UNIVERSITY OF CALIFORNIA SAN DIEGO

Paraspinal Gene Expression in Patients Undergoing Lumbar Spine Surgery is Associated with Pre- and Post-Operative Muscle Health

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Bradley Anderson

Committee in charge:

Professor Bahar Shahidi, Chair
Professor Yishi Jin, Co-Chair
Professor Randolph Hampton
Professor Simon Schenk

2021

The thesis of Bradley Anderson is approved and accepted in quality and form for publication on microfilm and electronically.

University of California San Diego

2021

DEDICATION

In recognition of those who have made this work possible.

For friends and loved ones for supporting me and reminding me to relax after a long week.

For the various teachers, mentors, and others, who have believed in me and given me a part of themselves, whether knowledge, time, or friendship.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Bahar Shahidi for her generosity and support over the last few years. She has been a remarkable influence on me, my goals, my curiosity, and my journey. I would not be who or where I am today without the relationship we have built. Her guidance and mentorship have been invaluable, and she will always remain a lifelong friend.

I would like to acknowledge Samuel Ward, Joe Behun, Severin Rouss, and Erin Englund, who have helped me learn the ins and outs of orthopedic research – especially how to actually read and interpret a scientific paper; and Shannon Bremner, David Berry, Katie Fisch, Jesal Parekh, and Mary Esparza, without whom my research would have no doubt taken five times as long. Additionally, I would like to acknowledge Dr. Yishi Jin, Dr. Simon Schenk, and Dr. Randy Hampton, for believing in me and my project, and helping support me throughout this process.

Finally, I would like to acknowledge the UC San Diego Health orthopedic spine team, Dr. Allen, Dr. Garfin, Dr. Zlomislic, Dr. Attenello, Dr. Bagi, the orthopedic residents, and the rest of the orthopedics staff, who welcomed me into their operating rooms, gave me guidance, and strengthened my desire to pursue a future in orthopedic surgery. And I would like to especially thank the individuals who agreed to participate in this research study, without whom this research would not be possible.

This thesis, in part is currently being prepared for submission for publication of the material. Anderson, Bradley; Shahidi, Bahar. The thesis author was the primary author of this material.

ABSTRACT OF THE THESIS

Paraspinal Gene Expression in Patients Undergoing Lumbar Spine Surgery is Associated with Pre- and Post-Operative Muscle Health

by

Bradley Anderson

Master of Science in Biology

University of California San Diego, 2021

Professor Bahar Shahidi, Chair
Professor Yishi Jin, Co-Chair

Lumbar spine pathology is a complex and multifactorial condition associated with degenerative changes in the lumbar spinal muscles (multifidus and erector spinae) that are easily observable using conventional imaging techniques, and highly associated with poor surgical and nonsurgical outcomes. Although muscle degeneration is ubiquitous in this patient population, the underlying genetic and cellular mechanisms are not well understood, especially in relation to disease progression in humans. Therefore, we examined gene expression of 42 functionally important genes from relevant adipogenic/metabolic, atrophic, fibrogenic, inflammatory, and myogenic gene pathways by obtaining intraoperative multifidus muscle biopsies from patients

undergoing surgery for lumbar spine pathology. Multifidus muscle morphology was examined at biopsy side and vertebral level using T2-weighted magnetic resonance imaging and bi-gaussian distribution to determine total multifidus cross-sectional area, cross-sectional area of muscle and fat, and fat fraction of the multifidus, both pre-operatively and 6 months post-operatively. These measures were used to investigate the relationships between gene expression patterns, whole muscle health (size and quality), and post-operative recovery. We found that fibrogenic (COL3A1) and adipogenic/metabolic genes (PPARD) were related to pre-operative muscle quality, and myogenic genes (mTOR) were related to pre-operative muscle size. Additionally, we found trends for relationships between inflammatory (TNF-a) genes and muscle loss after surgery, and fibrogenic genes (TIMP1, TIMP3) and muscle growth after surgery. These findings provide insight into important pathways associated with muscle health and adaptation in the presence of lumbar spine pathology and establish a foundation for future research in preventing muscle degenerative changes before and after surgery.

INTRODUCTION

1. Low Back Pain

Low back pain (LBP) is the leading musculoskeletal condition resulting in disability and years lived with disability in the United States and globally, affecting anywhere from 65-85% of the U.S. population.[1–3] Surgical treatment paradigms are believed to relieve pain and restore functionality, however, an estimated 75% of individuals experience recurrent pathology, suboptimal outcomes, unresponsiveness to exercise-based rehabilitation, and often require further revision surgeries.[4–12] Individuals undergoing surgery account for over 75% of direct healthcare cost-burden associated with LBP, and often present with the most chronic and severe functional impairments.[8,13] The paraspinal musculature (erector spinae and multifidus muscles) play an important role in movement and stabilization of the spine. The multifidus muscle, in particular is thought to be one of the most significant stabilizing muscles due to its architectural features. Specifically, its large cross sectional area and short fiber length allow it to generate large amounts of force even with small movements.[14–16] Although the etiology of LBP is complex and often times multifactorial in nature, individuals typically demonstrate pathological changes in the multifidus muscles in comparison to healthy individuals.[17–21]

2. Pathological Skeletal Muscle

Commonly observed muscular adaptations in individuals with LBP include increased accumulation of fat within muscle, and increased accumulation of collagen and other fibrotic tissues within muscle (Figure 1); adipogenic and fibrogenic processes respectively.[17,17,19–25] Reductions in overall muscle cross-sectional area (CSA), muscle atrophy, have also been observed,[20,25–31] yet findings are inconclusive across the literature with some prior studies demonstrating no changes in muscle size or even muscle hypertrophy at the level of the whole

multifidus muscle as visualized using magnetic resonance imaging (MRI).[32–36] Regardless of the specific profile of degenerative muscular changes one individual may demonstrate, they are thought to progress and worsen over the course of lumbar spine pathology. Importantly, paraspinal muscle degeneration has been associated with increased likelihood of symptom recurrence, poor post-operative outcomes, and reduced strength, endurance, and function; and may be an important contributor to the poor prognoses of this patient population.[10,37–39]

2.1. Macroscopic Observations

On a whole muscle level, changes in muscle size and quality are clearly observed using imaging modalities such as MRI (Figure 1) or computed tomography (CT).[24,25,27,39,40] MRI and CT can effectively differentiate between fatty tissue and muscle, based on pixel, or voxel, intensity; with higher voxel intensities (brighter) correlating with fatty tissue, and lower voxel intensity (darker) correlating with muscle.[18,24,40–42] Prior imaging studies have demonstrated that it is relatively common to find a large portion of the multifidus or erector spinae, sometimes upwards of 50% of the muscle compartment, replaced by fatty or fibrotic tissue.[17,18] This high portion of muscle replacement by non-contractile tissue may have substantial consequences regarding paraspinal muscle function and spinal stability as well as capacity for recovery after injury or surgery.

2.2. Microscopic Observations

Histological analyses of paraspinal muscle in pathological human and animal models provide a way to validate imaging findings on a microscopic scale. Similar to larger scale observations, histological studies have demonstrated muscle fiber degeneration,[17,23,43] deposition of fibrotic tissues,[17,44,45] increased inflammatory biomarkers,[17,46] and reductions in vascularity[17,47] in multifidus and erector spinae biopsies from both human and animal models. However, like

macroscopic studies of muscle degeneration, investigations of muscle atrophy are also inconsistent; prior studies have demonstrated atrophic,[36,48–50] non-atrophic,[51,52] and even hypertrophic[17,53] changes in muscle in the presence of lumbar spine pathology.

3. Skeletal Muscle Adaptation and Recovery

Over time, in the presence of injury, and after surgery, paraspinal muscle adaptations required for recovery involve myogenic progenitor cells such as muscle satellite cells[54] and fibro-adipogenic progenitor cells (FAPs).[55] These cells control proliferation and differentiation of not only muscle cells, but also surrounding ultrastructural cells that make up the muscular microenvironment such as interstitial fat and collagen.[55,56] Other cells involved in recovery from injury include inflammatory and apoptotic cells, which help with clearance of damaged tissue.[57] Additionally, within the microenvironment surrounding myofibers exists fibroblasts, vascular-associated cells, motor neurons, and a variety of interstitial cells, which could likely all play a role in muscle adaptation and recovery.[58] Prior studies have identified several pathways including expression of myogenic, fibrogenic, adipogenic/metabolic, inflammatory, and atrophic genes, that mediate muscle health and adaptation in both healthy individuals and those with lumbar spine pathology.

3.1. Animal Studies of Lumbar Spine Pathology

Animal models such as sheep, mouse, rat, and rabbit are commonly used to investigate gene expression and cellular characteristics of lumbar spine pathology by introducing mechanical intervertebral disc lesions, or via rapid disc degeneration.[45,46,59–61] Following injury, animal studies have demonstrated increased fibrotic and adipose tissue deposition, increased tissue stiffness, and an increase in overall muscle size, fiber and bundle number.[61] These changes were observed in conjunction with upregulation of inflammatory (TNF-a, IL1, IL1B, IL6, and IGF-1),[59]

atrophic/myogenic (Phosphoinositide 3-kinase (PI3k), Protein kinase B (Akt1), mTOR, and MSTN),[45] adipogenic (ADIPOQ)[60] and fibrogenic (COL1A1, COL3A1, FN1, CTGF, TIMP1, and TIMP2) gene expression.[60]

3.2. Human Studies of Lumbar Spine Pathology

Few human studies exist to describe the genetic and cellular mechanisms underlying pathological muscle.[44,57,62,63] Even fewer studies have investigated the relationship between lumbar spine pathology and muscle adaptation.[44] Of the existing studies, intraoperative biopsies of the multifidus muscle in individuals undergoing surgery for lumbar spine pathology demonstrated myofiber membrane disruption, cellular infiltration, cells lacking nuclei, and hyper-contractile cell remnants as well as a prevalence of Platelet-derived growth factor receptor-beta (PDGFR-B) positive pericytes or progenitor cells as well as inflammatory cells.[63] Similarly, individuals with chronic symptoms (>6 months) demonstrated upregulation of fibrogenic genes such as CTGF and COL1A1, and downregulation of MMP1 and MMP9; suggesting increased fibrotic tissue deposition and reduced extracellular matrix (ECM) breakdown.[44]

While prior studies have investigated many relevant and significant correlations between genetic and cellular markers and paraspinal muscle degeneration in individuals with LBP, most are limited to genes from a single genetic pathway (i.e. inflammation).[46,64–67] Attempting to identify a single, or even a small group of genes in relation to a complex pathology such as LBP is a stark oversimplification of the many genetic pathways that likely regulate the underlying muscle adaptations to disease. To our knowledge only one prior study has looked cumulatively at adipogenic/metabolic, atrophic, fibrotic, inflammatory, and myogenic gene pathways using multifidus muscle biopsies from individuals undergoing lumbar spine surgery for LBP.[44] Additionally, to our knowledge, no prior study has investigated the relationship between gene

expression patterns and changes in paraspinal muscle degeneration over time in a prospective manner across all potentially implicated gene pathways, or following lumbar spine surgery.

4. Purpose

The goal of this study was to investigate the relationship between adipogenic/metabolic, atrophic, fibrotic, inflammatory, and myogenic genetic pathways and the health (CSA and fatty infiltration) of the multifidus muscle before and after lumbar spine surgery in individuals with LBP. We hypothesized that (1) individuals exhibiting greater total multifidus or muscle only CSA pre-operatively would demonstrate upregulation of the myogenic gene pathway; (2) individuals exhibiting lower total multifidus or muscle only CSA pre-operatively would demonstrate upregulation of atrophic, fibrogenic, and inflammatory gene pathways; (3) individuals exhibiting greater fatty infiltration pre-operatively would demonstrate upregulation of adipogenic gene pathways; and from pre- to post-operatively (4) individuals exhibiting muscle growth following surgery would demonstrate upregulation of the myogenic gene pathway; (5) individuals exhibiting muscle loss following surgery would demonstrate upregulation of atrophic, fibrotic, and inflammatory gene pathways; and (6) individuals exhibiting increased fatty infiltration following surgery would demonstrate upregulation of adipogenic gene pathways. These findings will potentially improve our understanding of lumbar spine pathology disease progression in both clinical and research settings and help to identify genetic and/or molecular pathways that may be barriers to muscle recovery in this patient population. Clinically, these findings may help highlight certain predictive patient characteristics to optimize decision making regarding conservative and surgical treatment, and ultimately, more appropriately target treatment strategies aimed at preventing paraspinal muscle degeneration over the course of disease progression.

METHODS

1. Cohort

This was a longitudinal prospective observational study (Level I) of 59 individuals undergoing posterior approach lumbar spine surgery for degenerative lumbar spine pathology. All patients underwent an informed consent process to obtain an intraoperative biopsy of the multifidus muscle in the lumbar spine and were included if they were undergoing surgery including decompression, laminoforaminotomies, laminectomies, discectomies, or fusions (1 to 2 levels). Patients with any diagnosed myopathy or systemic neurological condition were excluded. This study was performed in accordance with the Declaration of Helsinki and under approval of the UC San Diego Institutional Review Board (IRB111647). Demographic and condition-specific characteristics including age, gender, symptom duration, diagnosis, Oswestry disability index (ODI), and Numerical pain rating scale (NPRS) rating were collected at the pre-operative timepoint.

2. Muscle Biopsy

Multifidus muscle biopsies were obtained intraoperatively during the open posterior surgical approach (n=59). As a standard part of the surgical approach, the multifidus muscle is retracted outward from the spinous process, revealing the intervertebral column and disc. The muscle biopsy is collected from retracted multifidus muscle tissue 1cm lateral to the spinous process at the spinolaminar border. Biopsies were obtained at the level and side of the primary pathology. For patients with bilateral symptomatology, biopsies were obtained on the side of surgeon's approach preference. Biopsies were handled using sterile techniques and immediately pinned at in-vivo length longitudinally using sterile non-ferrous magnetic pins and cork, and flash frozen using liquid nitrogen-cooled isopentane. Biopsies were then transported on dry ice back to

the laboratory where they were stored at –80ºC until processing. This methodology has been previously described.[17,44]

2.1. RNA Isolation and Quantitative PCR

Approximately 25-50mg of a given muscle biopsy was homogenized in a round bottom bead tube (Navy, NextAdvance) with 1 milliliter of QIAzol (Qiagen). RNeasy spin columns (Qiagen) were used to extract ribonucleic acid (RNA) by following the RNeasy Lipid Tissue Mini Kit protocol for the aqueous phase by following the manufacturer's protocol. Extracted RNA were analyzed for concentration and quality using QIAxpert Analysis (Qiagen). After determining acceptable purity and concentration, one microgram of complimentary deoxynucleic acid (cDNA) was reverse transcribed using the iScript cDNA Synthesis Kits (BioRad). Quantitative polymerase chain reaction (qPCR) was performed on custom plates (BioRad) on a BioRad CFX384 Touch qPCR analyzer for a panel of 42 genes associated with adipogenic/metabolic, atrophic, fibrogenic, inflammatory, and myogenic pathways, and 40S Ribosomal Protein (RPS18) and Beta-Actin (ACTB) as controls (Table 1). All gene names and corresponding abbreviations can be found in Table 1. Cycle threshold values (Ct-values) were determined using a SYBR green fluorophore. On-plate quality assessment was performed to assess genomic DNA contamination and RNA quality.

