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ORIGINAL ARTICLE

ISSLS Prize in Basic science 2019: Physical activity attenuates fibrotic alterations to the multifidus muscle associated with intervertebral disc degeneration

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Abstract

Purpose Chronic low back pain causes structural remodelling and inflammation in the multifidus muscle. Collagen expression is increased in the multifidus of humans with lumbar disc degeneration. However, the extent and mechanisms underlying the increased fibrotic activity in the multifidus are unknown. Physical activity reduces local inflammation that precedes multifidus fibrosis during intervertebral disc degeneration (IDD), but its effect on amelioration of fibrosis is unknown. This study aimed to assess the development of fibrosis and its underlying genetic network during IDD and the impact of physical activity.

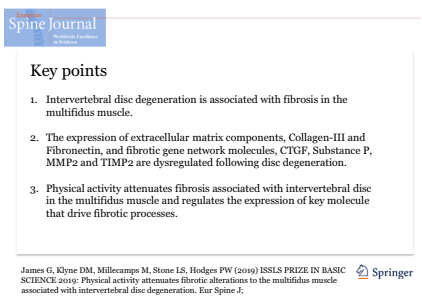
Methods Wild-type and SPARC-null mice were either sedentary or housed with a running wheel, to allow voluntary physical activity. At 12 months of age, IDD was assessed with MRI, and multifidus muscle samples were harvested from L2 to L6. In SPARC-null mice, the L1/2 and L3/4 discs had low and high levels of IDD, respectively. Thus, multifidus samples from L2 and L4 were allocated to low- and high-IDD groups compared to assess the effects of IDD and physical activity on connective tissue and fibrotic genes.

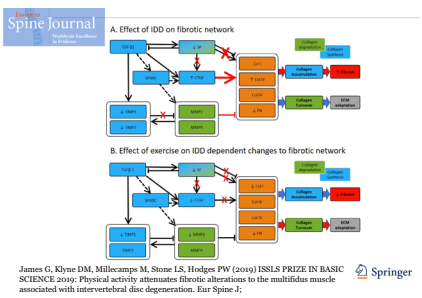
Results High IDD was associated with greater connective tissue thickness and dysregulation of collagen-III, fibronectin, CTGF, substance P, TIMP1 and TIMP2 in the multifidus muscle. Physical activity attenuated the IDD-dependent increased connective tissue thickness and reduced the expression of collagen-I, fibronectin, CTGF, substance P, MMP2 and TIMP2 in SPARC-null animals and wild-type mice. Collagen-III and TIMP1 were only reduced in wild-type animals.

Conclusions These data reveal the fibrotic networks that promote fibrosis in the multifidus muscle during chronic IDD. Furthermore, physical activity is shown to reduce fibrosis and regulate the fibrotic gene network.

Graphical abstract

These slides can be retrieved under Electronic Supplementary Material.





Keywords Exercise · Fibrosis · Intervertebral disc degeneration · Multifidus muscle · Low back pain

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Introduction

Low back pain (LBP) is the leading cause of disability internationally [1]. Treatment effects remain limited in part because the underlying mechanisms are multifactorial and vary in a time-dependant manner from the onset of injury to chronic stages [2–4]. Exercise is among the most efficacious treatments [5, 6], but incomplete understanding of the therapeutic mechanisms limits its targeted application.

LBP is accompanied by structural changes in the muscles that surround the spine, particularly the multifidus muscle [2, 7–13]. Localised acute atrophy [8] and then adipose accumulation (without atrophy) [14] in sub-acute LBP, followed by diffuse atrophy, fibrosis and adiposity in chronic LBP [15], have been identified in human studies. Ovine studies have shown increased cross-sectional area of connective tissue and expression of collagen-I (Col-I) in the multifidus during early chronic LBP [2]. These changes are characteristic of tissue fibrosis, which is a hallmark feature of various musculoskeletal conditions, and are associated with declines in sensory and motor function [16, 17], and once established, recovery is slow [18]. The extent and underlying mechanisms of fibrotic changes to the multifidus muscle during LBP are poorly characterised and require further investigation.

Connective tissue is highly adaptive and involves a delicate balance between extracellular matrix (ECM) synthesis and degradation [19, 20]. This balance is regulated by complex molecular networks (Fig. 1). Collagen synthesis is promoted by the upregulation of molecules such as transforming growth factor beta 1 (TGF-β1) [21, 22], connective tissue growth factor (CTGF) [23, 24], secreted protein acidic and rich in cysteine (SPARC) [25] and substance P (SP) [26, 27]. SP also has anti-fibrotic properties [28]. Collagen degradation is promoted by molecules including members of the matrix metalloproteinase (MMPs) family, which are in turn inhibited by tissue inhibitor of metalloproteinases (TIMPs) [29]. Although other molecules are involved, these networks are highly responsive to injury/pathology, and their dysregulation is a primary driver of fibrotic alterations in various tissues (e.g. skeletal muscle [29], liver [30], heart [31]).

Physical activity is a potent regulator of connective tissue in skeletal muscle [20, 32]. Short-term (acute) exercise stimulates both collagen synthesis and degradation to assist in its remodelling [33, 34], and long-term exercise prevents ageing-dependent fibrosis [35, 36]. The potential for exercise to reduce systemic and local inflammation [37–41] may partly explain its anti-fibrotic effect. In a model of LBP, physical activity attenuated increased pro-inflammatory cytokines and adipokines in multifidus associated with intervertebral disc degeneration (IDD) [42]. As inflammation precedes fibrosis, the anti-inflammatory effects of exercise highlight a possible pathway to prevent and/or reverse fibrotic changes.

