

Review

Potential Nociceptive Role of the Thoracolumbar Fascia: A Scope Review involving *in vivo* and *ex vivo* Studies

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Abstract: Nociceptive innervation of the thoracolumbar fascia (TLF) has been investigated over decades, however these studies have not been compiled or collectively appraised. The purpose of this scoping review was to assess current knowledge regarding nociceptive innervation of the TLF to better inform future mechanistic and clinical TLF research targeting low back pain (LBP) treatment. PubMed, ScienceDirect, Cochrane and Embase databases were searched in January 2021 using relevant descriptors encompassing fascia and pain. Eligible studies were: (a) published in English; (b) preclinical and clinical (*in vivo* and *ex vivo*) studies; (c) original data; (d) included quantification of at least one TLF nociceptive component. Two-phase screening procedures were conducted by a pair of independent reviewers, data were extracted and summarized from eligible studies. The search resulted in 257 articles of which 10 met inclusion criteria. Studies showed histological evidence of nociceptive nerve fibers terminating in low back fascia, suggesting a TLF contribution to LBP. Noxious chemical injection or electrical stimulation into fascia resulted in longer pain duration and higher pain intensities than injections into subcutaneous tissue or muscle. Pre-clinical and clinical research provides histological and functional evidence of nociceptive innervation of TLF. Greater knowledge of fascial neurological components could impact LBP treatment.

Keywords: fascia, *in vivo*, *ex vivo*, innervation, pain, thoracolumbar fascia, nociceptor, low back pain, scoping review.

Abbreviations

ASICs, acid-sensing ion channels;
 CFA, complete Freund's adjuvant;
 CGRP, calcitonin gene-related peptide;
 DRG, dorsal root ganglia;
 GDNF, glial-derived neurotrophic factor;
 HFS, high-frequency stimulation.
 LBP, low back pain
 MSK, musculoskeletal;
 NGF, nerve growth factor;
 NSE, neuron-specific enolase;
 PGP, protein gene product 9.5;
 SP, substance P;
 TH, tyrosine hydroxylase;
 TLF, thoracolumbar fascia;
 TRPA1, transient receptor potential ankyrin 1;
 TRPM8, transient receptor potential cation channel subfamily M member 8;
 TRPV1, transient receptor potential vanilloid 1;
 WHO, World Health Organization;

1. Introduction

The process by which intense thermal, mechanical or chemical stimuli are detected by a subpopulation of nociceptive peripheral nerve endings is called nociception [1-5]. Nociceptors have a peripheral and central axonal branch that innervates their target organ and spinal cord, respectively [1,2,6,7]. Their cell bodies are located in the dorsal root ganglia (DRG) for the trunk and extremities and in the trigeminal ganglion for the face [8-10]. There are two major classes of nociceptors [1-3,6,7]. The first includes medium diameter myelinated (A δ) afferents that mediate acute, well-localized nociception [2-4,6,7]. The second class of nociceptor includes small diameter unmyelinated "C" fibers that convey poorly localized nociception. Most C fibers are polymodal (heat and mechanical sensitive) [2-4,6,7]. Often, heat-responsive unmyelinated afferents develop mechanical sensitivity only in the injury setting [11]. These afferents are more responsive to chemical stimuli (capsaicin or histamine) and are likely to come into play when the chemical milieu of inflammation changes their properties [1,2,12-14]. However, not all C fibers are nociceptors. Some respond to innocuous cooling, while others respond to innocuous stroking of the hairy skin and appear to mediate pleasant touch [15].

C nociceptive fibers project to the superficial dorsal horn (laminae I & II) of the spinal cord, which is organized into anatomically and electrophysiological distinct laminae [1,2,6,7]. The heterogeneity of C fibers has been demonstrated by their neuroanatomical and molecular characterization [16]. For example, the so-called peptidergic population of C nociceptors release the neuropeptides, substance P, and calcitonin-gene related peptide (CGRP); they also express the TrkA neurotrophin receptor, which responds to nerve growth factor (NGF) [1,2,17]. The nonpeptidergic population of C nociceptors express the c-Ret neurotrophin receptor that is targeted by glial-derived neurotrophic factor (GDNF) [1,2,17]. Finally, the differentiated expression of nociceptor membrane channels can also

distinguish them by conferring sensitivity to heat (TRPV1), cold (TRPM8), acidic milieu (ASICs), and a host of chemical irritants (TRPA1) [1,2,14].