3. Magnetic Resonance Imaging

Pre-operative T2-weighted clinical MRIs (n=54) were obtained of the lumbar spine within the 6-months preceding lumbar spine surgery. For a subset of individuals, post-operative clinical MRI's (n=31) were obtained within at least 6-months following surgery. Individuals with pre-operative MRI composed the pre-group, and individuals with pre- and post-operative MRI composed the longitudinal Δ-group (Figure 2). Pre- and post-operative MRI image analyses for

each patient were performed on images matched as closely as possible to the same vertebral level to maintain reliability in MRI outcome measures between the pre- and post-operative timepoints.

3.1. Image Analyses

Open-source MRI processing software (Horos) was used to view and analyze axial MRI images. The image slice corresponding to the level of muscle biopsy specific to each patient was identified and saved as an anatomical reference dicom (DCM) in greyscale. Regions of interest (ROIs) were drawn on the anatomical reference image for both right and left multifidus. Anatomical reference DCMs and ROIs were arranged in a patient specific manner and analyzed for fat fraction (FF), the percent of fatty tissue within the muscle compartment, and total multifidus cross-sectional area (total CSA). As previously described,[18] custom MatLab code using a bi-gaussian distribution was applied to find the intercept between voxel intensity for water and fat, in order to differentiate between the two tissue types. After processing a given sample, we are left with an approximation of total CSA (cm^2) and FF (%) for each multifidus muscle compartment (Figure 3).

4. Statistical Analyses

Demographic characteristics were compared between the pre-operative only (Pre-group) and the longitudinal (Δ-group) cohorts using independent t-tests for continuous variables, and chi-square tests for binary or categorical variables.

Raw Ct-values were obtained from all samples (n=59) and read into a qPCR expression set using the R Bioconductor package high-throughput qPCR (HTqPCR).[68] Ct-values were then quantile normalized to the mean Ct-value to obtain gene expression values for each gene. A maximum Ct-value of 39 was applied to all genes of interest to allow for statistical comparison, with lower values indicating higher gene expression.[68] Unsupervised hierarchical clustering using

Euclidean distance was applied to the normalized expression values to generate heat maps and visualize potential patterns of gene expression across patients.

Total CSA and FF of the multifidus were then used to calculate the CSA of fat only (F-CSA) (cm^2) and the CSA of muscle only (M-CSA) (cm^2) within the given multifidus muscle compartment specific to the side of muscle biopsy. Mean FF and mean total CSA were then calculated between right and left multifidus. Total CSA, M-CSA, F-CSA, and FF describe measurements of muscle size and quality at the side of muscle biopsy, while mean total CSA and mean FF describe measurements of muscle size and quality between both right and left multifidus. This same MRI processing was repeated for both pre-operative and post-operative MRI. Change-scores for the Δ-group were then calculated by subtracting pre-operative MRI outcome measure from post-operative MRI outcome measure to give the relative morphological change for each given MRI outcome measure following lumbar spine surgery. A positive change-score for a given MRI outcome measure indicates an increase from pre-operative MRI to post-operative MRI, and vice versa.

Associations between gene expression and pre-group MRI imaging measures (n=54) and gene expression and Δ-group MRI imaging measures (n=31) were analyzed using SPSS (IBM). For each group, univariate analyses were performed between all genes and all MRI imaging measures. Raw p-values from univariate correlations were adjusted for multiple comparisons within each gene group using the Benjamini & Hochberg method.[69] Significance was set at an adjusted p-value threshold of $p<0.05$, and trends were defined as adjusted p-values<0.1. Data are reported as mean (±standard deviation).

RESULTS

Of the 59 patients with muscle biopsies, 54 patients had matching MRI imaging required for the pre-group, and 31 had matching MRI imaging required for the Δ-group (Table 2). Individuals in the pre-group were middle-aged, 51.5 (16.9) years old, with moderate to severe pre-operative disability, 44.9 (21.1), and moderate pre-operative pain, 5.6 (2.8). Individuals in the Δ-group were also middle-aged, 48.2 (17.4) years old, moderate to severe pre-operative disability, 39.1 (21.6), and moderate pre-operative pain, 5.6 (2.8). Age (p=0.43), disability (p=0.24), and pain (p=0.96) were similar between groups. The median age was 57 years old and 55 years old in the pre-group and Δ-group respectively. There were 22 females and 32 males in the pre-group, compared to 13 females and 22 males in the Δ-group. There were 20 patients with symptomatology duration shorter than 6-months (acute) and 34 patients with symptomatology greater than 6-months (chronic) in the pre-group, and 10 acute and 21 chronic patients in the Δ-group. Gender and duration of symptomatology were similar between groups (p=0.92, and p=0.66 respectively), and within both the pre-group (p=0.34, and p=0.17 respectively), and the Δ-group (p=0.52, and p=0.16 respectively). In the pre-group and Δ-group, diagnoses were similar, and included lumbar spinal stenosis (n=50, and n=28 respectively, p=0.95) with leg pain or weakness (n=45, and n=24 respectively, p=0.86), and with concomitant lumbar disc herniation (n=18, and n=12 respectively, p=0.76), spondylolisthesis or spondylosis (n=10, and n=6 respectively, p=0.94), degenerative disc disease (n=5, and n=4 respectively, p=0.65), and scoliosis or kyphoscoliosis (n=3, and n=1 respectively, p=0.65). Diagnoses were missing for two subjects in both the pre-group and Δ-group. In the pre-group and Δ-group, muscle biopsies were obtained from the pathological vertebral level L4 (n=23, and n=11 respectively, p=0.77), L3 (n=16, and n=9 respectively, p=0.98), L5 (n=14, and n=11 respectively, p=0.63), and L1 (n=1, and n=1

respectively, p=0.46). There were no significant differences in the distribution of pathological levels between groups.

Hierarchical clustering of normalized expression values by gene category for all patients (n=59) revealed apparent upregulation of pro-fibrogenic genes, and downregulation of anti-fibrogenic and inflammatory genes which was prevalent among most subjects (Figure 4).

Relationships between gene expression and MRI in the pre-group (n=54) revealed significant associations between downregulation of PPARD (Figure 5a-b) and upregulation of COL3A1 (Figure 5c-d) and lower FF (p=0.018, and p=0.047 respectively) and lower mean FF (p=0.073, and p=0.028 respectively). Additionally, downregulation of mTOR (Figure 5e-f) was related to greater total CSA and greater mean total CSA (p=0.045, and p=0.032 respectively). Finally, there was a trend for an association between upregulation of CASP3 and MSTN gene expression and greater F-CSA (p=0.097, and p=0.097 respectively).

In the Δ-group, followed longitudinally after surgery (n=31), we observed an average increase in total CSA by 0.18 (2.7) cm^2 and of M-CSA by 0.9 (2.1) cm^2, and an average decrease of F-CSA by 0.7 (1.5) cm^2 and of FF by 7.7 (11.9) %. Hierarchical clustering of normalized expression values by gene category for patients in the longitudinal group (n=31), classified by post-operative muscle growth (Figure 6), revealed upregulation of pro-fibrogenic genes, and downregulation of anti-fibrogenic and inflammatory genes. Specific relationships between gene expression and MRI demonstrated a trend for an association between upregulation of TIMP1 (Figure 7a) with increased M-CSA (p=0.098) and upregulation of TIMP3 (Figure 7b) with decreased mean FF (p=0.052). Additionally, there was a trend for upregulation of TNF-a with decreased total CSA and decreased mean total CSA (Figure 7c-d) (p=0.072, and p=0.079 respectively).

DISCUSSION

The broad aim of this study was to investigate the relationship between gene expression patterns of functional groups of genes and paraspinal muscle health before and after spine surgery for LBP. Across all patients, hierarchical analyses of gene expression trends indicated upregulation of pro-fibrogenic (COL1A1, COL3A1, CTGF, FN1, TIMP1, and TIMP3), pro-adipogenic (ADIPOQ, CEBPA, and FABP4), pro-atrophic (FBXO32, TRIM63, and FOXO3), myogenic (CSRP3), and downregulation of inflammatory (TNF-a, IL1B, IL6, and IL10) and anti-fibrogenic genes (MMP1, MMP3, and MMP9). Collectively, these observations suggest that individuals in this patient population have upregulation of skeletal muscle atrophy,[70–75] autophagy,[70,71,76–80] fibrotic tissue deposition,[44,60,67,81–84] and fatty infiltration;[60,85–88] which is in agreement with previously described muscle degeneration in the presence of lumbar spine pathology.[17,40,45,59–61,63,89]

We found that pre-operatively, individuals with higher levels of multifidus fatty infiltration demonstrated upregulation of adipogenic/metabolic genes, downregulation of fibrogenic genes, and a trend for upregulation of atrophic genes. We also observed that individuals with larger multifidus CSA demonstrated downregulation of myogenic genes. Longitudinally, although we did not find any significant associations between gene expression and changes in muscle size or fatty infiltration ≥6 months after surgery, we did observe trends for relationships between increased fibrogenic gene expression and muscle growth as well as fat loss, and trends for increased inflammatory gene expression and muscle loss.

Within the pre-operative MRI group, we observed that the adipogenic/metabolic gene PPARD was related to fatty infiltration, which was not unexpected. However, the directionality of this association was contrary to our hypothesis. PPARD is a ligand inducible nuclear

receptor/transcription factor which regulates lipid homeostasis in a variety of tissues including muscle,[90] and was correlated with greater fatty infiltration in the pre-group. This is surprising given that a prior study of PPARD function in skeletal muscle has shown that PPARD triggers a switch from glucose/fatty acid to fatty acid metabolism only, leading to *reductions* in body fat.[91] One possible reason for our observation is that the demand for turnover of adipocytes in highly fatty tissue increases activation of pathways (and subsequently genes) involved in fat metabolism within muscle.[92] Additionally, upregulation of PPARD has been suggested to lead to increased metabolism of branch chain amino acids, muscle wasting, and regulate Signal transducer and activator of transcription 3 (STAT3) atrophic signaling.[92–95] Another possible reason for our observation is that PPARD-mediated muscle atrophy may occur quicker than adipocyte turnover, increasing the relative amount of fat within the muscle compartment. Prior literature also supports the concept that PPARD activation is associated with high fat diet, metabolic syndrome, and obesity.[96,97] Given that many musculoskeletal conditions including LBP have been shown to be more severe in individuals with high body mass index and metabolic syndrome,[98] this observation likely suggests that an individual's metabolic profile may influence their muscle health.

Additionally, we observed the upregulation of atrophic gene expression of CASP3 and MSTN with greater fatty infiltration. These findings were not hypothesized, but logical considering that upregulation of both CASP3[99–101] and MSTN[102–104] are associated with muscle apoptosis and skeletal muscle atrophy. Therefore, the atrophy of muscle tissue within the multifidus may result in increased proportion of adipose tissue within the muscle compartment. Additionally, however, expression of CASP3 has been shown to increase with increasing amounts of fatty tissue,[105] likewise, MSTN-deficient mice have demonstrated suppression of body fat accumulation in appendicular muscles;[106] suggesting atrophic gene expression may influence

adipogenic processes. Interestingly, both CASP3 and MSTN are downstream targets of STAT3 signaling, which is in-turn activated via PPARD-mediated phosphorylation, [93–95] and MSTN upregulation has been demonstrated alongside increases in multifidus adipose tissue before and after lumbar spine surgery in mouse models.[45] Further supporting a relationship between atrophic and adipogenic processes in multifidus of individuals with lumbar spine pathology.

Also contrary to our hypothesis regarding pre-operative muscle quality, upregulation of fibrogenic gene expression of COL3A1 correlated with lower fatty infiltration in the pre-group. COL3A1 is one of various collagen isoforms that compose the ECM, secreted by muscle fibroblasts present within the ECM[107] in response to muscle injury[17,44,60] and with increasing mechanical loading.[108] Therefore, we initially hypothesized that increased fibrogenic gene expression would correlate with worse pre-operative muscle quality (higher fatty infiltration). Prior studies have shown upregulation of COL1A1 in chronic human patients,[44] and upregulation of COL3A1 in a mouse model following SPARC-null mediated disc degeneration,[60] however neither demonstrated significant associations with lower fat fraction. One possible reason for our observations is that conventional T2-weighted MRI techniques are unable to effectively differentiate between muscle and fibrosis due to relaxometry times of fibrotic tissue.[109] Specifically, both muscle and collagen have short signal decay times and appear dark grey on MRI. Given that our bi-gaussian analytical methodology was designed to discriminate between high intensity (white) and low intensity (grey) signal in MRI, the inability to distinguish between muscle and fibrosis using this method may have resulted in an overestimation of true muscle tissue within the multifidus muscle compartment. Therefore, COL3A1 expression may reflect an increase in the amount of fibrotic tissue within the multifidus muscle compartment, but it is being counted as muscle using this methodology.[17] This hypothesis may also explain our observation of

downregulation of the myogenic gene mTOR in individuals with greater pre-operative multifidus muscle size. This finding was also contrary to our hypothesis regarding pre-operative muscle size. mTOR is a potent regulator of myogenesis via the Akt1/mTOR signaling pathway, and is downregulated during skeletal muscle atrophy and upregulated during skeletal muscle hypertrophy.[110] As such, we would expect to see *upregulation* of mTOR signaling alongside greater multifidus muscle size.

In the longitudinal Δ-group, hierarchical analyses of gene expression trends by post-operative muscle growth revealed similar findings to hierarchical analyses across all patients. Pro-fibrogenic, pro-adipogenic, pro-atrophic, and myogenic genes appeared to be upregulated and anti-fibrogenic and inflammatory genes appeared to be downregulated. Although the clusters did not appear to distinguish patients by muscle growth or loss, there did appear to be two sub-clusters arranged by opposing expression of pro-atrophic/myogenic genes, CASP3 and ASB15, and FBXO32 and CSRP3, respectively. CASP3 is an important regulator of skeletal muscle atrophy[99] and ASB15 is an important regulator of muscle satellite cell differentiation and development, and myoblast turnover.[111] Similarly, FBXO32 is an important regulator of skeletal muscle atrophy[72,75] and CSRP3 is an important regulator of skeletal muscle autophagy, muscle satellite cell differentiation and development, and the stretch receptor/microtubule-associated muscle lim protein.[76,78,80] Although speculative, these findings suggest the possibility of two differing cellular pathways underlying skeletal muscle atrophy and myogenesis in this patient population, or differences in time-course of muscle degeneration at the time of the biopsy.