This study addresses two issues. First, evidence for muscle fibrotic with IDD is derived from experimentally induced intervertebral disc (IVD) lesions. Whether similar processes accompany *spontaneous* IDD is unclear. Second, whether exercise ameliorates the fibrotic response is untested. These questions can be explored using a SPARC-null mouse model that develops spontaneous IDD, as we have previously described [42]. SPARC is required for ECM remodelling,

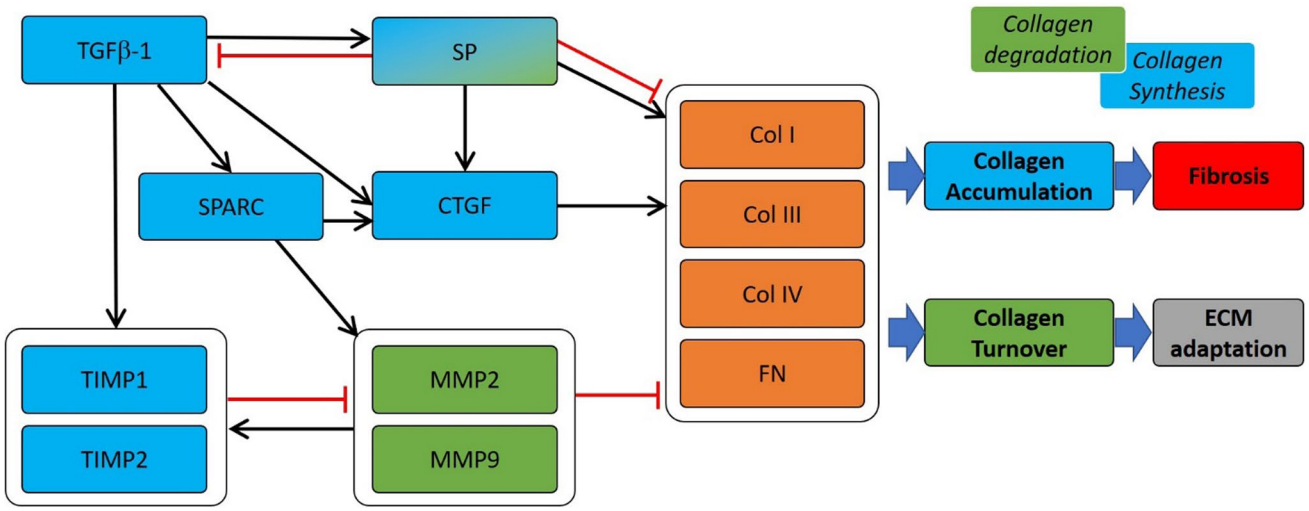

Fig. 1 Summary of the network of genes (although not exhaustive) that regulate fibrosis that have been investigated in this study. Factors that promote collagen synthesis and degradation are shown in blue and green boxes, respectively. Red arrows indicate an inhibitory function, whereas black arrows indicate a facilitatory role

and its absence results in spontaneous age-dependent IDD [43–47]. These animals provide an ideal model to explore: (1) whether fibrosis occurs during IDD, (2) whether the fibrotic gene network within multifidus are dysregulated in spontaneous IDD, (3) whether chronic physical activity ameliorates this dysregulation, and (4) the relationships between these genes.

Materials and methods

Experimental design

As SPARC has a pro-fibrotic role, we assessed the effects of IDD on fibrosis in the multifidus by comparison of a region of multifidus rostral to an IVD that consistently presented with IDD (adjacent to L4; 15 out of 16 had IDD in the L3/4 IVD) in SPARC mice against a region of multifidus rostral to an IVD with no/low levels of IDD (adjacent to L2; 3 of 16 had IDD in the L1/2 IVD) in the same mice, rather than use wild-type (WT) mice (Fig. 2a). The effect of physical activity on fibrotic networks in the multifidus were assessed in two ways: (1) using SPARC-null mice to examine the effect of physical activity on IDD-dependent alterations and (2) using WT mice (multifidus adjacent to L4 with no evidence of IDD.) to test the effects of physical activity in mice with a normal fibrotic network.

Animals and sample collection

All in vivo experiments were conducted with the approval of the faculty animal care committee at McGill University. Sixteen SPARC-null and 17 age-matched WT animals were used. The SPARC-null mice were developed on a mixed C57BL/6 × 129 SVJ background and backcrossed onto a standard C57BL/6 line for enough generations to be considered fully congenic [54]. Mice were housed with two-to-three littermates and separated into two groups: physical activity (PA) and sedentary (Sed). From 8 months of age, PA mice were housed with an Innowheel (Bio-serv, NJ, USA) that provided housing and a wheel for voluntary exercise. Voluntary exercise was selected to avoid stress-related