Musculoskeletal (MSK) pain is defined as acute or chronic pain that affects bones, muscles, ligaments, tendons, and even nerves, and in particular, low back pain (LBP) is the main contributor to disability worldwide [18-23]. According to the World Health Organization (WHO), 1.75 billion people has some form of chronic MSK pain [24]. Inadequately managed MSK pain can adversely affect quality of life and impose significant socioeconomic problems. However, the mechanisms that underlie its development remain poorly understood [19-23].

It has been demonstrated that there is nociceptive innervation of specialized connective tissues such as nerves [25], tendons [26], and joints [27-31]. Together these findings strongly suggest a role in the etiology of pain, and additional studies of nociceptive innervation of connective tissue will help to relate connective tissue dysfunction with LBP [32,33]. To date, it has been demonstrated that when healthy subjects underwent an injection of hypertonic saline into the thoracolumbar fascia (TLF) it resulted in pain of greater intensity, more unpleasant quality and spreading over a greater area of the back and lower limb, compared to similar injections into muscle or subcutaneous tissue [34-37]. Furthermore, this pain was associated with chemical stimulation rather than mechanical distention [35]. These studies suggest that the TLF makes a distinct contribution to nociception.

The TLF is a major connective tissue structure that can resist high tensile loads due to its fibers being oriented in multiple planes [38-40]. TLF consists of several layers, of which the posterior layer covers the deep muscles of the back and attaches with the spinous processes via the supraspinous ligament. This posterior layer of the TLF consists of two separate laminae. The superficial lamina forms a continuation of the aponeurosis of the latissimus dorsi muscle as well as of the aponeurosis of the gluteus maximus muscle. It usually contains many dense collagen fibers crossing to the contralateral side below L2, which constitutes a very rare feature within mammalian anatomy and is probably associated with a contralateral force transmission between latissimus dorsi on one side and the gluteus maximus of the opposite side during human locomotion. Anterior of it lies the deep lamina, which blends with the nuchal fascia cranially and with the long head of the biceps femoris muscle via the sacrotuberous ligament caudally [41,42]. The deep lamina is usually much thinner than the superficial lamina. Both laminae contain a multitude of smaller blood vessels, but do not contain any large arteries or veins [43,44]. Although the biomechanical properties of muscle fascia are extensively documented and discussed in the literature, the neural properties have relatively received very little attention to date [32].

Despite nociceptive innervation of the TLF having been investigated over decades in various animal models and humans using neuroanatomical and molecular techniques, these studies have not been compiled and their collective findings appraised. The purpose of this scoping review was to assess current scientific knowledge on the nociceptive innervation of the TLF in animal and human *in vivo* and *ex vivo* experiments to act as a resource for future clinical research targeting TLF in clinical treatment.

2. Materials and Methods

A scoping review methodology was selected to compile and evaluate data relating to the current state of scientific knowledge available in the literature related to TLF nociception and to identify the gaps that need to be addressed. The framework chosen was based on the guidelines provided by Arksey e O'Malley (2005) [45], and Preferred Reporting Items for Systematic reviews, and extensions of Meta-Analyzes, Scoping Reviews (PRISMA-ScR, **Supplementary Data Sheet 1**), and randomized clinical trials. Human and preclinical studies were included.