Examining specific gene and MRI relationships in the Δ-group, we observed trends for upregulation of fibrogenic gene expression of TIMP1 and TIMP3 with muscle growth and reduced fatty infiltration, respectively, following lumbar spine surgery. Both TIMP1 and TIMP3 are pro-

fibrogenic regulators of fibrosis and ECM remodeling, and negative regulators of the MMP family of anti-fibrogenic genes. [67,81,84,112–114] Based on these functions, our hypothesis was that fibrogenic genes would be associated with muscle loss or reduced muscle quality post-operatively. However, there is some evidence that TIMP3 is downregulated during adipogenesis, and that upregulation of TIMP3 interferes with adipocyte differentiation and adipogenesis, supportive of our findings;[115,116] in addition to a role as a potent negative regulator of myogenesis via inhibition of TNF-a.[84,112] Similar to our pre-operative findings, our observations may be partially explained by the limitations in our MRI techniques for differentiating fibrotic and muscle tissue.[109] Perhaps pro-fibrogenic gene expression is influencing the amount of fibrotic tissue within the muscle compartment, and, accordingly, increases in tissue identified as 'muscle' would lead to relative decreases in ratio measures of fatty infiltration like fat fraction over time. Interestingly, animal studies of TIMPs in aged sarcopenic rats versus aged and young controls demonstrated chronic upregulation of TIMPs in the sarcopenic group alone,[114] suggesting that the combination of aging and sarcopenia may be a phenotype linked with demonstrating fibrogenic upregulation. Similarly, previous human studies have demonstrated upregulation of fibrogenic genes in patients with chronic lumbar spine pathology.[44] Given that this population is older, and most participants had a symptom duration of >6 months, the upregulation of these genes is logical.

Finally, in agreement with our hypothesis regarding muscle loss, we found a trend for a relationship between upregulation of inflammatory gene expression of TNF-a and muscle loss following surgery. In lumbar spine pathology, animal models have demonstrated upregulation of TNF-a following induction of lumbar spine pathology alongside longitudinal decreases in slow-twitch muscle fibers and muscle fiber turnover.[46] Human studies have also demonstrated upregulation of serum TNF-a within two-weeks of the acute onset of LBP, and that upregulation

of TNF-a was associated with poor prognoses at 6-months.[62] Additionally, upregulation of TNF-a has been demonstrated to coincide with morphological and histological increases in adipose and fibrotic tissue.[45] Our findings indicate that TNF-a is facilitating post-surgical atrophy of the multifidus muscle. However, other studies in skeletal muscle have demonstrated a pro-myogenic role of TNF-a in response to muscle injury, leading to muscle satellite cell differentiation.[57,117] However, this role as a myogenic or atrophic cytokine may depend on the duration of TNF-a signaling; where-as chronic signaling has been shown to prolong early stages of muscle satellite cell differentiation.[117] It is also suggested that TNF-a is synthesized by myoblasts as an autocrine/paracrine signal which may lead to catabolism of muscle, dysfunction of contractile mechanisms, and disruption of myogenesis[118] as such, the mechanism of TNF in skeletal muscle is conflicting.[57,117–121] As a whole, our results support the concept that TNF-a is involved in muscle atrophy and degeneration after surgery.

This study did have some limitations. Primarily, our MRI acquisition techniques lacked the ability to fully differentiate between muscle and fibrosis. This may have contributed to the significance of our findings regarding upregulation of pro-fibrogenic genes (COL3A1, TIMP1, TIMP3) and greater muscle size pre-operatively, and muscle growth and decreased fatty infiltration following surgery. It is likely that future studies using methods such as ultra-short echo-time MRI, able to differentiate between muscle and fibrosis due to extremely short image acquisition times, would clarify the directionality and significance of these findings.[109] Additionally, in the future, these findings may be corroborated with histological analyses of this same patient population to more readily visualize muscle fibrosis. Another limitation of this study is pre-operative to post-operative MRI mismatch. While both pre-operative and post-operative images for a given patient were matched as closely as possible to the pathological level, between

timepoints, researcher matching could not account for variations in MRI slice angle, respiratory artifacts, or researcher error.[122] This could possibly be addressed by using volumetric analysis, where every MRI acquisition in the region of interest (say the lumbar spine or specific vertebral level) is cumulatively processed and analyzed, in order to correlate with gene expression findings. However, this type of analysis may result in the loss of significance with level or region specific gene and morphological associations.[123] Additionally, while our findings are a good step towards understanding the genetic mechanisms underlying muscle health in this patient population, it is difficult to ascribe function to particular gene findings based on associations with morphological outcomes, especially without understanding the association between those genes and actual cellular activity. As such, a future direction of this research will likely include protein analyses in order to better understand the relationship between gene expression, protein expression/activity, and muscle health. Finally, our cohort only included individuals with lumbar spine pathology undergoing lumbar spine surgery. Future research should include a control group of healthy individuals without pathology. Additionally, future research will likely include individuals with LBP undergoing surgery with a pre-operative exercise component; to explore how exercise influences gene expression patterns in the presence of lumbar spine pathology.

CONCLUSION

This study is the first to evaluate the relationships between multiple gene pathways and changes in paraspinal muscle health over time following surgery in humans with lumbar spine pathology. Our findings indicate that adipogenesis, atrophy, fibrogenesis, inflammation, and myogenesis, collectively influence the degenerative phenotype in this patient population, with fibrogenic genes influencing both pre- and post-surgical muscle health. Although future research

is needed to elucidate the temporal features of these processes – and the influence of specific disease and patient-specific characteristics such as vertebral level, pathology, age, and gender – these findings highlight the importance of studying functional groups of genes and provide important information regarding our understanding of muscle degeneration in human lumbar musculoskeletal disease. The influence of fibrogenic and other processes on pre- and post-surgical muscle health suggests important potential targets to improve patient function, recovery, and prognosis in this population.

This thesis, in part is currently being prepared for submission for publication of the material. Anderson, Bradley; Shahidi, Bahar. The thesis author was the primary author of this material.

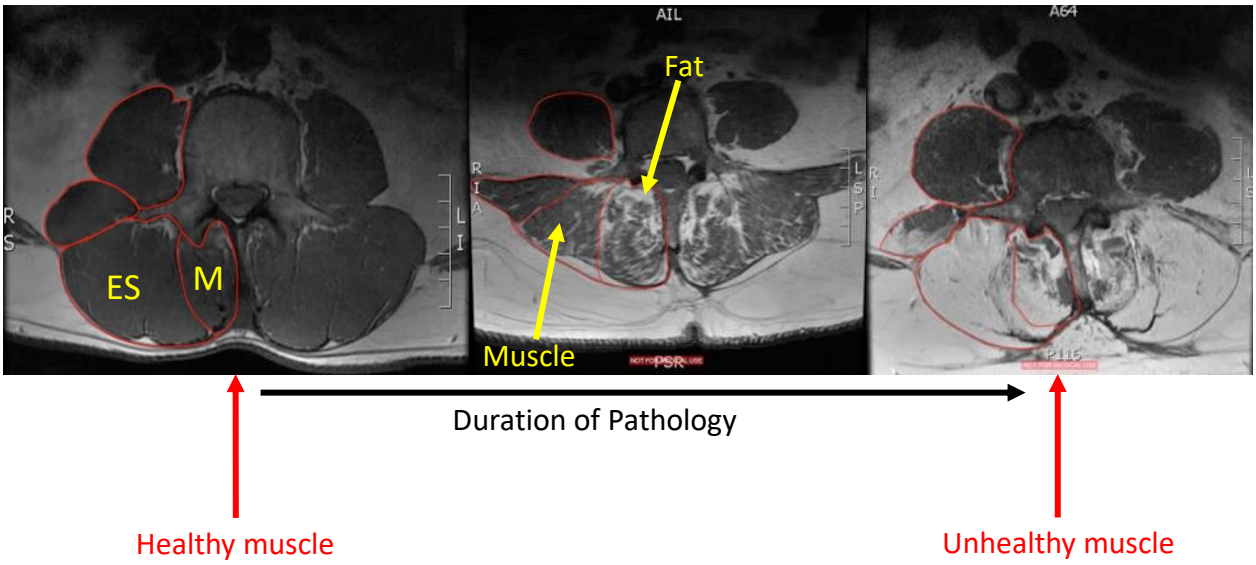

Figure 1. Pathological Muscle Adaptations
T2-weighted axial MRIs of the lumbar paraspinal muscles from three different individuals, depicting muscle degeneration which is suggested to progress over the duration of LBP pathology. Moving from left (healthy muscle) to right (unhealthy muscle) the erector spinae (ES) and multifidus (M) muscle compartments become increasingly infiltrated by non-contractile fatty tissue and fibrosis. The erector spinae sit lateral the multifidus at the posterior border of the spinal vertebrae. In conventional T2-weighted MRI imaging, muscle and fibrosis both appear dark and fatty tissue appears bright, as depicted in the middle panel.

Table 1. Genes and Gene Categories

Panel of 42 functional genes associated with adipogenic/metabolic, atrophic, fibrogenic, inflammatory, and myogenic pathways in skeletal muscle. Genes were examined using qPCR on custom cDNA plates containing these 42 genes of interest, and RPS18 and ACTB as controls.

Gene Category	**Adipogenic/ Metabolic**	**Atrophic**	**Fibrogenic**	**Inflammatory**	**Myogenic**
Gene Name (Abbreviation)	Peroxisome Proliferator-Activated Receptor Gamma **(PPARG),** PPARG Coactivator 1 Alpha **(PPARGC1A),** Peroxisome Proliferator-Activated Receptor Delta **(PPARD),** Fatty Acid Binding Protein 4 (adipocyte specific) **(FABP4),** CCATT/Enhancer Binding Protein Alpha **(CEBPA),** Adiponectin **(ADIPOQ),** Wnt Family Member 10B **(WNT10B)** Protein Tyrosine Phosphatase Non-Receptor Type 4 **(PTPN4)**	Myostatin/ Growth Differentiation Factor 8 **(MSTN),** Activin Receptor 2B **(ACVR2B),** Tripartite Motif Containing 63/E3 Ubiquitin Ligase **(TRIM63),** Forkhead Box O3 (**FOXO3),** F-box only protein 32 **(FBXO32)** Caspase-3 **(CASP3),** Caspase-1 **(CASP1)**	Platelet-Derived Growth Factor Receptor Alpha **(PDGFRA),** Tissue Inhibitor of Metalloproteinase 3 **(TIMP3),** Tissue Inhibitor of Metalloproteinase 1 **(TIMP1),** Matrix Metalloproteinase 9 **(MMP9),** Matrix Metalloproteinase 3 **(MMP3),** Matrix Metalloproteinase 1 **(MMP1),** Lysyl Oxidase **(LOX),** Fibronectin 1 **(FN1),** Connective Tissue Growth Factor **(CTGF),** Collagen Type III Alpha 1 Chain **(COL3A1),** Collagen type I Alpha Chain **(COL1A1),** Transforming Growth Factor Beta 1 **(TGFB1)**	Tumor Necrosis Factor **(TNF-a),** Interleukin-6 **(IL6),** Interleukin-10 **(IL10),** Interleukin-1 Beta **(IL1B)**	Embryonic Myosin Heavy Chain **(MYH3),** Myosin Heavy Chain – Type 1 **(MYH1),** Insulin-like Growth Factor I **(IGF-1),** Cysteine and Glycine Rich Protein 3/ Muscle LIM Protein **(CSRP3),** Ankyrin Repeat and SOCS Box Containing 15 **(ASB15),** Ankyrin Repeat Domain 2-Stretch Responsive Muscle **(ANKRD2),** Paired Box 7 Transcription Factor (**PAX7),** Myogenin/Myogenic Factor (**MYOG),** Myogenic Differentiation 1/Myogenic Factor 3 (**MYOD1),** Myogenic Factor 5 **(MYF5),** Mammalian Target of Rapamycin (**MTOR)**

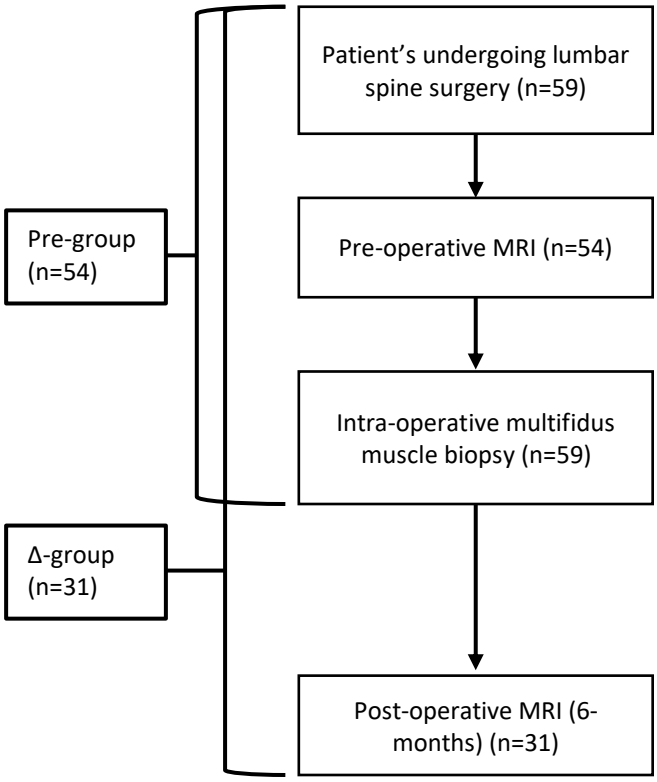

Figure 2. Schematic of Patient Groups
59 individuals undergoing lumbar spine surgery were consented for intra-operative multifidus muscle biopsy. 54 of these same individuals obtained pre-operative magnetic resonance imaging of the lumbar spine within 6-months preceding surgery and composed the pre-group. Following surgery, 31 of these individuals obtained post-operative magnetic resonance imaging of the lumbar spine 6-months post-operatively and composed the Δ-group.

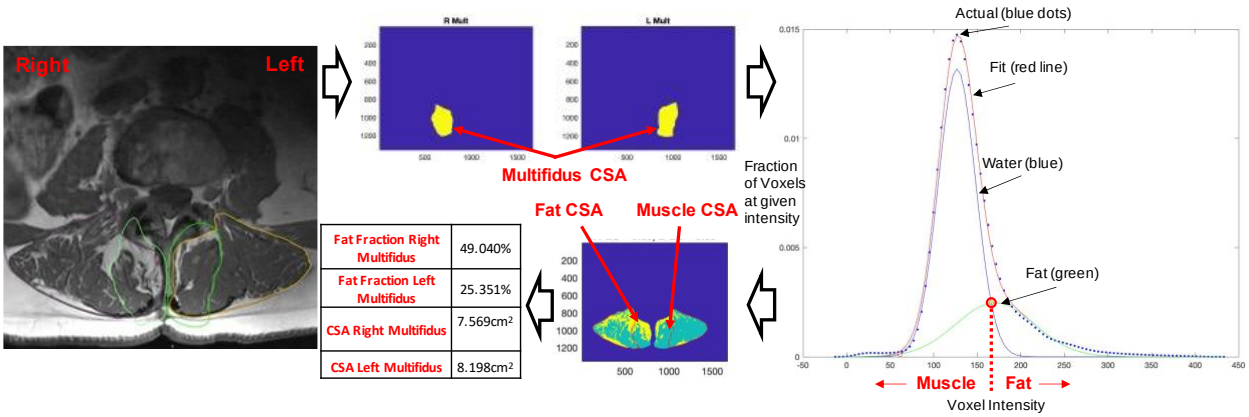

Figure 3. MRI Image Processing

Multifidus total cross-sectional area and fat fraction were determined using a custom MatLab code to differentiate between muscle and fat. Regions of interest were drawn around right and left multifidus (left panel, green outlines) in T2-weighted axial MRIs of the lumbar spine. Right and left multifidus were evaluated for muscle size (middle top panel), then, a bi-gaussian distribution of voxel intensity (right panel) was used to determine the intercept (red circle) between water (blue line) and fat (green line). The intercept is used as a threshold; greater voxel intensities are designated as fat and lower voxel intensities are designated as muscle. This analysis highlights muscle as teal and fat as yellow (middle bottom panel) and provides a calculation for both right and left multifidus cross-sectional area and fat fraction (middle bottom table).