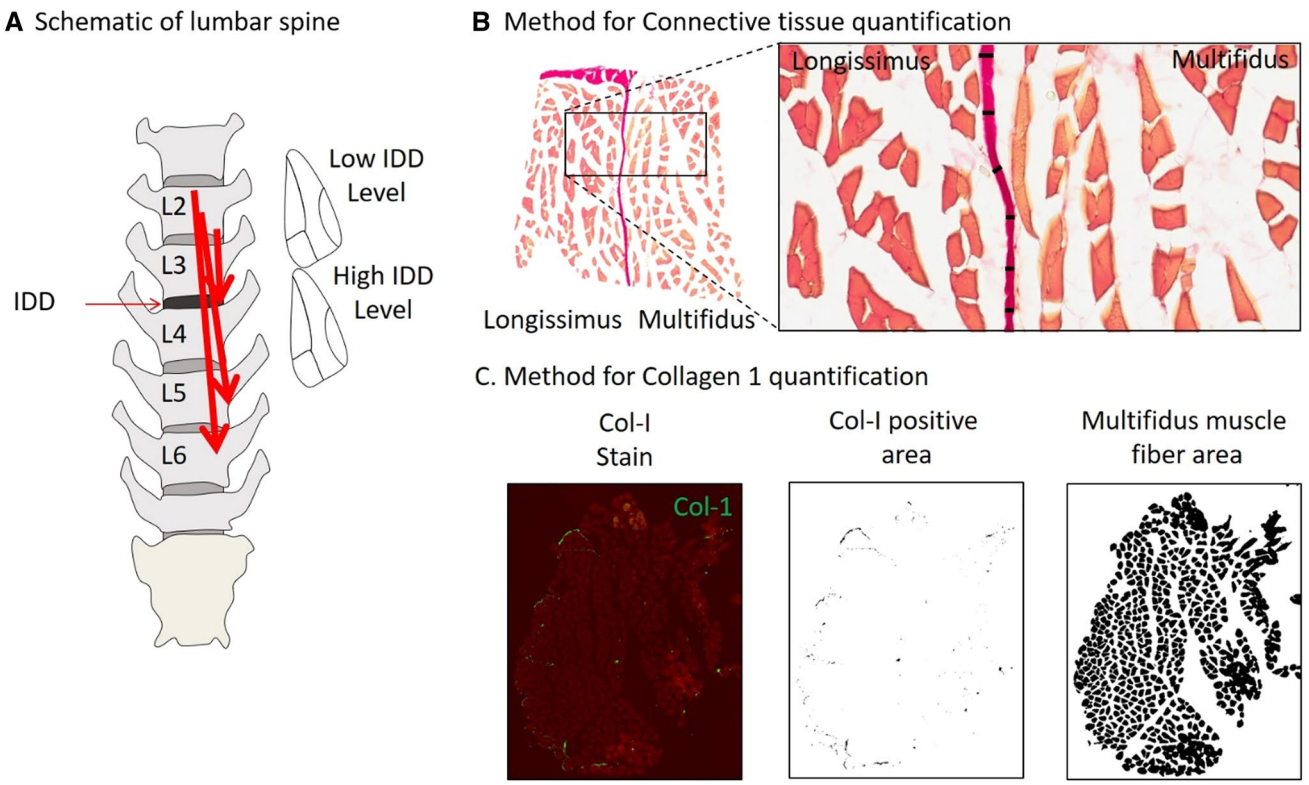

Fig. 2 **a** Schematic of the lumbar spine showing multifidus muscle fascicles (red), which can cross up to four intervertebral discs. The L1/2 and L3/4 intervertebral disc had low and high, respectively, proportions of intervertebral disc disease (IDD) in SPARC-null animals. Multifidus muscle samples harvested adjacent to L2 were deemed the low-IDD group and those adjacent to L4 were in the high-IDD group. **b** Quantification of the thickness of the epimysium between the multifidus and longissimus muscle using a Van Gieson's stain. The thickness was measured at multiple points along the connective tissue (pink tissue), indicated by black lines, and averaged. **c** Quantification of Collagen-1 (Col-1) expression in immunofluorescence assays. Images were separated into two images based on Col-1 positive area and muscle fibre area. The total area of each was quantified and the area of Col-1 was divided by the total muscle fibre area

problems and changes in circadian rhythm associated with forced exercise [48, 49]. Housing for the Sed animals was identical except the wheel was fixed in place with a screw preventing rotation and therefore unusable for voluntary exercise. At 12 months of age, IDD was assessed using MRI (see [42]). Multifidus muscle samples (L2–L6) were harvested adjacent to the spinous processes from the left and right sides and were stored in RNA later at − 20 °C or fixed overnight in 4% paraformaldehyde, then stored in 30% sucrose in phosphate-buffered saline. Fixed tissue was sectioned at 20 µm and mounted onto Superfrost Plus slides (Thermofisher) and stored at − 20 °C.

Van Gieson's stain

Slides containing multifidus muscle from L2 (non-IDD) and L4 (IDD) were incubated in running water for 2 min, Weigert's haematoxylin for 10 min and Van Gieson's solution for 1 min before dehydration and mounting. Slides were imaged (ImageScope, Leica), and thickness of the connective tissue separating the multifidus and adjacent longissimus muscles was measured (Fig. 2b) (ImageJ software, NIH).

Immunofluorescence assay

Multifidus muscle sections on slides from L2 and L4 were immersed in acetone for 10 min, blocked in 5% bovine serum albumin (Sigma) and incubated overnight at 4 °C with anti-collagen 1 (1:400, AB6308, Abcam). Sections were incubated for 1 h at room temperature with goat anti-mouse IgG1 conjugated to FITC (1:1000, AB97239, Abcam) and mounted with Fluroshield mounting medium (AB104139, Abcam). The area positive for *Col-I* expression was measured and divided by the total muscle fibre area using ImageJ software (NIH) (Fig. 2c).

Quantitative polymerase chain reaction (qPCR) assay

RNA extraction, cDNA synthesis and qPCRs were performed as previously described [42]. Primer pairs are listed in Table 1. We quantified gene expression of the ECM components [*Col-I, III, IV* and *fibronectin* (*Fn*)], as well as molecules involved with collagen synthesis (*SP*, *CTGF*) and collagen degradation (*MMP2*, *MMP9*, *TIMP1* and *TIMP2*). GAPDH was used as a housekeeping gene.

Statistical analysis

The role of IDD on fibrosis in the multifidus was tested by comparison of high- and low-IDD samples (low IDD vs. high IDD) and activity levels (Sed vs. PA) in SPARC-null animals using a two-way ANOVA and Duncan's post hoc analyses. A one-way ANOVA tested the effect of exercise (PA vs. Sed) for WT mice. Pearson's correlation was used to evaluate relationships between fibrotic and ECM gene expression in low-IDD and high-IDD groups from SPARC-null animals. Coefficients were interpreted as weak (0.3–0.5), moderate (0.5–0.7) or strong (0.7–1). Significance was set at $P < 0.05$. All data are presented as mean ± SEM. Statistica (StatSoft, USA) was used for all statistical analysis.

