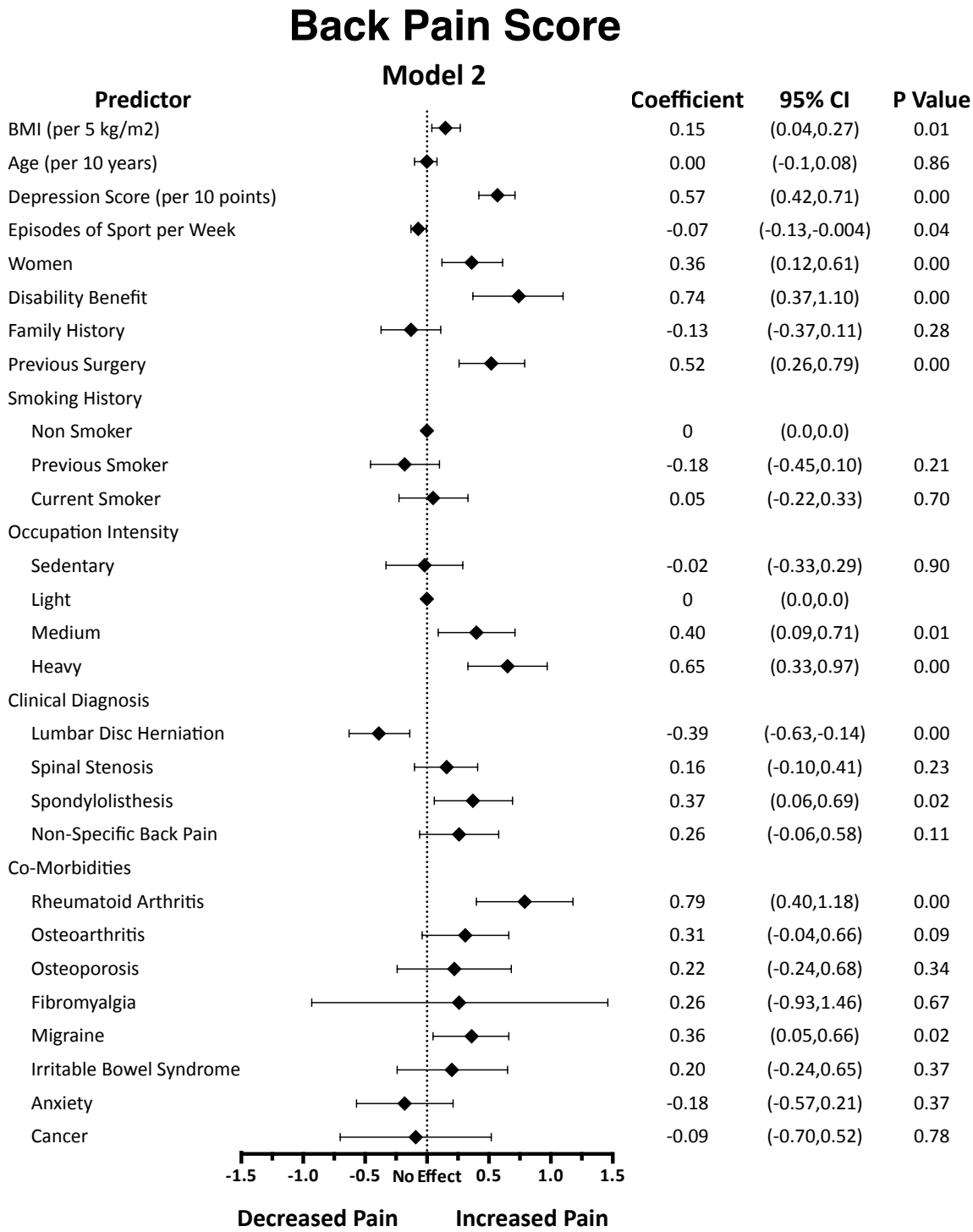


Figure 6.6: ***Confidence interval plot showing regression coefficients of back pain multivariate model 2 (hypertension and diabetes excluded)*** *Models fitted to 50 multiple imputation datasets (n=2636). Coefficient is the regression coefficient. A positive coefficient represents higher levels pain and a negative coefficient represents lower levels pain. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

	Back Pain Score			Leg Pain Score		
	Coefficient	(95% CI)	P value	Coefficient	(95% CI)	P value
Univariate	0.26	(0.14,0.37)	0.0	0.35	(0.23,0.47)	0.0
Multivariate 1	0.10	(-0.02,0.22)	0.10	0.19	(0.15,0.31)	0.00
Multivariate 2	0.15	(0.04,0.27)	0.01	0.22	(0.10,0.33)	0.0

Table 6.2: ***Univariate and multivariate regression coefficients for the effect of BMI upon back and leg pain scores.*** *Coefficient is the regression coefficient. Each coefficient represents a change in pain score for a 5-unit increase in BMI. A positive coefficient represents increased pain. Models were fitted to 50 multiple imputation datasets (n=2636). Multivariate model 1 is adjusted for all confounders as described in the main text. In multivariate model 2, hypertension and diabetes were excluded.*

Leg Pain Score

In the multivariate model for LP, BMI was associated with a 0.22 unit (95% CI 0.10,0.33) increase in pain (Table 6.2). There was very little change in this coefficient with the removal of hypertension and diabetes from the model (Figures 6.7 and 6.8).

6.3.3 Other Confounders

The regression coefficients with associated confidence intervals from multivariate models for both back and leg pain are illustrated in Figures 6.6 and 6.8. Female gender, depression (Zung Depression Score), rheumatoid arthritis, heavy workload and previous surgery were all significant positive predictors of greater back and leg. Sport was a significant negative predictor for both BP (-0.07 [95% CI -0.13,-0.004]) and LP (-0.10 [95% CI -0.16,-0.04]). Specifically, for BP, the strongest positive predictors were receiving benefit for disability (0.74 units [95% CI 0.37, 1.10]), a heavy workload (0.65 units [95% CI 0.33,0.97]) and rheumatoid arthritis (0.79 units [95% CI 0.40,1.18]). By contrast, the strongest association for LP was a diagnosis of lumbar disc herniation (1.08 units [95% CI 0.84,1.33]).

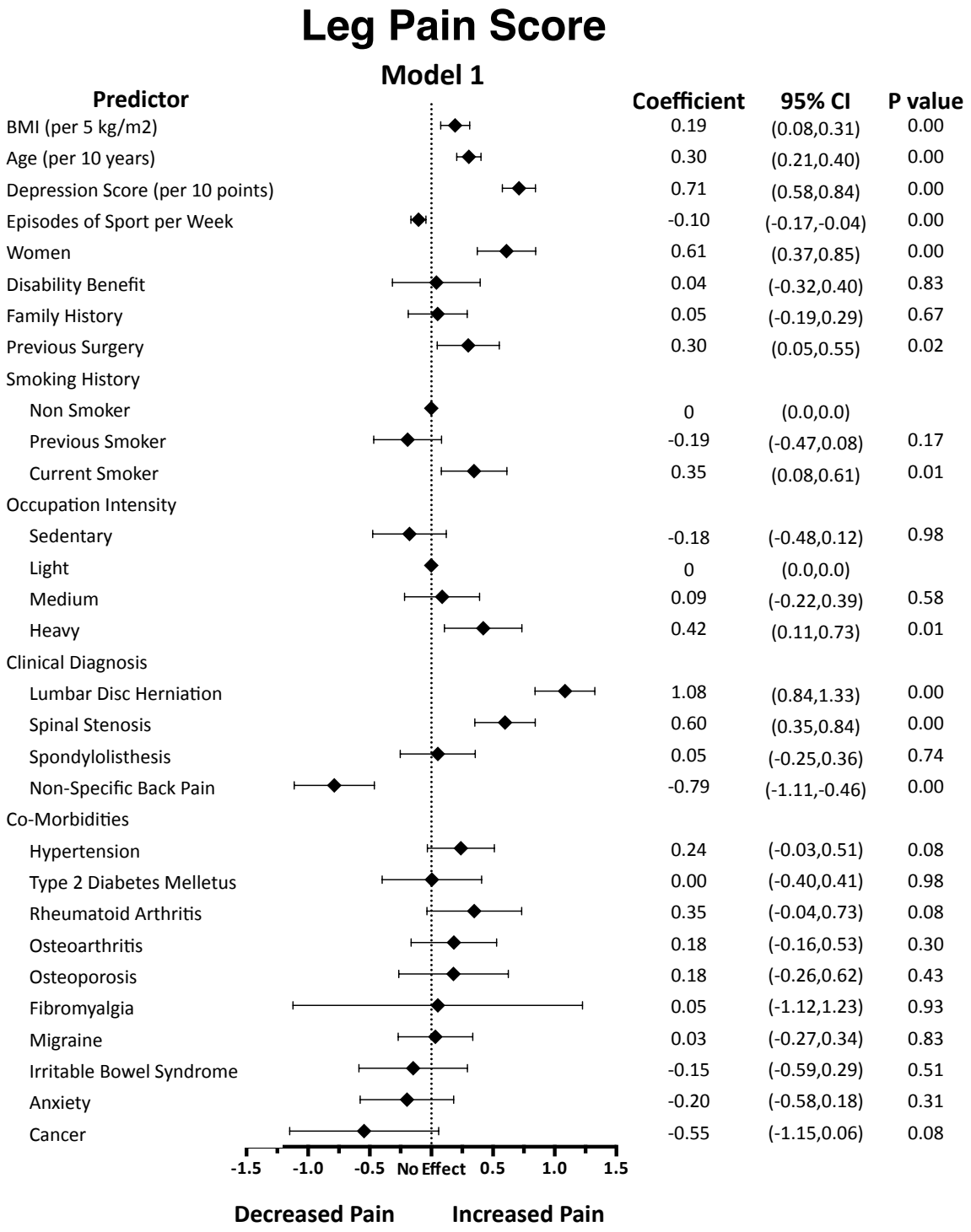


Figure 6.7: ***Confidence interval plot showing regression coefficients of leg pain multivariate model 1 (hypertension and diabetes included)*** *Models fitted to 50 multiple imputation datasets (n=2636). Coefficient is the regression coefficient. A positive coefficient represents higher levels pain and a negative coefficient represents lower levels pain. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

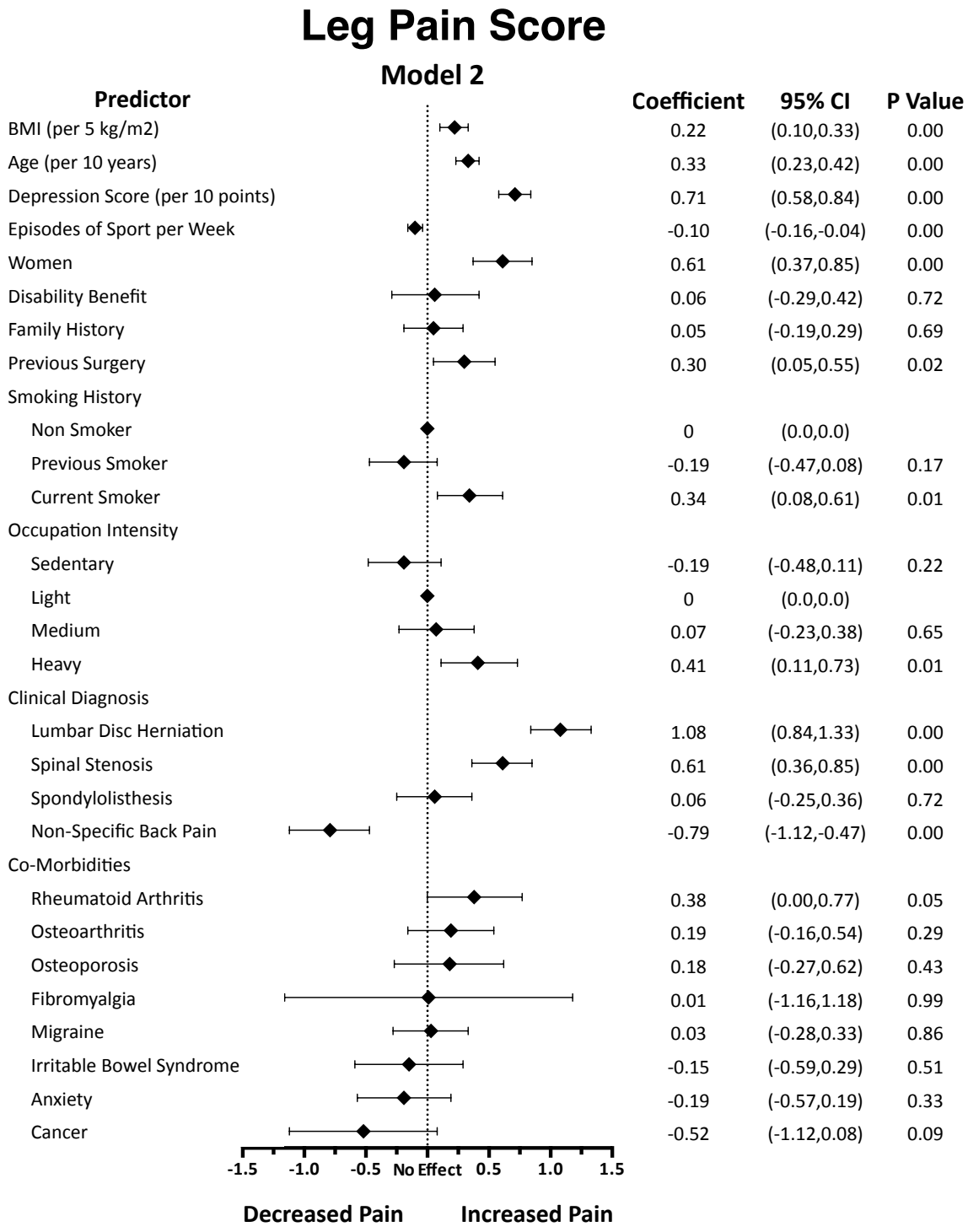


Figure 6.8: ***Confidence interval plot showing regression coefficients of leg pain multivariate model 2 (hypertension and diabetes excluded)*** *Models fitted to 50 multiple imputation datasets (n=2636). Coefficient is the regression coefficient. A positive coefficient represents higher levels pain and a negative coefficient represents lower levels pain. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

6.4 Discussion

In this population, increased BMI was significantly associated with an increase in both back and leg pain scores (Figures 6.4, 6.6, and 6.8). Other factors that were associated with an increase in either BP or LP were female gender, previous spine surgery, heavy workload, rheumatoid arthritis and depression (Figures 6.6 and 6.8). Back pain was associated with a greater number of significant predictors than LP possibly because it is a nebulous symptom with many potential contributors. However, leg pain, below the knee, usually has a clear underlying pathoanatomical lesion. This is supported in the LP model, where the diagnosis of disc herniation had a strong positive association, increasing LP score by 1.08 units (95% CI 0.84,1.33).

Hypertension and Diabetes In the analysis, when hypertension and diabetes were considered confounders, the effect of BMI upon BP score, but not LP score, was markedly weakened with minimal change in any of the other confounders.

The relationship between the cardiovascular risk factors and spine related pain is unclear as it is difficult to identify a direct effect. Certain authors have described an atherosclerotic hypothesis to explain a causal relationship between hypertension and diabetes and BP. This relationship has been established primarily in cadaveric studies[257,258] and also in an occupation-based epidemiological study.[259] Within this occupational study, the authors showed that over a 28-year period patients with higher blood pressure at baseline had increased risk of BP. This could explain why hypertension and diabetes only confound the relationship between BMI and BP, and not LP.

However, it is well recognised that both hypertension and diabetes are associated with obesity as part of the metabolic syndrome.[260] The data presented supports a positive association between increasing prevalence of both diabetes and hypertension with increasing BMI (Table 6.1). Given this, these confounders most likely lie on the causal pathway between obesity and BP and hence adjusting for these may not be appropriate. As such, the effect of hypertension and diabetes is interpreted to be primarily related to BMI and multivariate model 2 (Figures 6.6 and 6.8) is more valid.

A recent meta-analysis by Shiri *et al.*, found that people with increased BMI had greater odds of developing BP, increased prevalence of BP and were more likely to have chronic BP.[80] This relationship was dose dependant, with obese people (BMI>30kg/m^2) exhibiting greater levels of pain than those who were overweight (BMI 25-29kg/m^2). Another meta-analysis, by the same group, showed a similar dose dependent relationship between increasing BMI and self reported LP symptoms.[134] A limitation of these meta-analyses and of the original studies is that both BP and LP were considered as binary "yes/no" variables. This current study is in agreement with these studies but adds information to that in the literature by showing a similar relationship exists in BP and LP for patients seeking tertiary care. It also shows the effect of increasing BMI upon the severity of both BP and LP.

Shiri also noted that overweight (BMI 25-29.9 kg/m^2) or obese patients (BMI>30.0 kg/m^2) were more likely to "seek care" for their BP.[80] Based on this information the Genodisc population should have a higher mean BMI than the general population. However, when the mean BMI within our population (27.2 kg/m^2) was compared to those reported in population-based studies such as the English Longitudinal Study of Aging (27.9 kg/m^2),[261] the United Kingdom Biobank (27.4 kg/m^2)[262] or a large Hungarian cohort (25.9 kg/m^2),[263] there were minimal differences. This is important, as although overweight and obese people appear more likely to seek primary care, it appears that they do not filter through to tertiary clinics, based on the mean BMI in this study. This is despite their more severe pain (Figure 6.4) and longer duration of pain symptoms, suggesting a longer period before tertiary care consultation (Figure 6.1). A hypothetical explanation could be negative institutional attitudes for the health care of the obese[264] leads to restriction of access to tertiary care, similar to that seen in osteoarthritis.[265]

The findings for BP are similar to those of Fanuele *et al.*, who, though not looking at pain directly, used the American National Spine Network data to model the effect of BMI upon disability arising from low back pain.[266] Here, the authors found that obesity was associated with decreased functional status and increased disability. Unfortunately, given the nature of their dataset the authors were not able to correct for depressive symptoms, a significant confounder in our study, nor did they consider LP as an outcome in their adjusted model. Similarly, Heuch *et al.* recently showed, in a longitudinal study, that a BMI of greater than 30kg/m^2 increases the odds of developing BP,

further supporting the deleterious relationship between obesity and BP.[267]

As there appears a direct relationship between BMI and the severity of pain (Figure 6.4); the next question to ask is if weight loss will help reduce the pain. Recently, three separate small case-series of back pain patients have found that post bariatric surgery with resulting rapid weight loss, obese patients have less BP[268–270] and reduced spine related disability.[268,269] Surgical weight loss represents an extreme form of weight loss and is a treatment that may not be widely available but the results support the idea that weight loss could lead to decreased pain and this requires further investigation.

As well as weight loss, these results suggest that exercise is associated with less back pain (Figures 6.6 and 6.8). Within a population setting, Smuck *et al.*, in addition to finding a dose dependent increase of BP with BMI, showed that moderate physical was protective from back pain in an overweight (BMI 26-30 kg/m^2) and ultraobese (BMI>36 kg/m^2) population, but not in the obese group (BMI 31-35 kg/m^2).[271] However, when BMI was considered continuous, this study found physical activity conferred only a small protective effect. The Nord-Trøndelag Health study also found a small protective effect of physical activity upon back pain.[267] To an extent, these results are in keeping with this analysis where lower BP and LP scores were associated with greater physical activity (Figure 6.3). More specifically, the greatest decrease in pain score was noted between patients involved in only one episode of sport a week as compared to those who did none. Similarly, a higher BMI is associated with a lower number of episodes of sporting activity suggesting these patients are less likely to partake in potentially beneficial exercise (Figure 6.2). Alternatively, patients with greater pain may be less willing to exercise so whether exercise is protective cannot be fully assessed from this cross-sectional data.

Obesity has also been implicated or associated in other pain and psychiatric disorders such as fibromyalgia,[106] migraines[107,108] and depression.[109,110] For patients with depression and migraine headaches, a similar dose dependent relationship of more pain with increasing BMI is seen.[107,108,110] This linear relationship is less clear in fibromyalgia however it is suggested an important relationship between obesity, physical activity and symptoms exists.[106] Furthermore, altered adipokines have also been associated with migraine headaches[108] and fibromyalgia.[272] Taken together, this would suggest increased BMI is an important mediator in many pain related disorders and the effect could

be mediated by systemic biochemical mechanisms.

6.4.1 Strengths of this Analysis

Given the large sample size, the study carries considerable power and allows for adjustment for many potential participant and clinician reported confounders without limiting the validity of the results. Furthermore, the results are generalisable within the tertiary care setting as patients were recruited from six sites in four countries with a resulting heterogeneous population.

6.4.2 Limitations of this Analysis

The cross sectional nature of this study raises three important caveats when interpreting the results. Most importantly, as this is a cross-sectional study, causation cannot be established. Within the general population, there is evidence that obesity may be a factor directly leading to back and leg pain.[80,267] However, given these patients have been suffering from these symptoms for a considerable duration, it is also possible that the initial acute pain has now centralised[273] and as such, is causally independent of BMI albeit with a persistent cross-sectional association.

Furthermore, pain is a symptom, which is not constant and can change because of factors other than those relating to a biological or pathoanatomical process. It was attempted to acquire a more general picture of pain symptoms by asking participants to rate their pain over the previous week. To adequately address this and the question of causation, longitudinal studies are required.

The clinical relevance of these findings requires discussion. For the numerical pain rating scale, as used in this study, a reduction of two points or 30% is generally accepted as a clinically meaningful difference.[274–276] From this analysis, even a very large 15kg/m^2 increase in BMI (comparing a normal BMI of 20kg/m^2 to morbid obesity of 35kg/m^2) would not show a clinically meaningful difference in pain score using this definition. The small coefficients seen in this study may be statistically enhanced by the large sample size.

Although, the definition of a clinically important difference was derived from chronic pain populations including patients with low back and neuralgic pain, it is important to note that such dif-

ference represents a change in pain score, which is usually an intra-individual change within a longitudinal cohort or interventional study. Even though the Genodisc population is similar, the interpretation of clinical relevance is most likely informative rather than prescriptive. Similarly, our findings are consistent to what is seen in the literature suggesting a true result rather than a statically anomaly.

6.5 Conclusion

This study provides evidence supporting the hypothesis that obesity is independently associated with back and leg pain and that the effect of increasing BMI upon pain is linear. However, the magnitude of the association is small and hence the clinical relevance may be limited. It also provides information on other clinically important predictors of pain in spine patients, in particular female gender, heavy workload, rheumatoid arthritis, previous spine surgery, and depression.

KEY POINTS

- Increasing BMI is associated with greater back and leg pain in spinal patients.
- Other predictors of back pain were female gender, heavy occupation intensity, rheumatoid arthritis and a disability award.
- The most important predictor of leg pain was a diagnosis of lumbar disc herniation.
- Longitudinal studies are required to define casuation.

7

Spinal Diagnosis and Obesity

One of the main aims of the Genodisc project was to further the understanding of clinically relevant spine phenotypes. This chapter is focussed on investigating the relationship between obesity and clinically diagnosed phenotypes. The population is described within each diagnostic category and logistic regression was used to analyse the association. There was considerable overlap in diagnostic categories suggesting patient heterogeneity. Obesity was only associated with a diagnosis of lumbar spine stenosis.

7.1 Introduction

When making a diagnosis, a clinician takes into account the symptoms, examination findings and results of pertinent investigations. These factors are important in allowing a clinician to categorise and thus manage a patient appropriately. These diagnostic categories also direct research as they describe clinical phenotypes.

7.2 Analysis Plan

7.2.1 Hypothesis

That obesity is independently associated with a diagnosis of lumbar disc herniation, lumbar spine stenosis, degenerative spondylolisthesis, or non specific back pain.

7.2.2 Outcome

Within the Genodisc study, clinicians were asked to diagnose patients into four broad categories; lumbar disc herniation (LDH), lumbar spine stenosis (LSS), degenerative spondylolisthesis (DS) and non-specific back pain (NSBP). These binary variables were based on a complete clinical picture including clinical history, examination and MRI features. Patients can exhibit clinical features of more than one category and hence surgeons were allowed to categorise patients into more than one category.

7.2.3 Exposure

BMI was the exposure of interest and considered continuous.

7.2.4 Confounders

The comorbidities, fibromyalgia, migraine, IBS, anxiety and cancer, were not included in the models for LDH, LSS and DS but were included for NSBP. The relationship between these comorbidities and

either LDH, LSS or DS have not been established and hence inclusion would have been erroneous. However in the case of NSBP a relationship exists.[251]

7.2.5 Missing Data

7.2.6 Statistical Technique

As the outcome variables were binary, univariate and multivariate logistic regression models, as described in chapter 5, were used to model the relationship between BMI and clinical diagnosis. No interactions were found between gender or age and BMI.

7.3 Results

7.3.1 General Results

Patient Characteristics Table 7.1 describes the Genodisc population by their diagnostic category. LDH was the most common diagnosis (1551 patients) followed by LSS (983 patients), spondylolisthesis (408 patients) and finally NSBP (359 patients). Patients in the LDH (47.5 years) and NSBP (49.1 years) groups were younger than those in the LSS (59.7 years) and DS (55.6 years) groups (Figure 7.3). All four groups of patients were very similar in terms of BMI, smoking history and work intensity.

Table 7.1: ***Genodisc Population described by clinical diagnoses***[1]

	Lumbar Disc Herniation	Lumbar Spine Stenosis	Spondylolisthesis	Non-Specific Back Pain
Number of patients	1451	983	408	359
BMI (kg/m^2), mean (SD)	27.0 (4.7)	28.2 (4.8)	27.6 (4.8)	27.3 (4.9)
Age (years), mean (SD)	47.5 (13.5)	59.7 (13.2)	55.6 (14.7)	49.1 (12.9)
Female Gender, n (%)	711 (51%)	520 (55%)	248 (62%)	193 (56%)
Pain Score (VAS units)				
Back, mean (SD)	5.9 (2.9)	6.5 (2.8)	6.8 (2.7)	6.5 (2.7)
Leg, mean (SD)	6.9 (2.6)	7.2 (2.5)	6.7 (2.9)	5.7 (3.3)
Symptoms Duration (months)				

Continued

	Lumbar Disc Herniation	Lumbar Spine Stenosis	Spondylolis-thesis	Non-Specific Back Pain
Back, median [IQR]	6.0 [3,18]	12 [6,36]	15 [6,48]	13 [6,36]
Leg, median [IQR]	6.0 [3,12]	12 [6,24]	12 [6,24]	12 [5,24]
Zung Depression Score	40.0 (8.9)	40.7 (8.6)	40.9 (8.9)	39.9 (10.1)
Sport per week (episodes), median [IQR]	0 [0,2]	0 [0,1]	0 [0,2]	0 [0,2]
Disability Award, n (%)	122 (9%)	149 (15%)	48 (12%)	44 (13%)
Family History, n (%)	463 (33%)	242 (25%)	105 (27%)	120 (35%)
Previous Surgery, n (%)	359 (25%)	282 (29%)	86 (22%)	85 (24%)
Smoking Status, n (%)				
Non-Smoker	558 (42%)	397 (44%)	199 (53%)	158 (46%)
Previous Smoker	313 (24%)	291 (32%)	106 (28%)	104 (31%)
Current Smoker	457 (34%)	217 (24%)	72 (28%)	78 (23%)
Work Type, n (%)				
Sedentary	389 (28%)	256 (29%)	105 (28%)	100 (30%)
Light	323 (24%)	221 (25%)	101 (27%)	80 (24%)
Medium	327 (24%)	218 (24%)	75 (20%)	83 (25%)
Heavy	328 (24%)	203 (23%)	94 (25%)	70 (21%)
Comorbidities, n (%)				
Hypertension	337 (23%)	395 (40%)	145 (36%)	80 (22%)
Type 2 Diabetes Mellitus	105 (7%)	124 (13%)	38 (9%)	21 (6%)
Rheumatoid Arthritis	112 (8%)	118 (12%)	57 (14%)	25 (7%)
Osteoarthritis	121 (8%)	168 (17%)	70 (17%)	46 (13%)
Osteoporosis	82 (6%)	94 (10%)	40 (10%)	18 (5%)
Fibromyalgia	8 (1%)	7 (1%)	4 (1%)	3 (1%)
Migraine	211 (15%)	113 (14%)	63 (15%)	60 (17%)
Irritable Bowel Syndrome	74 (8%)	38 (9%)	61 (6%)	37 (10%)
Anxiety	124 (9%)	91 (9%)	91 (9%)	91 (9%)
Cancer	31 (2%)	44 (5%)	44 (5%)	44 (4%)

Pain Symptoms Figure 7.1 graphically represents the mean back and leg pain scores in each diagnostic category. Patients suffering from LDH and LSS had higher LP than BP, with the greatest BP seen in LSS (7.2 units [95% CI 7.0,7.4]). The opposite was seen in those with non-specific back pain. Patients with DS suffered from a similar intensity of BP and LP.