Table 2. Patient Demographics

Both the pre- and Δ-groups were similar in age, ODI, NPRS, gender, duration of symptomatology, and diagnoses. Gender and duration of symptomatology were also similar within groups. In both groups, a majority of patients underwent surgery for lumbar spinal stenosis, with other concomitant diagnoses. There were no significant differences between pathological vertebral level between groups. Significance was set at a p-value of $p<0.05$.
†Diagnoses were missing for two patients across both groups.

Patient Demographics	**Pre-group (n=54)**	**P-Value**	**Δ-group (n=31)**	**P-Value**
Age (Years)	51.5 (16.9)		48.2 (17.4)	0.43
ODI Baseline	44.9 (21.1)		39.1 (21.6)	0.24
NPRS Baseline	5.6 (2.8)		5.6 (2.8)	0.96
Gender				0.92
Female	22		13	
Male	32	0.34	18	0.52
Duration of Symptomatology				0.66
Acute (<6 months)	20		10	
Chronic (>6months)	34	0.17	21	0.16
†Diagnosis (# of patients)				0.98
Lumbar spinal stenosis	50		28	0.95
w/ leg pain/weakness	45		24	0.86
Lumbar disc herniation	18		12	0.76
Spondylolisthesis/Spondylosis	10		6	0.94
Degenerative disc disease	5		4	0.65
Scoliosis/kyphoscoliosis	3		1	0.65
Pathological Vertebral Level (# of patients)				0.70
L1	1		0	0.46
L2	0		0	NaN
L3	16		9	0.98
L4	23		11	0.77
L5	14		11	0.63

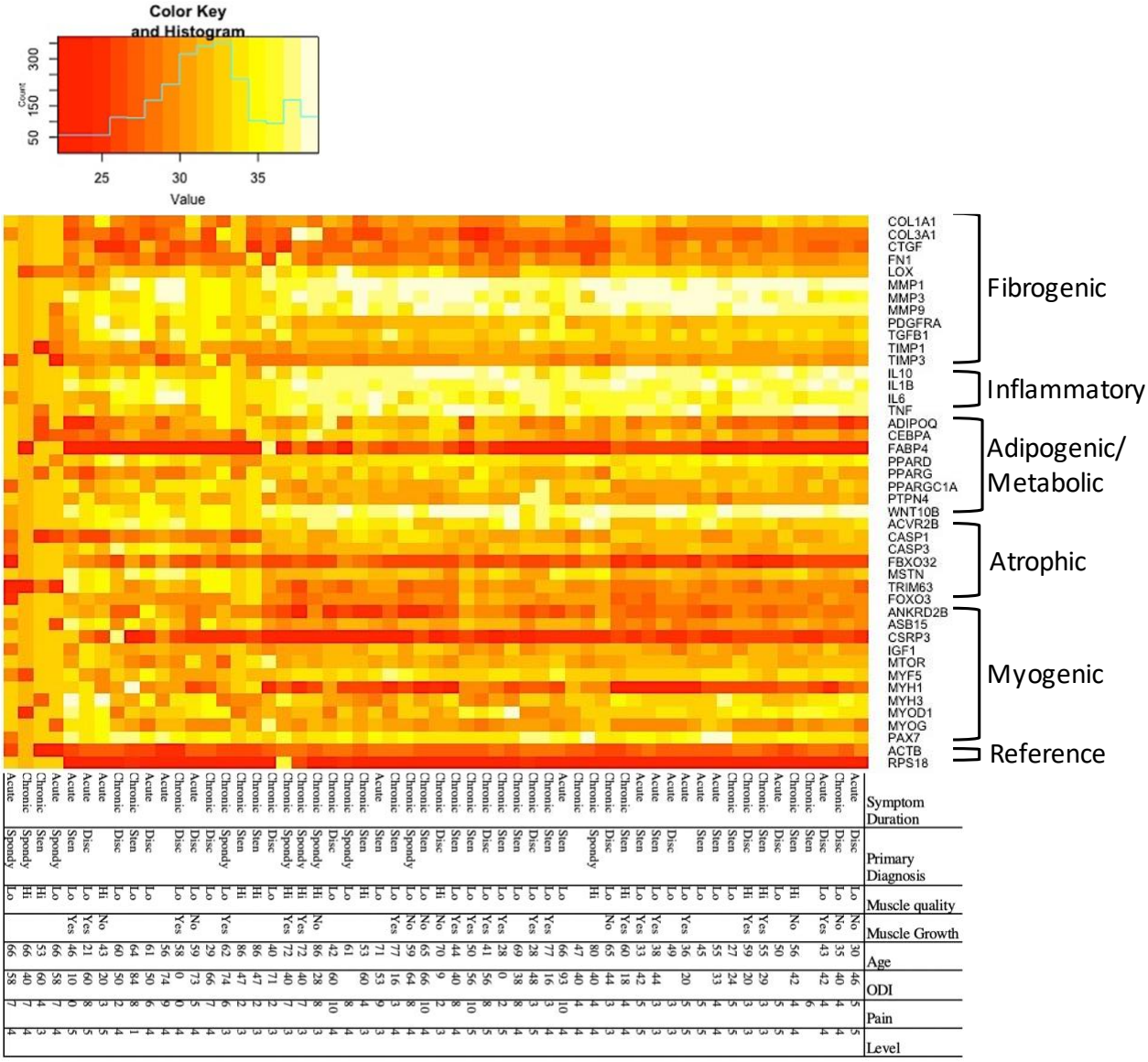

Figure 4. Gene Heatmap of Individuals Undergoing Surgery for Lumbar Spine Pathology
Hierarchical cluster analysis of quantile normalized gene expression values from intraoperative multifidus muscle biopsies (n=59). The expression levels of 42 genes from fibrogenic, inflammatory, adipogenic/metabolic, atrophic, and myogenic pathways were measured using qPCR. Patient groups such as symptom duration, diagnosis, high (<50% fat fraction) versus low (>50% fat fraction) muscle quality, and muscle growth (positive changes in muscle cross-sectional area post-operatively) and demographics (age, ODI, NPRS, and pathological vertebral level) are indicated on the x-axis. Gene abbreviations are indicated on the y-axis clustered by group (i.e., fibrogenic). Highly expressed genes are denoted by red coloring, and genes with low expression are denoted by yellow coloring.

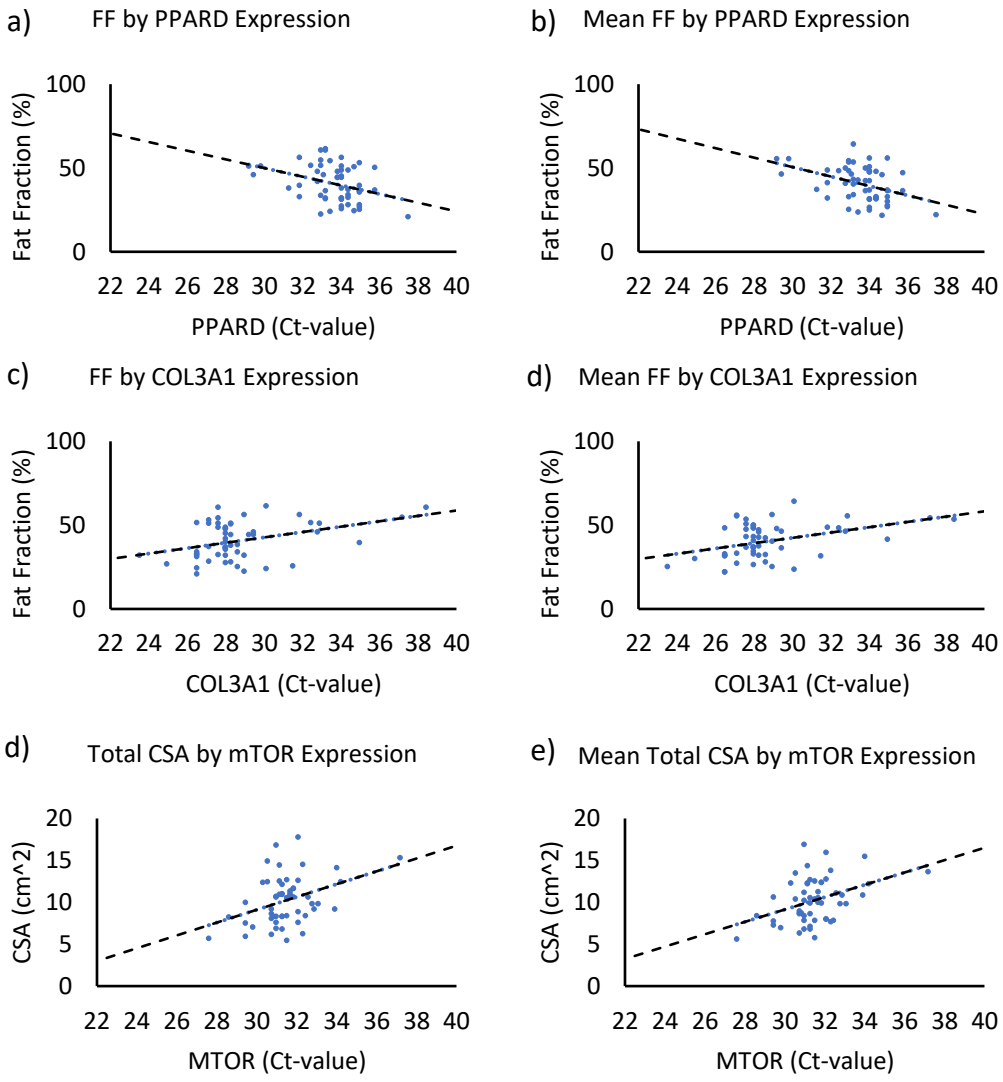

Figure 5. Associations Between Multifidus Gene Expression and Pre-operative Morphological Measures

Individuals with greater unilateral and mean fat fraction demonstrated (a, b) upregulation of adipogenic/metabolic gene expression of PPARD (p=0.018, r=-0.346, and p=0.073, r=-0.402, respectively), and (c, d) downregulation of fibrogenic gene expression of COL3A1 (p=0.047, r=0.386, and p=0.028, r=0.406, respectively). Individuals with lower unilateral and mean total multifidus cross-sectional area demonstrated (e, f) downregulation of myogenic gene expression of mTOR (p=0.045, r=0.388, and p=0.032, r=0.400, respectively). Multifidus muscle biopsies were collected intra-operatively (n=59) and gene expression was assessed using qPCR. Raw Ct-values were quantile normalized to the mean Ct-value; lower Ct-values representing higher gene expression and vice versa. Morphological measures were evaluated using pre-operative T2-weighted MRI of the lumbar spine at the level of multifidus biopsy (n=54). Univariate analyses were performed between each gene and MRI measure, and p-values were adjusted for multiple comparison using the Benjamini & Hochberg method with an adjusted p-value threshold of $p<0.05$, and trends were defined as adjusted p-values<0.1

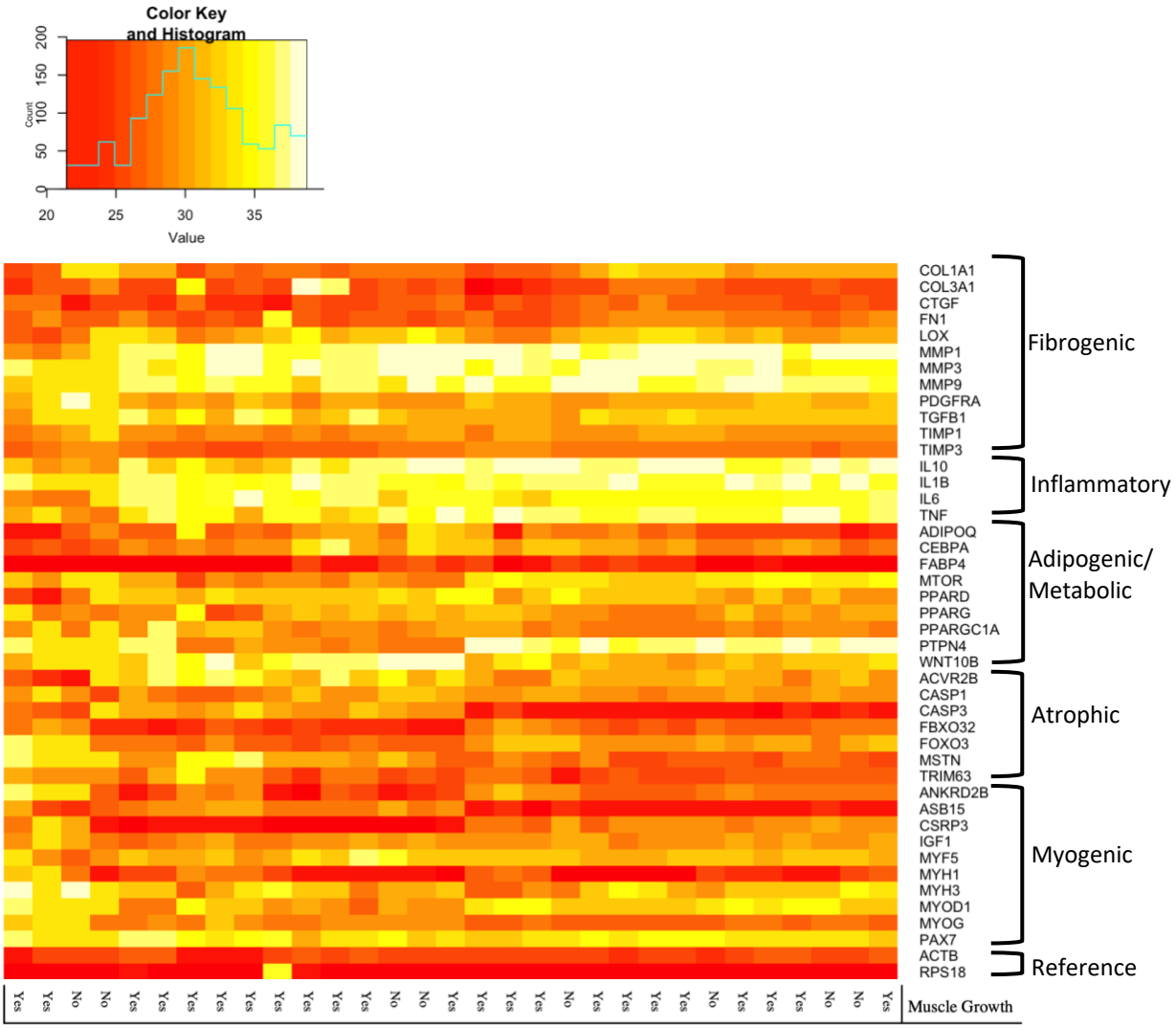

Figure 6. Gene Heatmap by Muscle Growth Following Surgery in Individuals with Lumbar Spine Pathology

Hierarchical cluster analysis of quantile normalized gene expression values from intraoperative multifidus muscle biopsies of patients demonstrating muscle growth ("Yes") and muscle loss ("No") following surgery (n=31). Muscle growth and muscle loss were determined by the pre-operative to post-operative change in muscle only cross-sectional area; positive changes in muscle only cross-sectional area were designated as muscle growth and vice versa. The expression levels of 42 genes from fibrogenic, inflammatory, adipogenic/metabolic, atrophic, and myogenic pathways were measured using qPCR. Patient groups (symptom duration, diagnosis, muscle quality, muscle growth) and demographics (age, ODI, NPRS, vertebral level) are indicated on the x-axis. Gene abbreviations are indicated on the y-axis clustered by group (i.e., fibrogenic). Highly expressed genes are denoted by red coloring, and genes with low expression are denoted by yellow coloring.