Table 1 Primer pairs used for quantitative PCR analysis

Gene name	Forward primer	Reverse primer
CTGF	5′TCCGGACACCTAAAATCGCC3′	5′TTCATGATCTCGCCATCGGG3′
SP	5′GGTCTGACCGCAAAATCGAAC3′	5′AAGATGCTCAAAGGG TCCG3′
MMP2	5′ACAAGTGGTCCGCGTAAAGT3′	5′GTAAACAAGGCTTCATGGGGG3′
MMP9	5′GCCGACTTTTGTGGTCTTCC3′	5′CTTCTCTCCCATCATCTGGGC3′
TIMP1	5′CCCCAGAAATCAACGAGACCA3′	5′ACTCTTCACTGCGGTTCTGG3′
TIMP2	5′TTTCTAGCCACACCAGGCAG3′	5′GCATGACGGGAGTAAGGGAG3′
Col-I	5′TTCTCCTGGCAAAGACGGAC3′	5′CGGCCACCATCTTGAGACTT3′
Col-III	5′AGTGGGCATCCAGGTCCTAT3′	5′GGGTGAAAAGCCACCAGACT3′
Col-IV	5′CCCTAACGGTTGGTCCTCAC3′	5′CGATGAATGGGGCGCTTCTA3′
FN	5′ACGGTTTCCCATTACGCCAT3′	5′TCATCCGCTGGCCATTTTCT3′
GAPDH	5′AGGTCGGTGTGAACGGATTTG3′	5′TGTAGACCATGTAGTTGAGGTCA3′

CTGF connective tissue growth factor, *SP* Substance P, *MMP* matrix metalloproteinase, *TIMP* tissue inhibitor of metalloproteinases, *Col* collagen, *FN* Fibronectin, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase

Results

Effect of IDD and physical activity on connective tissue

Connective tissue (CT) separating the multifidus and longissimus muscles (epimysium) was significantly thicker in L4 multifidus (high-IDD group) than at L2 (low-IDD group; Table 2, Fig. 3). Long-term exposure to physical activity reduced CT thickness in both SPARC-null and WT mice (Table 2, Fig. 3). Immunofluorescence staining for *Col-I* showed limited expression in the epimysium, and IDD or PA did not affect the area of *Col-I* as a percentage of the multifidus (Table 2, Fig. 3).

Effect of IDD and physical activity on ECM genes

Consistent with the impact of injury on fibrosis, *Col-III* gene expression was significantly higher in the multifidus at the high- than low-IDD levels (Table 3, Fig. 4). Dysregulation of ECM was also evidenced by lower *Fn* expression at the high-IDD level (Table 3, Fig. 4). Consistent with histological findings (above), IDD had no effect on *Col-I* or *Col-IV* expression (Table 3, Fig. 4). Physical activity had an opposite effect on *Col-III* in WT mice (lower in the PA than Sed group), but no effect in SPARC-null mice. Physical activity also lowered *Col-I* and *Fn*, but not *Col-IV*, in SPARC-null and WT mice (Table 3, Fig. 4).

Effect of IDD and physical activity on the fibrotic gene network

CTGF expression was significantly higher at the high- than at low-IDD levels in both Sed and PA groups (Table 4, Fig. 5). Physical activity attenuated the elevation of *CTGF* expression associated with high IDD in SPARC-null mice (Table 4, Fig. 5).

SP, *TIMP1* and *TIMP2* were significantly lower in the multifidus at high- than at low-IDD levels (Table 4, Fig. 5).

Table 2 Statistical analysis of extracellular matrix between groups (*G*; low IDD vs. high IDD) in SPARC-null animals and activity levels (*A*: sedentary vs. physical activity) in SPARC-null and wild-type animals

	ANOVA main effects and interaction	Post hoc[a]: Low IDD versus high IDD	Post hoc: Sed versus Ex in SPARC-null	ANOVA main effects and interaction	Post hoc: Sed versus Ex
Connective tissue thickness	***G*: < 0.001**	High IDD > low IDD	PA < Sed	***G*: < 0.0001**	PA < Sed
	***A*: 0.04**			***A*: < 0.001**	
	G×*A*: 0.9			*G*×A: 0.051	
Col-I area	*G*: 0.47			*G*: 0.67	
	A: 0.72			*A*: 0.3	
	G×*A*: 0.66			*G*×*A*: 0.68	

Significant main effects and interaction are shown in bold

SPARC SPARC-null, *WT* wild type, *Sed* sedentary, *PA* physical activity

[a]When interaction between *G*×*A* was significant, post hoc represents significant contrasts between relevant variables. When the main effect for *G* and/or *A* was significant, but not the interaction between them, post hoc shows the direction of difference in the main effect

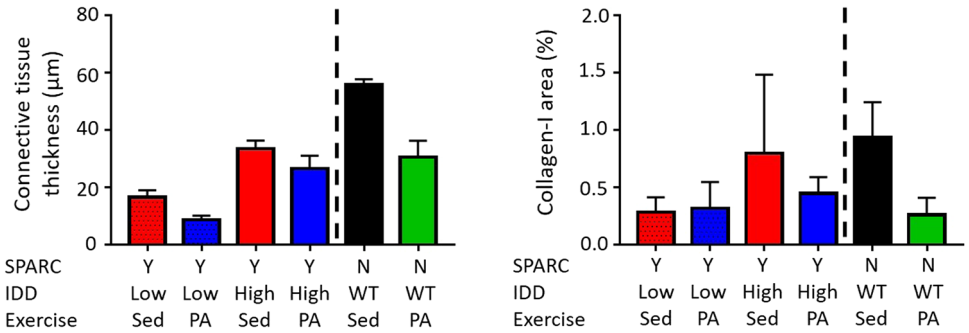

Fig. 3 Histological analysis of fibrosis in the multifidus muscle of SPARC-null animals. Connective tissue thickness and the percentage of multifidus positive for Col-1 expression were measured in the low IDD (SPARC "Y", IDD "Low"), high IDD (SPARC "Y", IDD "High") and wild-type (SPARC "N", IDD "WT") mice that were sedentary (Exercise "Sed") or physically active (Exercise "PA"). Data are presented as mean + SEM

Table 3 Statistical analysis of extracellular matrix component gene expression between groups (*G*; low IDD vs. high IDD) in SPARC-null animals and activity levels (*A*: sedentary vs. exercise) in SPARC-null and wild-type animals