[1]Data are n (%), mean (standard deviation, SD) or median [interquartile range,IQR]

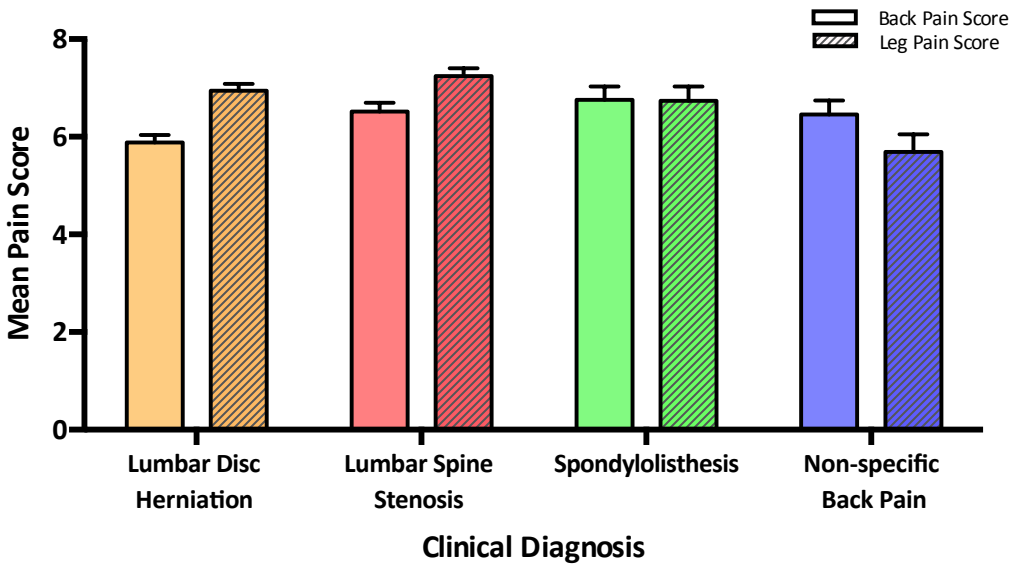


Figure 7.1: ***Mean back and leg pain scores for each clinical diagnosis.*** *Bars show mean and 95% confidence interval. Solid bars are back pain score, shaded bars are leg pain score.*

Duration of Symptoms Those with LDH had a considerably shorter duration of both back and leg pain (median 6 months) than those in the other three groups, who all presented after a median of 12 months or greater (Table 7.1).

Comorbidities The Zung depression score was comparable across all four group and the most common comorbidity was hypertension. The patients with LDH and NSBP had lower prevalence of hypertension, diabetes, RA, OA and OP than the LSS and spondylolisthesis groups. This findings is possibly a consequence of the age of patients within each group.

7.3.2 Overlap in Diagnosis

Figure 7.2 shows the considerable overlap between these four diagnostic categories. LDH was the most common diagnosis with 20% (293/1451) of these patients sharing a diagnosis of LSS. Similarly, 30% (293/983) of LSS patients and showed crossover with LDH. Those suffering from spondylolisthesis and NSBP also shared another diagnosis in 63% and 51% of cases respectively. A very small proportion of patients were categorised into three (19 patients) and four (9 patients) diagnostic groups.

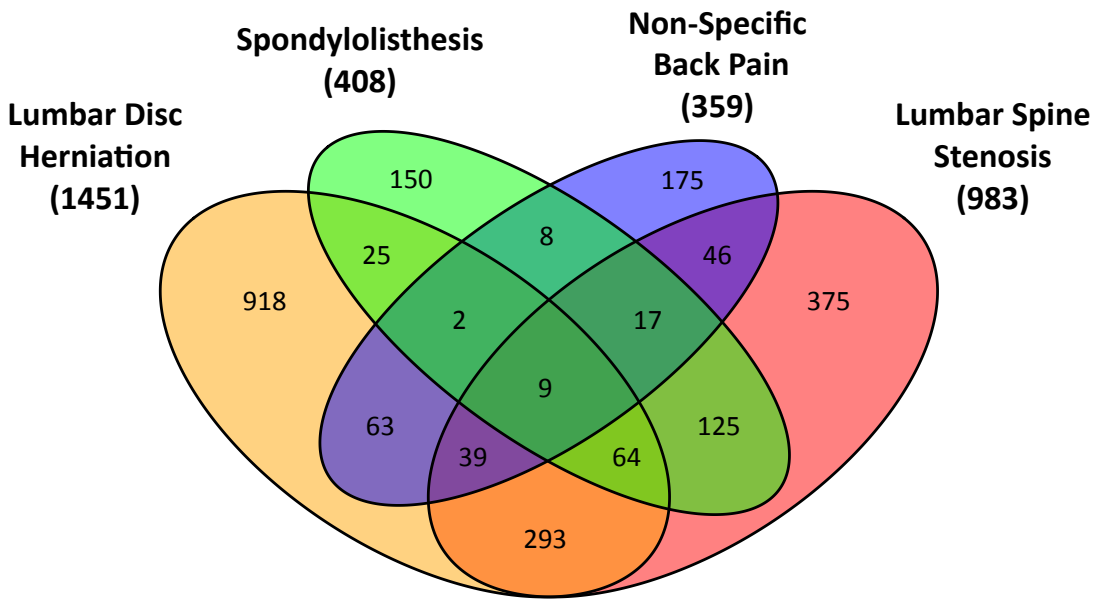


Figure 7.2: ***Venn diagram representing the overlap in diagnosis in the Genodisc population.***

7.3.3 Lumbar Disc Herniation

With increasing age, the prevalence of LDH decreased (Figure 7.3A). Conversely, with increasing BMI, there was little difference in prevalence (Figure 7.4A).

Figure 7.5 shows the confidence interval plot of the multivariate logistic regression model for LDH. There is no relationship between BMI and a diagnosis of LDH (1.01 [95% CI 0.93,1.11]) however in a univariate analysis BMI was a significant negative predictor with an OR of 0.90 [95% CI 0.83,0.98] (Table 7.2). This association was confounded by age (OR 0.72 [95% CI 0.67,0.77]), which was the strongest negative predictor of LDH. Other important positive predictors were a family history (1.23 [95% CI 1.02, 1.49]) and current smoking (1.66 [95% CI 1.34, 2.07]).

7.3.4 Lumbar Spine Stenosis

LSS showed an marked increase in prevalence with increase age and to a lesser extent with a greater BMI (Figures 7.3B and 7.4B).

In the multivariate analysis, both BMI (OR 1.21 [95% CI 1.10, 1.33]) and age (OR 2.04 [95% CI 1.88, 2.21]) were associated with greater odds of LSS (Figure 7.6). Similarly participation in sport

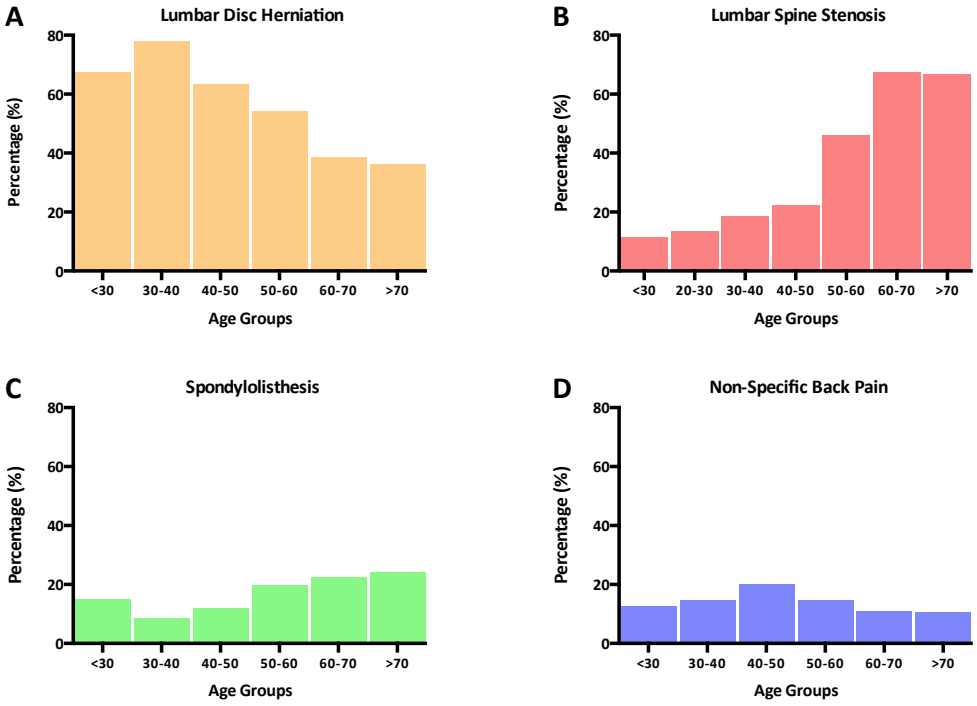


Figure 7.3: ***Prevalence of diagnoses within age groups*** *for a diagnosis of lumbar disc herniation (A), lumbar spine stenosis (B), degenerative spondylolisthesis (C), and non specific back pain (D). The y-axis scale is the same for all graphs.*

(OR 1.06 [95% CI 1.01, 1.12]) and smoking (OR 1.29 [95% CI 1.01, 1.64]) were weak but positive predictors.

7.3.5 Spondylolisthesis

The relationship between BMI and DS was not significant either in the univariate (Table 7.2) or multivariate analysis (Figure 7.7). Age (OR 1.25 [95% CI 1.14, 1.36]) and female gender (OR 1.52 [95% CI 1.19, 1.95]) were both associated with greater odds of a diagnosis of spondylolisthesis. Current smoking (OR 0.60 [95% CI 0.44, 0.81]) was a negative predictor.

7.3.6 Non-Specific Back Pain

Figure 7.8 shows the multivariate model for a diagnosis of NSBP. Age (0.88 [95% CI 0.81,0.97]) and previous spine surgery (0.75 [95% CI 0.56,0.99]) were associated with decreased odds of a diagnosis of back pain and were the only two significant predictors in this fully adjusted model. Not only were

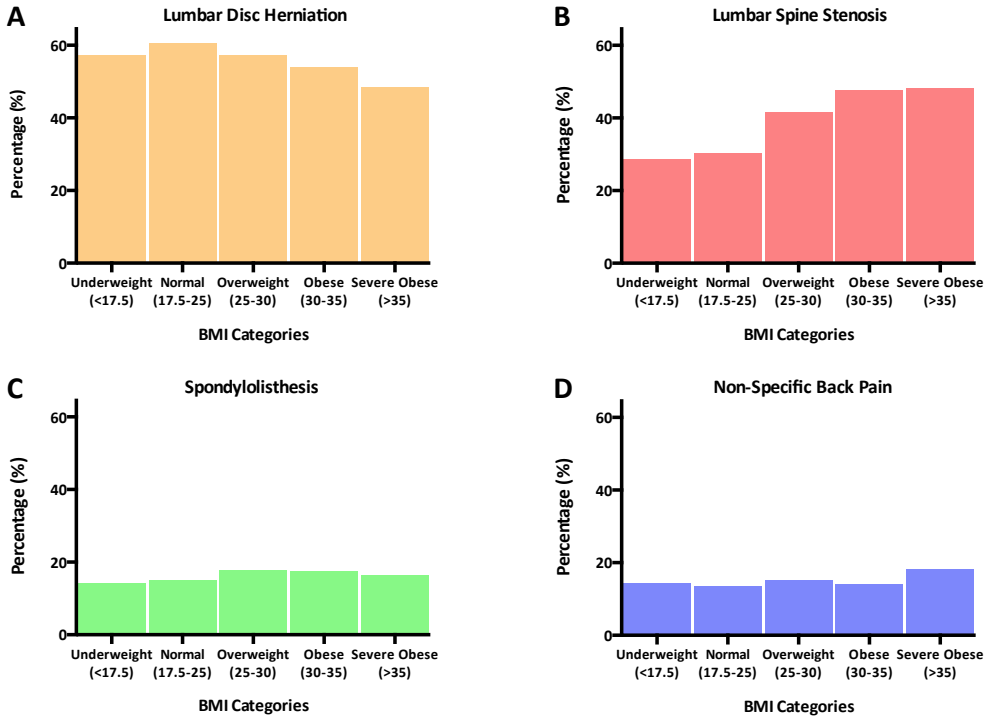


Figure 7.4: ***Prevalence of diagnoses within BMI groups*** *for a diagnosis of lumbar disc herniation (A), lumbar spine stenosis (B), degenerative spondylolisthesis (C), and non specific back pain (D). The y-axis scale is the same for all graphs.*

the other predictors non-significant, confidence intervals were considerably wider than in the other models, suggesting less precision.

	Univariate			Multivariate		
	Odds Ratio	(95% CI)	P value	Odds Ratio	(95% CI)	P value
Lumbar Disc Herniation	0.90	(0.83,0.98)	0.01	1.01	(0.93,1.11)	0.74
Lumbar Spine Stenosis	1.38	(1.27,1.51)	0.00	1.21	(1.10,1.33)	0.00
Spondylolisthesis	1.06	(0.95,1.18)	0.29	0.98	(0.87,1.10)	0.68
Non-Specific Back Pain	1.01	(0.90,1.12)	0.85	1.02	(0.91,1.15)	0.69

Table 7.2: ***Odds ratios (OR) from univariate and multivariate models for the effect of BMI upon clinical diagnoses.*** *Each OR is for a 5-unit increase in BMI. An OR greater than 1 suggests increased odds. Multivariate models were fitted to 50 multiple imputation datasets (n=2636).*

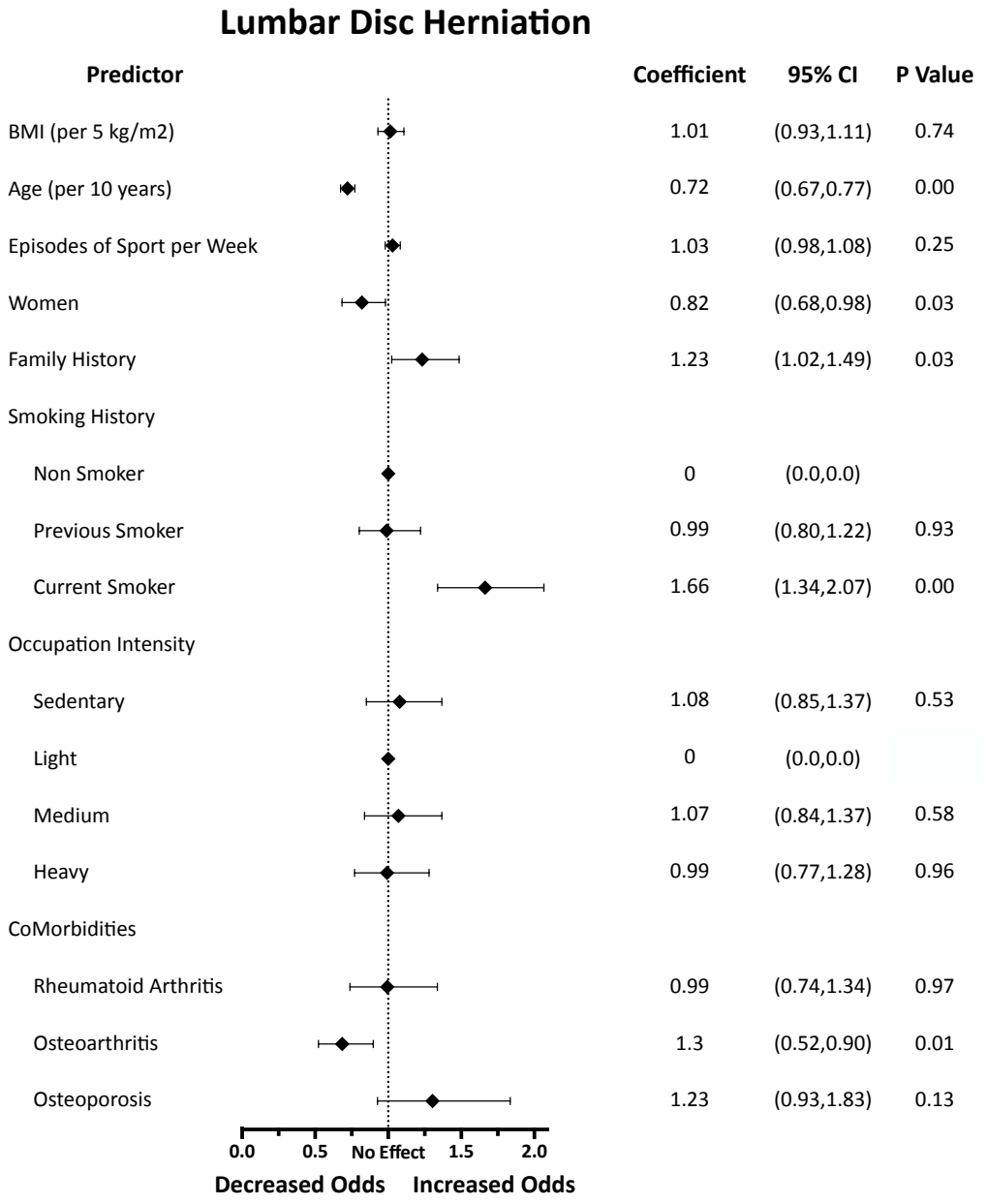


Figure 7.5: ***Confidence interval plot showing odds ratios (OR) for predictors of a diagnosis of lumbar disc herniation*** *Models fitted to 50 multiple imputation datasets (n=2636). An OR greater than one represents greater odds of lumbar disc herniation. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

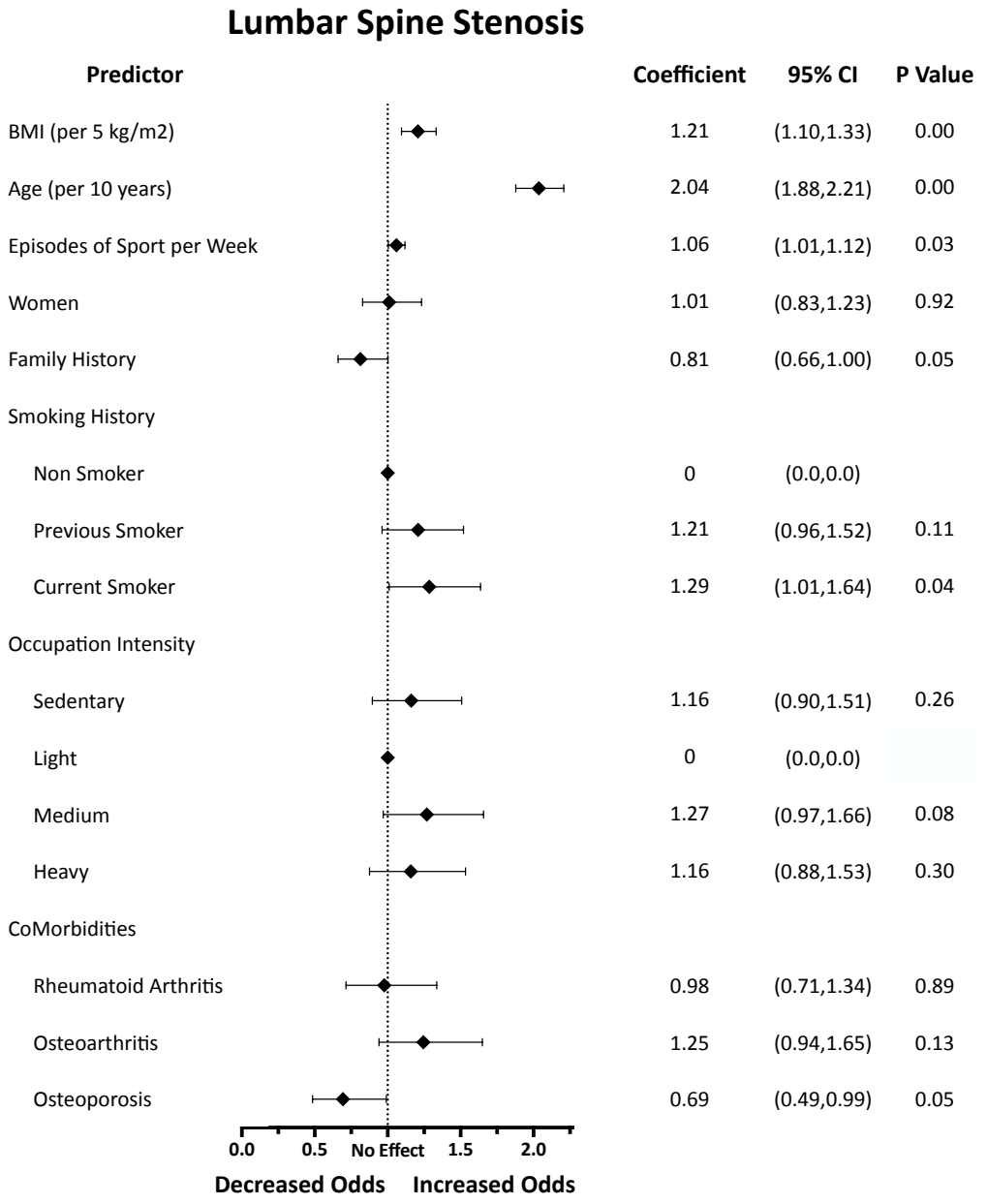


Figure 7.6: ***Confidence interval plot showing odds ratios (OR) for predictors of a diagnosis of lumbar spine stenosis*** *Models fitted to 50 multiple imputation datasets (n=2636). An OR greater than one represents greater odds of lumbar spine stenosis. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

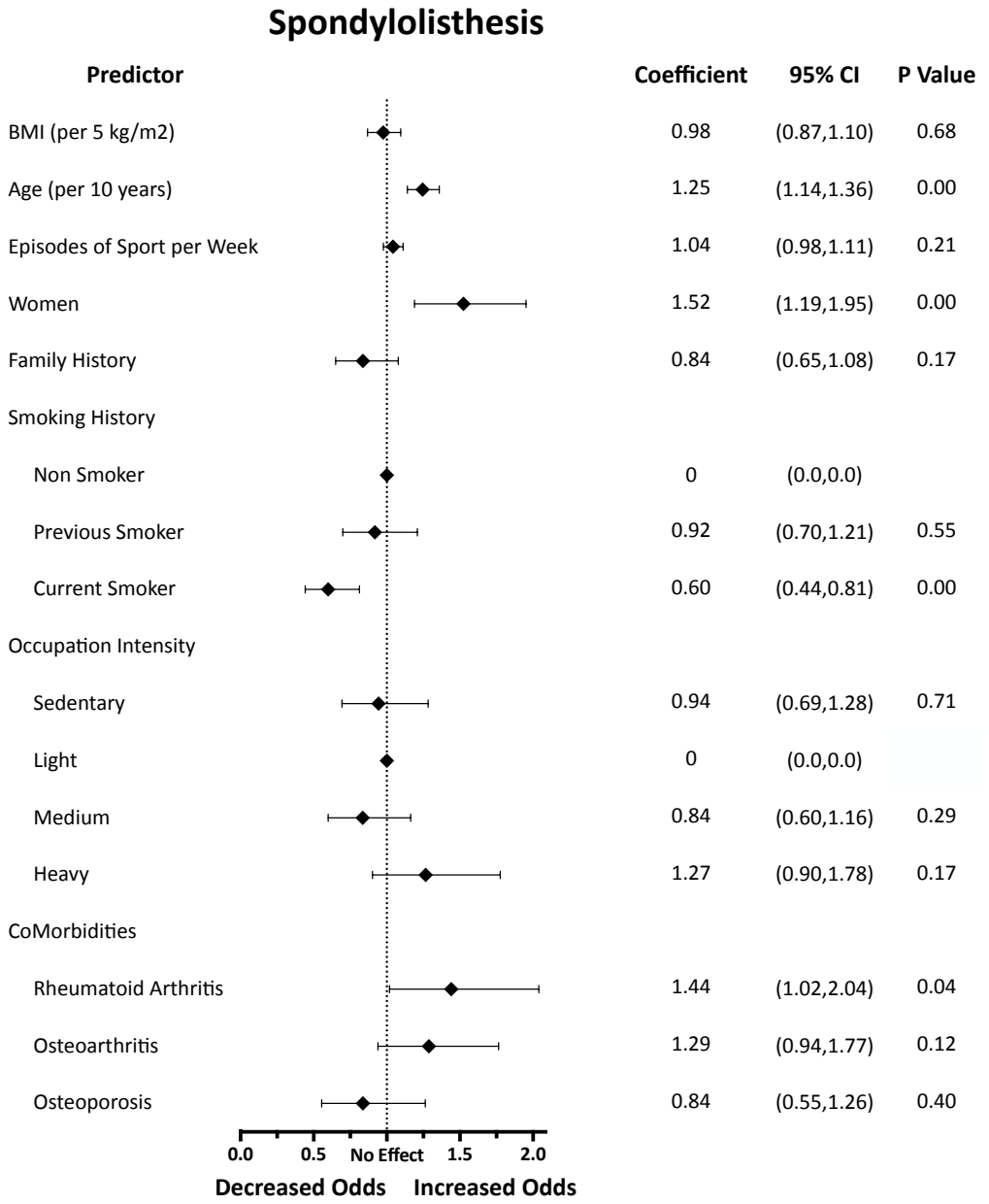


Figure 7.7: ***Confidence interval plot showing odds ratios (OR) for predictors of a diagnosis of spondylolisthesis*** *Models fitted to 50 multiple imputation datasets (n=2636). An OR greater than one represents greater odds of spondylolisthesis. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

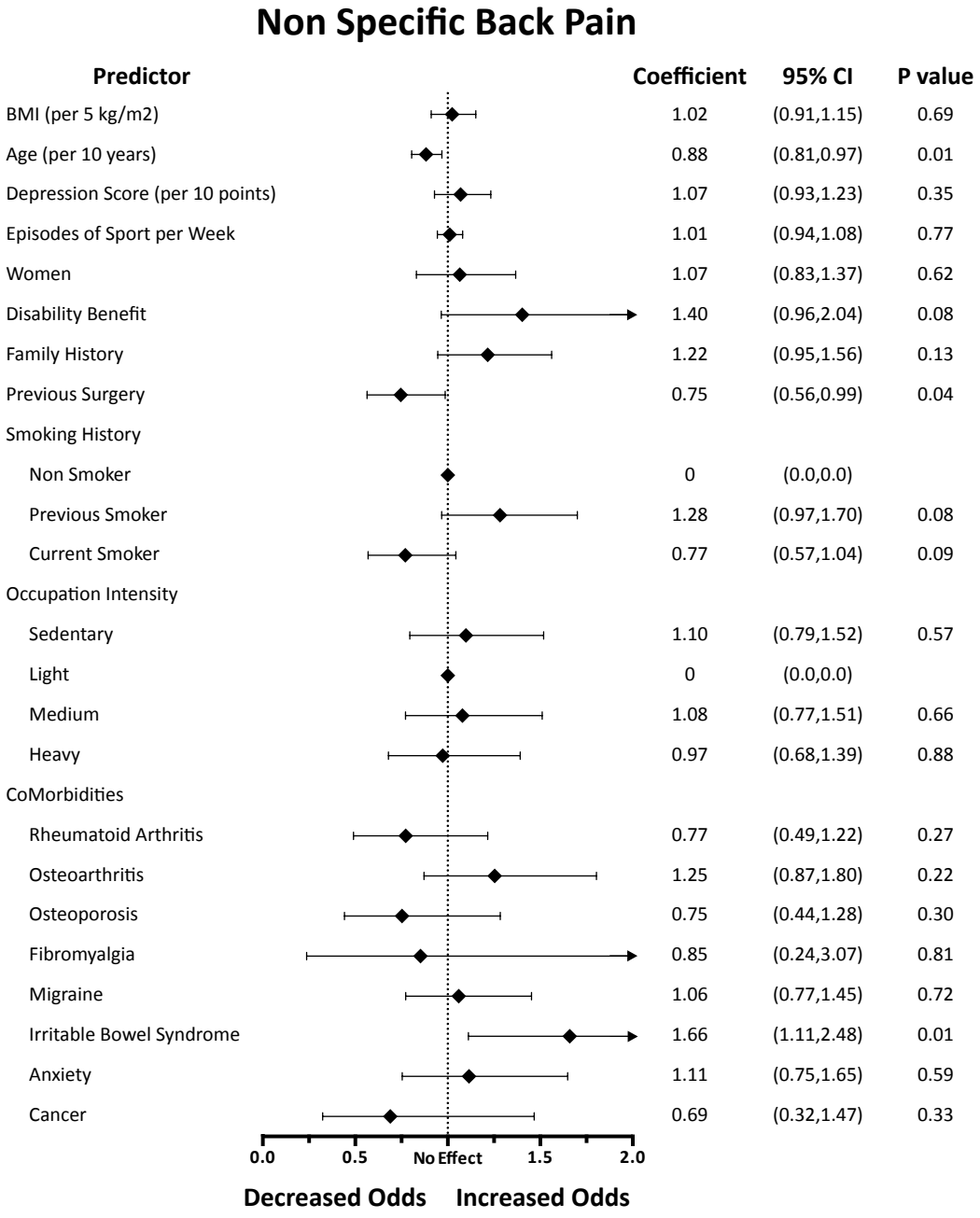


Figure 7.8: ***Confidence interval plot showing odds ratios (OR) for predictors of a diagnosis of non-specific back pain*** *Models fitted to 50 multiple imputation datasets (n=2636). An OR greater than one represents greater odds of non-specific back pain. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.*

7.4 Discussion

In this analysis, greater BMI was only associated with a diagnosis of LSS. Age was associated with greater odds of a diagnosis of LSS and spondylolisthesis, and lower odds of LDH and NSBP. Similarly, current smoking was correlated with greater odds of LDH and LSS but with lower odds of DS.