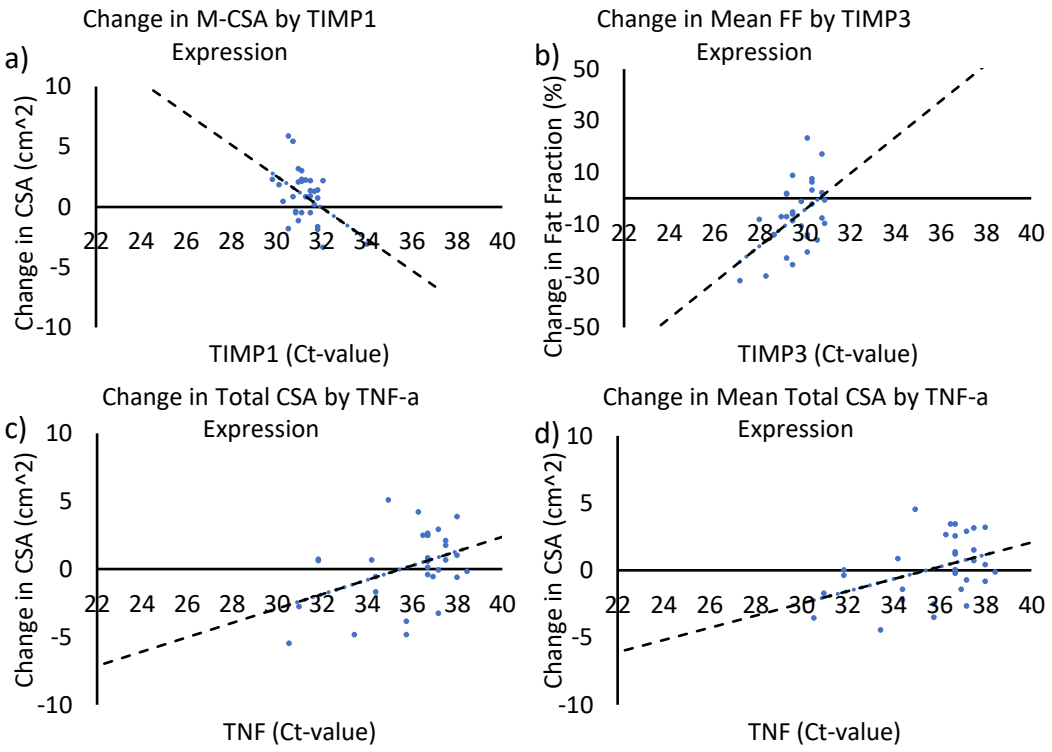

Figure 7. Associations Between Multifidus Gene Expression and Change in Morphological Measures Following Surgery

Following surgery, individuals with muscle growth demonstrated trends for (a) upregulation of fibrogenic gene expression of TIMP1 ($p=0.098$, $r=-0.466$), and individuals with lower fat fraction demonstrated trends for (b) upregulation of fibrogenic gene expression of TIMP3 ($p=0.052$, $r=-0.409$). Individuals with lower unilateral and mean total multifidus cross-sectional following surgery also demonstrated trends for (c,d) upregulation of inflammatory gene expression of TNF-a ($p=0.072$, $r=0.422$, and $p=0.079$, $r=0.417$ respectively). Multifidus muscle biopsies were collected intra-operatively ($n=59$) and gene expression was assessed using qPCR. Raw Ct-values were quantile normalized to the mean Ct-value; lower Ct-values representing higher gene expression and vice versa. Morphological measures were evaluated using pre-operative and 6-month post-operative T2-weighted MRI of the lumbar spine at the level of multifidus biopsy ($n=31$). Change-scores for each MRI measure were calculated by subtracting post-operative MRI measure from pre-operative MRI measure. Univariate analyses were performed using SPSS (IBM) between each gene and MRI change-score, and p-values were adjusted for multiple comparison using the Benjamini & Hochberg method with an adjusted p-value threshold of $p<0.05$, and trends were defined as adjusted p-values<0.1

REFERENCES

1. Wu, A., March, L., Zheng, X., Huang, J., Wang, X., Zhao, J., Blyth, F. M., Smith, E., Buchbinder, R. & Hoy, D. Global low back pain prevalence and years lived with disability from 1990 to 2017: estimates from the Global Burden of Disease Study 2017. *Ann Transl Med* **8,** 299–299 (2020).

2. Kyu, H. H., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, M., Abebe, Z., Abil, O. Z., Aboyans, V., Abrham, A. R., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Accrombessi, M. M. K., Acharya, D., Acharya, P., Ackerman, I. N., Adamu, A. A., Adebayo, O. M., Adekanmbi, V., Ademi, Z., Adetokunboh, O. O., Adib, M. G., Adsuar, J. C., Afanvi, K. A., Afarideh, M., Afshin, A., Agarwal, G., Agesa, K. M., Aggarwal, R., Aghayan, S. A., Agrawal, A., Ahmadi, A., Ahmadi, M., Ahmadieh, H., Ahmed, M. B., Ahmed, S., Aichour, A. N., Aichour, I., Aichour, M. T. E., Akinyemiju, T., Akseer, N., Al-Aly, Z., Al-Eyadhy, A., Al-Mekhlafi, H. M., Al-Raddadi, R. M., Alahdab, F., Alam, K., Alam, T., Alashi, A., Alavian, S. M., Alene, K. A., Alijanzadeh, M., Alizadeh-Navaei, R., Aljunid, S. M., Alkerwi, A., Alla, F., Allebeck, P., Alonso, J., Alsharif, U., Altirkawi, K., Alvis-Guzman, N., Aminde, L. N., Amini, E., Amiresmaili, M., Ammar, W., Amoako, Y. A., Anber, N. H., Andrei, C. L., Androudi, S., Animut, M. D., Anjomshoa, M., Ansha, M. G., Antonio, C. A. T., Anwari, P., Arabloo, J., Aremu, O., Ärnlöv, J., Arora, A., Arora, M., Artaman, A., Aryal, K. K., Asayesh, H., Ataro, Z., Ausloos, M., Avila-Burgos, L., Avokpaho, E. F. G. A., Awasthi, A., Ayala Quintanilla, B. P., Ayer, R., Azzopardi, P. S., Babazadeh, A., Badali, H., Balakrishnan, K., Bali, A. G., Banach, M., Banoub, J. A. M., Barac, A., Barboza, M. A., Barker-Collo, S. L., Bärnighausen, T. W., Barquera, S., Barrero, L. H., Bazargan-Hejazi, S., Bedi, N., Beghi, E., Behzadifar, M., Behzadifar, M., Bekele, B. B., Bekru, E. T., Belachew, A. B., Belay, Y. A., Bell, M. L., Bello, A. K., Bennett, D. A., Bensenor, I. M., Berhane, A., Bernabe, E., Bernstein, R. S., Beuran, M., Beyranvand, T., Bhala, N., Bhatt, S., Bhaumik, S., Bhutta, Z. A., Biadgo, B., Biehl, M. H., Bijani, A., Bikbov, B., Bilano, V., Bililign, N., Bin Sayeed, M. S., Bisanzio, D., Bjørge, T., Bleyer, A., Bobasa, E. M., Bou-Orm, I. R., Boufous, S., Bourne, R., Brady, O. J., Brant, L. C., Brayne, C., Brazinova, A., Breitborde, N. J. K., Brenner, H., Briant, P. S., Briko, A. N., Britton, G., Brugha, T., Buchbinder, R., Busse, R., Butt, Z. A., Cahuana-Hurtado, L., Campuzano Rincon, J. C., Cano, J., Cárdenas, R., Carrero, J. J., Carter, A., Carvalho, F., Castañeda-Orjuela, C. A., Castillo Rivas, J., Castro, F., Catalá-López, F., Cercy, K. M., Cerin, E., Chaiah, Y., Chang, J.-C., Charlson, F. J., Chattu, V. K., Chiang, P. P.-C., Chitheer, A., Choi, J.-Y. J., Christensen, H., Christopher, D. J., Chung, S.-C., Cicuttini, F. M., Cirillo, M., Collado-Mateo, D., Cooper, C., Cortesi, P. A., Cortinovis, M., Cousin, E., Criqui, M. H., Cromwell, E. A., Cross, M., Crump, J. A., Daba, A. K., Dachew, B. A., Dadi, A. F., Dandona, L., Dandona, R., Dargan, P. I., Daryani, A., Das Gupta, R., Das Neves, J., Dasa, T. T., Davitoiu, D. V., De La Hoz, F. P., De Leo, D., De Neve, J.-W., De Steur, H., Degefa, M. G., Degenhardt, L., Deiparine, S., Demoz, G. T., Denova-Gutiérrez, E., Deribe, K., Dervenis, N., Des Jarlais, D. C., Dey, S., Dharmaratne, S. D., Dhimal, M., Dinberu, M. T., Dirac, M. A., Djalalinia, S., Doan, L., Dokova, K., Doku, D. T., Dorsey, E. R., Doyle, K. E., Driscoll, T. R., Dubey, M., Dubljanin, E., Duken, E. E., Duncan, B. B., Duraes, A. R., Ebrahimi, H., Ebrahimpour, S., Echko, M. M., Edessa, D., Edvardsson, D., Effiong, A.,

Eggen, A. E., Ehrlich, J. R., El Bcheraoui, C., El-Khatib, Z., Elyazar, I. R. F., Enayati, A., Endalifer, M. L., Endries, A. Y., Er, B., Erskine, H. E., Eskandarieh, S., Esteghamati, A., Esteghamati, S., Fakhim, H., Faramarzi, M., Fareed, M., Farhadi, F., Farid, T. A., Farinha, C. S. E. sá, Farioli, A., Faro, A., Farzadfar, F., Fazaeli, A. A., Feigin, V. L., Fentahun, N., Fereshtehnejad, S.-M., Fernandes, E., Fernandes, J. C., Ferrari, A. J., Ferreira, M. L., Filip, I., Fischer, F., Fitzmaurice, C., Foigt, N. A., Foreman, K. J., Frank, T. D., Fukumoto, T., Fullman, N., Fürst, T., Furtado, J. M., Gakidou, E., Gall, S., Gallus, S., Ganji, M., Garcia-Basteiro, A. L., Gardner, W. M., Gebre, A. K., Gebremedhin, A. T., Gebremichael, T. G., Gelano, T. F., Geleijnse, J. M., Genova-Maleras, R., Geramo, Y. C. D., Gething, P. W., Gezae, K. E., Ghadami, M. R., Ghadiri, K., Ghasemi-Kasman, M., Ghimire, M., Ghoshal, A. G., Gill, P. S., Gill, T. K., Ginawi, I. A., Giussani, G., Gnedovskaya, E. V., Goldberg, E. M., Goli, S., Gómez-Dantés, H., Gona, P. N., Gopalani, S. V., Gorman, T. M., Goulart, A. C., Goulart, B. N. G., Grada, A., Grosso, G., Gugnani, H. C., Guillemin, F., Guo, Y., Gupta, P. C., Gupta, R., Gupta, R., Gupta, T., Gutiérrez, R. A., Gyawali, B., Haagsma, J. A., Hachinski, V., Hafezi-Nejad, N., Haghparast Bidgoli, H., Hagos, T. B., Hailegiyorgis, T. T., Haj-Mirzaian, A., Haj-Mirzaian, A., Hamadeh, R. R., Hamidi, S., Handal, A. J., Hankey, G. J., Hao, Y., Harb, H. L., Harikrishnan, S., Haririan, H., Haro, J. M., Hassankhani, H., Hassen, H. Y., Havmoeller, R., Hay, R. J., Hay, S. I., Hedayatizadeh-Omran, A., Heibati, B., Hendrie, D., Henok, A., Heredia-Pi, I., Herteliu, C., Heydarpour, F., Heydarpour, P., Hibstu, D. T., Hoek, H. W., Hoffman, H. J., Hole, M. K., Homaie Rad, E., Hoogar, P., Hosgood, H. D., Hosseini, S. M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Hotez, P. J., Hoy, D. G., Hsairi, M., Htet, A. S., Huang, J. J., Iburg, K. M., Ikeda, C. T., Ilesanmi, O. S., Irvani, S. S. N., Irvine, C. M. S., Islam, S. M. S., Islami, F., Jacobsen, K. H., Jahangiry, L., Jahanmehr, N., Jain, S. K., Jakovljevic, M., James, S. L., Jayatilleke, A. U., Jeemon, P., Jha, R. P., Jha, V., Ji, J. S., Johnson, C. O., Jonas, J. B., Jonnagaddala, J., Jorjoran Shushtari, Z., Joshi, A., Jozwiak, J. J., Jungari, S. B., Jürisson, M., Kabir, Z., Kadel, R., Kahsay, A., Kalani, R., Kanchan, T., Kar, C., Karami, M., Karami Matin, B., Karch, A., Karema, C., Karimi, N., Karimi, S. M., Kasaeian, A., Kassa, D. H., Kassa, G. M., Kassa, T. D., Kassebaum, N. J., Katikireddi, S. V., Kaul, A., Kawakami, N., Kazemi, Z., Karyani, A. K., Keighobadi, M. M., Keiyoro, P. N., Kemmer, L., Kemp, G. R., Kengne, A. P., Keren, A., Khader, Y. S., Khafaei, B., Khafaie, M. A., Khajavi, A., Khalid, N., Khalil, I. A., Khan, E. A., Khan, M. S., Khan, M. A., Khang, Y.-H., Khater, M. M., Khazaei, M., Khoja, A. T., Khosravi, A., Khosravi, M. H., Kiadaliri, A. A., Kidanemariam, Z. T., Kiirithio, D. N., Kim, C.-I., Kim, D., Kim, Y.-E., Kim, Y. J., Kimokoti, R. W., Kinfu, Y., Kisa, A., Kissimova-Skarbek, K., Knudsen, A. K. S., Kocarnik, J. M., Kochhar, S., Kokubo, Y., Kolola, T., Kopec, J. A., Kosen, S., Kotsakis, G. A., Koul, P. A., Koyanagi, A., Krishan, K., Krishnaswami, S., Krohn, K. J., Kuate Defo, B., Kucuk Bicer, B., Kumar, G. A., Kumar, M., Kuzin, I., Lad, D. P., Lad, S. D., Lafranconi, A., Lalloo, R., Lallukka, T., Lami, F. H., Lang, J. J., Langan, S. M., Lansingh, V. C., Latifi, A., Lau, K. M.-M., Lazarus, J. V., Leasher, J. L., Ledesma, J. R., Lee, P. H., Leigh, J., Leili, M., Leshargie, C. T., Leung, J., Levi, M., Lewycka, S., Li, S., Li, Y., Liang, X., Liao, Y., Liben, M. L., Lim, L.-L., Lim, S. S., Limenih, M. A., Linn, S., Liu, S., Looker, K. J., Lopez, A. D., Lorkowski, S., Lotufo, P. A., Lozano, R., Lucas, T. C. D., Lunevicius, R., Lyons, R. A., Ma, S., Macarayan, E. R. K., Mackay, M. T., Maddison, E. R., Madotto, F., Maghavani, D. P., Mai, H. T., Majdan, M., Majdzadeh, R., Majeed, A., Malekzadeh, R., Malta, D. C., Mamun, A. A., Manda, A.-L., Manguerra, H., Mansournia, M. A., Mantilla Herrera, A. M., Mantovani,