	ANOVA main effects and interaction	Post hoc[a]: Low IDD versus high IDD	Post hoc: Sed versus Ex in SPARC-null	ANOVA main effects and interaction	Post hoc: Sed versus Ex
Col-I	*G*: 0.11		PA < Sed	*G*: 0.19	PA < Sed
	***A*: < 0.01**			***A*: < 0.001**	
	G×*A*: 0.35			*G*×*A*: 0.63	
Col-III	***G*: 0.02**	High IDD > low IDD		***G*: 0.002**	PA < Sed
	A: 0.13			***A*: < 0.001**	
	G×*A*: 0.12			***G*×*A*: 0.002**	
Col-IV	*G*: 0.37			*G*: 0.59	
	A: 0.28			*A*: 0.09	
	G×*A*: 0.35			*G*×*A*: 0.55	
Fn	***G*: 0.03**	Low IDD > high IDD	PA < Sed	*G*: 0.08	PA < Sed
	***A*: < 0.01**			***A*: < 0.001**	
	G×*A*: 0.29			*G*×*A*: 0.39	

Significant main effects and interaction are shown in bold

SPARC SPARC-null, *WT* wild type, *Sed* sedentary, *PA* physical activity

[a]When interaction between *G*×*A* was significant, post hoc represents significant contrasts between relevant variables. When the main effect for *G* and/or *A* was significant, but not the interaction between them, post hoc shows the direction of difference in the main effect

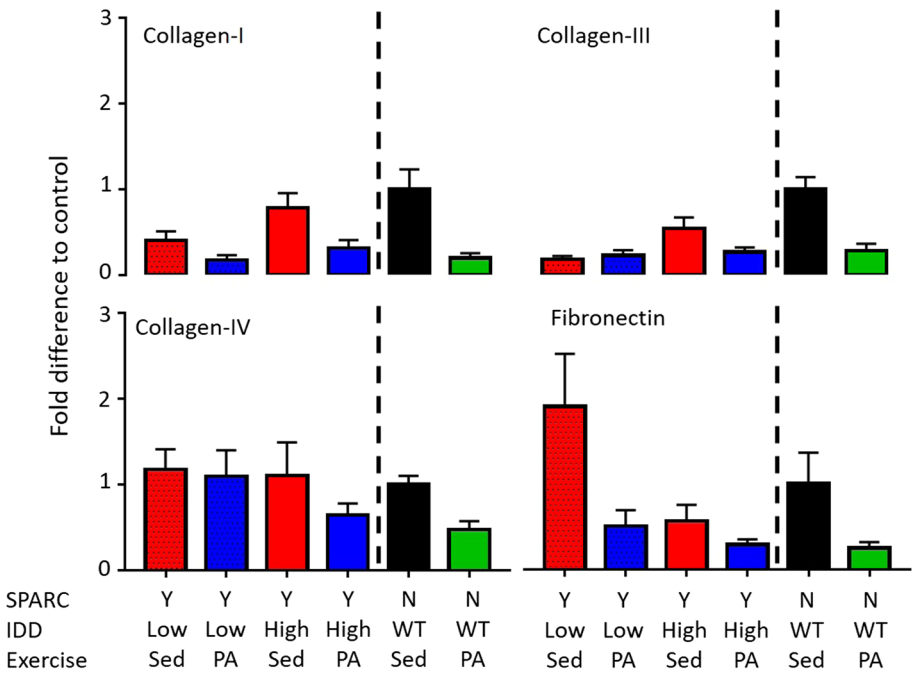

Fig. 4 Effect of IDD and physical activity on the expression of ECM components. The expression of collagen-I, collagen-III, collagen-IV and fibronectin were assessed in wild-type (SPARC "N", IDD "WT") and SPARC-null (SPARC "Y") animals that had low (IDD "Low") or high (IDD "High") levels of IDD. Furthermore, mice were either sedentary (Exercise "Sed") or physical activity (Exercise "PA"). Data are presented at mean + SEM

In contrast, *MMP2* and *MMP9* expression were not altered by IDD (Table 4, Fig. 5). Physical activity reduced the expression of *SP* and *MMP2* in WT and SPARC-null mice. *TIMP1* and *TIMP2* were reduced by physical activity in WT and SPARC-null mice, respectively (Table 4, Fig. 5).

Correlations between fibrotic and ECM gene expression in SPARC-null animals

CTGF, *Col-I* and *Col-III,* were moderately positively correlated in the SPARC-null animals (Table 5). *SP*, *MMP2*, *TIMP1*, *TIMP2* and *Fn* displayed moderate or greater

Table 4 Statistical analysis of fibrosis genetic pathway components between groups (*G*; low IDD vs. high IDD) in SPARC-null animals and activity levels (*A*: sedentary vs. exercise) in SPARC-null and wild-type animals

	ANOVA main effects and interaction	Post hoc[a]: Low IDD versus high IDD	Post hoc: Sed versus Ex in SPARC-null	ANOVA main effects and interaction	Post hoc: Sed versus Ex
CTGF	***G*: < 0.0001**	Sed high IDD > Sed low IDD: < 0.0001	Sed high	***G*: 0.001**	PA < Sed
	***A*: < 0.01**		IDD > Ex high	***A*: 0.01**	
	***G*×*A*: < 0.01**		IDD: < 0.001	*G*×*A*: 0.19	
		Ex high IDD > Ex low IDD: 0.04			
SP	***G*: < 0.05**	Low IDD > high IDD	PA < Sed	***G*: 0.02**	PA < Sed
	***A*: 0.02**			***A*: 0.003**	
	G×*A*:0.45			*G*×*A*:0.65	
MMP2	*G*: 0.09		PA < Sed	*G*: 0.17	PA < Sed
	***A*: < 0.01**			***A*: < 0.001**	
	G×*A*: 0.29			*G*×*A*: 0.51	
MMP9	*G*: 0.52			***G*: 0.003**	
	A: 0.06			*A*: 0.052	
	G×*A*:0.57			*G*×*A*:0.14	
TIMP1	***G*: 0.02**	Low IDD > high IDD		***G*: 0.005**	PA < Sed
	A: 0.06			***A*: 0.02**	
	G×*A*: 0.16			*G*×*A*: 0.16	
TIMP2	***G*: 0.03**	Low IDD > high	PA < Sed	*G*: 0.1	
	***A*: 0.04**	IDD		*A*: 0.07	
	G×*A*: 0.09			*G*×*A*: 0.15	