7.4.1 General

Within a populations setting, the incidence of LDH, LSS and DS has been estimated at 2.2[277]-3.3%,[278] 7.3[279]-9.3%,[28] 3.2% (symptomatic)[280]-6.3% respectively.[281] These proportions may seem large but this is likely because the authors considered both symptomatic and asymptomatic participants.[280] Furthermore, as discussed in chapter 1 the global point prevalence of BP has recently been estimated at 9.4-11.9%.[2,3]

It should be clear that these diagnoses are defined by the treating clinician who uses a combination of patient characteristics, clinical assessment and imaging. Hence, factors contributing to a diagnosis are not limited to anatomical lesions and the characterisation of the type of pain experienced is important. The influence on obesity on specific degenerative features of the spine is discussed in chapter 8.

The patient demographics are in keeping with other spinal epidemiological studies. The Spine Patient Outcomes Research Trial (SPORT) recruited 1417 patients (743 LDH, 363 LSS and 303 spondylolisthesis) and is the most comparable study to Genodisc.[29] In this population, as with Genodisc, patients with LDH were younger, healthier and had a shorter duration of symptoms. Unfortunately no information on BMI was provided.

However, unlike the SPORT study, patients in Genodisc could be allocated to one or more diagnostic categories. This is likely a more pragmatic approach as patients can have overlapping clinical diagnoses and isolating a single category can be difficult and inappropriate. For example a patient with a diagnosis of LDH, primarily causing leg pain, may have concomitant BP, over and above what would be expected with LDH and as such is allocated to both LDH and NSBP. Clinically, this can be seen with patients with LDH who fail to respond as expected to surgical discectomy.[282] Similarly,

LDH and LSS can occur jointly.[283] Hence, it is more clinically relevant not to exclusively categorise patients into a single diagnostic group.

7.4.2 Lumbar Disc Herniation

A positive relationship between obesity and LDH has been noted in previous studies. A recent study on 259 Han Chinese males found that the odds of LDH in obese was 1.98 times greater than in the non-obese group.[284] This would suggest obesity may have large influence on LDH. However, this analysis was not corrected for any confounders and the homogenous Chinese study population has limited generalisability.

Venkatesan *et al.*, in a retrospective case series, also showed a similar positive relationship between massive disc herniation, defined as a disc herniation causing cauda equina syndrome and BMI.[285] The odds of a massive disc herniation, compared to patients undergoing an elective discectomy for symptomatic LDH, was 2.2 times greater for a 5 point increase in BMI and 3.1 times higher for patients with a BMI greater than 25kg/m^2. However, as all patients in that study had a diagnosis of LDH, the authors were essentially investigating the relationship between BMI and severity of the diagnosis rather than looking specifically at predictors of LDH. Their study is further limited by the authors only adjusting for age and gender and retrospective data collection. In a similar study design, Lee and Lee showed that the BMI of women with severe LDH (managed surgically) was significantly different from those with less severe LDH (managed non-operatively).[286] However, the absolute difference in BMI was only 1 kg/$m^2$and likely not clinically relevant.

Studies comparing patients to a healthy population have shown mixed results for the relationship between obesity and LDH. In one of the earliest epidemiological studies on LDH, published by Kelsey in 1975, no difference in BMI was seen between patients and controls.[287] Conversely, in a larger study of 1129 LDH patients, Böstman showed that patients presenting to a single spinal unit had a greater BMI than that of the local population.[288] This analysis was not adjusted for confounders, rather the author reported the 99% CIs. This statistical technique is not appropriate as the finite study population would have inevitably introduced sampling bias thus limiting the generalisability of the results. Furthermore, the control population BMI was assessed two decades earlier in a

separate population study.

Heliövaara similarly showed BMI as a significant predictor of LDH in men.[278] Importantly, significance was only reached when the population was segmented by gender and into BMI categories of 2kg/m^2. Furthermore, the analysis was adjusted for body height which is already accounted for in the calculation of BMI and thus an inappropriate confounder.

Given the limitations of the current literature and the variable results it is possible that there is no relationship between BMI and a diagnosis LDH. In this analysis, although the initial univariate analysis showed a negative relationship, BMI was not a significant predictor in the adjusted multivariate model. The relationship was confounded by age, with a 10 year increase in age associated with 28% lower odds of LDH. Similarly, Venkatesan *et al.* showed a 23% lower odds of massive LDH for a 10 year increase in age.[285] As patients age, degenerative changes are superimposed upon anatomical herniation thus modifying the clinical symptoms.[289,290] These symptoms include but are not limited to back and leg pain which are in a similar pattern but more severe in LSS than LDH (Figure 7.1). Furthermore, degenerated discs are less likely to herniate.[291]

7.4.3 Lumbar Spine Stenosis

Age was the most important predictor for LSS with a 10 year increase in age associated with 2-fold greater odds of LSS. The mean age of patients with LSS was 60 years compared to 48 years for those with LDH. This is in keeping with the SPORT study[29] and the Wakayama Spine study,[28] a Japanese population study. Although the population of the Wakayama study was genetically homogenous, the authors showed the prevalence of symptomatic LSS increased with increasing age. In opposition, Hirano *et al.*, in a study on community living Japanese adults demonstrated that age was not associated with a diagnosis of LSS, most likely because the authors were able to adjust for sagittal balance which was strongly correlated with age.[292]

BMI was another significant predictor of LSS (Figure 7.6). However there is limited information about this relationship in the existing literature. The Wakayama Spine study and an analysis by Kim *et al.*, adjusted for BMI as a covariate, but did not publish the specific odds ratio. A request was made for this data but no reply was received from the authors. The only study which looked at

the association between BMI and a diagnosis of LSS was by Hirano *et al.*[292] They showed that BMI was associated with 12% greater odds of LSS, a smaller effect than the Genodisc findings. Taken together, this would suggest that BMI could be an important predictor for LSS however further work is required given the limited data.

7.4.4 Degenerative Spondylolisthesis

With respect to DS, age and female gender were two important positive predictors, smoking was the only significant negative predictor and BMI was not associated with the diagnosis. From the literature, it is known that the prevalence of spondylolisthesis is greater in females and older patients.[29,281] However the relationship between obesity and spondylolisthesis is less clear. Kalichman *et al.*, utilising a subset for the the Framingham heart Study, showed BMI was not associated with spondylolisthesis.[280] Similarly, Denard *et al.* found no relationship in men.[293]

However, Schuller *et al.* showed that patients presenting for surgical treatment of spondylolisthesis, had a significantly higher BMI than controls.[294] This finding is limited by the retrospective study design and the associated risk of control patient selection bias. Jacobsen *et al.* also showed a positive association, only in women, between the development of lumbar spondylolisthesis and index BMI, measured 17 years prior.[281] The authors used the Copenhagen Osteoarthritis Cohort and given the longitudinal nature of the data, the study carries considerable weight. However, the odds ratio were not provided, only described as ”slim” suggesting a small magnitude. Furthermore, in their multivariate logistic regression models, height, weight and BMI were included. Given that BMI is derived from the former two variables, these will be highly collinear and as such it is inappropriate to adjust for all three. Unlike Jacobsen *et al.*, the analysis presented in Figure 7.7 did not analyse males and females separately as no interaction was found between BMI and gender. Given the mixed results, it is possible that, like LDH, there is no relationship between obesity and a clinical diagnosis DS.

7.4.5 Non-Specific Back Pain

By definition, NSBP is a clinical diagnosis with an unknown cause.[33] Hence, the lack of predictors and the wide confidence intervals seen in the multivariate model (Figure 7.8) is not surprising. It is interesting that the predictors of back pain (chapter 6), a symptom, are not the same as those of the diagnosis of NSBP. The strongest positive association was seen with irritable bowel syndrome and this could be explained by a common psychological pathway.[295] Psychological factors, such as maladaptive pain coping and non-organic signs, have been shown to be important predictors of BP, especially in a chronic (greater than 12 weeks) pain.[296] Unfortunately, these factors were not assessed as part of the Genodisc study.

Another striking feature of NSBP was the considerable overlap in diagnosis with 51% of patients diagnosed with NSBP sharing one or more other diagnoses. This would suggests these patients have a mixture of symptoms part of which fit a clear clinical picture and others which the treating clinician cannot attribute to a specific diagnostic group and are unexplained.

7.4.6 Strengths of this Analysis

As discussed in chapter 6, the main strength of this analysis is the comprehensive dataset which allowed for correction of a number of clinically relevant confounders. This is also one of larger studies with a heterogeneous and thus generalisable population.

7.4.7 Limitations of this Analysis

The most important consideration is the diagnoses were not mutually exclusive and a patient could have more than one as evidenced in Figure 7.2. Allowing clinicians the opportunity to categorise patients as clinically appropriate is a pragmatic approach and generalisable to clinical practice. Patients with multiple diagnoses were present across all recruitment sites suggesting that this is neither a site nor a clinician specific practice but rather highlights patient complexity. However, an idealist could argue to the contrary.

A familial predisposition may influence a persons risk for developing certain spinal conditions

during their lifetime,[297] and hence family history was included as a confounder. However, the survey question may have caused confusion by asking about a family history of the ”same condition”. This could be problematic if patients are not aware of their specific diagnosis or diagnoses.

7.5 Conclusion

This analysis provides evidence that obesity is associated with a diagnosis of LSS but not LDH, DS, or NSBP. Information of other predictors of these diagnoses are also shown. Within a clinical setting, this information will allow a clearer understanding and differentiation of complex spine patients.

Key Points

- Patients with a diagnosis of lumbar disc herniation or non specific back pain were younger and healthier than those with a diagnosis of lumbar spine stenosis or degenerative spondylolisthesis.
- A large proportion of patients have more than one diagnosis, suggesting heterogeneity in symptoms and presentation.
- Only lumbar spine stenosis was associated with BMI, however age was the strongest predictor of this diagnosis.
- As a diagnosis, non specific back pain was nebulous but importantly different to back pain as a symptom.

8

Obesity and Pathological Features on Lumbar Spine MRI

This analysis builds on chapters 6 and 7, working to identify the relationship between obesity and underlying anatomical changes. Changes, which can contribute to pain and a clinical phenotype/diagnosis. Degenerative features of interest are intervertebral disc degeneration, disc herniation and stenosis. For these analyses, the spine is considered as a whole and dichotomised into upper and lower segments. Increased BMI was associated with disc degeneration, spine stenosis and disc herniation on MRI.

8.1 Introduction

Obesity can contribute to back and leg pain but the literature linking obesity to degenerative features on the lumbar spine is limited, especially at a population level. Advancing this understanding is important as it may have implications on prevention and management of spinal degeneration. Furthermore, a link at a population level will add clinical support to the findings of chapter 3, where a potential biochemical link between obesity and disc degeneration was described.

8.2 Analysis Plan

8.2.1 Hypothesis

It is predicted that obesity will be independently related to features of degeneration in the lumbar spine. These are disc degeneration, disc herniation and spinal stenosis.

8.2.2 Population

Of the total Genodisc population of 2636, 558 MRIs were of poor quality and 394 patients were excluded because of incomplete questionnaires resulting in a population for analysis of 1684. Multiple imputation was not used for this analysis as the missing questionnaire data was only 19% unlike in previous analysis where missingness was up to 50%.

8.2.3 MRI Data

MRIs were obtained as discussed in chapter 5. A single experienced radiologist read all MRIs in an anonymous and blinded fashion. A total of 121 random MRIs were double read by the same radiologist to assess intra-observer reliability. The kappa statistic was used to assess reliability for dichotomous variables and Lin's concordance coefficient for ordinal variables. The reliability analysis was performed by a collaborator (Prof Michele Crites-Battie, University of Alberta, Canada). Generally, reliability was greater than 0.70 and thus acceptable. Exceptions were Modic I & II changes

and endplate sclerosis (Table 8.1). A likely explanation is the low prevalence of these features and these estimates are in keeping with the literature.[298]

Variable	Reliability	(95% CI)
Ordinal		
Disc Degeneration Rating	0.91	(0.89,0.93)
Disc Herniation	0.81	(0.73,0.89)
Associated nerve root compression (DH)	0.82	(0.74,0.91)
Central canal stenosis	0.66	(0.47,0.86)
Upper Endplate defect	0.66	(0.55,0.78)
Lower Endplate defect	0.75	(0.66,0.84)
Dichotomous		
Any stenosis	0.75	(0.66,0.83)
Upper endplate defect	0.65	(0.56,0.75)
Lower endplate defect	0.69	(0.60,0.77)
Upper Modic I Changes	0.71	(0.59,0.83)
Lower Modic I Changes	0.76	(0.66,0.87)
Upper Modic II Changes	0.72	(0.63,0.81)
Lower Modic II Changes	0.66	(0.56,0.76)
Upper Modic III Changes	0.43	(0.25,0.61)
Lower Modic III Changes	0.32	(0.12,0.52)
Upper Vertebral Endplate Sclerosis	0.42	(0.14,0.71)
Lower Vertebral Endplate Sclerosis	0.57	(0.29,0.84)

Table 8.1: ***Intra-observer reliability estimates of MRI data*** *Kappa coefficient was used for dichotomous variables and Lin's concordance coefficient for ordinal variables. Confidence intervals were obtained using standard errors from nonparametric bootstrapping, with 1000 replications, clustered on person to account for any correlation between the six disc ratings per person. Analysis performed by Prof Michele Crites-Battie, University of Alberta, Canada.*

8.2.4 Outcome

Intervertebral Disc Degeneration

Disc degeneration was scored using the commonly utilised Pfirrmann rating system, which accounts for disc height, structure, signal intensity and nucleus pulposus-annulus fibrosus distinction.[55] The score ranges from grade 1, which represents a homogenous disc taken as non-degenerate, with bright signal intensity and normal height, to grade 5, which is a collapsed hypointense disc with no

distinction between the nucleus pulposus and annulus fibrosis (Figure 8.1). Disc narrowing was not considered as a separate variable as the Pfirrmann score encompasses this feature.

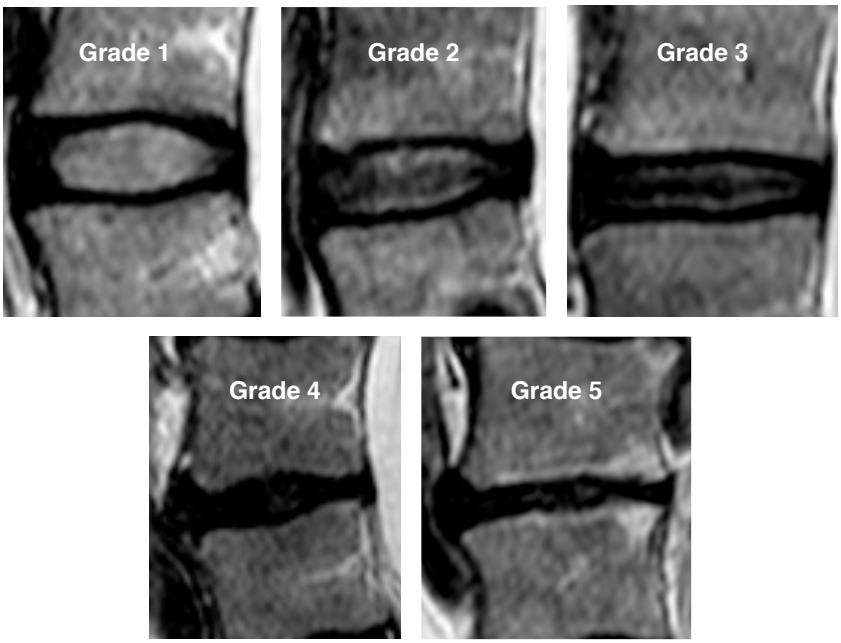


Figure 8.1: ***Disc Degeneration Grading Scale (Pfirrmann)*** *A normal disc (Grade 1) appears as a homogenous bright white disc with preserved height. With progressive degeneration there is loss of disc signal, inhomogeneity, and collapsing disc space. Modic changes are visible on the Grade 5 disc but this is strictly not part of the Pfirrmann grading system.*

To obtain an overall assessment of degeneration the score was averaged over all six, upper three and lower three levels. The upper and lower lumbar spine were considered separately as degeneration in these different segments may represent a specific phenotype.[9] Another approach to handling the degenerative score is to sum the variable across all levels.[9] To ensure appropriate data handling, a sensitivity analysis was performed using a summed degenerative score with theoretical maximum of 30 (over 6 levels).

Linear regression was used to model the relationship between the degenerative score and BMI. The score was also dichotomised to create three indicator variables and logistic regression was used to model this relationship. The indicator variables created were severe (grade 5) degeneration at any level, multiple levels of degeneration (grade 3,4 or 5), and multiple levels of severe (grade 5) degeneration.

Disc Herniation

Disc herniation is defined as the extrusion of disc material into the spinal canal. It was classified by size and this ordinal variable was dichotomised to either disc greater than 2mm or 10mm. Multivariate logistic regression was then used to model the relationship between BMI and herniation greater than:

- 2mm at any level,
- 2mm at the upper 3 levels,
- 2mm at the lower 3 levels,
- 2mm at multiple levels,
- 10mm at any level, and
- 2mm with associated nerve root compression.

Spinal Stenosis

Spinal stenosis occurs when there is reduced space within the spinal canal and anatomically, this is caused by a combination of disc herniation, ligament hypertrophy and facet joint hypertrophy.[27,297,299] Central spinal stenosis was classified qualitatively by severity (absent, mild, moderate and severe). In the analysis, stenosis was dichotomised and two indicator variables were created. These were any stenosis (mild, moderate or severe) and severe only. Logistic regression was then used to analyse the relationship between BMI and spinal stenosis at:

- any level (severe stenosis analysed separately),
- upper 3 levels,
- lower 3 levels, and
- multiple levels.

8.2.5 Exposure

BMI was the predictor of interest and considered continuous.

8.2.6 Confounders

Clinical findings

As this analysis did not account for pain, only biologically plausible confounders such as age, sporting activity, gender, family history, smoking history,[300] occupation intensity,[301] and comorbidities (RA, OA and OP) were included. Other comorbidities, such as fibromyalgia, which primarily contribute to pain were not.

End Plate Changes

The end plate is critically important to the intervertebral disc as it provides an important pathway for disc nutrition and compromise can lead to matrix degradation, cell death and disc degeneration.[46] In this study, the term end plate changes encompass three pathologies: Modic changes, end plate defects and end plate sclerosis. End plate changes are not specific to disease but have been associated with spinal degeneration and hence are important confounders.

Modic Changes Modic changes are defined as end plate and bone marrow changes visible on MRI.[302] These changes have been shown to be present in up to 27.4% of patients with back pain,[303] and are associated with disc herniation.[304] There are three types of Modic changes, described in Table 8.2 and Figure 8.2.[302] Modic changes are thought to represent a cascade with acute inflammation (Type I) followed by fatty replacement and granulation (Type 2). At this stage, the Type 2 changes may stabilise or progress to sclerosis (Type 3).[303]

End Plate Defects End plate defects or Schmorl's nodes are herniations of the nucleus pulposus into the vertebral end plates that may be associated with disc degeneration.[305] An example of a large defect is shown in Figure 8.2.

End Plate Sclerosis Sclerosis is defined as increased bone mineral density and when this occurs in the end plate it can compromise intervertebral disc nutrition[45] and it has been associated with disc degeneration.[306,307]

Modic Type	MRI Feature		Pathology
	T1 images	T2 images	
Modic I	hypointense	hyperintense	Bony oedema
Modic II	hyperintense	isointense or hyperintense	Fatty replacement of bone marrow
Modic III/mixed	hypointense	hypointense	Bony Sclerosis

Table 8.2: ***Summary of Modic changes***[302]

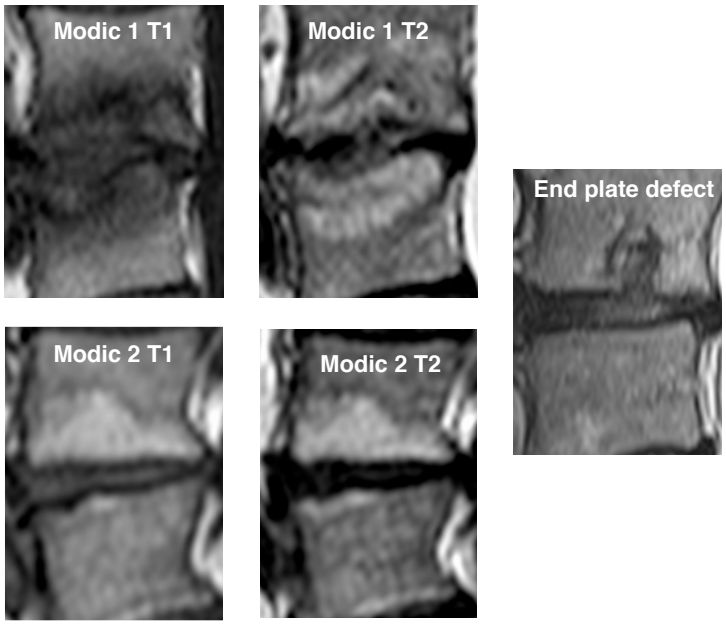


Figure 8.2: ***End plate changes*** *Modic 1 changes are hypointense on T1, hyperintense on T2 and represent bony oedemea. Modic 2 changes represent fatty replacement and are hyperintense on T1 and hyper- or isointense on T2. End plate defect is a defect in either the superior or inferior end plate with subsequent disc herniation.*

Inclusion in Models For the purposes of the model, all end plate changes were considered as binary variables, present or absent. All end plate changes were included in the model for intervertebral disc degeneration and only Modic changes for the herniation and stenosis models.

8.3 Results

8.3.1 General Findings

Figure 8.3 is a schematic showing the increasing prevalence of end plate features (blue) and disc herniation (purple) at the more caudal spinal levels. Similarly, the mean degeneration score (orange) is greater at the lower spinal levels.

The coefficients and odds ratios below relate to a 5kg/m^2or a 10 year difference BMI and age respectively.

8.3.2 Disc Degeneration

In the univariate analysis, BMI was a positive predictor of degeneration associated with 0.14 unit (95% CI 0.10,0.18) increase in degeneration (Figure 8.6). Age was also a positive predictor, a 10 year increase in age was associated with a 0.19 (95% CI 0.18,0.20) unit increase in degeneration. The fit of this relationship was striking with a R-squared of 0.44 compared to 0.03 for the relationship with BMI (Figure 8.5).

These findings persisted in the multivariate linear model however the effect of BMI was less and clinically insignificant (0.04 units) and that of age was larger (0.31 units) (Figure 8.7 and Table 8.3). Furthermore, all the end plate features (Modic, sclerosis and defects) were positive but less precise (wider CI) predictors of degeneration. The sensitivity analysis, using a summed degenerative score, produced regression coefficients of a similar magnitude to the mean degenerative score.

Table 8.3 summarises the findings for age and BMI when the upper and lower lumbar spine were considered separately. Age was still the most important positive predictor for both and of greater magnitude in the upper three levels 0.35 units per 10 years compared to 0.25 units in the lower

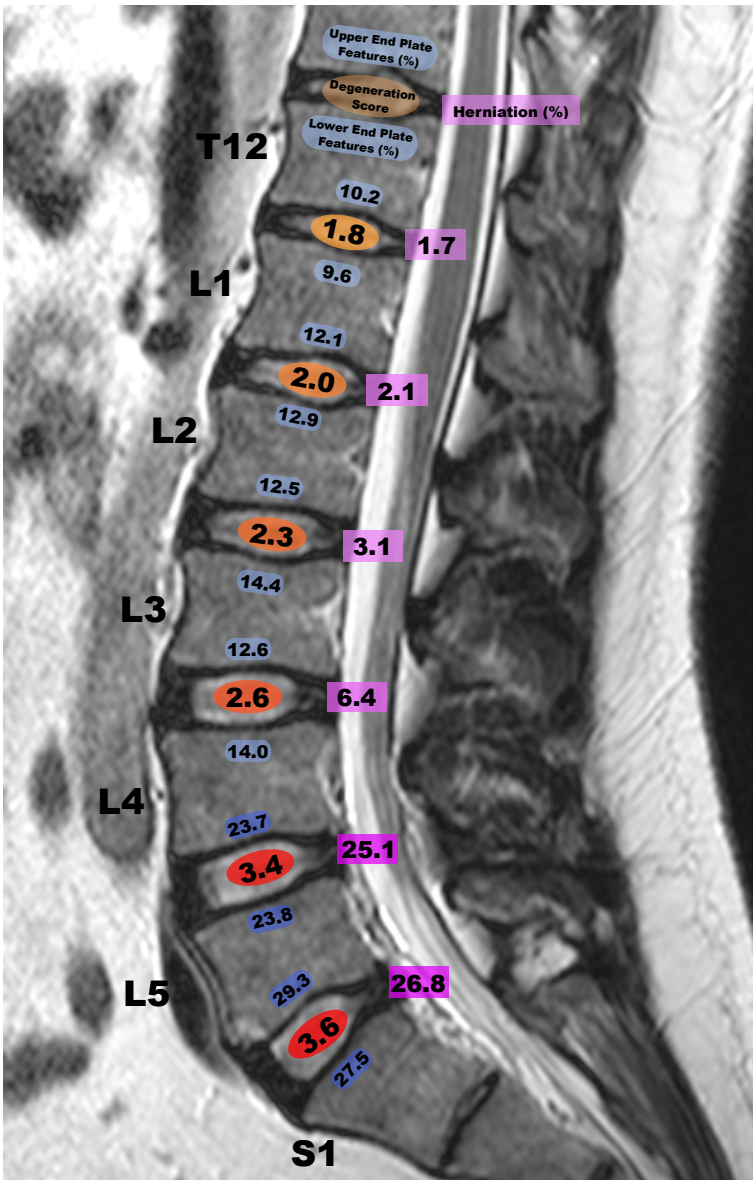


Figure 8.3: ***Schematic representing prevalence of MRI features at each spinal level*** *Blue boxes represent the prevalence of any upper or lower end plate changes, pink boxes is the prevalence of disc herniation >2.1mm and orange is the mean degeneration score (0-5).*

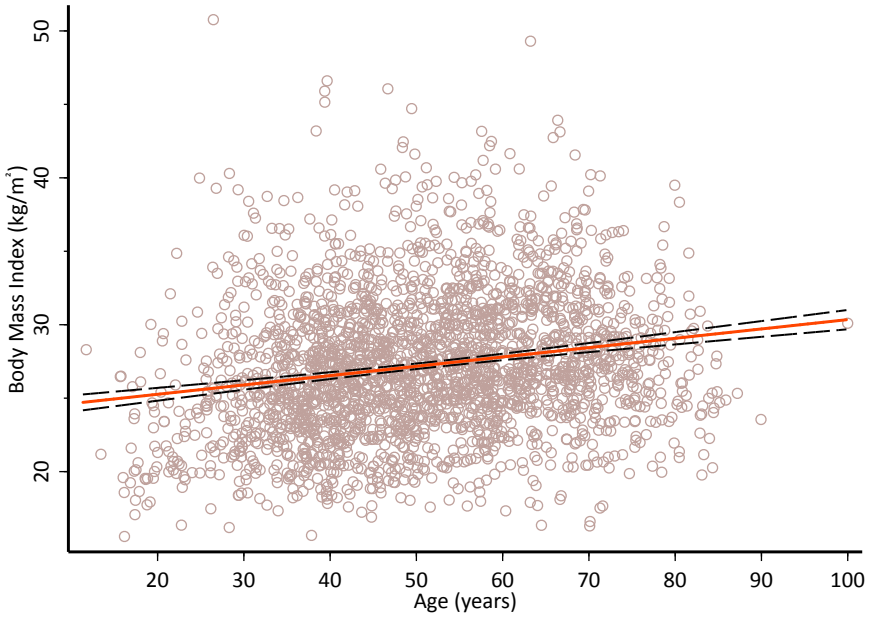


Figure 8.4: ***Scatterplot illustrating the relationship between body mass index and age*** *Red line represents the univariate linear regression line and dotted black lines the 95% confidence intervals.*

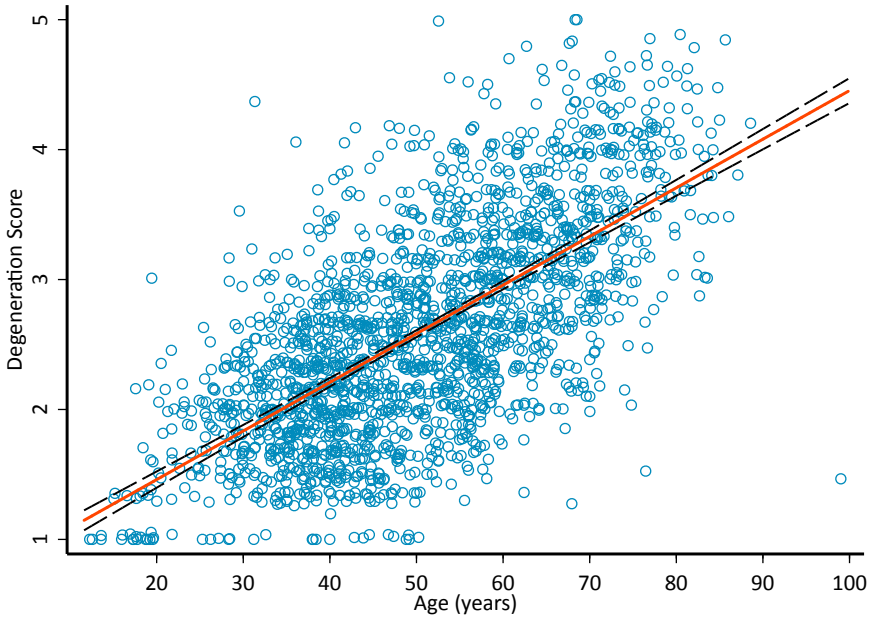


Figure 8.5: ***Scatterplot illustrating the relationship between age and disc degeneration*** *Red line represents the univariate linear regression line and dotted black lines the 95% confidence intervals.*

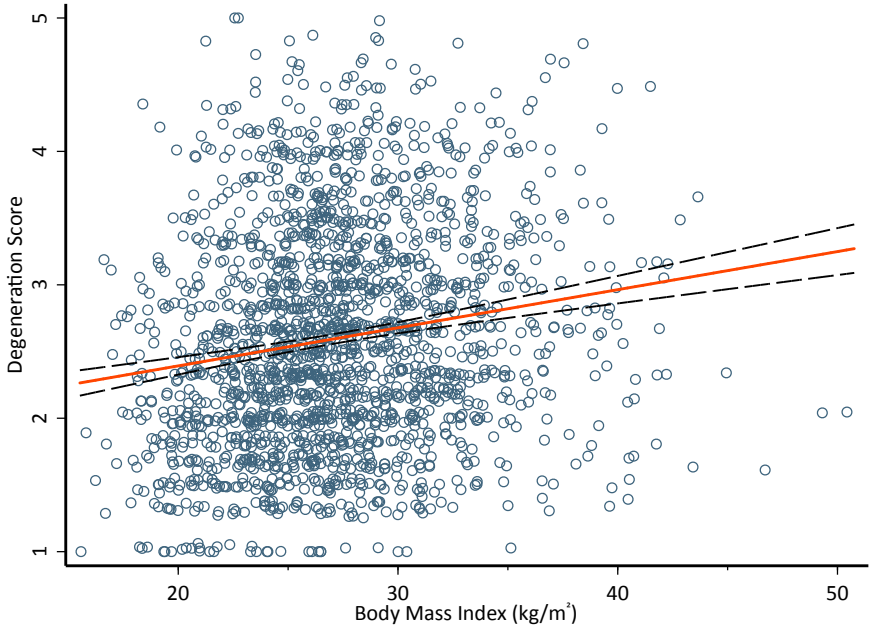


Figure 8.6: ***Scatterplot illustrating the relationship between body mass index and disc degeneration*** *Red line represents the univariate linear regression line and dotted black lines the 95% confidence intervals.*

spine. On the other hand, BMI was only significant in the lower lumbar spine albeit with a very small effect size (0.04 units [95% CI 0.00,0.08]). For the upper three levels, the effect of BMI was confounded by age.