L. G., Maravilla, J. C., Marcenes, W., Marks, A., Martins-Melo, F. R., Martopullo, I., März, W., Marzan, M. B., Massano, J., Massenburg, B. B., Mathur, M. R., Maulik, P. K., Mazidi, M., McAlinden, C., McGrath, J. J., McKee, M., McMahon, B. J., Mehata, S., Mehrotra, R., Mehta, K. M., Mehta, V., Mejia-Rodriguez, F., Mekonen, T., Melese, A., Melku, M., Memiah, P. T. N., Memish, Z. A., Mendoza, W., Mengistu, G., Mensah, G. A., Mereta, S. T., Meretoja, A., Meretoja, T. J., Mestrovic, T., Miazgowski, B., Miazgowski, T., Millear, A. I., Miller, T. R., Mini, G. K., Mirarefin, M., Mirica, A., Mirrakhimov, E. M., Misganaw, A. T., Mitchell, P. B., Mitiku, H., Moazen, B., Mohajer, B., Mohammad, K. A., Mohammadi, M., Mohammadifard, N., Mohammadnia-Afrouzi, M., Mohammed, M. A., Mohammed, S., Mohebi, F., Mokdad, A. H., Molokhia, M., Monasta, L., Montañez, J. C., Moosazadeh, M., Moradi, G., Moradi, M., Moradi-Lakeh, M., Moradinazar, M., Moraga, P., Morawska, L., Moreno Velásquez, I., Morgado-Da-Costa, J., Morrison, S. D., Moschos, M. M., Mousavi, S. M., Mruts, K. B., Muche, A. A., Muchie, K. F., Mueller, U. O., Muhammed, O. S., Mukhopadhyay, S., Muller, K., Mumford, J. E., Murthy, G. V. S., Musa, K. I., Mustafa, G., Nabhan, A. F., Nagata, C., Nagel, G., Naghavi, M., Naheed, A., Nahvijou, A., Naik, G., Najafi, F., Nam, H. S., Nangia, V., Nansseu, J. R., Neamati, N., Negoi, I., Negoi, R. I., Neupane, S., Newton, C. R. J., Ngunjiri, J. W., Nguyen, A. Q., Nguyen, G., Nguyen, H. T., Nguyen, H. L. T., Nguyen, H. T., Nguyen, L. H., Nguyen, M., Nguyen, N. B., Nguyen, S. H., Nichols, E., Ningrum, D. N. A., Nixon, M. R., Nomura, S., Noroozi, M., Norrving, B., Noubiap, J. J., Nouri, H. R., Shiadeh, M. N., Nowroozi, M. R., Nsoesie, E. O., Nyasulu, P. S., Odell, C. M., Ofori-Asenso, R., Ogbo, F. A., Oh, I.-H., Oladimeji, O., Olagunju, A. T., Olagunju, T. O., Olivares, P. R., Olsen, H. E., Olusanya, B. O., Olusanya, J. O., Ong, K. L., Ong, S. K., Oren, E., Ortiz, A., Ota, E., Otstavnov, S. S., Øverland, S., Owolabi, M. O., P A, M., Pacella, R., Pakhare, A. P., Pakpour, A. H., Pana, A., Panda-Jonas, S., Park, E.-K., Park, J., Parry, C. D. H., Parsian, H., Pasdar, Y., Patel, S., Patil, S. T., Patle, A., Patton, G. C., Paturi, V. R., Paudel, D., Paulson, K. R., Pearce, N., Pereira, A., Pereira, D. M., Perico, N., Pesudovs, K., Petzold, M., Pham, H. Q., Phillips, M. R., Pigott, D. M., Pillay, J. D., Piradov, M. A., Pirsaheb, M., Pishgar, F., Plana-Ripoll, O., Polinder, S., Popova, S., Postma, M. J., Pourshams, A., Poustchi, H., Prabhakaran, D., Prakash, S., Prakash, V., Prasad, N., Purcell, C. A., Qorbani, M., Quistberg, D. A., Radfar, A., Rafay, A., Rafiei, A., Rahim, F., Rahimi, K., Rahimi, Z., Rahimi-Movaghar, A., Rahimi-Movaghar, V., Rahman, M., Rahman, M. H. U., Rahman, M. A., Rahman, S. U., Rai, R. K., Rajati, F., Ranjan, P., Rao, P. C., Rasella, D., Rawaf, D. L., Rawaf, S., Reddy, K. S., Reiner, R. C., Reitsma, M. B., Remuzzi, G., Renzaho, A. M. N., Resnikoff, S., Rezaei, S., Rezai, M. S., Ribeiro, A. L. P., Roberts, N. L. S., Robinson, S. R., Roever, L., Ronfani, L., Roshandel, G., Rostami, A., Roth, G. A., Rothenbacher, D., Rubagotti, E., Sachdev, P. S., Sadat, N., Sadeghi, E., Saeedi Moghaddam, S., Safari, H., Safari, Y., Safari-Faramani, R., Safdarian, M., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sahraian, M. A., Sajadi, H. S., Salam, N., Salama, J. S., Salamati, P., Saleem, Z., Salimi, Y., Salimzadeh, H., Salomon, J. A., Salvi, S. S., Salz, I., Samy, A. M., Sanabria, J., Sanchez-Niño, M. D., Santomauro, D. F., Santos, I. S., Santos, J. V., Santric Milicevic, M. M., Sao Jose, B. P., Sardana, M., Sarker, A. R., Sarmiento-Suárez, R., Sarrafzadegan, N., Sartorius, B., Sarvi, S., Sathian, B., Satpathy, M., Sawant, A. R., Sawhney, M., Saxena, S., Schaeffner, E., Schmidt, M. I., Schneider, I. J. C., Schutte, A. E., Schwebel, D. C., Schwendicke, F., Scott, J. G., Sekerija, M., Sepanlou, S. G., Serván-Mori, E., Seyedmousavi, S., Shabaninejad, H., Shafieesabet, A., Shahbazi, M., Shaheen, A. A., Shaikh, M. A., Shams-Beyranvand, M.,

Shamsi, M., Sharafi, H., Sharafi, K., Sharif, M., Sharif-Alhoseini, M., Sharma, J., Sharma, R., She, J., Sheikh, A., Shi, P., Shibuya, K., Shiferaw, M. S., Shigematsu, M., Shiri, R., Shirkoohi, R., Shiue, I., Shokoohinia, Y., Shokraneh, F., Shoman, H., Shrime, M. G., Si, S., Siabani, S., Sibai, A. M., Siddiqi, T. J., Sigfusdottir, I. D., Sigurvinsdottir, R., Silva, D. A. S., Silva, J. P., Silveira, D. G. A., Singam, N. S. V., Singh, J. A., Singh, N. P., Singh, V., Sinha, D. N., Skiadaresi, E., Skirbekk, V., Sliwa, K., Smith, D. L., Smith, M., Soares Filho, A. M., Sobaih, B. H., Sobhani, S., Soofi, M., Sorensen, R. J. D., Soriano, J. B., Soyiri, I. N., Sposato, L. A., Sreeramareddy, C. T., Srinivasan, V., Stanaway, J. D., Starodubov, V. I., Stein, D. J., Steiner, C., Steiner, T. J., Stokes, M. A., Stovner, L. J., Subart, M. L., Sudaryanto, A., Sufiyan, M. B., Sulo, G., Sunguya, B. F., Sur, P. J., Sykes, B. L., Sylaja, P. N., Sylte, D. O., Szoeke, C. E. I., Tabarés-Seisdedos, R., Tabuchi, T., Tadakamadla, S. K., Tandon, N., Tassew, S. G., Tavakkoli, M., Taveira, N., Taylor, H. R., Tehrani-Banihashemi, A., Tekalign, T. G., Tekelemedhin, S. W., Tekle, M. G., Temsah, M.-H., Temsah, O., Terkawi, A. S., Tessema, B., Teweldemedhin, M., Thankappan, K. R., Theis, A., Thirunavukkarasu, S., Thomas, N., Tilahun, B., To, Q. G., Tonelli, M., Topor-Madry, R., Torre, A. E., Tortajada-Girbés, M., Touvier, M., Tovani-Palone, M. R., Towbin, J. A., Tran, B. X., Tran, K. B., Troeger, C. E., Tsadik, A. G., Tsoi, D., Tudor Car, L., Tyrovolas, S., Ukwaja, K. N., Ullah, I., Undurraga, E. A., Updike, R. L., Usman, M. S., Uthman, O. A., Vaduganathan, M., Vaezi, A., Valdez, P. R., Varavikova, E., Varughese, S., Vasankari, T. J., Venketasubramanian, N., Villafaina, S., Violante, F. S., Vladimirov, S. K., Vlassov, V., Vollset, S. E., Vos, T., Vosoughi, K., Vujcic, I. S., Wagnew, F. S., Waheed, Y., Wang, Y., Wang, Y.-P., Weiderpass, E., Weintraub, R. G., Weiss, D. J., Weldegebreal, F., Weldegwergs, K. G., Werdecker, A., West, T. E., Westerman, R., Whiteford, H. A., Widecka, J., Wijeratne, T., Williams, H. C., Wilner, L. B., Wilson, S., Winkler, A. S., Wiyeh, A. B., Wiysonge, C. S., Wolfe, C. D. A., Woolf, A. D., Wyper, G. M. A., Xavier, D., Xu, G., Yadgir, S., Yahyazadeh Jabbari, S. H., Yamada, T., Yan, L. L., Yano, Y., Yaseri, M., Yasin, Y. J., Yeshaneh, A., Yimer, E. M., Yip, P., Yisma, E., Yonemoto, N., Yoon, S.-J., Yotebieng, M., Younis, M. Z., Yousefifard, M., Yu, C., Zadnik, V., Zaidi, Z., Zaman, S. B., Zamani, M., Zandian, H., Zar, H. J., Zenebe, Z. M., Zhou, M., Zipkin, B., Zodpey, S., Zucker, I., Zuhlke, L. J. & Murray, C. J. L. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* **392,** 1859–1922 (2018).

3. Mokdad, A. H., Ballestros, K., Echko, M., Glenn, S., Olsen, H. E., Mullany, E., Lee, A., Khan, A. R., Ferrari, A. J., Kasaeian, A., Werdecker, A., Carter, A., Zipkin, B., Sartorius, B., Serdar, B., Sykes, L., Troeger, C., Fitzmaurice, C., Rehm, C. D., Santomauro, D., Kim, D., Colombara, D., Schwebel, D. C., Tsoi, D., Kolte, D., Nsoesie, E., Nichols, E., Oren, E., Charlson, F. J., Patton, G. C., Roth, G. A., Hosgood, H. D., Whiteford, H. A., Kyu, H., Erskine, H. E., Huang, H., Martopullo, I., Singh, J. A., Nachega, J. B., Sanabria, J. R., Ong, K., Tabb, K., Krohn, K. J., Degenhardt, L., Moses, M., Farvid, M., Griswold, M., Criqui, M., Bell, M., Nguyen, M., Mirarefin, M., Qorbani, M., Younis, M., Fullman, N., Liu, P., Briant, P., Gona, P., Havmoller, R., Leung, R., Kimokoti, R., Hay, S. I., Yadgir, S., Biryukov, S., Vollset, S. E., Alam, T., Frank, T., Farid, T., Vos, T., Bärnighausen, T., Telwelde, T., Yano, Y., Al-Aly, Z., Mehari, A., Kandel, A., Anderson, B., Biroscak, B., Mozaffarian, D., Dorsey, E. R., Ding, E. L., Park, E.-K., Wagner, G., Hu, G. & Chen, H.

The State of US Health, 1990-2016: Burden of Diseases, Injuries, and Risk Factors Among US States. 113

4. Cassidy, J. D., Côté, P., Carroll, L. J. & Kristman, V. Incidence and course of low back pain episodes in the general population. *Spine (Phila Pa 1976)* **30,** 2817–2823 (2005).

5. Hestbaek, L., Leboeuf-Yde, C. & Manniche, C. Low back pain: what is the long-term course? A review of studies of general patient populations. *Eur Spine J* **12,** 149–165 (2003).

6. Verkerk, K., Luijsterburg, P. A. J., Heymans, M. W., Ronchetti, I., Pool-Goudzwaard, A. L., Miedema, H. S. & Koes, B. W. Prognosis and course of disability in patients with chronic nonspecific low back pain: a 5- and 12-month follow-up cohort study. *Phys Ther* **93,** 1603–1614 (2013).

7. Carey, T. S., Garrett, J. M., Jackman, A. & Hadler, N. Recurrence and care seeking after acute back pain: results of a long-term follow-up study. North Carolina Back Pain Project. *Med Care* **37,** 157–164 (1999).

8. Andersson, G. B. Epidemiological features of chronic low-back pain. *The Lancet* **354,** 581–585 (1999).

9. Rundell, S. D., Sherman, K. J., Heagerty, P. J., Mock, C. N. & Jarvik, J. G. The clinical course of pain and function in older adults with a new primary care visit for back pain. *J Am Geriatr Soc* **63,** 524–530 (2015).

10. Airaksinen, O., Herno, A., Kaukanen, E., Saari, T., Sihvonen, T. & Suomalainen, O. Density of lumbar muscles 4 years after decompressive spinal surgery. *Eur Spine J* **5,** 193–197 (1996).

11. Storheim, K., Berg, L., Hellum, C., Gjertsen, Ø., Neckelmann, G., Espeland, A. & Keller, A. Fat in the lumbar multifidus muscles - predictive value and change following disc prosthesis surgery and multidisciplinary rehabilitation in patients with chronic low back pain and degenerative disc: 2-year follow-up of a randomized trial. *BMC Musculoskelet Disord* **18,** 145 (2017).

12. Steele, J., Fisher, J., Bruce-Low, S., Smith, D., Osborne, N. & Newell, D. Variability in Strength, Pain, and Disability Changes in Response to an Isolated Lumbar Extension Resistance Training Intervention in Participants with Chronic Low Back Pain. *Healthcare* **5,** 75 (2017).

13. Taylor, R. S. & Taylor, R. J. The economic impact of failed back surgery syndrome. *Br J Pain* **6,** 174–181 (2012).