Significant main effects and interaction are shown in bold

SPARC SPARC-null, *WT* wild type, *Sed* sedentary, *PA* physical activity

[a]When interaction between *G*×*A* was significant, post hoc represents significant contrasts between relevant variables. When the main effect for *G* and/or *A* was significant, but not the interaction between them, post hoc shows the direction of difference in the main effect

relationships with each other gene (Table 5). *Col-IV* was positively correlated with *Col-III*, *SP*, *MMP2* and *TIMP1* (Table 5). *MMP9* was not significantly correlated with any gene (Table 5).

Discussion

These results provide several new insights into the role of IDD in multifidus muscle fibrosis and the impact of physical activity. First, fibrosis (i.e. increased thickness of the CT between the multifidus and longissimus muscles) was present in muscle that crossed a degenerated disc. Second, expression of *Col-III* was higher, but *Fn* was lower in the multifidus at the high-IDD level. Third, the fibrotic gene network (*CTGF, SP, TIMP1* and *TIMP2*) was dysregulated in multifidus crossing a degenerated disc and correlated with changes in ECM gene expression. Fourth, physical activity attenuated IDD-dependent increases in *CTGF* expression but not *Col-III*, and reduced *Col-1*, *Fn*, *SP* and *MMP2* expression in WT and SPARC-null mice.

IDD is associated with multifidus fibrosis

Fibrotic changes in the multifidus are reported in chronic LBP [15] and in sub-acute/early chronic LBP following surgically induced IVD injury [2]. This study shows similar processes after spontaneous IDD and identifies candidate mechanisms that drive it.

Increased CT thickness between the multifidus and longissimus muscles in our model is consistent with results from an Ovine model of IDD. Those data showed increased CT in the multifidus during the sub-acute/early chronic period [2]. Consistent with our findings, that increase appeared limited to the outer sheath surrounding the muscle (epimysium). Increased epimysium thickness might increase multifidus muscle stiffness and alter the distribution of forces after IVD injury [50]. Further research is required to understand the clinical implications of the altered multifidus muscle CT.

Although histological findings from studies of different species are somewhat similar, the ECM genes that are dysregulated with IDD appear to differ. Here, *Col-III* and *Fn* expression, but not *Col-I,* is affected with IDD (Fig. 6a). Conversely, in the aforementioned Ovine model, *Col-I* was

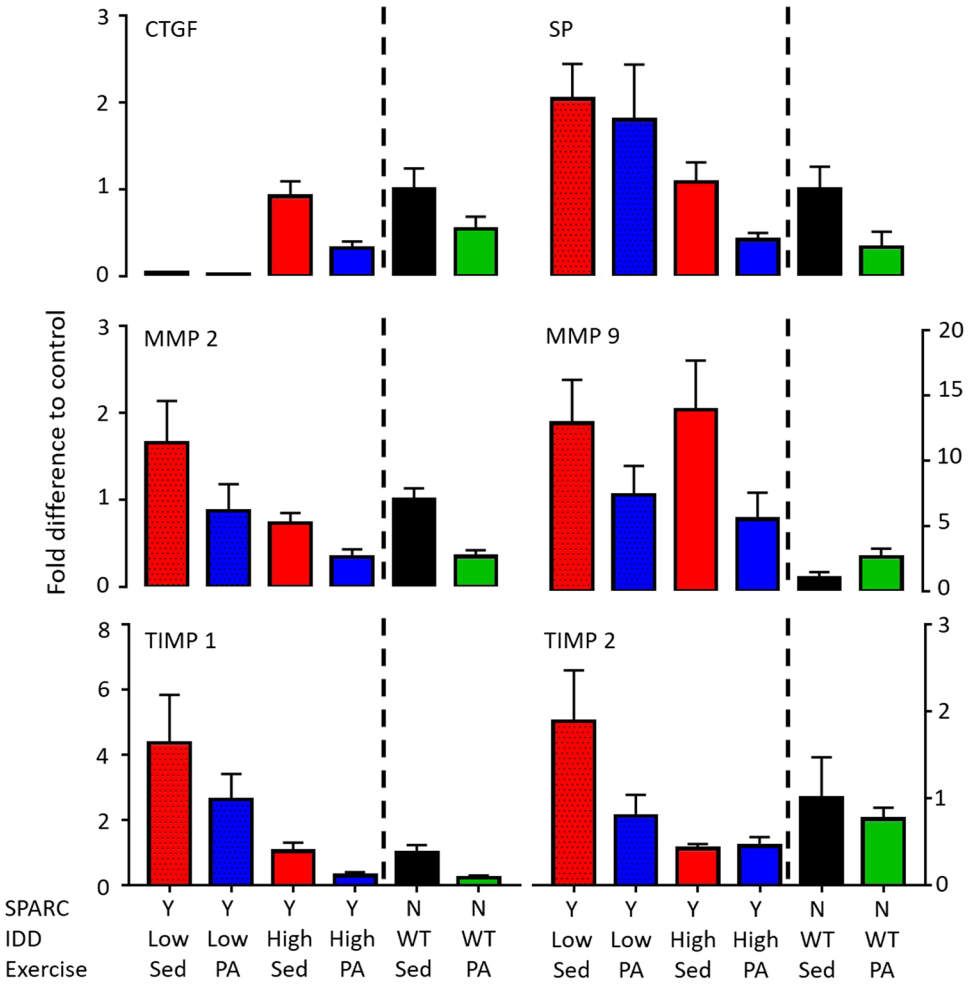

Fig. 5 Alterations to the fibrotic network in the multifidus muscle during IDD and physical activity. Multifidus muscle from SPARC-null animal's lumbar segments with low (SPARC "Y", IDD "Low") and high (SPARC "Y", IDD "High") proportions of IDD and wild-type (SPARC "N", IDD "WT") that were sedentary (Exercise "Sed") or were physically active (Exercise "PA") were compared for the expression of fibrotic networks genes. Data are presented as mean + SEM. *CTGF* connective tissue growth factor, *SP* Substance P, *MMP* matrix metalloproteinase, *TIMP* tissue inhibitor of metalloproteinases