2.1. Step 1: Identifying the research question

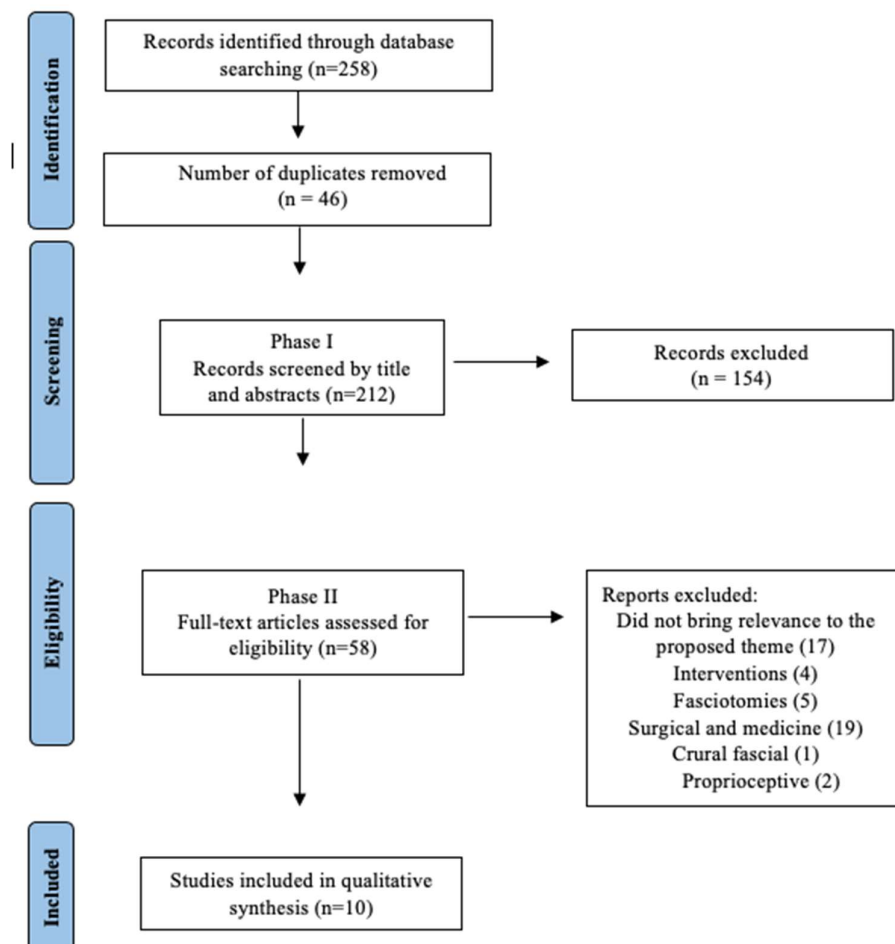
The aim of this scoping review was to identify the role of sensory innervation of the thoracolumbar fascia in musculoskeletal nociception.

2.2 Step 2: Identifying relevant studies

A search strategy was developed using a combination of relevant keywords (**Supplementary Data Sheet 2**). The search was conducted in January 2021 in the electronic databases of PubMed, ScienceDirect, Cochrane and Embase. Based on the scoping review methodology, the search filters used were: (a) pre-clinical studies; (b) clinical trials; (c) until the year 2021; (d) full text. Additional filters used were: (1) Species: human and animals; (2) Language: English; (3) Sex: female and male.

We selected the relevant descriptors that encompassed two main topics: pain and fascia. Animal models and human studies were included. A combination of DeCs descriptors in English was used: fascia, pain, and low back pain. After excluding duplicate articles, titles and abstracts were read for later selection to read the full text. Through the search for the DeCs descriptors: (a) fascia and pain we found 217 articles; (b) fascia and nociceptive pain 13 articles were found (all duplicates); (b) fascia and nociceptive pain and innervation - *in vivo* studies / *ex vivo* studies - 15 articles were found (all duplicates); (e) fascia and nociceptive pain and myofascial pain syndrome 3 articles (a single article duplicated); (f) fascia and nociceptive pain and low back pain 9 articles were found (all duplicates).

Figure 1: Flowchart diagram (file in attachment).