14. Brown, S. H. M., Banuelos, K., Ward, S. R. & Lieber, R. L. Architectural and morphological assessment of rat abdominal wall muscles: comparison for use as a human model: Rat abdominal muscle architecture. *Journal of Anatomy* **217,** 196–202 (2010).

15. Regev, G. J., Kim, C. W., Tomiya, A., Lee, Y. P., Ghofrani, H., Garfin, S. R., Lieber, R. L. & Ward, S. R. Psoas Muscle Architectural Design, In Vivo Sarcomere Length Range, and Passive Tensile Properties Support Its Role as a Lumbar Spine Stabilizer: *Spine* **36,** E1666–E1674 (2011).

16. Ward, S. R., Kim, C. W., Eng, C. M., Gottschalk, L. J., Tomiya, A., Garfin, S. R. & Lieber, R. L. Architectural Analysis and Intraoperative Measurements Demonstrate the Unique Design of the Multifidus Muscle for Lumbar Spine Stability: *The Journal of Bone and Joint Surgery-American Volume* **91,** 176–185 (2009).

17. Shahidi, B., Hubbard, J. C., Gibbons, M. C., Ruoss, S., Zlomislic, V., Allen, R. T., Garfin, S. R. & Ward, S. R. Lumbar multifidus muscle degenerates in individuals with chronic degenerative lumbar spine pathology: MULTIFIDUS DEGENERATION IN LOW BACK PAIN. *J. Orthop. Res.* **35,** 2700–2706 (2017).

18. Shahidi, B., Parra, C. L., Berry, D. B., Hubbard, J. C., Gombatto, S., Zlomislic, V., Allen, R. T., Hughes-Austin, J., Garfin, S. & Ward, S. R. Contribution of Lumbar Spine Pathology and Age to Paraspinal Muscle Size and Fatty Infiltration. *Spine* **42,** 616–623 (2017).

19. Hodges, P. W. & Danneels, L. Changes in Structure and Function of the Back Muscles in Low Back Pain: Different Time Points, Observations, and Mechanisms. *J Orthop Sports Phys Ther* **49,** 464–476 (2019).

20. Barker, K. L., Shamley, D. R. & Jackson, D. Changes in the cross-sectional area of multifidus and psoas in patients with unilateral back pain: the relationship to pain and disability. *Spine (Phila Pa 1976)* **29,** E515-519 (2004).

21. Demoulin, C., Crielaard, J.-M. & Vanderthommen, M. Spinal muscle evaluation in healthy individuals and low-back-pain patients: a literature review. *Joint Bone Spine* **74,** 9–13 (2007).

22. Alaranta, H., Tallroth, K., Soukka, A. & Heliövaara, M. Fat content of lumbar extensor muscles and low back disability: a radiographic and clinical comparison. *J Spinal Disord* **6,** 137–140 (1993).

23. Ng, J. K., Richardson, C. A., Kippers, V. & Parnianpour, M. Relationship between muscle fiber composition and functional capacity of back muscles in healthy subjects and patients with back pain. *J Orthop Sports Phys Ther* **27,** 389–402 (1998).

24. Wan, Q., Lin, C., Li, X., Zeng, W. & Ma, C. MRI assessment of paraspinal muscles in patients with acute and chronic unilateral low back pain. *Br J Radiol* **88,** 20140546 (2015).

25. Danneels, L. A., Vanderstraeten, G. G., Cambier, D. C., Witvrouw, E. E. & De Cuyper, H. J. CT imaging of trunk muscles in chronic low back pain patients and healthy control subjects. *Eur Spine J* **9,** 266–272 (2000).

26. Kamaz, M., Kireşi, D., Emlik, D. & Levendoğlu, F. CT measurement of trunk muscle areas in patients with chronic low back pain. *Diagn Interv Radiol* **13,** 144–148 (2007).

27. Kang, C. H., Shin, M. J., Kim, S. M., Lee, S. H. & Lee, C.-S. MRI of paraspinal muscles in lumbar degenerative kyphosis patients and control patients with chronic low back pain. *Clin Radiol* **62,** 479–486 (2007).

28. Hides, J., Gilmore, C., Stanton, W. & Bohlscheid, E. Multifidus size and symmetry among chronic LBP and healthy asymptomatic subjects. *Man Ther* **13,** 43–49 (2008).

29. Wallwork, T. L., Stanton, W. R., Freke, M. & Hides, J. A. The effect of chronic low back pain on size and contraction of the lumbar multifidus muscle. *Man Ther* **14,** 496–500 (2009).

30. Kim, W. H., Lee, S.-H. & Lee, D. Y. Changes in the cross-sectional area of multifidus and psoas in unilateral sciatica caused by lumbar disc herniation. *J Korean Neurosurg Soc* **50,** 201–204 (2011).

31. Chan, S.-T., Fung, P.-K., Ng, N.-Y., Ngan, T.-L., Chong, M.-Y., Tang, C.-N., He, J.-F. & Zheng, Y.-P. Dynamic changes of elasticity, cross-sectional area, and fat infiltration of multifidus at different postures in men with chronic low back pain. *Spine J* **12,** 381–388 (2012).

32. Fortin, M., Lazáry, À., Varga, P. P., McCall, I. & Battié, M. C. Paraspinal muscle asymmetry and fat infiltration in patients with symptomatic disc herniation. *Eur Spine J* **25,** 1452–1459 (2016).

33. Goubert, D., Oosterwijck, J. V., Meeus, M. & Danneels, L. Structural Changes of Lumbar Muscles in Non-specific Low Back Pain: A Systematic Review. *Pain Physician* **19,** E985–E1000 (2016).

34. Goubert, D., De Pauw, R., Meeus, M., Willems, T., Cagnie, B., Schouppe, S., Van Oosterwijck, J., Dhondt, E. & Danneels, L. Lumbar muscle structure and function in chronic versus recurrent low back pain: a cross-sectional study. *Spine J* **17,** 1285–1296 (2017).

35. Ranger, T. A., Cicuttini, F. M., Jensen, T. S., Peiris, W. L., Hussain, S. M., Fairley, J. & Urquhart, D. M. Are the size and composition of the paraspinal muscles associated with low back pain? A systematic review. *Spine J* **17,** 1729–1748 (2017).

36. Cooley, J. R., Walker, B. F., M Ardakani, E., Kjaer, P., Jensen, T. S. & Hebert, J. J. Relationships between paraspinal muscle morphology and neurocompressive conditions of the lumbar spine: a systematic review with meta-analysis. *BMC Musculoskelet Disord* **19,** 351 (2018).

37. Kaser, L., Mannion, A. F., Rhyner, A., Weber, E., Dvorak, J. & Muntener, M. Active Therapy for Chronic Low Back Pain. 11

38. Käser, L., Mannion, A. F., Rhyner, A., Weber, E., Dvorak, J. & Müntener, M. Active therapy for chronic low back pain: part 2. Effects on paraspinal muscle cross-sectional area, fiber type size, and distribution. *Spine (Phila Pa 1976)* **26,** 909–919 (2001).

39. D'hooge, R., Cagnie, B., Crombez, G., Vanderstraeten, G., Dolphens, M. & Danneels, L. Increased intramuscular fatty infiltration without differences in lumbar muscle cross-sectional area during remission of unilateral recurrent low back pain. *Manual Therapy* **17,** 584–588 (2012).

40. Kjaer, P., Bendix, T., Sorensen, J. S., Korsholm, L. & Leboeuf-Yde, C. Are MRI-defined fat infiltrations in the multifidus muscles associated with low back pain? *BMC Med* **5,** 2 (2007).

41. Zhi-Jun, H., Wen-Bin, X., Shuai, C., Zhi-Jie, Z., Feng-Dong, Z., Xiao-Jing, Y., Ji-Ying, W., Li-Li, H., Feng, J., Guo-Xiang, F., Dan-Ju, W., Shun-Wu, F. & Xiang-Qian, F. Accuracy of Magnetic Resonance Imaging Signal Intensity Ratio Measurements in the Evaluation of Multifidus Muscle Injury and Atrophy Relative to That of Histological Examinations. *Spine* **39,** (2014).

42. Commean, P. K., Tuttle, L. J., Hastings, M. K., Strube, M. J. & Mueller, M. J. Magnetic resonance imaging measurement reproducibility for calf muscle and adipose tissue volume. *J Magn Reson Imaging* **34,** 1285–1294 (2011).

43. Mannion, A. F., Weber, B. R., Dvorak, J., Grob, D. & Müntener, M. Fibre type characteristics of the lumbar paraspinal muscles in normal healthy subjects and in patients with low back pain. *J Orthop Res* **15,** 881–887 (1997).

44. Shahidi, B., Fisch, K. M., Gibbons, M. C. & Ward, S. R. Increased Fibrogenic Gene Expression in Multifidus Muscles of Patients With Chronic Versus Acute Lumbar Spine Pathology. *Spine* **45,** E189–E195 (2020).

45. Hodges, P. W., James, G., Blomster, L., Hall, L., Schmid, A., Shu, C., Little, C. & Melrose, J. Multifidus Muscle Changes After Back Injury Are Characterized by Structural Remodeling of Muscle, Adipose and Connective Tissue, but Not Muscle Atrophy: Molecular and Morphological Evidence. *Spine* **40,** 1057–1071 (2015).

46. Hodges, P. W., James, G., Blomster, L., Hall, L., Schmid, A. B., Shu, C., Little, C. & Melrose, J. Can Proinflammatory Cytokine Gene Expression Explain Multifidus Muscle Fiber Changes After an Intervertebral Disc Lesion?: *Spine* **39,** 1010–1017 (2014).

47. Hiepe, P., Gussew, A., Rzanny, R., Kurz, E., Anders, C., Walther, M., Scholle, H.-C. & Reichenbach, J. R. Age-related structural and functional changes of low back muscles. *Exp Gerontol* **65,** 23–34 (2015).

48. Yoshihara, K., Shirai, Y., Nakayama, Y. & Uesaka, S. Histochemical changes in the multifidus muscle in patients with lumbar intervertebral disc herniation. *Spine (Phila Pa 1976)* **26,** 622–626 (2001).

49. Yoshihara, K., Nakayama, Y., Fujii, N., Aoki, T. & Ito, H. Atrophy of the multifidus muscle in patients with lumbar disk herniation: histochemical and electromyographic study. *Orthopedics* **26,** 493–495 (2003).

50. Zhao, W. P., Kawaguchi, Y., Matsui, H., Kanamori, M. & Kimura, T. Histochemistry and morphology of the multifidus muscle in lumbar disc herniation: comparative study between diseased and normal sides. *Spine (Phila Pa 1976)* **25,** 2191–2199 (2000).

51. Ford, D., Bagnall, K. M., McFadden, K. D., Greenhill, B. & Raso, J. Analysis of vertebral muscle obtained during surgery for correction of a lumbar disc disorder. *Acta Anat (Basel)* **116,** 152–157 (1983).

52. Agten, A., Stevens, S., Verbrugghe, J., Timmermans, A. & Vandenabeele, F. Biopsy samples from the erector spinae of persons with nonspecific chronic low back pain display a decrease in glycolytic muscle fibers. *Spine J* **20,** 199–206 (2020).

53. Bajek, S., Bobinac, D., Bajek, G., Vranić, T. S., Lah, B. & Dragojević, D. M. Muscle fiber type distribution in multifidus muscle in cases of lumbar disc herniation. *Acta Med Okayama* **54,** 235–241 (2000).

54. Mauro, A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* **9,** 493–495 (1961).

55. Joe, A. W. B., Yi, L., Natarajan, A., Le Grand, F., So, L., Wang, J., Rudnicki, M. A. & Rossi, F. M. V. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol* **12,** 153–163 (2010).

56. Uezumi, A., Fukada, S., Yamamoto, N., Takeda, S. & Tsuchida, K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat Cell Biol* **12,** 143–152 (2010).

57. Morris, P., Ali, K., Merritt, M., Pelletier, J. & Macedo, L. G. A systematic review of the role of inflammatory biomarkers in acute, subacute and chronic non-specific low back pain. *BMC Musculoskelet Disord* **21,** 142 (2020).

58. Ciciliot, S. & Schiaffino, S. Regeneration of mammalian skeletal muscle. Basic mechanisms and clinical implications. *Curr Pharm Des* **16,** 906–914 (2010).

59. James, G., Millecamps, M., Stone, L. S. & Hodges, P. W. Dysregulation of the Inflammatory Mediators in the Multifidus Muscle After Spontaneous Intervertebral Disc

Degeneration SPARC-null Mice is Ameliorated by Physical Activity. *Spine (Phila Pa 1976)* **43,** E1184–E1194 (2018).

60. James, G., Klyne, D. M., Millecamps, M., Stone, L. S. & Hodges, P. W. ISSLS Prize in Basic science 2019: Physical activity attenuates fibrotic alterations to the multifidus muscle associated with intervertebral disc degeneration. *Eur Spine J* **28,** 893–904 (2019).

61. Brown, S. H. M., Gregory, D. E., Carr, J. A., Ward, S. R., Masuda, K. & Lieber, R. L. ISSLS Prize Winner: Adaptations to the Multifidus Muscle in Response to Experimentally Induced Intervertebral Disc Degeneration. *Spine* **36,** 1728–1736 (2011).

62. Klyne, D. M., Barbe, M. F., van den Hoorn, W. & Hodges, P. W. ISSLS PRIZE IN CLINICAL SCIENCE 2018: longitudinal analysis of inflammatory, psychological, and sleep-related factors following an acute low back pain episode—the good, the bad, and the ugly. *Eur Spine J* **27,** 763–777 (2018).

63. Shahidi, B., Gibbons, M. C., Esparza, M., Zlomislic, V., Allen, R. T., Garfin, S. R. & Ward, S. R. Cell populations and muscle fiber morphology associated with acute and chronic muscle degeneration in lumbar spine pathology. *JOR Spine* **3,** (2020).64. Attia, E., Brown, H., Henshaw, R., George, S. & Hannafin, J. A. Patterns of Gene Expression in a Rabbit Partial Anterior Cruciate Ligament Transection Model: The Potential Role of Mechanical Forces. *Am J Sports Med* **38,** 348–356 (2009).

65. Gibbons, M. C., Fisch, K. M., Pichika, R., Cheng, T., Engler, A. J., Schenk, S., Lane, J. G., Singh, A. & Ward, S. R. Heterogeneous muscle gene expression patterns in patients with massive rotator cuff tears. *PLoS One* **13,** e0190439 (2018).

66. Morales, M. G., Cabello-Verrugio, C., Santander, C., Cabrera, D., Goldschmeding, R. & Brandan, E. CTGF/CCN-2 over-expression can directly induce features of skeletal muscle dystrophy. *J Pathol* **225,** 490–501 (2011).

67. Sun, G., Haginoya, K., Chiba, Y., Uematsu, M., Hino-Fukuyo, N., Tanaka, S., Onuma, A., Iinuma, K. & Tsuchiya, S. Elevated plasma levels of tissue inhibitors of metalloproteinase-1 and their overexpression in muscle in human and mouse muscular dystrophy. *Journal of the Neurological Sciences* **297,** 19–28 (2010).