upregulated, whereas *Col-III* expression was independent of IDD [2]. In humans with lumbar IVD herniation, *Col-I*, *Col-III* and *Fn* are upregulated in the multifidus compared to controls [51]. These differences highlight the variable nature of fibrosis associated with IDD and that treatments cannot be tailored until this is understood.

Reduced *Fn* expression with IDD could have an important impact on multifidus muscle health because it is required to maintain and regenerate muscle stem cells [52]. *Fn* expression and the regenerative capacity of muscle decline with age, but the reintroduction of *Fn* expression in aged muscle restores its regenerative ability to levels comparable to young animals [52]. Hence, loss of *Fn* expression might reduce the capacity of muscle stem cells to regenerate damaged tissue in the multifidus, contributing to its degeneration in chronic LBP.

IDD is associated with changes to the genetic networks that drive fibrosis

CTGF is a major driver of fibrosis in various tissues [23, 24, 53] and musculoskeletal conditions [24, 54]. Its increase during IDD and positive relationship with *Col-I* and *Col-III* support this role in the multifidus during chronic LBP (Fig. 6a). Although *CTGF* expression is reported to be regulated by TGF-β1 [55], SPARC [56] and/or SP [57], its upregulation here was not correlated with TGF-β1 or SP, and it remained elevated in the absence of SPARC. One explanation is that *CTGF* is regulated differently during IDD. *CTGF* is a highly stress-responsive gene and is markedly upregulated during mechanical stress without accompanying increases in TGF-β1 and SP [58, 59]. It is therefore possible that changes in the mechanical forces in local tissues as a result of IDD could upregulate CTGF independent of TGF-β1 or SP.

SP is a neuropeptide that traditionally produces a strong pro-fibrotic function [17, 26, 27, 57], has a key role in nociception [60] and is upregulated in painful diseases such as fibromyalgia [61]. There is also contrasting evidence that it has anti-fibrotic [28] and anti-nociceptive [62] functions in muscle. The relationships between *SP, MMP2*, *TIMP1* and *TIMP2* suggest that SP plays a role in regulation of collagen degradation during IDD. Reduced SP expression could lower collagen degradation leading to its accumulation and

Table 5 Correlation analysis of fibrotic and ECM gene expression in SPARC-null animals

	Col-I	*Col-III*	*Col-IV*	*Fn*	*CTGF*	*SP*	*MMP2*	*MMP9*	*TIMP1*	*TIMP2*
Col-I	–	–	–	–	–	–	–	–	–	–
Col-III	**0.6 (0.001)**	–	–	–	–	–	–	–	–	–
Col-IV	0.32 (0.1)	**0.44 (0.02)**	–	–	–	–	–	–	–	–
Fn	−0.02 (0.92)	−0.22 (0.31)	−0.06 (0.77)	–	–	–	–	–	–	–
CTGF	**0.57 (0.002)**	**0.5 (0.009)**	0.17 (0.39)	−0.17 (0.43)	–	–	–	–	–	–
SP	0.05 (0.81)	0.04 (0.82)	**0.4 (0.03)**	**0.68 (<0.001)**	−0.25 (0.21)	–	–	–	–	–
MMP2	0.05 (0.78)	0.05 (0.81)	**0.55 (0.001)**	**0.48 (0.015)**	−0.2 (0.32)	**0.81 (<0.001)**	–	–	–	–
MMP9	−0.1 (0.61)	−0.09 (0.67)	−0.06 (0.77)	0.18 (0.39)	0.02 (0.93)	0.03 (0.87)	0.37 (0.06)	–	–	–
TIMP1	0.13 (0.5)	−0.02 (0.94)	**0.46 (0.015)**	**0.47 (0.03)**	−0.34 (0.1)	**0.73 (<0.001)**	**0.77 (<0.001)**	0.07 (0.74)	–	–
TIMP2	0.11 (0.6)	0.04 (0.83)	0.21 (0.27)	**0.64 (0.001)**	−0.34 (0.08)	**0.65 (<0.001)**	**0.65 (<0.001)**	0.17 (0.39)	**0.87 (<0.001)**	–

Correlation coefficients highlighted in bold are statistically significant

subsequent fibrosis. Conversely, its reported anti-fibrotic function involves inhibition of collagen synthesis [28]. The potential anti-fibrotic role of SP in promoting collagen degradation and subsequent turnover requires investigation.

Physical activity regulates fibrosis

Acute and long-term exercise alters the ECM [20, 32, 63]. Effects of physical activity on the ECM depend on the activity type and duration, and demographics of the study population, e.g. age [63]. Our model of long-term physical activity (3 months of voluntary aerobic exercise in middle-aged mice) sheds new light onto the role of physical activity in regulating CT in healthy and chronic IDD groups.

Physical activity attenuated IDD-dependent fibrosis by reducing epimysium thickness. However, physical activity had no effect on IDD-dependent increases in *Col-III* expression in SPARC-null animals, despite a decrease in *Col-III* expression in WT mice. This reveals that although physical activity is capable of regulating *Col-III* expression, it is unable to attenuate *Col-III* expression associated with IDD. This could indicate that although physical activity attenuates increased epimysium thickness after IDD, it does not prevent changes to the underlying ECM components. This may impact the mechanics of the CT, and subsequently the multifidus. More detailed examination of the collagen components of the epimysium, perimysium and endomysium during IDD and physical activity are required.