When degeneration was considered as a binary variable, age remained as the most important predictor increasing the odds of multilevel degeneration (OR 1.37, [95% CI 1.24,1.50]), multilevel severe degeneration (OR 1.98, [95% CI 1.73,2.26]) and severe (grade 5) degeneration (OR 2.10, [95% CI 1.79,2.47]). For all these outcomes, the effect of BMI was non-significant and confounded by age.

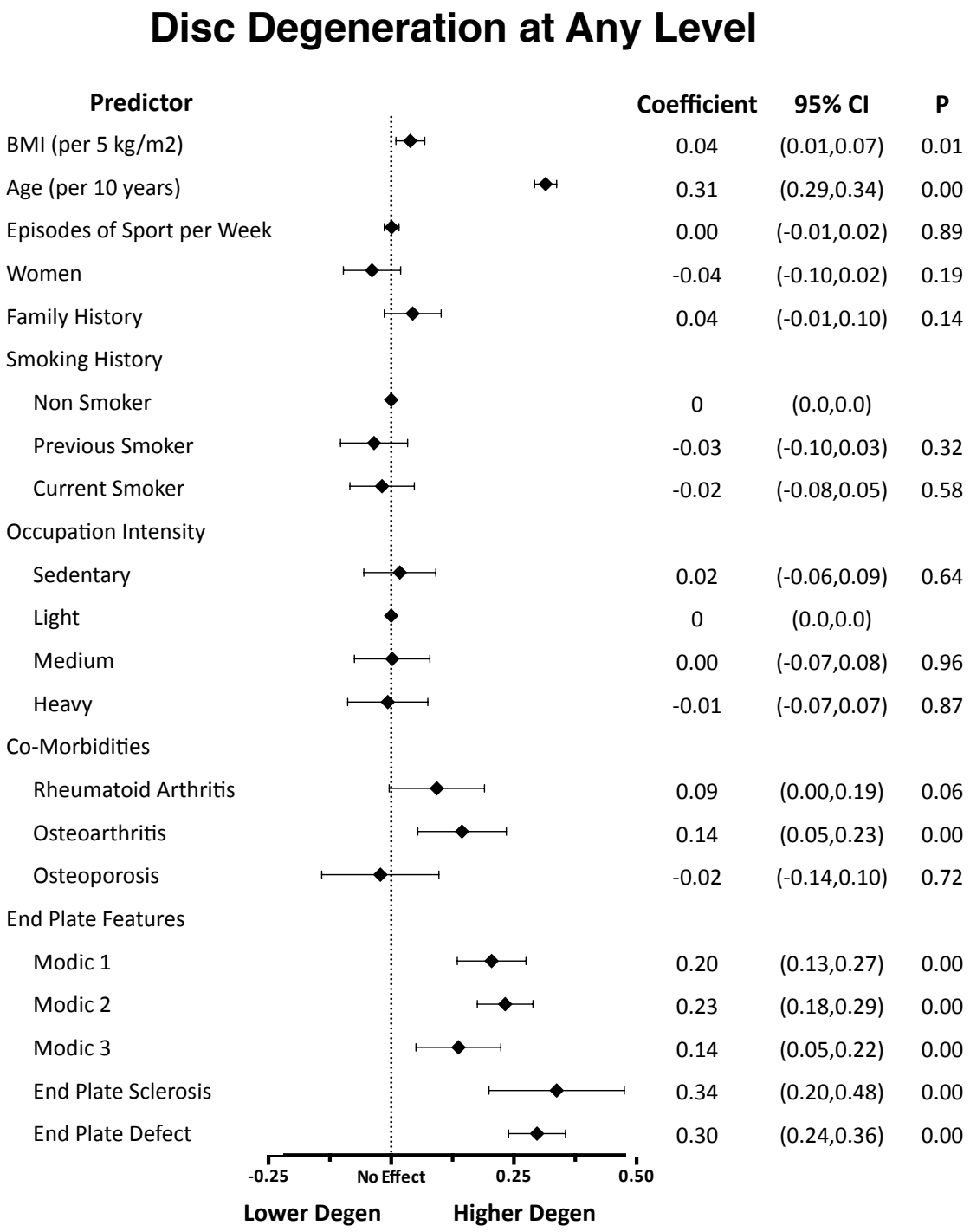


Figure 8.7: ***Confidence interval plot showing regression coefficients for predictors of disc degeneration***
A positive coefficient represents greater disc degeneration. The solid diamond is the effect and errors bars are the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.

Disc Degeneration Over:	Descriptive	BMI (per 5 kg/m^2)			Age (per 10 years)		
	mean (SD)	Coefficient	(95% CI)	P value	Coefficient	(95% CI)	P value
All 6 Levels	2.6 (0.8)	0.04	(0.01,0.07)	0.01	0.31	(0.29,0.34)	0.00
Upper 3 Levels	2.0 (1.0)	0.02†	(-0.02,0.06)	0.26	0.35	(0.32,0.39)	0.00
Lower 3 Levels	3.2 (0.8)	0.04	(0.00,0.08)	0.03	0.25	(0.23,0.28)	0.00

Table 8.3: ***Coefficients from multivariate linear regression models showing the relationship between BMI or age and intervertebral disc degeneration.*** *Each coefficients is for a 5-unit increase in BMI or a 10-unit increase in age. A coefficient greater than 0 suggests greater degeneration. †The relationship between BMI and disc degeneration was significant in univariate analysis but was confounded by age in the multivariate model. Each line represents a different outcome variable fitted to a model with the same covariates as in Figure 8.7 (n=1684)*

Disc Degeneration At:	Descriptive	BMI (per 5 kg/m^2)			Age (per 10 years)		
	n (%)	Odds Ratio	(95% CI)	P value	Odds Ratio	(95% CI)	P value
Any Level Severe (Grade 5)	646 (38%)	1.06†	(0.94,1.20)	0.36	1.37	(1.24,1.50)	0.00
Multiple Levels (Grade 3, 4 or 5)	1386 (82%)	1.12†	(0.96,1.30)	0.15	1.98	(1.73,2.26)	0.00
Multiple Levels Severe (Grade 5)	181 (11%)	1.09†	(0.90,1.32)	0.38	2.10	(1.79,2.47)	0.00

Table 8.4: ***Odds ratios from multivariate logistic regression models showing the relationship between BMI or age and intervertebral disc degeneration*** *Each OR is for a 5-unit increase in BMI or a 10-unit increase in age. An OR greater than 1 suggests increased odds of degeneration.†The relationship between BMI and disc degeneration was significant in univariate analysis but was confounded by age in the multivariate model. Each line represents a different outcome variable fitted to a model with the same covariates as in Figure 8.7 (n=1684)*

8.3.3 Disc Herniation

Figure 8.8 describes the prevalence of MRI diagnosed disc herniation within the Genodisc population. Not only was the prevalence of DH greater at the lower spine levels (Figure 8.3), so was the proportion of larger DHs (Figure 8.8A) and the severity of nerve root compression (Figure 8.8B). L4-5 and L5-S1 showed a similar prevalence of DH. In contrast to degeneration, there was a lower proportion of DH with greater BMI and, more markedly, with older age (Figure 8.8C&D).

In univariate assessment, with DH at any level as the outcome, BMI was not a significant predictor (OR 1.09 [95% CI 0.99,1.20]) whereas age was associated with 29% lower odds of DH (OR 0.71 [95% CI 0.66,0.76]) (Table 8.5). However, in the multivariate model (Figure 8.9), the relationship between BMI and DH became significant with an odds ratio of 1.19 (95% CI 1.07,1.33). Age was the primary confounder of this relationship with addition of this variable resulting in a significant relationship between DH and BMI.

The same confounding effect of age was also seen when the outcome variable was DH at the lower three levels, DH greater than 10mm at any level and DH at any level with nerve root compression. Contrary to this pattern, herniation in the upper lumbar spine showed the strongest relationship to BMI, which was significant in both univariate and multivariate analysis (OR 1.39 [95% CI 1.10,1.77]) and was not related to age.

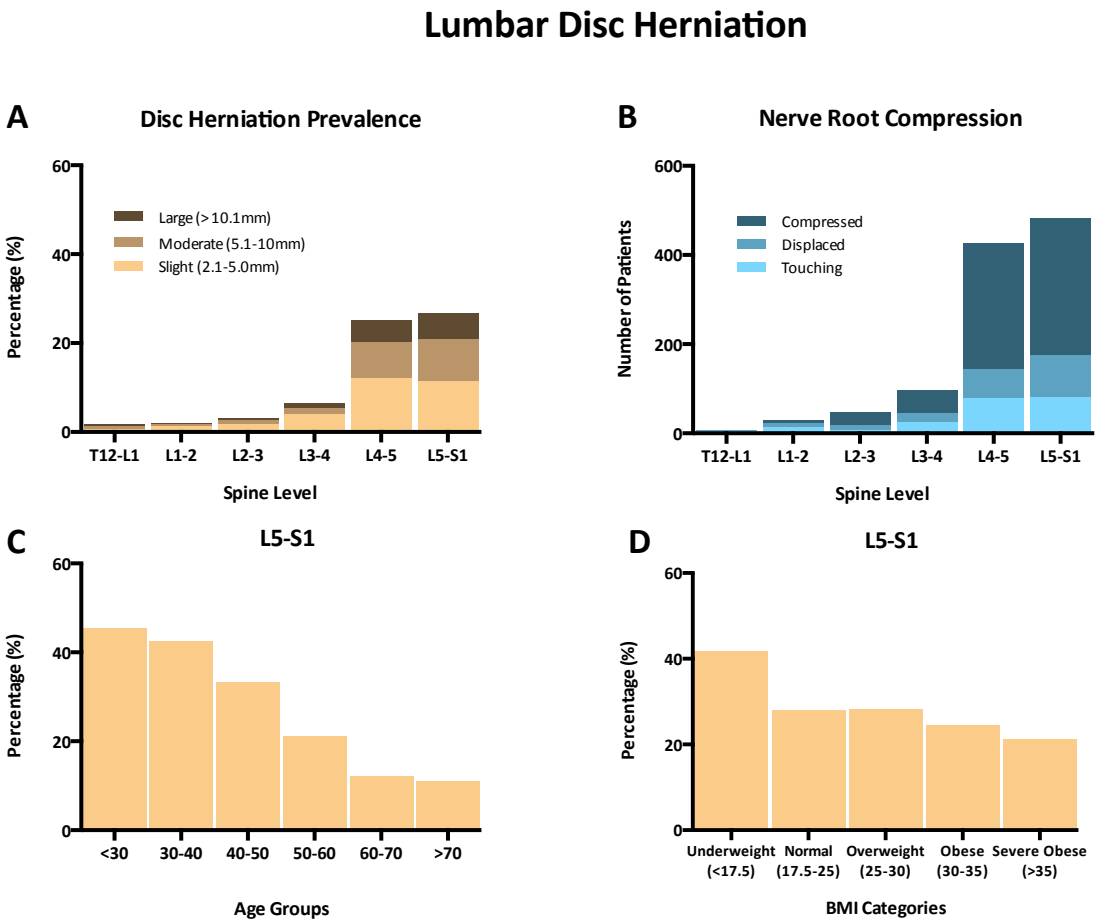


Figure 8.8: ***Prevalence of disc herniation on MRI*** *A: Size of disc herniation at all spinal levels. Bars are stacked. B: Severity of nerve root compression at all spinal levels. Bars are stacked. C: Prevalence of disc herniation (>2mm) by age groups. D:Prevalence of disc herniation (>2mm) by BMI groups.*

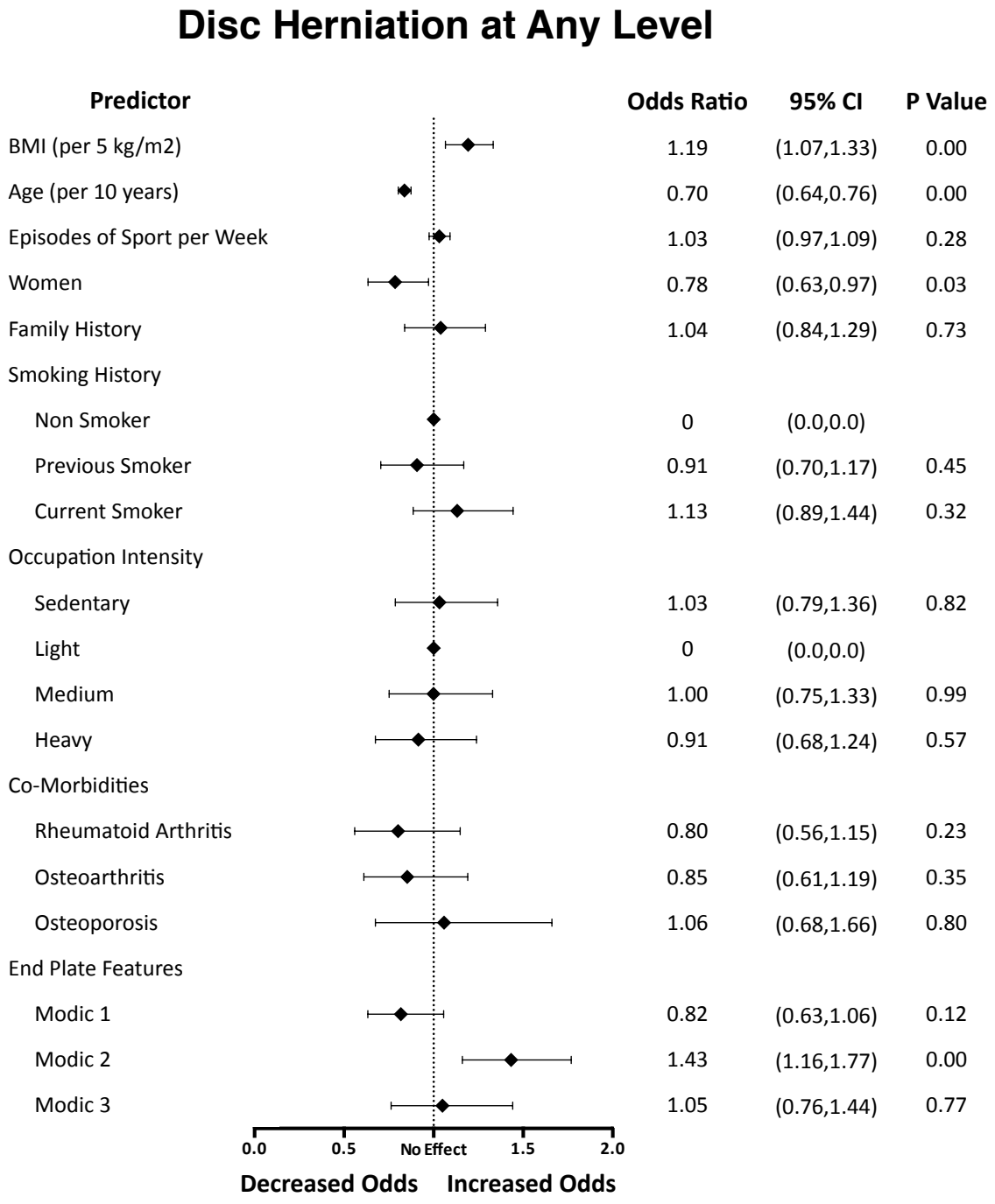


Figure 8.9: ***Confidence interval plot showing odds ratios for predictors of lumbar disc herniation on MRI***
An OR greater than one represents greater odds of disc herniation. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.

Disc Herniation at:	Descriptive	BMI (per 5 kg/m^2)			Age (per 10 years)		
	n (%)	Odds Ratio	(95% CI)	P value	Odds Ratio	(95% CI)	P value
Any Level (>2mm)	900 (53%)	1.19‡	(1.07,1.33)	0.00	0.70	(0.64,0.76)	0.00
Multiple Levels (>2mm)	186 (11%)	1.14	(0.97,1.35)	0.11	0.76	(0.66,0.87)	0.00
Upper 3 Levels (>2mm)	81 (5%)	1.39	(1.10,1.77)	0.01	1.17	(0.98,0.46)	0.07
Lower 3 Levels (>2mm)	860 (51%)	1.15‡	(1.03,1.29)	0.01	0.67	(0.61,0.73)	0.00
Any level, severe (>10mm)	258 (12%)	1.13‡	(1.01,1.27)	0.03	0.76	(0.69,0.83)	0.00
Any Level (>2mm) with nerve root compression	560 (33%)	1.16‡	(1.04,1.30)	0.01	0.73	(0.67,0.79)	0.00

Table 8.5: ***Odds ratios from multivariate logistic regression models showing the relationship between BMI or age and disc herniation*** *ORs are for a 5-unit increase in BMI or a 10-unit increase in age. An OR greater than 1 suggests greater odds of a herniated disc. ‡The relationship between BMI and disc herniation was not significant in univariate analysis but was confounded by age in the multivariate model and thus became a significant predictor. Each line represents a different outcome variable fitted to a model with the same covariates as in Figure 8.9 (n=1684)*

8.3.4 Lumbar Stenosis

The prevalence of stenosis increased with more caudal levels, but unlike degeneration and DH, peaked at L4-L5 with 17% of patients (Figure 8.10A). Furthermore, with increasing age, there was a higher proportion of stenosis, with 52% those greater than 70 years exhibiting reduced spinal canal space (Figure 8.10C). With respect to BMI, there was a general trend from normal to obese for increase in stenosis. In the underweight group, 25% of patients had stenosis on MRI, comparable to 23% in the overweight group and 27% in the obese group (Figure 8.10D). Furthermore, the proportion of multilevel stenosis peaked in the overweight group with 13% of patients exhibiting stenosis at two or more levels. Surprisingly, none of the patients the underweight group had stenosis at more than one level (Figure 8.10B) despite 17% of these patients exhibiting severe stenosis, the largest for all the BMI groups (Figure 8.10D).

Both BMI, OR 1.24 (95% CI [1.07,1.44]) and age, OR 2.56 (95% CI [2.25,2.92]) were the only two positive predictors of stenosis on MRI (Figure 8.11). Table 8.6 summaries the pertinent findings from the other multivariate models. Age was the strongest positive predictor of stenosis, except in multilevel stenosis, where age was associated with 22% lower odds (OR 0.78 [95% CI 0.69,0.89]). Similarly, BMI was associated with greater stenosis but of a generally lower magnitude than age. The largest relationship for BMI was with stenosis in the upper three lumbar levels (OR 1.65 [95% CI 1.27,2.16]). Finally, BMI was not a significant predictor of severe stenosis (OR 1.11 [95% CI 0.92,1.35]) with this relationship confounded by age.

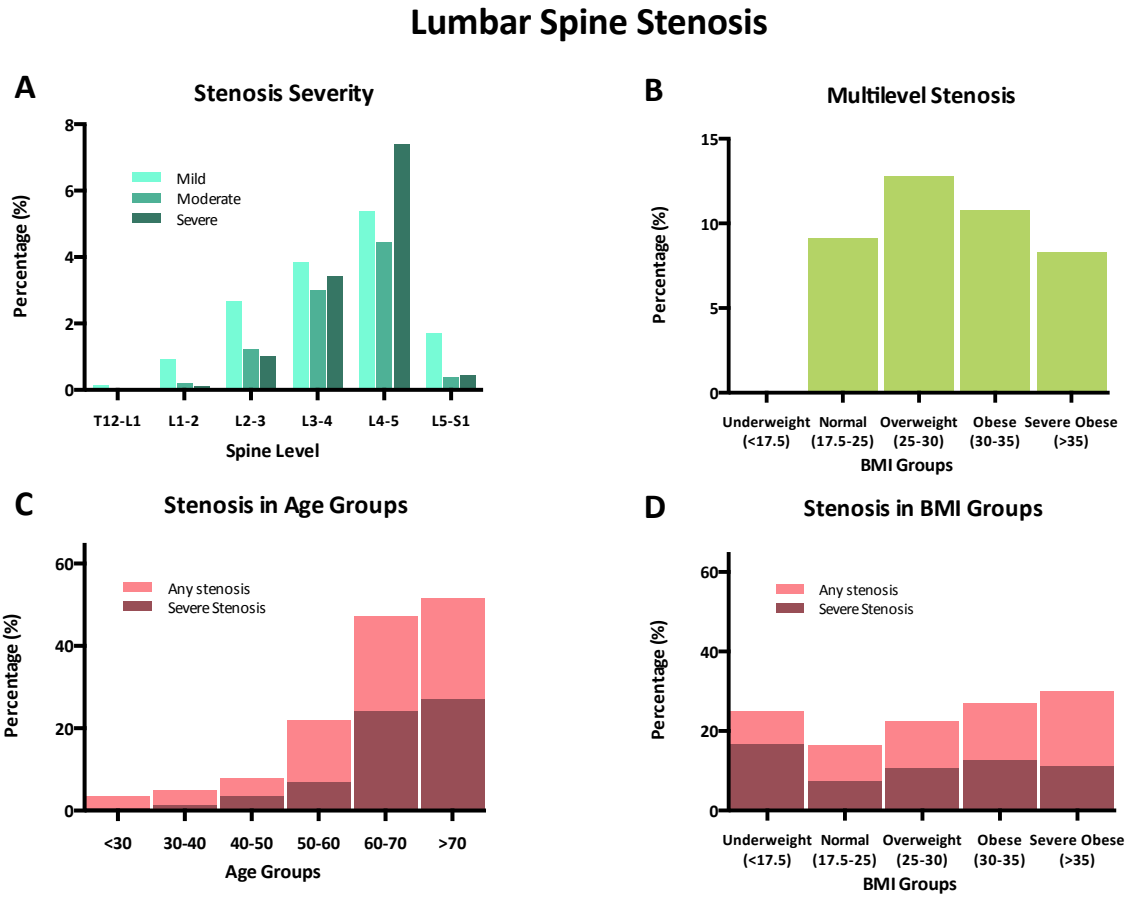


Figure 8.10: ***Prevalence of spinal stenosis on MRI*** *A:Severity of spinal stenosis at all spinal levels. B: Prevalence of multilevel spinal stenosis by BMI categories. C: Prevalence of any spinal stenosis by age groups. Severe stenosis is superimposed on stenosis of any severity. D.Prevalence of any spinal stenosis of any severity by BMI groups.Severe stenosis is superimposed on stenosis of any severity. Percentage refers to percent of patients within an x-axis category displaying stenosis*

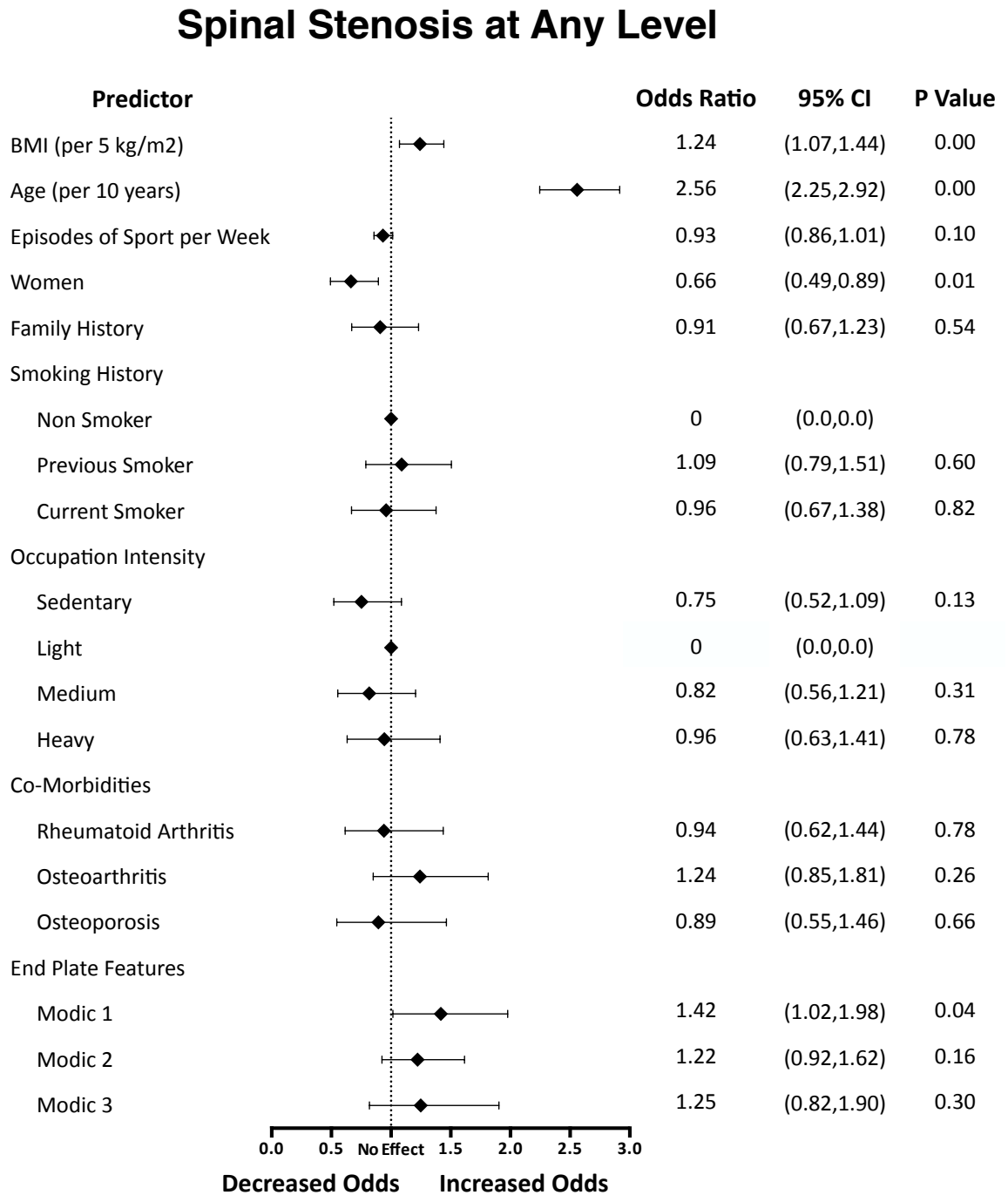


Figure 8.11: ***Confidence interval plot showing odds ratios for predictors of lumbar spine stenosis on MRI***
An OR greater than one represents greater odds of spinal stenosis. The solid diamond represents the effect and the error bars the 95% confidence interval. If the confidence interval does not cross the "No Effect" dotted line the predictor is significant.