2.3 Step 3: Study selection

2.3.1 Inclusion and exclusion criteria

The inclusion criteria consisted of: (a) studies published in English; (b) complete studies (c) preclinical and clinical (*in vivo* and *ex vivo*) studies; (d) original data studies; (e) studies involving the relationship between fascia and pain; (f) included quantification of at least one analysis of any nociceptive component of thoracolumbar fascia.

Studies were excluded if classified as: studies involving the relationship between fascia and proprioception, usage protocols, clinical intervention, fasciotomy, surgical procedures and medicine, practical guidelines, unpublished manuscripts, dissertations, reviews, expert comments, chapters of books and / or books, registered reports, conference proceedings.

Records screened by title and abstracts numbered 212. After reading the abstracts 154 were excluded for failure to meet inclusion criteria. Fully read articles numbered 58, and of these 48 were excluded because the content did not address the proposed theme, included interventions, fasciotomy, surgical procedures associated with the use of medication, crural fascial or proprioceptive innervation of facial tissue (Figure 1). In the end, two tables containing a total of 10 articles were developed: (a) Table 1 *ex vivo* studies and (b) Table 2 *in vivo* studies.

2.4 Screening and agreement

The research articles were reviewed and selected in two phases. Phase I consisted of screening titles and abstracts to include possible relevant studies and to exclude irrelevant studies. Phase II consisted of screening full texts of studies previously identified as possibly relevant to select eligible studies. Screenings, in phases I and II, were performed by two independent reviewers (L.S., M.A.) and any discrepancy in relation to the study's eligibility was mediated by a third reviewer (D.F.M., n = 2).

2.5 Step 4: Data Charting

The following data were extracted from eligible studies: (a) references; (b) animals, disease model; (c) method; (d) main results (Table 1 and Table 2). The extraction parameters were defined jointly by two authors. Data extraction was performed by one author and verified by a second author to minimize any discrepancy.

2.6 Step 5: Collating, summarizing, and reporting the results

The data were summarized descriptively according to the following items:

1. Preclinical: including *in vivo* and *ex vivo* studies related to nociceptive investigation of TLF.
2. Clinical: including *in vivo* and *ex vivo* studies related to nociceptive investigation of TLF.

3. Results

3.1 Basic numerical analysis

The search of the database carried out on January 11, 2021 resulted in 258 articles. After the removal of duplicates, 212 articles had their titles and abstracts screened in Phase I and those articles considered relevant for eligibility in Phase II were screened. Fifty-eight articles were selected to be read in full, and of these 48 were excluded due to failure to meet eligibility requirements. Thus, a total of 10 articles were included in this review, all of which were published prior to the year 2021. These 10 articles were divided into: 1) *Ex vivo* studies and 2) *In vivo* studies.

3.2 *Ex vivo* studies

A total of 5 studies evaluated and quantified the nociceptive nerve fibers terminating within the nonspecialized connective tissues in the low back. Specific immunohistochemistry assays included: immunofluorescence (5/5), calcitonin gene-related peptide (CGRP) immunoreactivity (5/5), substance P (SP) immunoreactivity (4/5) and retrograde labeling (2/5). Species selected for ex vivo analysis included: mice (1/5), rats (4/5) and humans (2/5). Anatomical structures analyzed included the TLF (5/5), low back muscles (5/5), and DRG (2/5). Detailed information regarding the immunohistochemistry assay, species, and outcome measures utilized can be found in Table 1.