Reduced *CTGF* following long-term exercise is a likely mechanism to explain the effectiveness of physical activity in attenuating fibrosis (Fig. 6b). Inhibition of CTGF ameliorates fibrosis and inflammation in a mouse model of Duchenne muscular dystrophy [64]. Further investigation of treatments targeting CTGF during chronic LBP requires investigation.

Exercise prevents ageing-dependent fibrotic changes in skeletal muscle [65–67], but this is based on studies that compare young and old animals [35, 65]. Mice in this study are considered middle age (38–47 in human years [68]). We show that exercise reduced the quantity of CT and ECM genes in middle-aged WT mice (Fig. 6b). This is suggestive of age-dependent fibrosis attenuation and requires further investigation. Taken together, exercise appears to be a potent regulator of the ECM in the multifidus muscle and is a promising treatment option due to its ability to attenuate fibrotic changes.

Methodological considerations

As supported by our data, SPARC is a pro-fibrotic molecule [25] (Fig. 1). To control for the potential influence of SPARC on our findings, analyses with respect to fibrosis were performed by limiting comparisons to multifidus muscle within

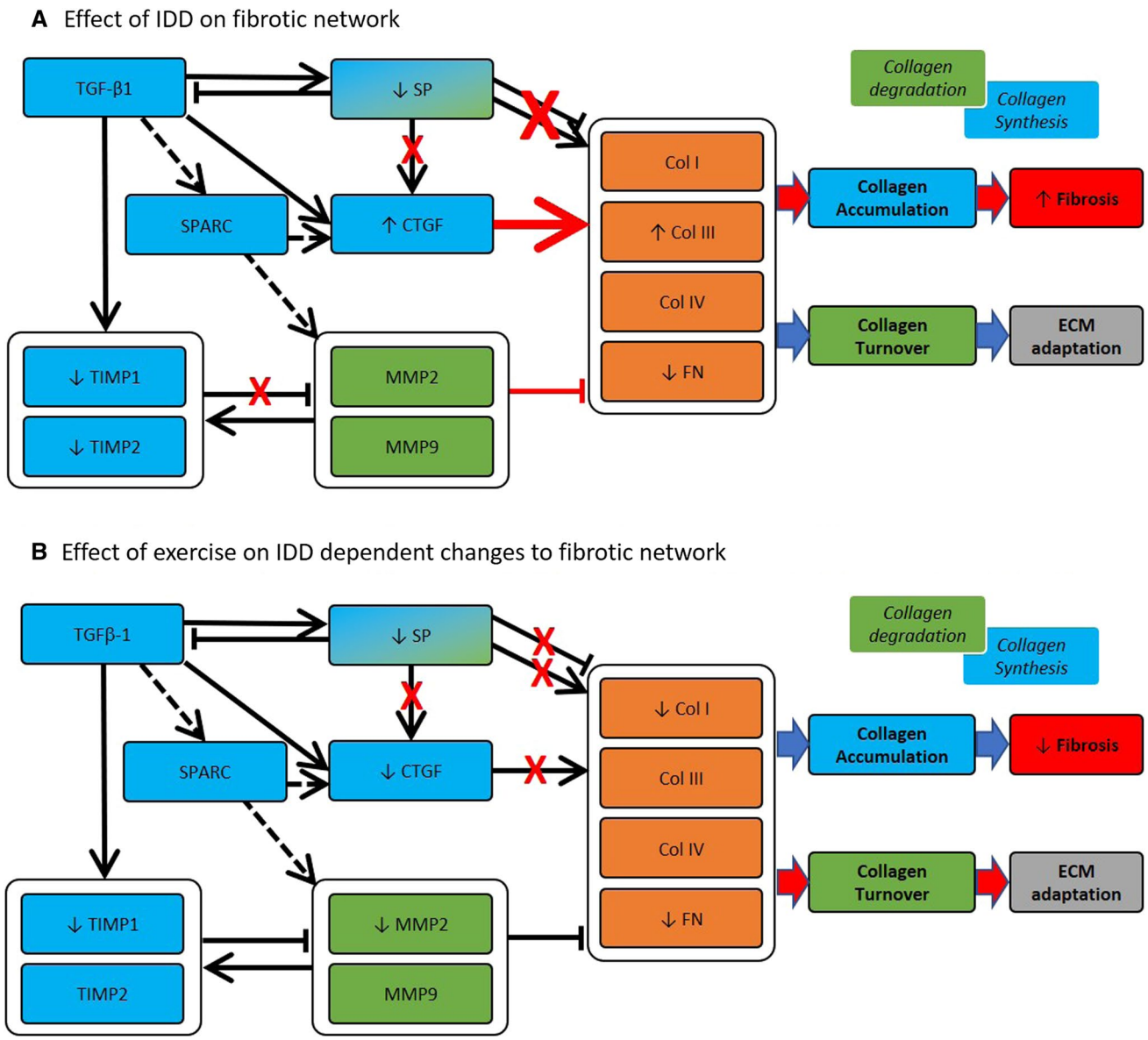

Fig. 6 Effect of IDD (**a**) and physical activity (**b**) on the fibrotic network in the multifidus muscle. Arrows in the boxes indicate alterations to gene expression or histological features. Red arrows and cross indicate pathways that up promoted or inhibited, respectively, during IDD or physical activity

the same SPARC-null mice, but in muscle rostral to IVDs with high IDD versus low IDD. The influence of the absence of SPARC on interactions in the fibrosis network requires consideration.

Conclusions

This study has provided novel data on the extent and nature of the fibrosis in the multifidus muscle in association with IDD. Further, we identified a range of biomarkers, such as CTGF, that could be targeted to improve muscle health and outcomes in individuals with IDD and chronic LBP. Our data build on evidence for the positive impact of physical activity in the prevention/treatment of age- and IDD-dependent fibrosis of the multifidus muscle.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest related to this work.

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