Spinal Stenosis at:	Descriptive	BMI (per 5 kg/m^2)			Age (per 10 years)		
	n (%)	Odds Ratio	(95% CI)	P value	Odds Ratio	(95% CI)	P value
Any Level	354 (21%)	1.24	(1.07,1.44)	0.01	2.56	(2.25,2.92)	0.00
Multiple Levels	186 (11%)	1.18	(1.00,1.39)	0.05	0.78	(0.69,0.89)	0.00
Upper 3 Levels	79 (5%)	1.65	(1.27,2.16)	0.00	3.19	(2.45,4.15)	0.00
Lower 3 Levels	340 (20%)	1.24	(1.07,1.44)	0.00	2.54	(2.24,2.90)	0.00
Any Level (Severe)	161 (20%)	1.11†	(0.92,1.35)	0.28	2.55	(2.16,3.02)	0.00

Table 8.6: ***Odds ratios from multivariate logistic regression models showing the relationship between BMI or age and lumbar spine stenosis.*** *Each OR is for a 5-unit increase in BMI or a 10-unit increase in age. An OR greater than 1 suggests greater odds of stenosis. †The relationship between BMI and stenosis was significant in univariate analysis but was confounded by age in the multivariate model. Each line represents a different outcome variable fitted to a model with the same covariates as in Figure 8.11 (n=1684)*

8.4 Discussion

This analysis presents many interesting findings. Firstly, degeneration progressively worsened with age. The prevalence of DH and stenosis was greatest at more caudal spinal levels. In the multivariate models, age was an important predictor for all three features, positive for degeneration and stenosis and negative for DH. Greater BMI was also associated with greater disc degeneration, odds of DH and odds of stenosis, when the respective feature was considered present at any spinal level. In certain cases, age and BMI shared an interesting interrelationship with age confounding the association with BMI, removing significance for outcomes of degeneration and stenosis, and adding it for DH. It was also surprising that other predictors such as work intensity, smoking and comorbidities showed no relationship in this large patient group.

8.4.1 Disc Degeneration

The pattern of disc degeneration is clearly defined in the lumbar spine and the results shown here are in keeping with those of Cheung *et al.*,[9] Takatalo *et al.*,[135] and the Wakayama Spine Study.[136] However, literature surrounding disc degeneration and obesity is varied, there are few large MRI based studies, and many of the reports have not identified the confounding interrelationship between age and BMI. This analysis has shown that when degeneration was considered continuous, the effect of BMI although statistically significant was marginal and unlikely to be clinically relevant. Furthermore, BMI was not related to end stage or multilevel degeneration.

In the largest MRI study to date, Samartzis *et al.* showed that BMI was associated with the presence of degeneration, number of degenerated levels and severe degeneration.[82] They also found a similar relationship between degeneration and age with a R-squared of 0.40 in univariate analysis but the odds ratio for age in the multivariate model was not provided. Similarly, the Wakayama spine study found a significant association between age, BMI and degeneration.[136] These results are supported by a smaller Japanese study, which also showed a significant relationship between degeneration, age and BMI.[308] However, in a study of 200 healthy Japanese volunteers, Kanayama *et al.* did not show a relationship between degeneration and BMI but found age to be the primary

contributor.[309] A general caveat about generalisability of these four studies is required because these results arise from the respective homogenous Southern Chinese and Japanese populations.

Liuke *et al.*, in a longitudinal MRI based study, found that sustained BMI greater than 25 kg/m^2 over a 4 year period was associated with 4.3 times greater odds of disc degeneration.[85] They also found that BMI greater than 25kg/m^2 in the young (less than 25 years old) was associated with 3.8 times greater odds of degeneration, no relationship was seen for those over 40. This could be due to the confounding effect of age similar to that seen in the Genodisc population (Table 8.3). It can also be due to the relatively crude method used to score degeneration, a disc was classified as degenerate simply if the nucleus pulposus signal intensity was less than that of cerebrospinal fluid (CSF). Another longitudinal study, using the Finnish Birth cohort, showed morbid obesity was associated with greater odds of disc degeneration.[310] This study was surprising as the authors showed that even at 21 years old, 54% of their cohort had some form of disc degeneration and 17% were severely degenerate.

The use of computer tomography (CT) and plain images have also been used to assess the relationship between obesity and disc degeneration. As a surrogate for direct disc assessment, which is limited by the poor soft tissue visualisation on CT, authors have used disc height or narrowing. Disc height is part of the grading system used in this analysis (Pfirrmann grading)[55] and reduced disc height is correlated with features of degeneration.[311] Kalichiman *et al.* showed that although there was a strong relationship between age and degeneration, the association between disc height and BMI was not significant.[312] Hassett *et al.* analysed the lumbar spine radiographs from the Chingford study and found that after a nine year interval, age but not BMI predicted progression of disc space narrowing and other degenerative features.[313] However, Urquhart *et al.* recently showed that obesity may be associated with reduced disc height especially at L1-2 and L3-4.[133]

In contrast, some authors believe obesity to be protective of disc degeneration.[86,314] Videman *et al.*, using 44 monozygotic twins, showed the heavier sibling had significantly higher disc signal variation but not disc height. However, the magnitude of difference in signal variation was only 5% between twins and when fully adjusted the significance of this association was lost. The authors also showed the potential ”protective” effect of greater body mass was only apparent when the difference in weight between siblings weight was less than 18kg.[314] Although this was an interesting

finding, it further complicates the understanding of obesity and disc degeneration.

All the studies discussed above were conducted on healthy individuals whereas the Genodisc study was focussed on spinal patients presenting to tertiary spinal centres. Irrespective, it would appear age is the primary contributor to disc degeneration for both patients and the population. The relationship between BMI and disc degeneration is less clear. It is possible that obesity or BMI is an inappropriate measure of body fat or adiposity. Takatalo *et al.* showed truncal obesity, measured by abdominal and sagittal diameter on MRI, and body fat percentage, were associated with greater disc degeneration in males.[84] This could be explained by increased local adipokines contributing to degeneration (chapter 3).

It is disappointing that obesity is not a major contributor to disc degeneration as weight loss could be an intervention to prevent or hasten disc degeneration, certainly reversing ageing is not a simple solution. Nevertheless, a promising result was described by Lidar *et al.* who showed a mean restoration of 2mm disc height at L4-5 after a 13kg/m^2 reduction in BMI. Although this is a small change in disc height, which could be a result of measurement error, it is an encouraging finding.

Finally, the association between degeneration and end plate changes requires a brief discussion. In this analysis, degeneration was significantly associated with all end plate changes albeit with wide confidence intervals. In the Genodisc Study, the prevalence of end plate changes is greatest at the lower spinal levels similar to that found by Albert and colleagues.[303] The authors also showed that of all spine pathologies, degeneration at L4-L5 and L5-S1 had the strongest association with both Modic I and II changes. Although the progression of Modic changes can be predicted, the inciting processes are not known. Certain authors have shown degeneration to be associated with the development of Modic changes, predominantly type I and given the strong association it is possible that the development of Modic changes are a biomechanical phenomenon.[315] Furthermore, as Modic II changes thought to be fatty deposits and associated with obesity[316] it is possible these may serve a source of adipokines initiating and potentiating disc degeneration and pain.

8.4.2 Disc Herniation

In contrast to degeneration, BMI was associated with greater odds of DH whereas increasing age was associated with lower odds. These relationships were present for all outcome variables including DH at any level, upper or lower three levels, with nerve root compression, and severe herniation.

The largest study looking at DH was published by Jhawar et el.[317] Using the Nurses' Health Study, the authors showed an increased risk of developing a DH with a linear increase in BMI. Given the large sample size and longitudinal data collection, this study carries considerable strength. However, cardiovascular risk factors were adjusted for in their multivariate model and given the collinearity between obesity and these risk factors, it may have attenuated the magnitude of effect of BMI, similar to that seen in chapter 6.

The upper and lower lumbar spine, although anatomically similar, appear to have different risk factors for DH as shown by Saberi *et al.*[318] The authors studied 514 patients with back pain and found increasing age to only be associated with herniation only in the upper lumbar spine. These results were supported by Dammers and Koehler who demonstrated that the mean age of patients with DH at the upper lumbar levels was significantly greater than at L5-S1.[319] This analysis above shows a non-significant trend for greater odds of DH in the upper lumbar spine with an increase in age (Table 8.3). However, with all the other DH outcome variables, age was a negative predictor and of greatest magnitude in the lower lumbar spine. Recently, Ma *et al.* also showed that DH tends to decrease with increasing age.[320] Additionally, patients with DH in both the SPORT study and Hong Kong Spine cohort were younger.[9,29] These results suggest a biological explanation. Disc degeneration is strongly correlated with ageing and the process of degeneration results in loss of disc proteoglycan, desiccation and reduction in disc height which leaves little material to physically herniate.[15]

Modic changes have also been associated with disc herniation. Albert *et al.* noted a longitudinal relationship between DH and the development of Modic 1 changes.[304] In the Genodisc study, patients with a MRI diagnosed herniation suffered back pain symptoms for a mean of 23 months. Initially, the end plate changes could have been Modic I but with the chronicity of the lesion, these converted to a more stable Modic II and hence a significant association is seen.

8.4.3 Lumbar Stenosis

On imaging, there are three pathological changes, which contribute to spinal stenosis. These are disc degeneration with loss of disc height and bulging, ligamentum flavum thickening/buckling and facet joint hypertrophy which together produce circumferential narrowing of the spinal canal.[30,297] Given the contribution of disc degeneration to its development, it is not surprising that stenosis is associated with ageing. The data from the Genodisc population shows a clear increase in prevalence as well as greater odds of stenosis (for all outcome variables except for multilevel stenosis) with increasing age.

The Wakayama spine study, the largest radiographic study of spinal stenosis, showed a clear age-dependent increase in radiographic stenosis.[30] Furthermore, a recent study by Gandhi *et al.*, showed that patients with severe stenosis were generally older, but the authors did not clearly define the imaging modality used.[321] Similarly, Kalichman found a relationship between ageing and mild stenosis (<12mm) but not severe stenosis (<10mm).[279] The significance of this relationship was lost in a model adjusted for BMI and gender. Furthermore, the small sample size (15 stenosis patients out of 187) and arbitrary cut off for stenosis may have limited the analysis.[312] BMI was an important predictor for stenosis whether at any level, at the upper three or the lower lumbar levels. The Wakayama study supports these results in only men.[30]

8.4.4 General

The effect of BMI upon both DH and stenosis was of greatest magnitude in upper lumbar spine. Using cluster analysis, two separate research groups described a possible upper lumbar degenerative pattern with Cheung hypothesising a non-mechanical cause given this part of the spine is subject to less mechanical load.[9,322] Biological studies also support a distinct upper lumbar phenotype.[323] From the clinical literature, is unclear if BMI influences the upper lumbar spine via biomechanical or biochemical factors. Battie showed that heavy workload and thus greater loading was the only significant predictor of upper spine degeneration.[301] However, workload was not a determinant in any of the models described here and given the results of the *in vitro* study (chapter 3), a biochemical link may exist. Another possible link to an upper lumbar phenotype is age. DH in the upper spine

was the only DH variable that had a positive but non-significant trend with age. Furthermore, age had the largest magnitude of effect with stenosis in the upper spine. This finding is supported by Werndle *et al.* who showed that in patients older than 65 years, DH was more common in the upper lumbar spine.[324]

The exact mechanisms underlying a link observed between obesity and the MRI features discussed here are unknown. It is possible that adipokines, as described in chapter 3 could mediate this relationship. Adipokines could also act upon the annulus to initiate failure and promote herniation. In addition to degeneration, leptin has been implicated in posterior longitudinal ligament hypertrophy which contributes to spinal stenosis.[325] However, biomechanical factors are also possible.[326]

The association between BMI and degeneration, DH or stenosis at multiple levels was not significant, possibly because the underlying cause multilevel disease is genetic. For example, an aggrecan gene with a short variable number of tandem repeats has been implicated in both severe and multilevel disc degeneration but not multilevel DH.[327] Similarly, a vitamin D allele (Tt) has been associated with severe and multilevel disc degeneration and multilevel herniation.[328] The collagen-9A3 (COL9A3) gene polymorphism is also inovolved in multilevel disc degeneration and bulging. The authors also found a synergistic and interactive effect between BMI and the COL9A3.[329] Battie *et al.* also recently showed central lumbar stenosis was highly genetic with a heritability estimate, the percentage of phenotypic variation resulting from genetic factors, of 64%.[297] Genetic analyses of the Genodisc patients are still pending.

Another similarity from all analyses was that neither smoking nor work intensity were predictors of MRI features. This was intriguing because both current smoking and heavy workload were associated with back and leg pain (chapter 6) and smoking but not workload was associated with a diagnosis of LDH and LSS (chapter 7). Taken together, this suggests smoking and workload are possible drivers of pain which contribue to a diagnosis. Liuke *et al.* and the Chingford study showed that smoking and occupation were not associated with degeneration.[85,313] Furthermore, the relationship between smoking and chronic pain or back pain is well documented[330,331] and the causal mechanism suggested by Goldberg *et al.* could be due to underlying neuropathic processes or a systemic pro-inflammatory state[332] rather than degeneration.[333] Smoking could also influence mental

state and thus pain,[330] however the analysis in chapter 6 showed both smoking and depression were independently associated with pain.

Finally, there are clear gender differences with female gender associated with lower odds of anatomical herniation and stenosis but greater levels of back and leg pain (chapter 6). This discordance could indicate gender differences in pain sensitivity or pain processing. Using the pain sensitivity questionnaire, Kim *et al.* recently showed that women with spinal stenosis had higher sensitivity even after adjusting for spinal degeneration.[334]

8.4.5 Strengths of this Analysis

This analysis adds to the current literature by providing information on predictors of MRI defined spinal degeneration in patients. To date, the larger studies have been population or community based,[28,82,136] or used a less precise imaging modality[313] and have been focused primarily on disc degeneration.[82,136,313] The analysis also adds information on different patterns of degeneration, stenosis and DH.

8.4.6 Limitations of this Analysis

The most important caveat with this analysis relates to the MRI reading, as all scans were read by a single radiologist. However, the assessment was performed in a random, anonymised fashion and independent of the primary study investigators. The radiologist was a specialist musculoskeletal radiologist with over 30 years of clinical experience, followed a standardised protocol when assessing the scans and his intra-observer reliability was within accepted limits.

8.5 Conclusion

From this analysis, BMI is an important predictor of spinal stenosis and disc herniation. For disc degeneration, age was the most important factor and although increasing BMI was associated with greater disc degeneration this is unlikely to be clinically relevant. The results further support an

upper spinal phenotype. Finally, given the link between obesity, adipokines are a potential contributing factor.

Key Points

- Obesity was associated with disc degeneration, disc herniation, and spinal stenosis on MRI, however the association with degeneration is unlikely to be clinically relevant.

- Age was the strongest positive predictor of degeneration and stenosis and a negative predictor of herniation.

- Neither BMI nor age were important predictors of multiple level degeneration raising the question of a genetic pathogenesis.

- The upper lumbar phenotype requires further investigation as the results of this study support those from previous investigators.

9
General Discussion

9.1 Summary of Findings

This thesis provides a greater understanding of the effects of obesity upon the lumbar spine. This relationship was initially explored at a cellular level in an *in vitro* study described in chapter 3. This was followed by an *ex vivo* clinical study in chapter 4 which was designed to validate the *in vitro* results. Finally, a detailed epidemiological analysis was performed to further delineate the association between obesity and multiple indicators of lumbar spine pathology. These included pain, clinical diagnoses and degeneration quantified on MR imaging.

Leptin is a potential mediator of disc degeneration

The first objective of this thesis was to understand the biochemical effects of leptin, an adipokine, upon the IVD. Using a bovine IVD model, it was shown that leptin can increase the production and expression of proteases and pro-inflammatory cytokines (chapter 3). Within a pro-inflammatory milieu, especially in the presence of IL-6, these effects were amplified along with the production of pain-generating molecules such as lactate and NO. These findings support a hypothesis that leptin could mediate a biochemical link between obesity and intervertebral disc degeneration.

Local adipokines, plasma cytokines and potential biomarkers

The next objective was to investigate if adipokines and cytokines from local adipose tissue were related to symptoms in spine patients (chapter 4). No local cytokines, including leptin and pro-inflammatory mediators, were clearly related to symptoms or specific clinical diagnoses. However, the adipose tissue from patients was not taken from sites adjacent to the disc and hence before discounting a possible relationship, further investigations with appropriate controls and adipose samples from sites adjacent to the disc are required.

Results here also found that plasma cytokines, particularly IL-6 and TNF-α, though not adipokines, were associated with higher pain levels and spinal stenosis. This further indicates that IL-6 plays an important role in disc degeneration (chapter 3) and back pain. Further detailed analysis of the plasma proteins revealed that clusterin and complement proteins, may have an important role in

spine patients and could be potential biomarkers.

Obesity is related to back and leg pain

The thesis also aimed to understand the role of obesity in the lumbar spine at an epidemiological level in patients with spinal conditions. The Genodisc population, a large heterogenous patient population, was utilised. The first analysis (chapter 6) showed that obesity was related to both back and leg pain. However, the magnitude relationship was small. Other important predictors of back and leg pain such as female gender, rheumatoid arthritis, previous surgery, and depression were also identified in this population.

Obesity is associated with a diagnosis of lumbar spine stenosis

The second analysis was performed to assess the relationship between obesity and clinical diagnoses (chapter 7). From this study, it was found that there was considerable overlap in clinical diagnoses. This demonstrates the heterogeneity in patient presentation and highlights the difficulty in clinical assessment. Obesity was found to be an important predictor of a clinical diagnosis of lumbar spinal stenosis but not of lumbar disc herniation, degenerative spondylolisthesis or non-specific back pain.

Obesity is a predictor of spinal stenosis and disc herniation on MRI

Finally, the role of obesity in MRI-defined degeneration was investigated (chapter 8). For disc degeneration, obesity was a positive predictor but of small magnitude. This finding does not negate obesity as a predictor, but rather results from the finding that as disc degeneration is primarily a factor of ageing and this overshadows other effects. Increased BMI was however associated with presence of herniated discs and spinal stenosis which may indicate a mismatch between pathology on MRI and clinical diagnoses. Furthermore, obesity was identified as a potentially important mediator of an upper lumbar spine phenotype for herniation and stenosis.[9,322]

9.2 Clinical Application of this Research

The clinical implications of this thesis are threefold. Firstly, therapeutic opportunities for intervention will arise if the role of leptin in induction and progression of disc degeneration is validated by more comprehensive studies. Secondly, the results here, though only on a small cohort of patients, suggest identification of specific serum biomarkers for LSS and LBP is possible and these could be an aid to diagnosis and to following the progress of interventions. Moreover, as pro-inflammatory cytokines seem to be associated in particular with LSS and given that exogenous administered pro-inflammatory molecules are already utilised in the spine, treatment with appropriate cytokine blockers could be a promising strategy for the management of LSS patients and pain.[23–25,75] Finally, the epidemiological findings provide clinicians with a greater understanding of spine patients, the drivers of their pain, predictors of a clinical diagnosis and associations with degeneration.

9.3 Future Work and Further Questions

In addition to adding to the literature, this thesis raises important questions which require further investigation. One that needs clarification is the clinical correlation between both local and systemic adipokines and patient symptoms, as described in chapter 4.

At the local adipose tissue level, the first step to further define such a relationship is to conduct a similar but larger study with appropriate controls. Controls should include fat from another site in the same patient, as an internal control, and as an external control, fat from the same site in asymptomatic participants. In addition to the immunoassays described in chapter 4, the secreted proteome of the fat can also be analysed.[335] This will answer the question, with greater statistical power, as to whether spinal adipose tissue is different in patients and whether it can secrete potentially degradative or inflammatory molecules. Such work would thus provide a more detailed understanding spinal adipose tissue.

Leptin is not only produced by adjacent adipose tissue, it is produced by the disc itself.[119,120,179] Unfortunately, the actual concentrations have not been quantified. Evaluation is required to determine if levels are ever high enough to promote degeneration. Leptin concentrations can be

assayed in two ways. Firstly, leptin could be assayed in tissue samples taken from patients during surgical procedures. Secondly, the secreted proteins can be assessed after a period of culture. It would be useful to stratify samples by different stages of disc degeneration as well as obesity categories. Controls can be obtained from post-mortems. The difficulty with such a study will be obtaining enough clinical samples to achieve statistical power and a multi-centre study may be required. Other adipokines may also mediate degeneration. Adiponectin is the second most studied adipokine and is raised in RA[87] and induces inflammatory changes in OA.[336] A similar cell culture model can be used to study this in the IVD.

Although this thesis has concentrated on the role of leptin in disc degeneration , leptin acts in an ubiquitous fashion and has effects on multiple other tissues.[337] An interesting finding from both the *ex vivo* (chapter 4) and epidemiology (chapters 7 and 8) work was the role of pro-inflammatory cytokines and obesity in LSS. LSS is a condition which involves degeneration of the osseoligamentous structures of the spine as well as the IVD.[27] Given the strong epidemiological association between obesity and LSS, it is possible that leptin influences bony or ligamentous degeneration in the spine. Leptin has been implicated in osteophyte formation[338] and ossification of both the posterior longitudinal ligament of the spine[339] and the ligamentum flavum.[340] All of these will compromise the canal diameter and contribute to spinal stenosis.

It is also important to understand the relationship between genetics, obesity and spine pathology. This thesis has identified that obesity may be an important mediator of a previously identified upper lumbar spine phenotype.[9,322] A systematic review, currently in press, showed that genetics may underlie the relationship between obesity and LBP.[341] The TwinsUK cohort is an ideal population to study this further given the comprehensive phenotypic and genotypic analysis as well as clinical assessments and lumbar spine MRIs at two separate time points; all this information is currently available.[342]

The UK Biobank is another avenue for large population study of back pain. It has accumulated data on over 500,000 participants from a genetically diverse population with longitudinal information now being collected.[262] The specific question for the Biobank data will be the role of leptin and other cytokines in back pain and spine-related health-seeking behaviour, given the Biobank is linked to hospital episode statistics. Furthermore, an analysis with respect to genetic relationships

is also possible.

Finally, the epidemiological data presented in chapters 6, 7 and 8 is cross-sectional and hence causation cannot be established. Longitudinal cohort studies are required to show whether obesity leads to degeneration or pain. Following up the Genodisc population, if possible will be able to yield such answers. It will also consist of sub-populations of those who underwent surgery and those who have not. From this, the relationship between obesity and clinical outcomes can also be modelled. Furthermore, once genetic data is available, analyses can be performed and comparisons made to a UK Biobank population of asymptomatic individuals. The benefits of using the Genodisc population are established study group and infrastructure, proven willing participants, and available baseline data; all of which are considerable barriers to establishing a cohort study.

9.4 Conclusions

In summary, these findings will provide a constructive and useful addition to the current understanding of obesity and the lumbar spine. Little was known about the effect of leptin upon the IVD; here it was shown that if present in the disc, particularly under inflammatory conditions, it can contribute to the degradative process. However, obesity is not the only factor and other pro-inflammatory cytokines and plasma proteins may be important. These include the upregulation of the pro-inflammatory cytokine IL-6 in LSS patients, and clusterin and complement in LBP patients. Finally, the epidemiological investigations showed that obesity was related to pain, LSS, disc degeneration, and herniation. Further investigations are required particularly into the IVD production of leptin, novel plasma proteins which may influence pain or degeneration, and longitudinal studies with the hypothesis that obesity directly leads to back pain and degeneration.

Original Contributions of this Thesis

1. Leptin can initiate and potentiate intervertebral disc degeneration.

2. The pro-inflammatory cytokines, IL-6 and TNF-α, are related to back pain and spinal stenosis.

3. The plasma proteins, clusterin and complement, are found in spine patients and not controls.

4. In spine patients obesity is
   (a) related to back and leg pain,
   (b) associated with a diagnosis of spinal stenosis, and
   (c) a predictor of disc degeneration, disc herniation and spinal stenosis on MRI.

References

[1] Vos, T. et al. “Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010.” In: *Lancet* 380.9859 (Dec. 2012), pp. 2163–2196.

[2] Hoy, D. et al. “The global burden of low back pain: estimates from the Global Burden of Disease 2010 study.” In: *Annals of the Rheumatic Diseases* 73.6 (June 2014), pp. 968–974.

[3] Hoy, D. et al. “A systematic review of the global prevalence of low back pain.” In: *Arthritis & Rheumatism* 64.6 (June 2012), pp. 2028–2037.

[4] Maniadakis, N. and Gray, A. “The economic burden of back pain in the UK.” In: *Pain* 84.1 (Jan. 2000), pp. 95–103.

[5] Gaskin, D. J. and Richard, P. “The Economic Costs of Pain in the United States”. In: *Relieving Pain in America*. National Academies Press (US), 2011.

[6] Luoma, K. et al. “Low back pain in relation to lumbar disc degeneration.” In: *Spine* 25.4 (Feb. 2000), pp. 487–492.

[7] Salminen, J. J., Erkintalo, M., Laine, M., and Pentti, J. “Low back pain in the young. A prospective three-year follow-up study of subjects with and without low back pain.” In: *Spine* 20.19 (Oct. 1995),

[8] Schepper, E. I. T. de et al. “The association between lumbar disc degeneration and low back pain: the influence of age, gender, and individual radiographic features.” In: *Spine* 35.5 (Mar. 2010), pp. 531–536.

[9] Cheung, K. M. C. et al. “Prevalence and pattern of lumbar magnetic resonance imaging changes in a population study of one thousand forty-three individuals.” In: *Spine* 34.9 (Apr. 2009), pp. 934–940.

[10] Brinjikji, W. et al. “Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations.” In: *AJNR. American journal of neuroradiology* (Nov. 2014).

[11] Jensen, M. C. et al. “Magnetic resonance imaging of the lumbar spine in people without back pain.” In: *NEJM* 331.2 (July 1994), pp. 69–73.

[12] Boden, S. D., Davis, D. O., Dina, T. S., Patronas, N. J., and Wiesel, S. W. “Abnormal magnetic-resonance scans of the lumbar spine in asymptomatic subjects. A prospective investigation.” In: *Journal of Bone and Joint Surgery* 72.3 (Mar. 1990), pp. 403–408.

[13] Savage, R. A., Whitehouse, G. H., and Roberts, N. “The relationship between the magnetic resonance imaging appearance of the lumbar spine and low back pain, age and occupation in males”. In: *European Spine Journal* 6.2 (1997), pp. 106–114.

[14] Jarvik, J. J., Hollingworth, W., Heagerty, P., Haynor, D. R., and Deyo, R. A. "The Longitudinal Assessment of Imaging and Disability of the Back (LAIDBack) Study: baseline data." In: *Spine* 26.10 (May 2001), pp. 1158–1166.

[15] Urban, J. P. and Roberts, S. "Degeneration of the intervertebral disc". In: *Arthritis Research & Therapy* 5.3 (2003), pp. 120–138.

[16] Risbud, M. V. and Shapiro, I. M. "Role of cytokines in intervertebral disc degeneration: pain and disc content." In: *Nature Publishing Group* 10.1 (Jan. 2014), pp. 44–56.

[17] Deyo, R. A. and Tsui-Wu, Y. "Descriptive epidemiology of low-back pain and its related medical care in the United States." In: *Spine* 12.3 (1987), p. 264.

[18] Rihn, J. A. et al. "Duration of symptoms resulting from lumbar disc herniation: effect on treatment outcomes: analysis of the Spine Patient Outcomes Research Trial (SPORT)." In: *The Journal of bone and joint surgery American volume* 93.20 (Oct. 2011), pp. 1906–1914.

[19] Rajasekaran, S., Bajaj, N., Tubaki, V., Kanna, R. M., and Shetty, A. P. "ISSLS Prize winner: The anatomy of failure in lumbar disc herniation: an in vivo, multimodal, prospective study of 181 subjects." In: *Spine* 38.17 (Aug. 2013), pp. 1491–1500.

[20] Wade, K. R., Robertson, P. A., Thambyah, A., and Broom, N. D. "'Surprise' Loading in Flexion Increases the Risk of Disc Herniation Due to Annulus-Endplate Junction Failure: A Mechanical And Microstructural Investigation." In: *Spine* (Mar. 2015).

[21] Kang, J. D. et al. "Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2." In: *Spine* 21.3 (Feb. 1996), pp. 271–277.

[22] Genevay, S. et al. "Elevated levels of tumor necrosis factor-alpha in periradicular fat tissue in patients with radiculopathy from herniated disc." In: *Spine* 33.19 (Sept. 2008), pp. 2041–2046.

[23] Genevay, S. et al. "Adalimumab in severe and acute sciatica: A multicenter, randomized, double-blind, placebo-controlled trial". In: *Arthritis & Rheumatism* 62.8 (Aug. 2010), pp. 2339–2346.

[24] Genevay, S., Stingelin, S., and Gabay, C. "Efficacy of etanercept in the treatment of acute, severe sciatica: a pilot study." In: *Annals of the Rheumatic Diseases* 63.9 (Sept. 2004), pp. 1120–1123.

[25] Freeman, B. J. C. et al. "Randomized, Double-blind, Placebo-Controlled, Trial of Transforaminal Epidural Etanercept for the Treatment of Symptomatic Lumbar Disc Herniation". In: *Spine* 38.23 (Nov. 2013), pp. 1986–1994.

[26] Hess, A. et al. "Blockade of TNF-α rapidly inhibits pain responses in the central nervous system." In: *Proceedings of the National Academy of Sciences of the United States of America* 108.9 (Mar. 2011), pp. 3731–3736.

[27] Martinelli, T. A. and Wiesel, S. W. "Epidemiology of spinal stenosis." In: 41 (1992), pp. 179–181.

[28] Ishimoto, Y. et al. “Prevalence of symptomatic lumbar spinal stenosis and its association with physical performance in a population-based cohort in Japan: the Wakayama Spine Study.” In: *Osteoarthritis and Cartilage* 20.10 (Oct. 2012), pp. 1103–1108.

[29] Cummins, J. et al. “Descriptive epidemiology and prior healthcare utilization of patients in the Spine Patient Outcomes Research Trial’s (SPORT) three observational cohorts: disc herniation, spinal stenosis, and degenerative spondylolisthesis.” In: *Spine* 31.7 (Apr. 2006), pp. 806–814.