Table 1. *Ex vivo* studies

Studies (Authors, years)	Specie, condition, n	Method	Main findings
Corey et al., 2011 [46]	Rats, Naïve, n = 17	<ul style="list-style-type: none"> • Three-dimensional reconstructions of thick tissue sections (low back L1-L6) • Fast Blue retrograde labeling (low back muscle - DRG) and immunofluorescence with CGRP antibody 	<ul style="list-style-type: none"> • 60-88% DRG cells with terminations within the collagen matrix of connective tissue in the low back also expressed CGRP
Barry et al., 2015 [47]	C57Bl/6 mouse, Naïve. 4-8 per group	<ul style="list-style-type: none"> • Immunohistochemistry: TLF were multiple-labeled using antibodies to the pan-neuronal marker neuron-specific enolase (NSE), and CGRP and SP • Multiple-labeling immunofluorescence and retrograde axonal tracing 	<ul style="list-style-type: none"> • The TLF contained the same proportions of nerve fiber subpopulations found in back muscles. • The TLF contained a higher density of sensory nerves, with three times more CGRP-immunoreactive fibers compared to back muscles (latissimus dorsi). However, around half of these lack SP-immunoreactive.
Mense et al., 2016 [48]	Rats, <ul style="list-style-type: none"> • Naïve, n = 5 • Fascia inflammation - CFA, n = 5 • Dorsal root electrical stimulation, n = 4 	<ul style="list-style-type: none"> • Immunohistochemical antibodies to CGRP, SP and TRPV1 • Functional data Neurogenic plasma extravasation (Evans Blue) 	In naïve rats <ul style="list-style-type: none"> • CGRP-immunopositive fibers in the outer and inner layer of TLF • SP-containing nerve fibers existed exclusively in the outer layer of TLF In inflamed (CFA) rats <ul style="list-style-type: none"> • The CGRP-immunopositive fiber length increased significantly only in the inner layer of TLF • SP-positive structures were found in the inner layer of TLF In inflamed (electrical stimulation) rats <ul style="list-style-type: none"> • Neurogenic plasma extravasation in form of dark patches of Evans blue in the TLF

Tesarz et al., 2011 [41]	<ul style="list-style-type: none">• Rats, Naïve, n = 8	<ul style="list-style-type: none">• Immunohistochemical antibodies to PGP 9.5, TH, CGRP and SP	Rats
	<ul style="list-style-type: none">• Specimens Humans TLF n = 3		<ul style="list-style-type: none">• Fibers immunoreactive to PGP 9.5, TH, CGRP and SP were found in the subcutaneous tissue and the outer layer of TLF
Mense et al., 2019 [32]	<ul style="list-style-type: none">• Rats, Naïve and Fascia inflammation - CFA, n = NI	<ul style="list-style-type: none">• Immunohistochemical antibodies to PGP 9.5, TH, CGRP and SP	Rats
	<ul style="list-style-type: none">• Specimens Humans TLF n = NI		<ul style="list-style-type: none">• Fibers immunoreactive to PGP 9.5, TH, CGRP and SP were found in the TLF• The inflamed fascia exhibited a higher density of CGRP- immunoreactive and SP- immunoreactive units
			Humans
			<ul style="list-style-type: none">• Fibers immunoreactive to CGRP were the most frequent ones

Note: CFA: complete Freund’s adjuvant; CGRP: calcitonin-gene related peptide; DRG: dorsal root ganglia; SP: substance P; TLF: thoracolumbar fascia; TRPV1: transient receptor potential vanilloid 1; TH: tyrosine hydroxylase; NI: no informed.

3.3 Nociceptive innervation of TLF: histological evidences

A total of 5 studies characterized the distribution of the nociceptive nerve fibers terminating within the TLF [32,41,46,47,48]. All these studies investigated whether fasciae associated with muscle are also innervated by peptidergic nociceptive sensory fibers. Collectively, these studies reported that “naïve” fascia (mice, rats and human) contained in the following: (a) 60-88% DRG cells with terminations within the collagen matrix of connective tissue in the low back also expressed calcitonin gene-related peptide (CGRP) immunoreactivity in rats [46], (b) The TLF contained a higher density of sensory nerves, with three times more CGRP-immunoreactive fibers compared to back muscles (latissimus dorsi). However, around half of these lack SP-immunoreactive in mice [47], (c)