68. Dvinge, H. & Bertone, P. HTqPCR: high-throughput analysis and visualization of quantitative real-time PCR data in R. *Bioinformatics* **25,** 3325–3326 (2009).

69. Reiner, A., Yekutieli, D. & Benjamini, Y. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics* **19,** 368–375 (2003).

70. Mammucari, C., Milan, G., Romanello, V., Masiero, E., Rudolf, R., Del Piccolo, P., Burden, S. J., Di Lisi, R., Sandri, C., Zhao, J., Goldberg, A. L., Schiaffino, S. & Sandri, M. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* **6,** 458–471 (2007).

71. Fitzwalter, B. E. & Thorburn, A. FOXO3 links autophagy to apoptosis. *Autophagy* **14,** 1467–1468 (2018).

72. Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S. H. & Goldberg, A. L. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117,** 399–412 (2004).

73. Sandri, M., Barberi, L., Bijlsma, A. Y., Blaauw, B., Dyar, K. A., Milan, G., Mammucari, C., Meskers, C. G. M., Pallafacchina, G., Paoli, A., Pion, D., Roceri, M., Romanello, V., Serrano, A. L., Toniolo, L., Larsson, L., Maier, A. B., Muñoz-Cánoves, P., Musarò, A., Pende, M., Reggiani, C., Rizzuto, R. & Schiaffino, S. Signalling pathways regulating muscle mass in ageing skeletal muscle: the role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology* **14,** 303–323 (2013).

74. Csibi, A., Leibovitch, M. P., Cornille, K., Tintignac, L. A. & Leibovitch, S. A. MAFbx/Atrogin-1 controls the activity of the initiation factor eIF3-f in skeletal muscle atrophy by targeting multiple C-terminal lysines. *J Biol Chem* **284,** 4413–4421 (2009).

75. Bodine, S. C. & Baehr, L. M. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. *Am J Physiol Endocrinol Metab* **307,** E469-484 (2014).

76. Rashid, M. M., Runci, A., Russo, M. A. & Tafani, M. Muscle Lim Protein (MLP)/CSRP3 at the crossroad between mechanotransduction and autophagy. *Cell Death Dis* **6,** e1940–e1940 (2015).

77. Vafiadaki, E., Arvanitis, D. A. & Sanoudou, D. Muscle LIM Protein: Master regulator of cardiac and skeletal muscle functions. *Gene* **566,** 1–7 (2015).

78. Han, S., Cui, C., Wang, Y., He, H., Liu, Z., Shen, X., Chen, Y., Li, D., Zhu, Q. & Yin, H. Knockdown of CSRP3 inhibits differentiation of chicken satellite cells by promoting TGF-β/Smad3 signaling. *Gene* **707,** 36–43 (2019).

79. Jensen, J. H., Conley, L. N., Hedegaard, J., Nielsen, M., Young, J. F., Oksbjerg, N., Hornshøj, H., Bendixen, C. & Thomsen, B. Gene expression profiling of porcine skeletal muscle in the early recovery phase following acute physical activity. *Exp Physiol* **97,** 833–848 (2012).

80. Vafiadaki, E., Arvanitis, D. A., Papalouka, V., Terzis, G., Roumeliotis, T. I., Spengos, K., Garbis, S. D., Manta, P., Kranias, E. G. & Sanoudou, D. Muscle lim protein isoform negatively regulates striated muscle actin dynamics and differentiation. *FEBS J* **281,** 3261–3279 (2014).

81. Chen, X. & Li, Y. Role of matrix metalloproteinases in skeletal muscle: migration, differentiation, regeneration and fibrosis. *Cell Adh Migr* **3,** 337–341 (2009).

82. Mackey, A. L., Brandstetter, S., Schjerling, P., Bojsen-Moller, J., Qvortrup, K., Pedersen, M. M., Doessing, S., Kjaer, M., Magnusson, S. P. & Langberg, H. Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *FASEB J* **25,** 1943–1959 (2011).

83. Morales, M. G., Acuña, M. J., Cabrera, D., Goldschmeding, R. & Brandan, E. The pro-fibrotic connective tissue growth factor (CTGF/CCN2) correlates with the number of necrotic-regenerative foci in dystrophic muscle. *J Cell Commun Signal* **12,** 413–421 (2018).

84. Liu, H., Chen, S.-E., Jin, B., Carson, J. A., Niu, A., Durham, W., Lai, J.-Y. & Li, Y.-P. TIMP3: a physiological regulator of adult myogenesis. *J Cell Sci* **123,** 2914–2921 (2010).

85. Lee, Y.-S., Kim, J.-Y., Oh, K.-S. & Chung, S. W. Fatty acid-binding protein 4 regulates fatty infiltration after rotator cuff tear by hypoxia-inducible factor 1 in mice. *J Cachexia Sarcopenia Muscle* **8,** 839–850 (2017).

86. Li, X. Z., Yan, Y., Zhang, J. F., Sun, J. F., Sun, B., Yan, C. G., Choi, S. H., Johnson, B. J., Kim, J. K. & Smith, S. B. Oleic acid in the absence of a PPARγ agonist increases adipogenic gene expression in bovine muscle satellite cells1. *J Anim Sci* **97,** 4114–4123 (2019).

87. Qiao, L., Zou, C., Shao, P., Schaack, J., Johnson, P. F. & Shao, J. Transcriptional regulation of fatty acid translocase/CD36 expression by CCAAT/enhancer-binding protein alpha. *J Biol Chem* **283,** 8788–8795 (2008).

88. Cavanaugh, E. J. & DiMario, J. X. C/EBPα represses slow myosin heavy chain 2 gene expression in developing avian myotubes. *Biochim Biophys Acta* **1860,** 2355–2362 (2016).

89. Albert, H. B., Briggs, A. M., Kent, P., Byrhagen, A., Hansen, C. & Kjaergaard, K. The prevalence of MRI-defined spinal pathoanatomies and their association with modic changes in individuals seeking care for low back pain. *Eur Spine J* **20,** 1355–1362 (2011).

90. Fürnsinn, C., Willson, T. M. & Brunmair, B. Peroxisome proliferator-activated receptor-δ, a regulator of oxidative capacity, fuel switching and cholesterol transport. *Diabetologia* **50,** 8–17 (2006).

91. Riserus, U., Sprecher, D., Johnson, T., Olson, E., Hirschberg, S., Liu, A., Fang, Z., Hegde, P., Richards, D., Sarov-Blat, L., Strum, J. C., Basu, S., Cheeseman, J., Fielding, B. A., Humphreys, S. M., Danoff, T., Moore, N. R., Murgatroyd, P., O'Rahilly, S., Sutton, P., Willson, T., Hassall, D., Frayn, K. N. & Karpe, F. Activation of Peroxisome Proliferator-Activated Receptor (PPAR) Promotes Reversal of Multiple Metabolic Abnormalities, Reduces Oxidative Stress, and Increases Fatty Acid Oxidation in Moderately Obese Men. *Diabetes* **57,** 332–339 (2008).

92. Roberts, L. D., Murray, A. J., Menassa, D., Ashmore, T., Nicholls, A. W. & Griffin, J. L. The contrasting roles of PPARδ and PPARγ in regulating the metabolic switch between oxidation and storage of fats in white adipose tissue. *Genome Biol* **12,** R75 (2011).

93. Silva, K. A. S., Dong, J., Dong, Y., Dong, Y., Schor, N., Tweardy, D. J., Zhang, L. & Mitch, W. E. Inhibition of Stat3 Activation Suppresses Caspase-3 and the Ubiquitin-Proteasome System, Leading to Preservation of Muscle Mass in Cancer Cachexia. *Journal of Biological Chemistry* **290,** 11177–11187 (2015).

94. Romanowska, M., Reilly, L., Palmer, C. N. A., Gustafsson, M. C. U. & Foerster, J. Activation of PPARbeta/delta causes a psoriasis-like skin disease in vivo. *PLoS One* **5,** e9701 (2010).

95. Mao, F., Xu, M., Zuo, X., Yu, J., Xu, W., Moussalli, M. J., Elias, E., Li, H. S., Watowich, S. S. & Shureiqi, I. 15-Lipoxygenase-1 suppression of colitis-associated colon cancer through inhibition of the IL-6/STAT3 signaling pathway. *FASEB J* **29,** 2359–2370 (2015).

96. Wagner, N. & Wagner, K.-D. The Role of PPARs in Disease. *Cells* **9,** (2020).

97. Beyaz, S., Mana, M. D. & Yilmaz, Ö. H. High-fat diet activates a PPAR-δ program to enhance intestinal stem cell function. *Cell Stem Cell* **28,** 598–599 (2021).

98. Goode, A. P., Cleveland, R. J., George, S. Z., Schwartz, T. A., Kraus, V. B., Renner, J. B., Gracely, R. H., DeFrate, L. E., Hu, D., Jordan, J. M. & Golightly, Y. M. Predictors of Lumbar Spine Degeneration and Low Back Pain in the Community: The Johnston County Osteoarthritis Project. *Arthritis Care Res (Hoboken)* (2021). doi:10.1002/acr.24643

99. Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., Price, S. R. & Mitch, W. E. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J. Clin. Invest.* **113,** 115–123 (2004).

100. Plant, P. J., Bain, J. R., Correa, J. E., Woo, M. & Batt, J. Absence of caspase-3 protects against denervation-induced skeletal muscle atrophy. *Journal of Applied Physiology* **107,** 224–234 (2009).

101. Zhu, S., Nagashima, M., Khan, M. A. S., Yasuhara, S., Kaneki, M. & Martyn, J. A. J. Lack of caspase-3 attenuates immobilization-induced muscle atrophy and loss of tension generation along with mitigation of apoptosis and inflammation. *Muscle Nerve* **47,** 711–721 (2013).

102. Welle, S., Cardillo, A., Zanche, M. & Tawil, R. Skeletal muscle gene expression after myostatin knockout in mature mice. *Physiological Genomics* **38,** 342–350 (2009).

103. Carnac, G., Vernus, B. & Bonnieu, A. Myostatin in the pathophysiology of skeletal muscle. *Curr Genomics* **8,** 415–422 (2007).

104. Durieux, A.-C., Amirouche, A., Banzet, S., Koulmann, N., Bonnefoy, R., Pasdeloup, M., Mouret, C., Bigard, X., Peinnequin, A. & Freyssenet, D. Ectopic Expression of Myostatin Induces Atrophy of Adult Skeletal Muscle by Decreasing Muscle Gene Expression. *Endocrinology* **148,** 3140–3147 (2007).

105. Wilson, C. H. & Kumar, S. Caspases in metabolic disease and their therapeutic potential. *Cell Death Differ* **25,** 1010–1024 (2018).

106. McPherron, A. C. & Lee, S.-J. Suppression of body fat accumulation in myostatin-deficient mice. *J. Clin. Invest.* **109,** 595–601 (2002).

107. Sanderson, R. D., Fitch, J. M., Linsenmayer, T. R. & Mayne, R. Fibroblasts promote the formation of a continuous basal lamina during myogenesis in vitro. *J Cell Biol* **102,** 740–747 (1986).

108. Fry, C. S., Kirby, T. J., Kosmac, K., McCarthy, J. J. & Peterson, C. A. Myogenic Progenitor Cells Control Extracellular Matrix Production by Fibroblasts during Skeletal Muscle Hypertrophy. *Cell Stem Cell* **20,** 56–69 (2017).

109. Schuijf, J. D., Ambale-Venkatesh, B., Kassai, Y., Kato, Y., Kasuboski, L., Ota, H., Caruthers, S. D. & Lima, J. A. Cardiovascular ultrashort echo time to map fibrosis—promises and challenges. *BJR* **92,** 20190465 (2019).

110. Bodine, S. C., Stitt, T. N., Gonzalez, M., Kline, W. O., Stover, G. L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J. C., Glass, D. J. & Yancopoulos, G. D. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* **3,** 1014–1019 (2001).

111. McDaneld, T. G., Hannon, K. & Moody, D. E. Ankyrin repeat and SOCS box protein 15 regulates protein synthesis in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **290,** R1672-1682 (2006).

112. Federici, M., Hribal, M. L., Menghini, R., Kanno, H., Marchetti, V., Porzio, O., Sunnarborg, S. W., Rizza, S., Serino, M., Cunsolo, V., Lauro, D., Mauriello, A., Smookler, D. S., Sbraccia, P., Sesti, G., Lee, D. C., Khokha, R., Accili, D. & Lauro, R. Timp3 deficiency in insulin receptor-haploinsufficient mice promotes diabetes and vascular inflammation via increased TNF-alpha. *J Clin Invest* **115,** 3494–3505 (2005).

113. Urso, M. L., Szelenyi, E. R., Warren, G. L. & Barnes, B. R. Matrix metalloprotease-3 and tissue inhibitor of metalloprotease-1 mRNA and protein levels are altered in response to traumatic skeletal muscle injury. *Eur J Appl Physiol* **109,** 963–972 (2010).

114. Zhu, S., Lü, A., Zhao, Y., Pu, D., Liao, Z., Sun, Y., Chen, J. & Xiao, Q. [Expressions of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinases 1 in skeletal muscles of aged rats with sarcopenia]. *Nan Fang Yi Ke Da Xue Xue Bao* **40,** 104–109 (2020).

115. Bernot, D., Barruet, E., Poggi, M., Bonardo, B., Alessi, M.-C. & Peiretti, F. Down-regulation of tissue inhibitor of metalloproteinase-3 (TIMP-3) expression is necessary for adipocyte differentiation. *J Biol Chem* **285,** 6508–6514 (2010).

116. Fenech, M., Gavrilovic, J. & Turner, J. Effect of tissue inhibitor of metalloproteinases 3 on DLK1 shedding in cultured human pre-adipocytes and implications for adipose tissue remodelling. *Lancet* **385 Suppl 1,** S35 (2015).

117. Tidball, J. G. & Villalta, S. A. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol* **298,** R1173-1187 (2010).

118. Li, Y. P. & Reid, M. B. Effect of tumor necrosis factor-alpha on skeletal muscle metabolism. *Curr Opin Rheumatol* **13,** 483–487 (2001).

119. Collins, R. A. & Grounds, M. D. The role of tumor necrosis factor-alpha (TNF-alpha) in skeletal muscle regeneration. Studies in TNF-alpha(-/-) and TNF-alpha(-/-)/LT-alpha(-/-) mice. *J Histochem Cytochem* **49,** 989–1001 (2001).

120. Li, Y.-P. TNF-alpha is a mitogen in skeletal muscle. *Am J Physiol Cell Physiol* **285,** C370-376 (2003).

121. Zhou, J., Liu, B., Liang, C., Li, Y. & Song, Y.-H. Cytokine Signaling in Skeletal Muscle Wasting. *Trends in Endocrinology & Metabolism* **27,** 335–347 (2016).

122. Zaitsev, M., Maclaren, J. & Herbst, M. Motion artifacts in MRI: A complex problem with many partial solutions. *J Magn Reson Imaging* **42,** 887–901 (2015).

123. Cho, S.-M., Kim, S.-H., Ha, S.-K., Kim, S.-D., Lim, D.-J., Cha, J. & Kim, B.-J. Paraspinal muscle changes after single-level posterior lumbar fusion: volumetric analyses and literature review. *BMC Musculoskelet Disord* **21,** 73 (2020).