[30] Ishimoto, Y. et al. “Associations between radiographic lumbar spinal stenosis and clinical symptoms in the general population: the Wakayama Spine Study.” In: *Osteoarthritis and Cartilage* 21.6 (June 2013), pp. 783–788.

[31] Sengupta, D. K. and Herkowitz, H. N. “Degenerative spondylolisthesis: review of current trends and controversies.” In: *Spine* 30.6 Suppl (Mar. 2005), S71–81.

[32] Matsunaga, S., Sakou, T., Morizono, Y., Masuda, A., and Demirtas, A. M. “Natural history of degenerative spondylolisthesis. Pathogenesis and natural course of the slippage.” In: *Spine* 15.11 (Nov. 1990), pp. 1204–1210.

[33] Balagué, F., Mannion, A. F., Pellisé, F., and Cedraschi, C. “Non-specific low back pain”. In: *The Lancet* 379.9814 (Feb. 2012), pp. 482–491.

[34] Battié, M. C. et al. “Disc degeneration-related clinical phenotypes.” In: *European Spine Journal* 23 Suppl 3 (June 2014), S305–14.

[35] Sivan, S. S., Wachtel, E., and Roughley, P. “Structure, function, aging and turnover of aggrecan in the intervertebral disc”. In: *Biochimica et Biophysica Acta (BBA)* 1840.10 (Oct. 2014), pp. 3181–3189.

[36] Roberts, S., Menage, J., Duance, V., Wotton, S., and Ayad, S. “1991 Volvo Award in basic sciences. Collagen types around the cells of the intervertebral disc and cartilage end plate: an immunolocalization study.” In: *Spine* 16.9 (Sept. 1991), pp. 1030–1038.

[37] Eyre, D. R. and Muir, H. “Quantitative analysis of types I and II collagens in human intervertebral discs at various ages”. In: *Biochimica et Biophysica Acta (BBA)* 492.1 (May 1977), pp. 29–42.

[38] Johnstone, B. and Bayliss, M. T. “The large proteoglycans of the human intervertebral disc. Changes in their biosynthesis and structure with age, topography, and pathology.” In: *Spine* 20.6 (1995), p. 674.

[39] Urban, J. P. and Maroudas, A. “Swelling of the intervertebral disc in vitro.” In: *Connective tissue research* 9.1 (1981), pp. 1–10.

[40] Marchand, F. and Ahmed, A. M. “Investigation of the laminate structure of lumbar disc anulus fibrosus.” In: *Spine* 15.5 (May 1990), pp. 402–410.

[41] Yu, J. et al. “Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc.” In: *Journal of anatomy* 210.4 (Apr. 2007), pp. 460–471.

[42] Bruehlmann, S. B., B Rattner, J., R Matyas, J., and A Duncan, N. "Regional variations in the cellular matrix of the annulus fibrosus of the intervertebral disc". In: *Journal of anatomy* 201.2 (Aug. 2002), pp. 159–171.

[43] Roberts, S., Menage, J., and Urban, J. P. "Biochemical and structural properties of the cartilage end-plate and its relation to the intervertebral disc." In: *Spine* 14.2 (Feb. 1989), pp. 166–174.

[44] Benneker, L. M., Heini, P. F., Alini, M., Anderson, S. E., and Ito, K. "2004 Young Investigator Award Winner: vertebral endplate marrow contact channel occlusions and intervertebral disc degeneration." In: *Spine* 30.2 (Jan. 2005), pp. 167–173.

[45] Roberts, S., Urban, J. P., Evans, H., and Eisenstein, S. "Transport properties of the human cartilage endplate in relation to its composition and calcification." In: *Spine* 21.4 (Feb. 1996), pp. 415–420.

[46] Urban, J. P. G., Smith, S., and Fairbank, J. C. T. "Nutrition of the intervertebral disc." In: *Spine* 29.23 (Dec. 2004), pp. 2700–2709.

[47] Nachemson, A., Lewin, T., Maroudas, A., and Freeman, M. A. "In vitro diffusion of dye through the end-plates and the annulus fibrosus of human lumbar inter-vertebral discs." In: *Acta orthopaedica Scandinavica* 41.6 (1970), pp. 589–607.

[48] Bernick, S. and Cailliet, R. "Vertebral end-plate changes with aging of human vertebrae." In: *Spine* 7.2 (Mar. 1982), pp. 97–102.

[49] Rajasekaran, S. et al. "ISSLS prize winner: A study of diffusion in human lumbar discs: a serial magnetic resonance imaging study documenting the influence of the endplate on diffusion in normal and degenerate discs." In: *Spine* 29.23 (Dec. 2004), pp. 2654–2667.

[50] Grünhagen, T., Wilde, G., Soukane, D. M., Shirazi-Adl, S. A., and Urban, J. P. G. "Nutrient supply and intervertebral disc metabolism." In: *Journal of Bone and Joint Surgery* 88 Suppl 2 (Apr. 2006), pp. 30–35.

[51] Ohshima, H. and Urban, J. "The effect of lactate and pH on proteoglycan and protein synthesis rates in the intervertebral disc". In: *Spine* 17.9 (1992), p. 1079.

[52] Razaq, S., Wilkins, R. J., and Urban, J. P. G. "The effect of extracellular pH on matrix turnover by cells of the bovine nucleus pulposus". In: *European Spine Journal* 12.4 (Aug. 2003), pp. 341–349.

[53] Wemmie, J. A., Price, M. P., and Welsh, M. J. "Acid-sensing ion channels: advances, questions and therapeutic opportunities". In: *Trends in neurosciences* 29.10 (2006), pp. 578–586.

[54] S R S Bibby, J. P. G. U. "Effect of nutrient deprivation on the viability of intervertebral disc cells". In: *European Spine Journal* 13.8 (Dec. 2004), pp. 695–701.

[55] Pfirrmann, C. W., Metzdorf, A., Zanetti, M., Hodler, J., and Boos, N. "Magnetic resonance classification of lumbar intervertebral disc degeneration." In: *Spine* 26.17 (Sept. 2001), pp. 1873–1878.

[56] Pockert, A. J. et al. “Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration”. In: *Arthritis & Rheumatism* 60.2 (Feb. 2009), pp. 482–491.

[57] Séguin, C. A., Pilliar, R. M., Roughley, P. J., and Kandel, R. A. “Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue.” In: *Spine* 30.17 (Sept. 2005), pp. 1940–1948.

[58] Le Maitre, C. L., Freemont, A. J., and Hoyland, J. A. “The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration.” In: *Arthritis Research & Therapy* 7.4 (2005), R732–45.

[59] Sivan, S. S. et al. “Biochemical composition and turnover of the extracellular matrix of the normal and degenerate intervertebral disc”. In: *European Spine Journal* 23.3 (Apr. 2013), pp. 344–353.

[60] Snoek-van Beurden, P. A. M. and Von den Hoff, J. W. “Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors.” In: *BioTechniques* 38.1 (Jan. 2005), pp. 73–83.

[61] Page-McCaw, A., Ewald, A. J., and Werb, Z. “Matrix metalloproteinases and the regulation of tissue remodelling”. In: *Nature reviews. Molecular cell biology* 8.3 (Mar. 2007), pp. 221–233.

[62] Khokha, R., Murthy, A., and Weiss, A. “Metalloproteinases and their natural inhibitors in inflammation and immunity”. In: *Nature Reviews Immunology* 13.9 (Sept. 2013), pp. 649–665.

[63] Roberts, S. et al. “Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc”. In: *Spine* 25.23 (2000), pp. 3005–3013.

[64] Weiler, C., Nerlich, A. G., Zipperer, J., Bachmeier, B. E., and Boos, N. “2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption.” In: *European Spine Journal* 11.4 (Aug. 2002), pp. 308–320.

[65] Le Maitre, C. L., Freemont, A. J., and Hoyland, J. A. “Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc”. In: *The Journal of pathology* 204.1 (Sept. 2004), pp. 47–54.

[66] Porter, S., Clark, I. M., Kevorkian, L., and Edwards, D. R. “The ADAMTS metalloproteinases.” In: *Biochemical Journal* 386.Pt 1 (Feb. 2005), pp. 15–27.

[67] Nagase, H. and Kashiwagi, M. “Aggrecanases and cartilage matrix degradation”. In: *Arthritis Research & Therapy* 5.2 (2003), p. 94.

[68] Pockert, A. J. et al. “Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration.” In: *Arthritis & Rheumatism* 60.2 (Feb. 2009), pp. 482–491.

[69] Kashiwagi, M. “TIMP-3 Is a Potent Inhibitor of Aggrecanase 1 (ADAM-TS4) and Aggrecanase 2 (ADAM-TS5)”. In: *Journal of Biological Chemistry* 276.16 (Jan. 2001), pp. 12501–12504.

[70] Purmessur, D. et al. “A role for TNFα in intervertebral disc degeneration: a non-recoverable catabolic shift.” In: *Biochemical and biophysical research communications* 433.1 (Mar. 2013), pp. 151–156.

[71] Hoyland, J. A., Le Maitre, C., and Freemont, A. J. “Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc”. In: *Rheumatology* 47.6 (Mar. 2008), pp. 809–814.

[72] Kepler, C. K. et al. “Substance P stimulates production of inflammatory cytokines in human disc cells.” In: *Spine* 38.21 (Oct. 2013), E1291–9.

[73] Wang, J. et al. “TNF-α and IL-1β Dependent Induction of CCL3 Expression by Nucleus Pulposus Cells Promotes Macrophage Migration through CCR1”. In: *Arthritis & Rheumatism* 65.3 (Mar. 2013), pp. 832–842.

[74] Roberts, S. et al. “TNFα-stimulated gene product (TSG-6) and its binding protein, IαI, in the human intervertebral disc: new molecules for the disc”. In: *European Spine Journal* 14.1 (Nov. 2004), pp. 36–42.

[75] Korhonen, T. et al. “Efficacy of Infliximab for Disc Herniation-Induced Sciatica”. In: *Spine* 29.19 (Oct. 2004), pp. 2115–2119.

[76] Battié, M. C. et al. “Occupational driving and lumbar disc degeneration: a case-control study”. In: *Lancet* 360.9343 (2002), pp. 1369–1374.

[77] Näkki, A., Battié, M. C., and Kaprio, J. “Genetics of disc-related disorders: current findings and lessons from other complex diseases”. In: *European Spine Journal* 23.3 (July 2013), pp. 354–363.

[78] Eskola, P. J. et al. “Genetic Association Studies in Lumbar Disc Degeneration: A Systematic Review”. In: *PLoS ONE* 7.11 (Nov. 2012), e49995.

[79] Urquhart, D. M. et al. “2011 Young Investigator Award winner: Increased fat mass is associated with high levels of low back pain intensity and disability.” In: *Spine* 36.16 (July 2011), pp. 1320–1325.

[80] Shiri, R., Karppinen, J., Leino-Arjas, P., Solovieva, S., and Viikari-Juntura, E. “The association between obesity and low back pain: a meta-analysis.” In: *American Journal of Epidemiology* 171.2 (Jan. 2010), pp. 135–154.

[81] Weiler, C. et al. “Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index”. In: *BMC research notes* 4.1 (2011), p. 497.

[82] Samartzis, D., Karppinen, J., Chan, D., Luk, K. D. K., and Cheung, K. M. C. “The association of lumbar intervertebral disc degeneration on magnetic resonance imaging with body mass index in overweight and obese adults: a population-based study.” In: *Arthritis & Rheumatism* 64.5 (May 2012), pp. 1488–1496.

[83] Samartzis, D. et al. “A population-based study of juvenile disc degeneration and its association with overweight and obesity, low back pain, and diminished functional status.” In: 93.7 (Apr. 2011), pp. 662–670.

[84] Takatalo, J. et al. “Association of abdominal obesity with lumbar disc degeneration - a magnetic resonance imaging study.” In: *PLoS ONE* 8.2 (2013), e56244.

[85] Liuke, M. et al. “Disc degeneration of the lumbar spine in relation to overweight”. In: *International journal of obesity* 29.8 (2005), pp. 903–908.

[86] Videman, T., Levalahti, E., and Battié, M. C. “The effects of anthropometrics, lifting strength, and physical activities in disc degeneration.” In: *Spine* 32.13 (June 2007), pp. 1406–1413.

[87] Lago, F., Dieguez, C., Gomez-Reino, J., and Gualillo, O. “Adipokines as emerging mediators of immune response and inflammation.” In: *Nature clinical practice. Rheumatology* 3.12 (Dec. 2007), pp. 716–724.

[88] Haslam, D. W. and James, W. P. T. “Obesity”. In: *The Lancet* 366.9492 (Oct. 2005), pp. 1197–1209.

[89] Kelly, T., Yang, W., Chen, C.-S., Reynolds, K., and He, J. “Global burden of obesity in 2005 and projections to 2030”. In: *International journal of obesity* 32.9 (Sept. 2008), pp. 1431–1437.

[90] Withrow, D. and Alter, D. A. “The economic burden of obesity worldwide: a systematic review of the direct costs of obesity.” In: *Obesity reviews* 12.2 (Feb. 2011), pp. 131–141.

[91] Kellgren, J. H. and Lawrence, J. S. “Osteo-arthrosis and disk degeneration in an urban population.” In: *Annals of the Rheumatic Diseases* 17.4 (Dec. 1958), pp. 388–397.

[92] Hartz, A. J. et al. “The association of obesity with joint pain and osteoarthritis in the HANES data”. In: *Journal of Chronic Diseases* 39.4 (Jan. 1986), pp. 311–319.

[93] Adams, M. A., Freeman, B. J., Morrison, H. P., Nelson, I. W., and Dolan, P. “Mechanical initiation of intervertebral disc degeneration.” In: *Spine* 25.13 (July 2000), pp. 1625–1636.

[94] Roffey, D. M., Wai, E. K., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of awkward occupational postures and low back pain: results of a systematic review”. In: *The Spine Journal* 10.1 (Jan. 2010), pp. 89–99.

[95] Roffey, D. M., Wai, E. K., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of occupational standing or walking and low back pain: results of a systematic review”. In: *The Spine Journal* 10.3 (Mar. 2010), pp. 262–272.

[96] Roffey, D. M., Wai, E. K., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of occupational pushing or pulling and low back pain: results of a systematic review”. In: *The Spine Journal* 10.6 (June 2010), pp. 544–553.

[97] Wai, E. K., Roffey, D. M., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of occupational bending or twisting and low back pain: results of a systematic review”. In: *The Spine Journal* 10.1 (Jan. 2010), pp. 76–88.

[98] Wai, E. K., Roffey, D. M., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of occupational lifting and low back pain: results of a systematic review”. In: *The Spine Journal* 10.6 (June 2010), pp. 554–566.

[99] Wai, E. K., Roffey, D. M., Bishop, P., Kwon, B. K., and Dagenais, S. “Causal assessment of occupational carrying and low back pain: results of a systematic review”. In: *The Spine Journal* 10.7 (July 2010), pp. 628–638.

[100] Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. "Elevated C-reactive protein levels in overweight and obese adults." In: *JAMA* 282.22 (Dec. 1999), pp. 2131–2135.

[101] Takaoka, M. et al. "Periadventitial adipose tissue plays a critical role in vascular remodeling." In: *Circulation Research* 105.9 (Oct. 2009), pp. 906–911.

[102] Sierra-Honigmann, M. R. et al. "Biological action of leptin as an angiogenic factor." In: *Science (New York, N.Y.)* 281.5383 (Sept. 1998), pp. 1683–1686.

[103] Sanna, V. et al. "Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses". In: *The Journal of clinical investigation* 111.111(2) (Jan. 2003), pp. 241–250.

[104] Tuzun, A., Uygun, A., Yesilova, Z., and Ozel, A. M. "Leptin levels in the acute stage of ulcerative colitis". In: *Journal of …* (2004).

[105] Gomez, R., Lago, F., Gomez-Reino, J., Dieguez, C., and Gualillo, O. "Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases." In: *Journal of molecular endocrinology* 43.1 (July 2009), pp. 11–18.

[106] Okifuji, A., Donaldson, G. W., Barck, L., and Fine, P. G. "Relationship between fibromyalgia and obesity in pain, function, mood, and sleep." In: *The journal of pain : official journal of the American Pain Society* 11.12 (Dec. 2010), pp. 1329–1337.

[107] Bigal, M. E., Liberman, J. N., and Lipton, R. B. "Obesity and migraine: a population study." In: *Neurology* 66.4 (Feb. 2006), pp. 545–550.

[108] Bigal, M. E. and Lipton, R. B. "Obesity is a risk factor for transformed migraine but not chronic tension-type headache." In: *Neurology* 67.2 (July 2006), pp. 252–257.

[109] Peterlin, B. L., Rapoport, A. M., and Kurth, T. "Migraine and obesity: epidemiology, mechanisms, and implications." In: *Headache* 50.4 (Apr. 2010), pp. 631–648.

[110] Luppino, F. S. et al. "Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies." In: *Archives of General Psychiatry* 67.3 (Mar. 2010), pp. 220–229.

[111] Rasouli, N. and Kern, P. A. "Adipocytokines and the metabolic complications of obesity." In: *The Journal of clinical endocrinology and metabolism* 93.11 Suppl 1 (Nov. 2008), S64–73.

[112] Ingalls, A. M., Dickie, M. M., and Snell, G. D. *Obese, a new mutation in the house mouse.* Vol. 4. Jan. 1996.

[113] Zhang, Y. et al. "Positional cloning of the mouse obese gene and its human homologue." In: *Nature* 372.6505 (Dec. 1994), pp. 425–432.

[114] Hoggard, N. et al. "Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta." In: *Proceedings of the National Academy of Sciences of the United States of America* 94.20 (Sept. 1997), pp. 11073–11078.

[115] Bado, A. et al. "The stomach is a source of leptin". In: *Nature* 394.6695 (Aug. 1998), pp. 790–793.

[116] Conde, J. et al. “Expanding the adipokine network in cartilage: identification and regulation of novel factors in human and murine chondrocytes”. In: *Annals of the Rheumatic Diseases* 70.3 (Feb. 2011), pp. 551–559.

[117] Reseland, J. E. et al. “Leptin Is Expressed in and Secreted from Primary Cultures of Human Osteoblasts and Promotes Bone Mineralization”. In: *Journal of Bone and Mineral Research* 16.8 (Aug. 2001), pp. 1426–1433.

[118] Wang, J., Liu, R., Hawkins, M., Barzilai, N., and Rossetti, L. “A nutrient-sensing pathway regulates leptin gene expression in muscle and fat”. In: *Nature* 393.6686 (June 1998), pp. 684–688.

[119] Zhao, C.-Q., Liu, D., Li, H., Jiang, L.-S., and Dai, L.-Y. “Expression of leptin and its functional receptor on disc cells: contribution to cell proliferation.” In: *Spine* 33.23 (Nov. 2008), pp. 858–864.

[120] Gruber, H. E., Ingram, J. A., Hoelscher, G. L., and Hanley Jr., E. N. “Leptin expression by annulus cells in the human intervertebral disc”. In: *The Spine Journal* 7.4 (July 2007), pp. 437–443.

[121] Enriori, P. J., Evans, A. E., Sinnayah, P., and Cowley, M. A. “Leptin resistance and obesity”. In: *Obesity* 14 (2006), 254S–258S.

[122] Considine, R. V. et al. “Serum immunoreactive-leptin concentrations in normal-weight and obese humans.” In: *NEJM* 334.5 (Feb. 1996), pp. 292–295.

[123] Otsuka, R. et al. “Perceived Psychological Stress and Serum Leptin Concentrations in Japanese Men”. In: *Obesity* 14.10 (Oct. 2006), pp. 1832–1838.

[124] Considine, R. V. et al. “Dexamethasone stimulates leptin release from human adipocytes: Unexpected inhibition by insulin”. In: *Journal of cellular biochemistry* 65.2 (May 1997), pp. 254–258.

[125] Vuolteenaho, K. et al. “Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage–mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production.” In: *Mediators of Inflammation* 2009 (2009), p. 345838.

[126] Koskinen, A., Vuolteenaho, K., Nieminen, R., Moilanen, T., and Moilanen, E. “Leptin enhances MMP-1, MMP-3 and MMP-13 production in human osteoarthritic cartilage and correlates with MMP-1 and MMP-3 in synovial fluid from OA patients.” In: *Clinical and experimental rheumatology* 29.1 (Jan. 2011), pp. 57–64.

[127] Simopoulou, T. et al. “Differential expression of leptin and leptin’s receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism”. In: *Osteoarthritis and Cartilage* 15.8 (Aug. 2007), pp. 872–883.

[128] Presle, N. et al. “Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production”. In: *Osteoarthritis and Cartilage* 14.7 (July 2006), pp. 690–695.

[129] Ku, J. H. et al. “Correlation of synovial fluid leptin concentrations with the severity of osteoarthritis.” In: *Clinical Rheumatology* 28.12 (Dec. 2009), pp. 1431–1435.

[130] Dumond, H. l. n. et al. “Evidence for a key role of leptin in osteoarthritis”. In: *Arthritis & Rheumatism* 48.11 (Nov. 2003), pp. 3118–3129.

[131] Hui, W. et al. “Leptin produced by joint white adipose tissue induces cartilage degradation via upregulation and activation of matrix metalloproteinases”. In: *Annals of the Rheumatic Diseases* 71 (Nov. 2011), pp. 455–462.

[132] Lim, G., Wang, S., Zhang, Y., Tian, Y., and Mao, J. “Spinal leptin contributes to the pathogenesis of neuropathic pain in rodents”. In: *The Journal of clinical investigation* (Jan. 2009).

[133] Urquhart, D. M. et al. “Obesity is associated with reduced disc height in the lumbar spine but not at the lumbosacral junction.” In: *Spine* 39.16 (July 2014), E962–6.

[134] Shiri, R., Lallukka, T., Karppinen, J., and Viikari-Juntura, E. “Obesity as a risk factor for sciatica: a meta-analysis.” In: *American Journal of Epidemiology* 179.8 (Apr. 2014), pp. 929–937.

[135] Takatalo, J. et al. “Prevalence of degenerative imaging findings in lumbar magnetic resonance imaging among young adults.” In: *Spine* 34.16 (July 2009), pp. 1716–1721.

[136] Teraguchi, M. et al. “Prevalence and distribution of intervertebral disc degeneration over the entire spine in a population-based cohort: the Wakayama Spine Study.” In: *Osteoarthritis and Cartilage* 22.1 (Jan. 2014), pp. 104–110.

[137] Horner, H. A. et al. “Cells from different regions of the intervertebral disc: effect of culture system on matrix expression and cell phenotype.” In: *Spine* 27.10 (May 2002), pp. 1018–1028.

[138] Guo, J. F., Jourdain, G. W., and MacCallum, D. K. “Culture and growth characteristics of chondrocytes encapsulated in alginate beads.” In: *Connective tissue research* 19.2-4 (1989), pp. 277–297.

[139] Hauselmann, H. J. et al. “Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads.” In: *Journal of cell science* 107 (Pt 1) (Jan. 1994), pp. 17–27.

[140] Urban, J. P., Holm, S., Maroudas, A., and Nachemson, A. “Nutrition of the intervertebral disc: effect of fluid flow on solute transport.” In: *Clinical orthopaedics and related research* 170 (Oct. 1982), pp. 296–302.

[141] Holm, S., Selstam, G., and Nachemson, A. “Carbohydrate metabolism and concentration profiles of solutes in the canine lumbar intervertebral disc.” In: *Acta physiologica scandinavica* 115.1 (May 1982), pp. 147–156.

[142] Farndale, R., Buttle, D., and Barratt, A. “Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue”. In: *Biochimica et Biophysica Acta (BBA)* 883.2 (Sept. 1986), pp. 173–177.

[143] Barbosa, I. et al. “Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies.” In: *Glycobiology* 13.9 (Sept. 2003), pp. 647–653.

[144] Lowry, O. H., Rosenbrough, A. L., Farr, A. L., and Randall, R. J. “Protein measurement with the Folin phenol reagent.” In: *Journal of Biological Chemistry* 193.1 (Nov. 1951), pp. 265–275.

[145] Hu, X. and Beeton, C. “Detection of functional matrix metalloproteinases by zymography.” In: *Journal of visualized experiments : JoVE* 45 (2010).

[146] Stryer, L. “Fluorescence energy transfer as a spectroscopic ruler”. In: *Annual review of biochemistry* (1978).

[147] Vandesompele, J. et al. “Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes”. In: *Genome Biology* 3.7 (2002), research0034.

[148] Thellin, O. et al. “Housekeeping genes as internal standards: use and limits.” In: *Journal of biotechnology* 75.2-3 (Oct. 1999), pp. 291–295.

[149] Minogue, B. M., Richardson, S. M., Zeef, L. A., Freemont, A. J., and Hoyland, J. A. “Transcriptional profiling of bovine intervertebral disc cells: implications for identification of normal and degenerate human intervertebral disc cell phenotypes.” In: *Arthritis Research & Therapy* 12.1 (2010), R22.

[150] Li, S., Duance, V. C., and Blain, E. J. “Zonal variations in cytoskeletal element organization, mRNA and protein expression in the intervertebral disc.” In: *Journal of anatomy* 213.6 (Dec. 2008), pp. 725–732.

[151] Chou, A. I., Reza, A. T., and Nicoll, S. B. “Distinct intervertebral disc cell populations adopt similar phenotypes in three-dimensional culture.” In: *Tissue engineering. Part A* 14.12 (Dec. 2008), pp. 2079–2087.

[152] Erwin, W. M., Ashman, K., O’Donnel, P., and Inman, R. D. “Nucleus pulposus notochord cells secrete connective tissue growth factor and up-regulate proteoglycan expression by intervertebral disc chondrocytes.” In: *Arthritis & Rheumatism* 54.12 (Dec. 2006), pp. 3859–3867.

[153] Korecki, C. L., MacLean, J. J., and Iatridis, J. C. “Characterization of an in vitro intervertebral disc organ culture system.” In: *European Spine Journal* 16.7 (July 2007), pp. 1029–1037.

[154] Cui, Y., Yu, J., Urban, J. P. G., and Young, D. A. “Differential gene expression profiling of metalloproteinases and their inhibitors: a comparison between bovine intervertebral disc nucleus pulposus cells and articular chondrocytes.” In: *Spine* 35.11 (May 2010), pp. 1101–1108.

[155] Seol, D. et al. “Selection of reference genes for normalization of quantitative real-time PCR in organ culture of the rat and rabbit intervertebral disc”. In: *BMC research notes* 4.1 (2011), p. 162.

[156] McCulloch, R. S., Ashwell, M. S., O’Nan, A. T., and Mente, P. L. “Identification of stable normalization genes for quantitative real-time PCR in porcine articular cartilage.” In: *Journal of animal science and biotechnology* 3.1 (2012), p. 36.

[157] Foldager, C. B. et al. “Validation of suitable house keeping genes for hypoxia-cultured human chondrocytes.” In: *BMC Molecular Biology* 10.1 (2009), p. 94.

[158] Peansukmanee, S. et al. “Effects of hypoxia on glucose transport in primary equine chondrocytes in vitro and evidence of reduced GLUT1 gene expression in pathologic cartilage in vivo.” In: *Journal of orthopaedic research* 27.4 (Apr. 2009), pp. 529–535.

[159] Bustin, S. A., Benes, V., Nolan, T., and Pfaffl, M. W. “Quantitative real-time RT-PCR–a perspective.” In: *Journal of molecular endocrinology* 34.3 (June 2005), pp. 597–601.

[160] Schmittgen, T. D. and Livak, K. J. “Analyzing real-time PCR data by the comparative CT method”. In: *Nature protocols* 3.6 (2008), pp. 1101–1108.

[161] Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P., and Bahouth, S. W. “Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans.” In: *Endocrinology* 145.5 (May 2004), pp. 2273–2282.

[162] Finehout, E. J. and Lee, K. H. “An introduction to mass spectrometry applications in biological research.” In: *Biochemistry and molecular biology education : a bimonthly publication of the International Union of Biochemistry and Molecular Biology* 32.2 (Mar. 2004), pp. 93–100.

[163] Lahm, H.-W. and Langen, H. “Mass spectrometry: A tool for the identification of proteins separated by gels”. In: *Electrophoresis* 21.11 (June 2000), pp. 2105–2114.

[164] Callesen, A. K. et al. “Serum protein profiling by solid phase extraction and mass spectrometry: a future diagnostics tool?” In: *PROTEOMICS* 9.6 (Mar. 2009), pp. 1428–1441.

[165] Anderson, N. L. and Anderson, N. G. “The human plasma proteome: history, character, and diagnostic prospects.” In: *Molecular & Cellular Proteomics* 1.11 (Nov. 2002), pp. 845–867.

[166] Fonslow, B. R. et al. “Improvements in proteomic metrics of low abundance proteins through proteome equalization using ProteoMiner prior to MudPIT.” In: *Journal of proteome research* 10.8 (Aug. 2011), pp. 3690–3700.

[167] Hu, S., Loo, J. A., and Wong, D. T. “Human body fluid proteome analysis.” In: *PROTEOMICS* 6.23 (Dec. 2006), pp. 6326–6353.

[168] Hartwig, S. et al. “Combinatorial hexapeptide ligand libraries (ProteoMiner): an innovative fractionation tool for differential quantitative clinical proteomics.” In: *Archives of physiology and biochemistry* 115.3 (July 2009), pp. 155–160.