CGRP-immunopositive fibers in the outer and inner layer of TLF and SP-containing nerve fibers existed exclusively in the outer layer of TLF in rats [48], (d) in rats, fibers immunoreactive to protein gene product (PGP) 9.5, tyrosine hydroxylase (TH), CGRP and SP were found in the subcutaneous tissue and the outer layer of TLF, and in human, the majority of peptidergic nerve endings were located in the subcutaneous tissue and in structures comparable with outer and inner layer of the rat TLF [41]. Two studies investigated nociceptive-related responses to experimental irritation of the TLF, that resulted in the following: (a) in rats with CFA injection made in the spinous processes L4 and L5, the CGRP-immunopositive fiber length increased significantly only in the inner layer of TLF and SP-positive structures were found in the inner layer of TLF [48], (b) in rats, inflammation was induced by electrically stimulating the dorsal roots L3-L6 at an intensity supramaximal for unmyelinated fibers. Specifically, SP and CGRP are known to cause plasma extravasation by increasing the permeability of capillaries close to the endings. Neurogenic plasma extravasation in form of dark patches of Evans blue in the TLF were observed [48].

3.4 *In vivo studies*

A total of 5 studies evaluated and quantified outcomes nociceptive after electrical and chemical stimulation of the TLF relating to its contribution to LBP [35-37,49,50]. Of these five studies, two investigated the role of chemical (3/5) and electrical (2/5) irritation of the TLF on measures related to nociceptive innervation of the low back as a potential source of LBP. Specific outcomes included: electrophysiological recordings from dorsal horn neurons in spinal (2/5), pressure pain threshold (algometer) (2/5), pain distribution (1/5), pain quality (1/5), electrical detection threshold (1/5). Two studies made systematic recordings from dorsal horn neurons in the spinal cord to obtain information about the spinal nociceptive processing from the TLF in naïve rats. Species selected for *in vivo* analysis included: rats (2/5) and humans (3/5). Anatomical site of analysis included the TLF (5/5), low back muscles (5/5), and dorsal horn neurons in spinal (2/5). Detailed information regarding the species and outcome measures can be found in Table 2.

Table 2. *In vivo* studies

Studies (Authors, years)	Specie, condition, n	Method	Main findings
Schilder et al., 2014 [35]	Healthy human, Ultrasound-guided bolus injections of hypertonic saline into the erector spinae muscle, the thoracolumbar fascia (TLF, posterior layer), and the overlying subcutis, n = 6 women n = 6 men	<ul style="list-style-type: none">• Pressure pain threshold (algometer)• Pain distribution (2-dimensional paper form body image)• Pain quality (Pain Perception Scale, “Schmerzempfindungs-Skala” [SES])	<ul style="list-style-type: none">• Hypertonic saline into the fascia resulted in longer pain duration and higher pain intensities than injections into subcutaneous tissue or muscle• Pain radiation and pain affect evoked by fascia injection exceeded those of the muscle and the subcutaneous tissue• Pain descriptors (burning, throbbing, and stinging) suggested innervation by both A- and C-fiber nociceptors.
	Healthy human,		Fascia vs muscle high-frequency stimulation (HFS):

Schilder et al., 2016 [37]	Stimulation electrical with high-frequency pulses in the multifidus muscle and the overlying TLF through bipolar concentric needle electrodes placed at lumbar level (L3/L4) n = 8 women n = 8 men	<ul style="list-style-type: none"> • Electrical detection threshold and pain threshold • Pain distribution (2-dimensional paper form body image) • Pain rating during HFS 	<ul style="list-style-type: none"> • Electrical pain thresholds lower and pain ratings higher for fascia • For both tissues, pain ratings increased significantly • Fascia HFS increased fascia pain ratings 2.17 times compared with control site • Muscle HFS decreased pain sensitivity of the overlying fascia by 20% <p>Potentialiation by fascia HFS was similar to that of skin HFS, followed over 60 minutes post-HFS.</p>
Schilder et al., 2018 [37]	Healthy human, Stimulation electrical with high-frequency pulses in the multifidus muscle and the overlying TLF through bipolar concentric needle electrodes placed at lumbar level (L3/L4) n = 8 women n = 8 men	<ul style="list-style-type: none"> • Pain quality (Pain Perception Scale, "Schmerzempfindungs-Skala" [SES]) 	<p>Factor analysis of the sensory descriptors revealed:</p> <ul style="list-style-type: none"> • Superficial thermal ("heat pain" identified by the items "burning," "scalding," and "hot") and superficial mechanical factors ("sharp pain" identified by the items "cutting," "tearing," and "stinging") was more pronounced for fascia than muscle • Deep pain (identified by the items "beating," "throbbing," and "pounding") was more pronounced for muscle than fascia
Hoheisel et al., 2011 [49]	Rats, Naïve, n = 12 Rats, Treated with CFA, n = 32	Were made systematic electrophysiological recordings from dorsal horn neurons in spinal (Th13-L5) to obtain information about the spinal nociceptive processing from the TLF	<p>In naïve rats</p> <ul style="list-style-type: none"> • 6 -14% of the neurons in the spinal segments Th13–L2 had nociceptive input from the TLF • No neurons responsive to input from the TLF were found in segments L3-L5. <p>In inflamed (multifidus) rats</p> <ul style="list-style-type: none"> • There was a significant increase from 4% to 15% in the proportion of neurons responsive to input from the TLF • Neurons in spinal segment L3 responded to fascia input in animals.