[169] Zolotarjova, N. et al. “Differences among techniques for high-abundant protein depletion”. In: *PROTEOMICS* 5.13 (Aug. 2005), pp. 3304–3313.

[170] Shen, Y. et al. “Characterization of the human blood plasma proteome.” In: *PROTEOMICS* 5.15 (Oct. 2005), pp. 4034–4045.

[171] Righetti, P. G., Boschetti, E., Lomas, L., and Citterio, A. “Protein Equalizer Technology : the quest for a ”democratic proteome”.” In: *PROTEOMICS* 6.14 (July 2006), pp. 3980–3992.

[172] Guerrier, L., Righetti, P. G., and Boschetti, E. “Reduction of dynamic protein concentration range of biological extracts for the discovery of low-abundance proteins by means of hexapeptide ligand library.” In: *Nature protocols* 3.5 (2008), pp. 883–890.

[173] Perkins, D. N., Pappin, D. J., Creasy, D. M., and Cottrell, J. S. "Probability-based protein identification by searching sequence databases using mass spectrometry data." In: *Electrophoresis* 20.18 (Dec. 1999), pp. 3551–3567.

[174] Johnson, W. E., Eisenstein, S., and Roberts, S. "Cell cluster formation in degenerate lumbar intervertebral discs is associated with increased disc cell proliferation." In: *Connective tissue research* 42.3 (2001), pp. 197–207.

[175] Li, Z. et al. "Leptin induces cyclin D1 expression and proliferation of human nucleus pulposus cells via JAK/STAT, PI3K/Akt and MEK/ERK pathways." In: *PLoS ONE* 7.12 (2012), e53176.

[176] Nakai, T., Mochida, J., and Sakai, D. "Synergistic role of c-Myc and ERK1/2 in the mitogenic response to TGF beta-1 in cultured rat nucleus pulposus cells". In: *Arthritis Research & Therapy* (2008).

[177] Koh, K. K., Park, S. M., and Quon, M. J. "Leptin and cardiovascular disease: response to therapeutic interventions." In: *Circulation* 117.25 (June 2008), pp. 3238–3249.

[178] Masaki, T. and Yoshimatsu, H. "Obesity, adipocytokines and cancer." In: *Translational Oncogenomics* 3 (2008), pp. 45–52.

[179] Koerner, J. D. et al. "Differential Gene Expression in Anterior and Posterior Annulus Fibrosus". In: *Spine* 39.23 (2014), pp. 1917–1923.

[180] Roberts, S., Ayad, S., and Menage, P. J. "Immunolocalisation of type VI collagen in the intervertebral disc." In: *Annals of the Rheumatic Diseases* 50.11 (Nov. 1991), pp. 787–791.

[181] Li, Z. et al. "Leptin Downregulates Aggrecan through the p38-ADAMST Pathway in Human Nucleus Pulposus Cells". In: *PLoS ONE* 9.10 (Oct. 2014), e109595.

[182] Jacobs, L. J., Vo, N., and Kang, J. D. "Identifying inflammatory targets for biologic therapies for spine pain." In: *Physical Medicine and Rehabilitation* 3.6 Suppl 1 (June 2011), S12–7.

[183] Liu, G. Z., Ishihara, H., Osada, R., Kimura, T., and Tsuji, H. "Nitric oxide mediates the change of proteoglycan synthesis in the human lumbar intervertebral disc in response to hydrostatic pressure." In: *Spine* 26.2 (Jan. 2001), pp. 134–141.

[184] Shamji, M. F. et al. "Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues". In: *Arthritis & Rheumatism* 62.7 (July 2010), pp. 1974–1982.

[185] Otero, M., Lago, R., Lago, F., Reino, J. J. G., and Gualillo, O. "Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1." In: *Arthritis Research & Therapy* 7.3 (2005), R581–91.

[186] Otero, M. et al. "Phosphatidylinositol 3-kinase, MEK-1 and p38 mediate leptin/interferon-gamma synergistic NOS type II induction in chondrocytes." In: *Life sciences* 81.19-20 (Oct. 2007), pp. 1452–1460.

[187] Scheller, J., Chalaris, A., Schmidt-Arras, D., and Rose-John, S. "The pro- and anti-inflammatory properties of the cytokine interleukin-6". In: *Biochimica et Biophysica Acta (BBA)* 1813.5 (May 2011), pp. 878–888.

[188] Frühbeck, G. “Intracellular signalling pathways activated by leptin”. In: *Biochemical Journal* 393.1 (Jan. 2006), p. 7.

[189] Agrawal, S., Gollapudi, S., Su, H., and Gupta, S. “Leptin activates human B cells to secrete TNF-α, IL-6, and IL-10 via JAK2/STAT3 and p38MAPK/ERK1/2 signaling pathway.” In: *Journal of clinical immunology* 31.3 (June 2011), pp. 472–478.

[190] Yang, W.-H. et al. “Leptin induces IL-6 expression through OBRl receptor signaling pathway in human synovial fibroblasts.” In: *PLoS ONE* 8.9 (2013), e75551.

[191] Tang, C.-H. et al. “Leptin-induced IL-6 production is mediated by leptin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, Akt, NF-kappaB, and p300 pathway in microglia.” In: *Journal of immunology (Baltimore, Md. : 1950)* 179.2 (July 2007), pp. 1292–1302.

[192] Sadagurski, M., Norquay, L., Farhang, J., and D’Aquino, K. “Human IL6 enhances leptin action in mice”. In: *Diabetologia* (2010).

[193] Stannus, O. P. et al. “The association between leptin, interleukin-6, and hip radiographic osteoarthritis in older people: a cross-sectional study.” In: *Arthritis Research & Therapy* 12.3 (2010), R95.

[194] Ruhl, C. E. and Everhart, J. E. “Leptin concentrations in the United States: relations with demographic and anthropometric measures.” In: *The American Journal of Clinical Nutrition* 74.3 (Sept. 2001), pp. 295–301.

[195] Iwamoto, J., Takeda, T., Sato, Y., and Matsumoto, H. “Serum Leptin Concentration Positively Correlates with Body Weight and Total Fat Mass in Postmenopausal Japanese Women with Osteoarthritis of the Knee”. In: *Arthritis* 2011.1, suppl (2011), pp. 1–6.

[196] Shamsuzzaman, A. S. M. “Independent Association Between Plasma Leptin and C-Reactive Protein in Healthy Humans”. In: *Circulation* 109.18 (May 2004), pp. 2181–2185.

[197] Li, Z. et al. “The role of leptin on the organization and expression of cytoskeleton elements in nucleus pulposus cells”. In: *Journal of orthopaedic research* (2013).

[198] Fowler Brown, A. et al. “The mediating effect of leptin on the relationship between body weight and knee osteoarthritis in older adults.” In: *Arthritis & Rheumatism* 67.1 (Jan. 2015), pp. 169–175.

[199] Karvonen-Gutierrez, C. A. et al. “Leptin Levels are Associated with Radiographic Knee Osteoarthritis Among a Cohort of Mid-Life Women”. In: *Arthritis Care & Research* 65.6 (June 2013), pp. 936–944.

[200] Staikos, C. et al. “The association of adipokine levels in plasma and synovial fluid with the severity of knee osteoarthritis.” In: *Rheumatology (Oxford, England)* 52.6 (June 2013), pp. 1077–1083.

[201] Berry, P. A., Jones, S. W., Cicuttini, F. M., Wluka, A. E., and Maciewicz, R. A. “Temporal relationship between serum adipokines, biomarkers of bone and cartilage turnover, and cartilage volume loss in a population with clinical knee osteoarthritis”. In: *Arthritis & Rheumatism* 63.3 (Mar. 2011), pp. 700–707.

[202] Filková, M. et al. “Increased serum adiponectin levels in female patients with erosive compared with non-erosive osteoarthritis.” In: *Annals of the Rheumatic Diseases* 68.2 (Feb. 2009), pp. 295–296.

[203] Yusuf, E. et al. “Association between leptin, adiponectin and resistin and long-term progression of hand osteoarthritis.” In: *Annals of the Rheumatic Diseases* 70.7 (July 2011), pp. 1282–1284.

[204] Choe, J.-Y. et al. “Serum resistin level is associated with radiographic changes in hand osteoarthritis: Cross-sectional study”. In: *Joint Bone Spine* 79.2 (Mar. 2012), pp. 160–165.

[205] Gosset, M. et al. “Crucial role of visfatin/pre–B cell colony-enhancing factor in matrix degradation and prostaglandin E2 synthesis in chondrocytes: Possible influence on osteoarthritis”. In: *Arthritis & Rheumatism* 58.5 (May 2008), pp. 1399–1409.

[206] Klein-Wieringa, I. R. et al. “The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype.” In: *Annals of the Rheumatic Diseases* 70.5 (May 2011), pp. 851–857.

[207] Ushiyama, T., Chano, T., Inoue, K., and Matsusue, Y. “Cytokine production in the infrapatellar fat pad: another source of cytokines in knee synovial fluids.” In: *Annals of the Rheumatic Diseases* 62.2 (Feb. 2003), pp. 108–112.

[208] Ruiz-Romero, C. and Blanco, F. J. “Proteomics role in the search for improved diagnosis, prognosis and treatment of osteoarthritis.” In: *Osteoarthritis and Cartilage* 18.4 (Apr. 2010), pp. 500–509.

[209] Cornier, M.-A. et al. “Assessing adiposity a scientific statement from the American Heart Association”. In: *Circulation* 124.18 (2011), pp. 1996–2019.

[210] Fairbank, J. C., Couper, J., Davies, J. B., and O’Brien, J. P. “The Oswestry low back pain disability questionnaire.” In: *Physiotherapy* 66.8 (Aug. 1980), pp. 271–273.

[211] Deyo, R. A. et al. “Outcome measures for low back pain research. A proposal for standardized use.” In: *Spine*. Department of Medicine, University of Washington, Seattle, USA. Sept. 1998, pp. 2003–2013.

[212] Fairbank, J. C. and Pynsent, P. “The Oswestry Disability Index.” In: *Spine* 25.22 (Nov. 2000),

[213] Monticone, M. et al. “Responsiveness of the Oswestry Disability Index and the Roland Morris Disability Questionnaire in Italian subjects with sub-acute and chronic low back pain”. In: *European Spine Journal* 21.1 (Jan. 2012), pp. 122–129.

[214] Leclaire, R., Blier, F., Fortin, L., and Proulx, R. “A cross-sectional study comparing the Oswestry and Roland-Morris Functional Disability scales in two populations of patients with low back pain of different levels of severity.” In: *Spine* 22.1 (Jan. 1997), pp. 68–71.

[215] Grotle, M., Brox, J. I., and Vollestad, N. K. “Cross-cultural adaptation of the Norwegian versions of the Roland-Morris Disability Questionnaire and the Oswestry Disability Index”. In: *Journal of Rehabilitation …* (2003).

[216] Mannion, A. F. et al. “Outcome assessment in low back pain: how low can you go?” In: *European Spine Journal* 14.10 (Dec. 2005), pp. 1014–1026.

[217] Miekisiak, G. et al. “Validation and Cross-Cultural Adaptation of the Polish Version of the Oswestry Disability Index”. In: *Spine* 38.4 (Feb. 2013), E237–E243.

[218] Valasek, T. et al. “Reliability and validity study on the Hungarian versions of the Oswestry Disability Index and the Quebec Back Pain Disability Scale”. In: *European Spine Journal* 22.5 (May 2013), pp. 1010–1018.

[219] Tilg, H. and Moschen, A. R. “Adipocytokines: mediators linking adipose tissue, inflammation and immunity”. In: *Nature Reviews Immunology* 6.10 (Sept. 2006), pp. 772–783.

[220] Ioan-Facsinay, A. and Kloppenburg, M. “An emerging player in knee osteoarthritis: the infrapatellar fat pad.” In: *Arthritis Research & Therapy* 15.6 (Dec. 2013), p. 225.

[221] Fain, J. N. et al. “Stimulation of leptin release by arachidonic acid and prostaglandin E2 in adipose tissue from obese humans”. In: *Metabolism* 50.8 (Aug. 2001), pp. 921–928.

[222] Fain, J. N. et al. “Comparison of PGE2, prostacyclin and leptin release by human adipocytes versus explants of adipose tissue in primary culture”. In: *Prostaglandins, Leukotrienes and Essential Fatty Acids* 67.6 (Dec. 2002), pp. 467–473.

[223] Fain, J. N. “Release of Inflammatory Mediators by Human Adipose Tissue Is Enhanced in Obesity and Primarily by the Nonfat Cells: A Review”. In: *Mediators of Inflammation* 2010 (2010), pp. 1–20.

[224] Sacks, H. S. and Fain, J. N. “Human epicardial adipose tissue: A review”. In: *American Heart Journal* 153.6 (June 2007), pp. 907–917.

[225] Distel, E. et al. “The infrapatellar fat pad in knee osteoarthritis: An important source of interleukin-6 and its soluble receptor”. In: *Arthritis & Rheumatism* 60.11 (Nov. 2009), pp. 3374–3377.

[226] Chen, T.-H. et al. “Evidence for a protective role for adiponectin in osteoarthritis”. In: *Biochimica et Biophysica Acta (BBA)* 1762.8 (Aug. 2006), pp. 711–718.

[227] Conde, J. et al. “Differential expression of adipokines in infrapatellar fat pad (IPFP) and synovium of osteoarthritis patients and healthy individuals.” In: *Annals of the Rheumatic Diseases* 73.3 (Mar. 2014), pp. 631–633.

[228] Lübbeke, A. et al. “Do synovial leptin levels correlate with pain in end stage arthritis?” In: *International Orthopaedics* 37.10 (Oct. 2013), pp. 2071–2079.

[229] Gandhi, R. et al. “Microarray Analysis of the Infrapatellar Fat Pad in Knee Osteoarthritis: Relationship with Joint Inflammation”. In: *The Journal of Rheumatology* 38.9 (Sept. 2011), pp. 1966–1972.

[230] Gandhi, R., Takahashi, M., Smith, H., Rizek, R., and Mahomed, N. N. “The synovial fluid adiponectin-leptin ratio predicts pain with knee osteoarthritis”. In: *Clinical Rheumatology* 29.11 (Mar. 2010), pp. 1223–1228.

[231] Trayhurn, P., Drevon, C. A., and Eckel, J. “Secreted proteins from adipose tissue and skeletal muscle – adipokines, myokines and adipose/muscle cross-talk”. In: *dx.doi.org* 117.2 (Apr. 2011), pp. 47–56.

[232] Raschke, S. and Eckel, J. “Adipo-myokines: two sides of the same coin—mediators of inflammation and mediators of exercise”. In: *Mediators of Inflammation* (2013).

[233] Hodges, P. W., James, G., Blomster, L., Hall, L., and Schmid, A. B. “Can proinflammatory cytokine gene expression explain multifidus muscle fiber changes after an intervertebral disc lesion?” In: *Spine* (2014).

[234] Weisberg, S. P. et al. “Obesity is associated with macrophage accumulation in adipose tissue. - PubMed - NCBI”. In: *The Journal of clinical investigation* 112.12 (Dec. 2003), pp. 1796–1808.

[235] Curat, C. A. et al. “Macrophages in human visceral adipose tissue: increased accumulatio... - PubMed - NCBI”. In: *Diabetologia* 49.4 (Feb. 2006), pp. 744–747.

[236] Wang, H., Schiltenwolf, M., and Buchner, M. “The Role of TNF-[alpha] in Patients With Chronic Low Back Pain-A Prospective Comparative Longitudinal Study”. In: *The Clinical journal of pain* 24.3 (2008), pp. 273–278.

[237] Wang, H. et al. “Influence of depression symptoms on serum tumor necrosis factor-α of patients with chronic low back pain.” In: *Arthritis Research & Therapy* 12.5 (2010), R186.

[238] Sturmer, T. et al. “Pain and high sensitivity C reactive protein in patients with chronic low back pain and acute sciatic pain.” In: *Annals of the Rheumatic Diseases* 64.6 (June 2005), pp. 921–925.

[239] Jones, S. “Clusterin”. In: *The International Journal of Biochemistry & Cell Biology* 34.5 (May 2002), pp. 427–431.

[240] Connor, J. “Clusterin expression in adult human normal and osteoarthritic articular cartilage”. In: *Osteoarthritis and Cartilage* 9.8 (Nov. 2001), pp. 727–737.

[241] Fandrids, E. et al. “Increased expression levels of apolipoprotein J/clusterin during primary osteoarthritis.” In: *In vivo (Athens, Greece)* 25.5 (Sept. 2011), pp. 745–749.

[242] Gruber, H. E., Hoelscher, G. L., Ingram, J. A., and Hanley Jr, E. N. “Genome-wide analysis of pain-, nerve-and neurotrophin-related gene expression in the degenerating human annulus”. In: *Mol Pain* (2012).

[243] Sarma, J. V. and Ward, P. A. “The complement system”. In: *Cell and Tissue Research* 343.1 (Sept. 2010), pp. 227–235.

[244] Wang, Q. et al. “Identification of a central role for complement in osteoarthritis”. In: *Nature Medicine* 17.12 (Dec. 2011), pp. 1674–1679.

[245] Sävendahl, L. and Underwood, L. E. “Fasting increases serum total cholesterol, LDL cholesterol and apolipoprotein B in healthy, nonobese humans.” In: *The Journal of Nutrition* 129.11 (Nov. 1999), pp. 2005–2008.

[246] Zung, W. W., Richards, C. B., and Short, M. J. “Self-rating depression scale in an outpatient clinic: further validation of the SDS”. In: *Archives of General Psychiatry* 13.6 (1965), p. 508.

[247] Pincus, T. et al. “A review and proposal for a core set of factors for prospective cohorts in low back pain: a consensus statement.” In: *Arthritis & Rheumatism* 59.1 (Jan. 2008), pp. 14–24.

[248] Sterne, J. A. C. et al. “Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls.” In: *BMJ (Clinical research ed.)* 338 (2009), b2393.

[249] White, I. R., Royston, P., and Wood, A. M. “Multiple imputation using chained equations: Issues and guidance for practice.” In: *Statistics in medicine* 30.4 (Feb. 2011), pp. 377–399.

[250] *Regression with STATA - Simple and Multiple Regression*. Sept. 2014.

[251] Hagen, E. M., Svensen, E., Eriksen, H. R., Ihlebaek, C. M., and Ursin, H. “Comorbid subjective health complaints in low back pain.” In: *Spine* 31.13 (June 2006), pp. 1491–1495.

[252] Mork, P. J., Vasseljen, O., and Nilsen, T. I. L. “Association between physical exercise, body mass index, and risk of fibromyalgia: longitudinal data from the Norwegian Nord-Trøndelag Health Study.” In: *Arthritis Care & Research* 62.5 (May 2010), pp. 611–617.

[253] Gariepy, G., Nitka, D., and Schmitz, N. “The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis.” In: *International journal of obesity* 34.3 (Mar. 2010), pp. 407–419.

[254] Whorwell, P. J., McCallum, M., Creed, F. H., and Roberts, C. T. “Non-colonic features of irritable bowel syndrome.” In: *Gut* 27.1 (1986), pp. 37–40.

[255] Delgado-Aros, S. et al. “Obesity is associated with increased risk of gastrointestinal symptoms: a population-based study.” In: *The American Journal of Gastroenterology* 99.9 (Sept. 2004), pp. 1801–1806.

[256] Shrive, F. M., Stuart, H., Quan, H., and Ghali, W. A. “Dealing with missing data in a multi-question depression scale: a comparison of imputation methods”. In: *BMC Medical Research Methodology* 6.1 (2006), p. 57.

[257] Kauppila, L. I. “Atherosclerosis and disc degeneration/low-back pain–a systematic review.” In: *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery* 37.6 (June 2009), pp. 661–670.

[258] Kauppila, L. I. “Prevalence of stenotic changes in arteries supplying the lumbar spine. A postmortem angiographic study on 140 subjects.” In: *Annals of the Rheumatic Diseases* 56.10 (Oct. 1997), pp. 591–595.

[259] Leino-Arjas, P., Solovieva, S., Kirjonen, J., Reunanen, A., and Riihimäki, H. “Cardiovascular risk factors and low-back pain in a long-term follow-up of industrial employees.” In: *Scandinavian journal of work, environment & health* 32.1 (Feb. 2006), pp. 12–19.

[260] Grundy, S. M. et al. “Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition.” In: *Circulation*. Lippincott Williams & Wilkins, Jan. 2004, pp. 433–438.

[261] Zaninotto, P., Pierce, M., Breeze, E., Oliveira, C. de, and Kumari, M. “BMI and waist circumference as predictors of well-being in older adults: findings from the English Longitudinal Study of Ageing.” In: *Obesity* 18.10 (Oct. 2010), pp. 1981–1987.

[262] *UK Biobank Data Showcase*. Apr. 2014.

[263] Vamos, E. P., Kopp, M. S., Keszei, A., Novak, M., and Mucsi, I. “Prevalence of diabetes in a large, nationally representative population sample in Hungary.” In: *Diabetes research and clinical practice* 81.3 (Sept. 2008), e5–8.

[264] Puhl, R. and Brownell, K. D. “Bias, discrimination, and obesity.” In: *Obesity research* 9.12 (Dec. 2001), pp. 788–805.

[265] Judge, A. et al. “Body mass index is not a clinically meaningful predictor of patient reported outcomes of primary hip replacement surgery: prospective cohort study.” In: *Osteoarthritis and Cartilage* 22.3 (Mar. 2014), pp. 431–439.

[266] Fanuele, J. C., Abdu, W. A., Hanscom, B., and Weinstein, J. N. “Association between obesity and functional status in patients with spine disease.” In: *Spine* 27.3 (Feb. 2002), pp. 306–312.

[267] Heuch, I., Heuch, I., Hagen, K., and Zwart, J.-A. “Body mass index as a risk factor for developing chronic low back pain: a follow-up in the Nord-Trøndelag Health Study.” In: *Spine* 38.2 (Jan. 2013), pp. 133–139.

[268] Khoueir, P. et al. “Prospective assessment of axial back pain symptoms before and after bariatric weight reduction surgery.” In: *The Spine Journal* 9.6 (June 2009), pp. 454–463.

[269] Melissas, J. et al. “The effect of surgical weight reduction on functional status in morbidly obese patients with low back pain.” In: *Obesity surgery* 15.3 (Mar. 2005), pp. 378–381.

[270] Lidar, Z. et al. “Intervertebral disc height changes after weight reduction in morbidly obese patients and its effect on quality of life and radicular and low back pain.” In: *Spine* 37.23 (Nov. 2012), pp. 1947–1952.

[271] Smuck, M. et al. “Does physical activity influence the relationship between low back pain and obesity?” In: *The Spine Journal* 14.2 (Feb. 2014), pp. 209–216.

[272] Ursini, F., Naty, S., and Grembiale, R. D. “Fibromyalgia and obesity: the hidden link.” In: *Rheumatology international* 31.11 (Nov. 2011), pp. 1403–1408.

[273] Roussel, N. A. et al. “Central sensitization and altered central pain processing in chronic low back pain: fact or myth?” In: *The Clinical journal of pain* 29.7 (July 2013), pp. 625–638.

[274] Farrar, J. T., Young, J. P., 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.” In: *Pain* 94.2 (Nov. 2001), pp. 149–158.

[275] Childs, J. D., Piva, S. R., and Fritz, J. M. “Responsiveness of the numeric pain rating scale in patients with low back pain.” In: *Spine* 30.11 (June 2005), pp. 1331–1334.

[276] Ostelo, R. W. J. G. et al. “Interpreting change scores for pain and functional status in low back pain: towards international consensus regarding minimal important change.” In: *Spine* 33.1 (Jan. 2008), pp. 90–94.

[277] Younes, M. et al. “Prevalence and risk factors of disk-related sciatica in an urban population in Tunisia.” In: *Joint Bone Spine* 73.5 (Oct. 2006), pp. 538–542.

[278] Heliövaara, M. “Body height, obesity, and risk of herniated lumbar intervertebral disc.” In: *Spine* 12.5 (June 1987), pp. 469–472.

[279] Kalichman, L. et al. “Spinal stenosis prevalence and association with symptoms: the Framingham Study.” In: *The Spine Journal* 9.7 (July 2009), pp. 545–550.

[280] Kalichman, L. et al. “Spondylolysis and spondylolisthesis: prevalence and association with low back pain in the adult community-based population.” In: *Spine* 34.2 (Jan. 2009), pp. 199–205.

[281] Jacobsen, S., Sonne-Holm, S., Rovsing, H., Monrad, H., and Gebuhr, P. “Degenerative lumbar spondylolisthesis: an epidemiological perspective: the Copenhagen Osteoarthritis Study.” In: *Spine* 32.1 (Jan. 2007), pp. 120–125.

[282] Weinstein, J. N. et al. “Surgical vs nonoperative treatment for lumbar disk herniation: the Spine Patient Outcomes Research Trial (SPORT): a randomized trial.” In: *JAMA* 296.20 (Nov. 2006), pp. 2441–2450.

[283] Modic, M. T., Masaryk, T., Boumphrey, F., Goormastic, M., and Bell, G. “Lumbar herniated disk disease and canal stenosis: prospective evaluation by surface coil MR, CT, and myelography.” In: *American Journal of Roentgenology* 147.4 (Oct. 1986), pp. 757–765.

[284] Cong, L., Zhu, Y., Pang, H., and Guanjun, T. U. “The interaction between aggrecan gene VNTR polymorphism and obesity in predicting incident symptomatic lumbar disc herniation.” In: *Connective tissue research* (Sept. 2014), pp. 1–7.

[285] Venkatesan, M., Uzoigwe, C. E., Perianayagam, G., Braybrooke, J. R., and Newey, M. L. “Is cauda equina syndrome linked with obesity?” In: *The Journal of bone and joint surgery British volume* 94.11 (Nov. 2012), pp. 1551–1556.

[286] Lee, D. Y. and Lee, S.-H. “Effect of anthropometric features on the severity of lumbar disk herniation”. In: *Neurologia medico-chirurgica* 49.3 (2009), pp. 104–107.

[287] Kelsey, J. L. “An epidemiological study of acute herniated lumbar intervertebral discs.” In: *Rheumatology and rehabilitation* 14.3 (Aug. 1975), pp. 144–159.

[288] Böstman, O. M. “Body mass index and height in patients requiring surgery for lumbar intervertebral disc herniation”. In: *Spine* 18.7 (1993), pp. 851–854.

[289] Jonsson, B. and Stromqvist, B. “Influence of age on symptoms and signs in lumbar disc herniation”. In: *European Spine Journal* 4.4 (1995), pp. 202–205.

[290] Rainville, J. and Lopez, E. “Comparison of Radicular Symptoms Caused by Lumbar Disc Herniation and Lumbar Spinal Stenosis in the Elderly”. In: *Spine* 38.15 (2013), pp. 1282–1287.

[291] Lama, P. et al. “Do intervertebral discs degenerate before they herniate, or after?” In: *The bone & joint journal* 95-B.8 (Aug. 2013), pp. 1127–1133.

[292] Hirano, K., Imagama, S., Hasegawa, Y., Muramoto, A., and Ishiguro, N. “Impact of spinal imbalance and BMI on lumbar spinal canal stenosis determined by a diagnostic support tool: cohort study in community-living people”. In: *Archives of Orthopaedic and Trauma Surgery* 133.11 (2013), pp. 1477–1482.

[293] Denard, P. J. et al. “Lumbar spondylolisthesis among elderly men: prevalence, correlates, and progression.” In: *Spine* 35.10 (May 2010), pp. 1072–1078.

[294] Schuller, S., Charles, Y. P., and Steib, J.-P. "Sagittal spinopelvic alignment and body mass index in patients with degenerative spondylolisthesis". In: *European Spine Journal* 20.5 (Dec. 2010), pp. 713–719.

[295] Whitehead, W. E., Palsson, O., and Jones, K. R. "Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications?" In: *Gastroenterology* 122.4 (Apr. 2002), pp. 1140–1156.

[296] Chou, R. and Shekelle, P. "Will this patient develop persistent disabling low back pain?" In: *JAMA* 303.13 (Apr. 2010), pp. 1295–1302.

[297] Battié, M. C. et al. "Lumbar spinal stenosis is a highly genetic condition partly mediated by disc degeneration." In: *Arthritis & Rheumatism* (Aug. 2014).

[298] Jensen, T. S., Sorensen, J. S., and Kjaer, P. "Intra- and Interobserver Reproducibility of Vertebral Endplate Signal (Modic) Changes in the Lumbar Spine: The Nordic Modic Consensus Group Classification". In: *Acta radiologica* 48.7 (Jan. 2007), pp. 748–754.

[299] Kreiner, D. S. et al. *An evidence-based clinical guideline for the diagnosis and treatment of degenerative lumbar spinal stenosis (update).* July 2013.

[300] Battié, M. C. et al. "1991 Volvo Award in clinical sciences. Smoking and lumbar intervertebral disc degeneration: an MRI study of identical twins." In: *Spine* 16.9 (1991), p. 1015.

[301] Battié, M. C. et al. "1995 Volvo Award in clinical sciences. Determinants of lumbar disc degeneration. A study relating lifetime exposures and magnetic resonance imaging findings in identical twins." In: *Spine* 20.24 (Dec. 1995), pp. 2601–2612.

[302] Zhang, Y.-H., Zhao, C.-Q., Jiang, L.-S., Chen, X.-D., and Dai, L.-Y. "Modic changes: a systematic review of the literature." In: *European Spine Journal* 17.10 (Oct. 2008), pp. 1289–1299.

[303] Albert, H. B. et al. "The prevalence of MRI-defined spinal pathoanatomies and their association with Modic changes in individuals seeking care for low back pain". In: *European Spine Journal* 20.8 (May 2011), pp. 1355–1362.

[304] Albert, H. B. and Manniche, C. "Modic changes following lumbar disc herniation." In: *European Spine Journal* 16.7 (July 2007), pp. 977–982.

[305] Sonne-Holm, S., Jacobsen, S., Rovsing, H., and Monrad, H. "The epidemiology of Schmorl's nodes and their correlation to radiographic degeneration in 4,151 subjects". In: *European Spine Journal* 22.8 (Mar. 2013), pp. 1907–1912.

[306] Wang, Y., Battié, M. C., Boyd, S. K., and Videman, T. "The osseous endplates in lumbar vertebrae: thickness, bone mineral density and their associations with age and disk degeneration." In: *Bone* 48.4 (Apr. 2011), pp. 804–809.

[307] Pye, S. R. et al. "Lumbar disc degeneration: association between osteophytes, end-plate sclerosis and disc space narrowing." In: *Annals of the Rheumatic Diseases* 66.3 (Mar. 2007), pp. 330–333.

[308] Hangai, M. et al. "Factors associated with lumbar intervertebral disc degeneration in the elderly." In: *The Spine Journal* 8.5 (Sept. 2008), pp. 732–740.

[309] Kanayama, M., Togawa, D., Takahashi, C., Terai, T., and Hashimoto, T. “Cross-sectional magnetic resonance imaging study of lumbar disc degeneration in 200 healthy individuals.” In: *Journal of neurosurgery. Spine* 11.4 (Oct. 2009), pp. 501–507.

[310] Takatalo, J. et al. “Body mass index is associated with lumbar disc degeneration in young Finnish males: subsample of Northern Finland birth cohort study 1986”. In: *BMC musculoskeletal disorders* 14.1 (2013), p. 87.

[311] Benneker, L. M., Heini, P. F., Anderson, S. E., Alini, M., and Ito, K. “Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration.” In: *European Spine Journal* 14.1 (Feb. 2005), pp. 27–35.

[312] Kalichman, L., Guermazi, A., Li, L., and Hunter, D. J. “Association between age, sex, BMI and CT-evaluated spinal degeneration features.” In: *Journal of back and musculoskeletal rehabilitation* 22.4 (2009), pp. 189–195.

[313] Hassett, G., Hart, D. J., Manek, N. J., Doyle, D. V., and Spector, T. D. “Risk factors for progression of lumbar spine disc degeneration: the Chingford Study.” In: *Arthritis & Rheumatism* 48.11 (Nov. 2003), pp. 3112–3117.

[314] Videman, T., Gibbons, L. E., Kaprio, J., and Battié, M. C. “Challenging the cumulative injury model: positive effects of greater body mass on disc degeneration.” In: *The Spine Journal* 10.1 (Jan. 2010), pp. 26–31.

[315] Jensen, T. S. et al. “Predictors of new vertebral endplate signal (Modic) changes in the general population”. In: *European Spine Journal* 19.1 (Nov. 2009), pp. 129–135.

[316] Arana, E. et al. “Modic changes and associated features in Southern European chronic low back pain patients.” In: *The Spine Journal* 11.5 (May 2011), pp. 402–411.

[317] Jhawar, B. S., Fuchs, C. S., Colditz, G. A., and Stampfer, M. J. “Cardiovascular risk factors for physician-diagnosed lumbar disc herniation.” In: *The Spine Journal* 6.6 (Nov. 2006), pp. 684–691.

[318] Saberi, H., Rahimi, L., and Jahani, L. “A comparative MRI study of upper and lower lumbar motion segments in patients with low back pain.” In: *Journal of spinal disorders & techniques* 22.7 (Oct. 2009), pp. 507–510.

[319] Dammers, R. and Koehler, P. J. “Lumbar disc herniation: level increases with age”. In: *Surgical neurology* 58.3 (2002), pp. 209–212.

[320] Ma, D. et al. “Trend of the incidence of lumbar disc herniation: decreasing with aging in the elderly”. In: *Clinical interventions in aging* 8 (2013), p. 1047.

[321] Gandhi, R., Woo, K. M., Zywiel, M. G., and Rampersaud, Y. R. “Metabolic syndrome increases the prevalence of spine osteoarthritis.” In: *Orthopaedic surgery* 6.1 (Feb. 2014), pp. 23–27.

[322] Jensen, R. K., Jensen, T. S., Kjaer, P., and Kent, P. “Can pathoanatomical pathways of degeneration in lumbar motion segments be identified by clustering MRI findings.” In: *BMC musculoskeletal disorders* 14.1 (2013), p. 198.

[323] Adams, M. A. and Dolan, P. “Intervertebral disc degeneration: evidence for two distinct phenotypes”. In: *Journal of anatomy* 221.6 (2012), pp. 497–506.

[324] Werndle, M. C., Reza, A., Wong, K., and Papadopoulos, M. C. "Acute disc herniation in the elderly." In: *British journal of neurosurgery* 26.2 (Apr. 2012), pp. 255–257.

[325] Ikeda, Y., Nakajima, A., Aiba, A., Koda, M., and Okawa, A. "Association between serum leptin and bone metabolic markers, and the development of heterotopic ossification of the spinal ligament in female patients with …". In: *European Spine Journal* (2011).

[326] Callaghan, J. P. and McGill, S. M. "Intervertebral disc herniation: studies on a porcine model exposed to highly repetitive flexion/extension motion with compressive force". In: *Clinical Biomechanics* (2001).

[327] Kawaguchi, Y. et al. "Association between an aggrecan gene polymorphism and lumbar disc degeneration." In: *Spine* 24.23 (Dec. 1999), pp. 2456–2460.

[328] Kawaguchi, Y. et al. "The association of lumbar disc disease with vitamin-D receptor gene polymorphism." In: *The Journal of bone and joint surgery American volume* 84-A.11 (Nov. 2002), pp. 2022–2028.

[329] Solovieva, S. et al. "COL9A3 gene polymorphism and obesity in intervertebral disc degeneration of the lumbar spine: evidence of gene-environment interaction." In: *Spine* 27.23 (Dec. 2002), pp. 2691–2696.

[330] Vogt, M. T., Hanscom, B., Lauerman, W. C., and Kang, J. D. "Influence of smoking on the health status of spinal patients: the National Spine Network database." In: *Spine* 27.3 (Feb. 2002), pp. 313–319.

[331] Goldberg, M. S., Scott, S. C., and Mayo, N. E. "A review of the association between cigarette smoking and the development of nonspecific back pain and related outcomes." In: *Spine* 25.8 (Apr. 2000), pp. 995–1014.

[332] Yanbaeva, D. G., Dentener, M. A., Creutzberg, E. C., Wesseling, G., and Wouters, E. F. M. "Systemic effects of smoking." In: *Chest* 131.5 (May 2007), pp. 1557–1566.

[333] Akmal, M. et al. "Effect of nicotine on spinal disc cells: a cellular mechanism for disc degeneration." In: *Spine* 29.5 (Mar. 2004), pp. 568–575.

[334] Kim, H.-J. et al. "Gender difference of symptom severity in lumbar spinal stenosis: role of pain sensitivity." In: *Pain physician* 16.6 (Nov. 2013), E715–23.

[335] Lim, J.-M., Wollaston-Hayden, E. E., Teo, C. F., Hausman, D., and Wells, L. "Quantitative secretome and glycome of primary human adipocytes during insulin resistance". In: *Clinical Proteomics* 11.1 (May 2014), p. 20.

[336] Francin, P. J., Abot, A., Guillaume, C., and Moulin, D. "Association between adiponectin and cartilage degradation in human osteoarthritis". In: *Osteoarthritis and …* (2014).

[337] Ouchi, N., Parker, J. L., Lugus, J. J., and Walsh, K. "Adipokines in inflammation and metabolic disease". In: *Nature Reviews Immunology* 11.2 (Jan. 2011), pp. 85–97.

[338] Karvonen-Gutierrez, C. A., Harlow, S. D., Jacobson, J., Mancuso, P., and Jiang, Y. "The relationship between longitudinal serum leptin measures and measures of magnetic resonance imaging-assessed knee joint damage in a population of mid-life women." In: *Annals of the Rheumatic Diseases* 73.5 (May 2014), pp. 883–889.

[339] Li, H., Jiang, L.-S., and Dai, L.-Y. “Hormones and growth factors in the pathogenesis of spinal ligament ossification”. In: *European Spine Journal* 16.8 (Aug. 2007), pp. 1075–1084.

[340] Fan, D., Chen, Z., Chen, Y., and Shang, Y. “Mechanistic roles of leptin in osteogenic stimulation in thoracic ligament flavum cells.” In: *Journal of Biological Chemistry* 282.41 (Oct. 2007), pp. 29958–29966.

[341] Dario, A. B. et al. “The relationship between obesity, low back pain, and lumbar disc degeneration when genetics and the environment are considered: a systematic review of twin studies.” In: *The Spine Journal* (Feb. 2015).

[342] Moayyeri, A., Hammond, C. J., Valdes, A. M., and Spector, T. D. “Cohort Profile: TwinsUK and healthy ageing twin study.” In: *International Journal of Epidemiology* 42.1 (Feb. 2013), pp. 76–85.

APPENDICES

A

Materials List

Reagent	Product Code	Supplier
1,9-Dimethyl-Methylene Blue zinc chloride (DMMB)	341088	Sigma-Aldrich
2-Mercaptoethanol	M6250	Sigma-Aldrich
10% Ready Gel Zymogram Gel	161-1167	Bio-Rad Laboratories
12% Mini-Protean TGX Gel	456-1043	Bio-Rad Laboratories
Alginic acid (sodium salt) from brown algae	71238	Sigma-Aldrich
Antibiotic-Antimycotic (Gibco)	15240-062	Life Technologies
Amersham ECL Rabbit IgG, HRP-linked whole Ab (from donkey)	NA934	GE Life Sciences
Amersham ECL Western blotting detection reagent	RPN2106	GE Life Sciences
Amersham Hyperfilm ECL	28-9068-36	GE Life Sciences
Amersham ECL Blocking Agent	RPN2125	GE Life Sciences
Anti-MMP1 antibody (rabbit)	ab137332	abcam
Anti-MMP3 antibody (rabbit)	ab53015	abcam
Anti-MMP13 antibody (rabbit)	ab39012	abcam
Bos tarus geNorm- kit	ge-SY-6-bt	PrimerDesign
Bright White real-time PCR 96-well plates	BW-96AB1	PrimerDesign
Chondroitin sulfate A sodium salt from bovine trachea	C9819	Sigma-Aldrich
Collagenase Type I from *Clostridium histolyticum*	C0130	Sigma-Aldrich
Custom real-time PCR primer kit (sequences in Appendix C	SY-any-600	PrimerDesign
DC Protein Assay	500-0116	Bio-Rad Laboratories
Dulbecco's Modified Eagle Medium /Nutrient Mixture F-12 (DMEM/F-12)	11330-032	Life Technologies
Dulbecco's Modified Eagle Medium (DMEM) with 25mM Hepes, 1mM sodium pyruvate, 1000mg/L glucose and pyroxidine (Gibco)	22320	Life Technologies
Foetal Bovine Serum	10106-169	Life Technologies
Human Adiponectin Kit	K151BXC	Meso Scale Diagnostics
Human Leptin Kit	K151BYC	Meso Scale Diagnostics
Human Proinflammatory Panel 1 (human) V-PLEX Kit	K15049D	Meso Scale Diagnostics

iBlot Gel Transfer Device	IB1001	Life Technologies
iBlot PVDF Gel Transfer Stack	IB401001	Life Technologies
InstantBlue Protein Stain	ISB1L	Expedeon
ISOLUTE PH 25 mg/1 ml Solid Phase Extraction Columns	360-0002-A	Biotage
Kaleidoscope Prestained Molecular Weight Standards	161-0324	Bio-Rad Laboratories
Lactate Assay Kit	735-10	Trinity Biotech
Laemmli Sample Buffer (2x)	161-0737	Bio-Rad
nanoScript 2 Reverse Transcription kit		
Papain from papaya latex	P4762	Sigma-Aldrich
Phosphate Buffered Saline pH 7.4 (Gibco)	10010-056	Life Technologies
Pierce Top 12 Abundant Protein Depletion Spin Columns	85164	Perbio Science UK
Precision DNase kit	DNASE-50	PrimerDesign
Precision MasterMix with ROX and SYBRgreen	Precision-R-SY	PrimerDesign
Precision nanoScript Reverse transcription kit	RT-nanoScript	PrimerDesign
Protease Type XIV from *Streptomyces griseus*	P5147	Sigma-Aldrich
ProteoMiner Protein Enrichment Small-Capacity Kit	163-3006	Bio-Rad Laboratories
QIAshredder	79654	Qiagen
Recombinant Bovine IL-1β	RP0106B	2BScientific
Recombinant Bovine IL-6	RP0014B	2BScientific
Recombinant Bovine TNF-α	RP0055B	2BScientific
Recombinant Bovine Leptin	MBS232445	MyBioSource
Recombinant Human Adiponectin/Acrp30	1065-AP-050	R & D Systems
Recombinant Human MMP-1 Western Blot Standard	WBC024	R & D Systems
Recombinant Human MMP-2	420-02	PeproTech
Recombinant Human MMP-3 Western Blot Standard	WBC015	R & D Systems
Recombinant Human MMP-13 Western Blot Standard	WBC020	R & D Systems
RNeasy Mini Kit	74104	Qiagen
SensoLyte 520 Generic MMP Assay Kit (Fluorimetric)	71158	AnaSpec
SimplyBlue SafeStain (Novex)	LC6060	Life Technologies
Sodium hypochlorite	11448842	Fisher Scientific
StrataClean Resin	400714	Aligent Technologies
Triton X-100	T8787	Sigma-Aldrich
Trypsin Gold (Mass Spectrometry Grade)	V5280	Promega UK
Zymogram Developing Buffer (Novex)	LC2671	Life Technologies
Zymogram Renaturing Buffer (Novex)	LC2670	Life Technologies

B
Buffers

Citrate buffer

- 55 mM sodium citrate
- 50 mM EDTA
- 0.15 mM sodium chloride
- at pH 7.4

Papain Buffer

- 1 mM EDTA
- 1mM cysteine HCl
- 50mM Trizma Buffer
- Make up to 100ml with ultra pure water
- adjust to pH 6.8

C

PCR Primers

Gene	Sense 5'-3'	Antisense 3'-5'
TNF-α	ATGATGCTGATTTGGTGACTGA	ACACTTTATTTCTCGCCACTGA
IL-1β	CACAGGAAATGAACCGAGAAGT	TTTCACACAAGACAGGTATAGATTC
IL-6	TGACCACTCCAGAGAAAACC	TTCTGCCAGTGTCTCCTTG
ACAN	CAGATGATTCAGAGGCAACCA	CGGGAAGTGGCGGTAACA
ADAMTS-1	ACTATGACACGGCGATTCTATT	GCTTCTGCTGGGATCACAT
ADAMTS-4	CCACTTTGACACAGCCATTCT	CGTCAGCCATGCCTAGAGT
ADAMTS-5	CCAGCATTGACGCATCCA	GGTAGGTAAAGCAAACAGT
COL1A1	GACTGTCCTAACGCCAAAGT	TTTCTTGGTCCGTGGGTGAT
COL2A1	CCAGCGTCCCCAAGAAGA	CCAGGTTGTCATCTCCATAGC
COL6A6	GGAAGCAGAGGAGCAAAGG	CCCAGCAGATACCGTCTTG
MMP-1	GCTTTCTCAGGACGACATTG	GCTTGCTATCACACACTTCTG
MMP-2	CCGTCGCCCATCATCAAAT	GCCGTAGAAGGTGTTTAGGTAT
MMP-7	TGAGGATGAACGCTGGACTG	TAGGTTGGATACATCACAGCATTAG
MMP-9	ATTAGCACGCACGACATCTT	CAGGGCACTTCAGGAGGT
MMP-11	AGAAGACGGACCTCACCTAC	TGACATCGCTCCACACCT
TIMP-1	TGCTGCTGGTTGTGAAGGAA	TGGAACCCCTTGTCAAGAGC
TIMP-2	TGGACTCATGGCAACGACAT	AGGAGGGGGGCTGTGTGTAGAT
TIMP-3	ATATCACCTGGGCTGTAACTG	CCGAAATTGGAGAGCATGTC
TIMP-4	GAAGCGGTATCTCCTGACTG	CAGATGGTAGTGGTGATTCAGA

D

Clinical Assessment Questionnaire

Extra Data Sheet

OMB Label

Demographics

1) Age _____

2) Gender _____

3) Height _____

4) Weight _____

5) Waist Circumference _____

6) Smoking □ Current □ Previous □ Never
_____/wk

7) Occupation _____

Clinical History

8) Diagnosis at Surgery

_____

9) Duration of Symptoms

<6 months 6-12 months >12 months NA

10) Previous Surgery

_____

11) Co-Morbidities

HTN IHD DM VTE OA Thyroid CA

12) Medications

Bisphos	Calcium	Phosphate	
Aspirin	NSAIDs	Opioids	
ACEI	Allopurinol	PPI	Statin

13) Other Comments

E

Oswestry Disability Index

Oswestry Disability Index 2.1a

Patient Label

First Name

Last Name

Date of Birth (mm/dd/yyyy)

Could you please complete this questionnaire? It is designed to give us information as to how your back (or leg) trouble affects your ability to manage in everyday life. Please answer ***every section.*** Fill in the ***one bubble only*** in each section that most closely describes you ***today.***

Section 1 - Pain intensity
- I have no pain at the moment.
- The pain is very mild at the moment.
- The pain is moderate at the moment.
- The pain is fairly severe at the moment.
- The pain is very severe at the moment.
- The pain is the worst imaginable at the moment.

Section 2 - Personal care (washing, dressing, etc.)
- I can look after myself normally without causing extra pain.
- I can look after myself normally but it is very painful.
- It is painful to look after myself and I am slow and careful.
- I need some help but manage most of my personal care.
- I need help every day in most aspects of self care.
- I do not get dressed, wash with difficulty and stay in bed.

Section 3 - Lifting
- I can lift heavy weights without extra pain.
- I can lift heavy weights but it gives extra pain.
- Pain prevents me from lifting heavy weights off the floor but I can manage if they are conveniently positioned, e.g. on a table.
- Pain prevents me from lifting heavy weights but I can manage light to medium weights if they are conveniently positioned.
- I can lift only very light weights.
- I cannot lift or carry anything at all.

Section 4 - Walking
- Pain does not prevent me walking any distance.
- Pain prevents me walking more than one mile.
- Pain prevents me walking more than a quarter of a mile.
- Pain prevents me walking more than 100 yards.
- I can only walk using a stick or crutches.
- I am in bed most of the time and have to crawl to the toilet.

Section 5 - Sitting
- I can sit in any chair as long as I like.
- I can sit in my favourite chair as long as I like.
- Pain prevents me from sitting more than 1 hour.
- Pain prevents me from sitting for more than half an hour.
- Pain prevents me from sitting for more than 10 minutes.
- Pain prevents me from sitting at all.

Section 6 - Standing
- I can stand as long as I want without extra pain.
- I can stand as long as I want but it gives me extra pain.
- Pain prevents me from standing for more than 1 hour.
- Pain prevents me from standing for more than half an hour.
- Pain prevents me from standing for more than 10 minutes.
- Pain prevents me from standing at all.

Section 7 - Sleeping
- My sleep is never disturbed by pain.
- My sleep is occasionally disturbed by pain.
- Because of pain I have less than 6 hours of sleep.
- Because of pain I have less than 4 hours of sleep.
- Because of pain I have less than 2 hours of sleep.
- Pain prevents me from sleeping at all.

Section 8 - Sex life (if applicable)
- My sex life is normal and causes no extra pain.
- My sex life is normal but causes some extra pain.
- My sex life is nearly normal but is very painful.
- My sex life is severely restricted by pain.
- My sex life is nearly absent because of pain.
- Pain prevents any sex life at all.

Section 9 - Social life
- My social life is normal and causes me no extra pain.
- My social life is normal but increases the degree of pain.
- Pain has no significant effect on my social life apart from limiting my more energetic interests, e.g., sport,etc.
- Pain has restricted my social life and I do not go out as often.
- Pain has restricted social life to my home.
- I have no social life because of pain.

Section 10 - Travelling
- I can travel anywhere without pain.
- I can travel anywhere but it gives extra pain.
- Pain is bad but I manage journeys over two hours.
- Pain restricts me to journeys of less than one hour.
- Pain restricts me to short necessary journeys under 30 minutes.
- Pain prevents me from travelling except to receive treatment.

References:
Fairbank JC, Pynsent PB. "The Oswestry Disability Index" Spine 2000; 25(22): 2940-2952
Fairbank JC, Couper J, Davies JB. "The Oswestry Low Back Pain Questionnaire" Physiotherapy 1980: 66: 271-273

F

GENODISC PATIENT SURVEY

Genodisc No. ☐

Participant Survey

Today's date: __ __/__ __/__ __ __ __ (day/month/year)

Gender: ☐ Female ☐ Male

Date of birth: __ __/__ __/__ __ __ __ (day/month/year)

What is your body weight? _____Kg **Standing height?** _____cm

Ethnicity: *Insert appropriate categories for your recruitment site here. It may be good to have Jaakko look at the categories you are planning to use in case he has further suggestions based on his experience.*

In the past 4 weeks, have you had pain in your low back?

☐ Yes ☐ No

If yes, how long ago did *this* episode of low back pain begin? ____months ago

Is the pain worse with coughing and sneezing? ☐ Yes ☐ No

About how many episodes of back pain lasting more than 1 day have you had over your lifetime? _____

In the past 4 weeks, have you had pain that goes down the leg? ☐ Yes ☐ No

If yes, does it go down the: ☐ left leg ☐ right leg ☐ both legs

If yes, does the pain go down below the knee? ☐ Yes ☐ No

If you have had pain that goes down the leg:

How long ago did this episode of leg pain begin? _____months ago

Is this the first episode of such leg pain you have had? ☐ Yes ☐ No

If no, about how many previous episodes of such leg pain have you had? _____

In the past week, how bothersome have the following symptoms been?

Using a scale from 0 to 10, where 0=no pain and 10=the worst pain imaginable:

Back pain _____

Leg pain _____

Did your back (or leg) pain problem begin within 24 hours following trauma (e.g., a fall, being struck by an object, a motor vehicle accident)? ☐ Yes ☐ No

The next few questions are designed to give us information as to how your back (or leg) trouble affects your ability to manage in everyday life.

Walking: (Mark one box that most closely describes you today)
- ☐ Pain does not prevent me walking any distance.
- ☐ Pain prevents me walking more than one mile (~=1Km).
- ☐ Pain prevents me walking more than a quarter of a mile (~=500m).
- ☐ Pain prevents me walking more than 100 yards (~=100m).
- ☐ I can only walk using a stick or crutches.
- ☐ I am in bed most of the time and have to crawl to the toilet.

(alternative distances for metric countries)

Because of back pain, do you prefer: (tick one response)

- ☐ walking fast
- ☐ walking slow
- ☐ standing

Because of back pain, for a small amount of shopping, do you prefer: (tick one response)

- ☐ pushing a shopping trolley
- ☐ carrying a shopping basket

During the past 4 weeks, how many days did low back pain or leg pain (sciatica) keep you from going to work or school? _____days

During the past 4 weeks, how much did the pain interfere with your normal work (including both work outside the home and housework)?

- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Have any of your parents or siblings had the same condition for which you are now being treated?
☐ Yes ☐ No

If yes, how many other members of your family (parents and siblings) have had the same condition? _____

How old were you when you had your first episode of low back pain lasting more than one day? ____years old

Have you had back surgery? ☐ Yes ☐ No

If yes, in what year? _____

Have you received a permanent disability award because of your back problem? ☐ Yes ☐ No

Have you had <u>neck pain</u> lasting more than one day over the past 12 months? ☐ Yes ☐ No

If yes, over the past 12 months, how many days have you had difficulty doing your daily work (at home, school or at the work site) due to neck problems? _____days

Now, we would like to ask you about other health conditions you may have.
Tick the box for each condition which applies to you.

Please indicate if you have any of the following conditions.	
Please tick box if Present	
☐	Arthritis: ☐ rheumatoid arthritis ☐ osteoarthritis: ☐ hip ☐ knee ☐ hand ☐ other,_____
☐	Osteoporosis
☐	Fibromyalgia
☐	Asthma
☐	Chronic obstructive pulmonary disease (COPD) Respiratory distress (ARDS) or emphysema
☐	Migraine or frequent headaches
☐	Angina / coronary heart disease
☐	High blood pressure or peripheral vascular disease
☐	Irritable bowel syndrome
☐	Upper gastrointestinal disease (ulcer, hernia, reflux)
☐	Depression
☐	Anxiety or panic disorders
☐	Diabetes
☐	Cancer, type:_____

Which of the following alternatives best characterizes your current smoking habits? (tick one response)

☐ Non-smoker (I have never smoked more than 100 cigarettes in my lifetime)
☐ I have smoked earlier, but I no longer smoke
☐ I smoke at most 10 cigarettes daily
☐ I smoke 11-20 cigarettes daily
☐ I smoke 21-30 cigarettes daily
☐ I smoke more than 30 cigarettes a day

If you smoke, how soon after you wake up do you smoke your first cigarette? (tick one response)

☐ more than 60 min
☐ 31-60 min
☐ 6-30 min
☐ 5 minutes or less

How many hours per day do you spend, on average, driving or as a passenger in motorized vehicles (i.e., car, tractor, truck, public transport, etc.)? ___ hours per day

Which of the following best describes the physical demands of the jobs you have held for the majority of your working years? (tick one response)

- ☐ sedentary work (spending nearly all day sitting, with little lifting)
- ☐ light physical demands (involves sitting and standing or walking activities, and light lifting (generally less than 5 kg) and little work in awkward twisted or bent positions)
- ☐ medium physical demands (lifting and handling of weights generally less than 20 kg and occasional work in awkward twisted or bent positions)
- ☐ heavy physical demands (frequent lifting of materials over 20 kg or work requiring hours during the day in awkward twisted or bent positions)

I am still a full-time student (elementary, high school or university student) ☐ Yes ☐ No

During the majority of your working years, have the physical demands on your back (e.g. lifting, twisting, bending, etc.) been greatest during your employment OR home and leisure activities (tick one response)?

- ☐ greatest during my regular work (employment) duties
- ☐ greatest during home and leisure activities

Over the past year, on average, how many times a week have you engaged in vigorous sports or leisure activities involving twisting, bending or lifting? ____ (times per week)

The next questions deal with how you are feeling.
Please read each statement and decide how much of the time the statement describes how you have been feeling during the past several days

Make check mark (☑) in appropriate column.	A little of the time	Some of the time	Good part of the time	Most of the time
1. I feel down-hearted and blue (sad)				
2. Morning is when I feel the best				
3. I have crying spells or feel like it				
4. I have trouble sleeping at night				
5. I eat as much as I used to				
6. I still enjoy sex				
7. I notice that I am losing weight				
8. I have trouble with constipation				
9. My heart beats faster than usual				
10. I get tired for no reason				
11. My mind is as clear as it used to be				
12. I find it easy to do the things I used to				
13. I am restless and can't keep still				
14. I feel hopeful about the future				
15. I am more irritable than usual				
16. I find it easy to make decisions				
17. I feel that I am useful and needed				
18. My life is pretty full				
19. I feel that others would be better off if I were dead				
20. I still enjoy the things I used to do				

Thank you for taking the time to complete this questionnaire and for your support of research on back disorders.

G

Awards and Abstracts

Awards

1. **Osteoarthritis Research Society International Young Investigators Award**
   Presented at the World Congress on Osteoarthritis 2015, Seattle, USA. Abstract entitled *"The Association of Obesity with Intervertebral Disc Degeneration, Disc Herniation and Spinal Stenosis: A MRI Study of 1,684 Patients"*

2. **European Spine Society Young Investigator Travel Award**
   Presented at the Eurospine 2013: The Annual meeting of the European Spine Society, Liverpool, UK. Abstract entitled *"Adipokines and the Intervertebral Disc: A Biochemical Link Between Obesity and Disc Degeneration"*

Accepted Abstracts at International Conferences

In addition to above, the following abstracts have been presented at international scientific meetings:

2015

World Congress on Osteoarthritis, Seattle, USA and the 42nd Annual Meeting of the International Society of the Study of the Lumbar Spine, San Francisco, USA:

1. Obesity is Associated with a Clinical Diagnosis of Lumbar Spine Stenosis but not Lumbar Disc Herniation or Degenerative Spondylolisthesis
2. The Influence of Obesity on Back and Leg Pain in Spinal Patients: A Study of 2,636 Patients
3. Multi-Array and Mass Spectrometric Analysis of Plasma from Patients with Spinal Disorders: Attempting to Identify Novel Biomarkers

2014

41st Annual Meeting of the International Society of the Study of the Lumbar Spine, Seoul, Korea

1. Leptin and the Intervertebral Disc: A Pro-Inflammatory Environment May Potentiate the Biochemical Effects of Obesity

World Congress on Osteoarthritis, Paris, France

1. Adipokines and the Intervertebral Disc: Does a Biochemical Link Exist Between Obesity and Intervertebral Disc Degeneration?