			In inflamed (Intrafascial CFA injection) rats
Hoheisel et al., 2015 [50]	Rats, Intrafascial CFA injection, n = 25	Were made extracellular recordings from dorsal horn neurons in the lumbar segment L3, identification of receptive fields and behavioural experiments	• 11.1% of neurons in the spinal segment L3 received input after TLF inflammation
			• Compared with control, the proportion of neurons having input from all deep somatic tissues rose from 10.8% to 33.3%
			• Moreover, many neurons acquired new deep receptive fields
			• Most of which were located in the hindlimb
			• Although the pressure pain threshold of the inflamed rats did not change, they presented a reduction in exploratory activity

Note: CFA: complete Freund’s adjuvant; TLF: thoracolumbar fascia; HFS: high-frequency stimulation.

3.5 Nociceptive innervation of TLF: functional evidences

A total of 5 studies evaluated the role the input nociceptive through electrical and chemical stimulation of the TLF in relation to LBP [35-37,49,50]. Of these five studies, three investigated the role of chemical irritation of the TLF on measures related to nociceptive innervation of the low back as a potential source of LBP. These studies reported that chemical irritation of fascia (in rats and humans) produced in the following: (a) in rats with bilateral complete Freund’s adjuvant (CFA)[49] injection made in the spinous processes L4 and L5 or intrafascial (L3) [50], there was a significant increase from 4% to 15% in the proportion of neurons responsive to input from the TLF and neurons in the L3 spinal segment - that did not receive input from the fascia in control animals, (b) healthy volunteers received ultrasound-guided bolus injections of hypertonic saline into the erector spinae muscle, the TLF (posterior layer), and the overlying subcutis. Hypertonic saline injection into the TLF resulted in longer pain duration and higher pain intensities than injections into subcutaneous tissue or muscle. Pain radiation and pain affect evoked by fascia injection exceeded those of the muscle and the subcutaneous tissue. Pain descriptors (burning, throbbing, and stinging) suggested innervation by both A- and C-fiber nociceptors [35].

Two studies [36,37] used electrical stimulation with high-frequency pulses in the multifidus muscle and the overlying TLF through bipolar concentric needle electrodes placed at lumbar level (L3/L4) to assess distinct differences between nociceptive innervation of low back fascia and muscle in humans. These studies reported the following: (a) lower electrical pain thresholds and higher pain ratings for fascia for both tissues. Fascia high-frequency stimulation increased fascia pain ratings 2.17 times compared to control. Muscle high frequency stimulation (HFS) decreased pain sensitivity of the overlying fascia by 20%. Potentiation by fascia HFS was similar to that of skin HFS, followed over 60 minutes post-HFS [36], (b) Factor analysis of the sensory descriptors revealed: Superficial thermal and superficial mechanical factors was more pronounced for fascia than muscle, whereas deep pain was more pronounced for muscle than fascia [37].

4. Discussion

The main objective of this scoping review was to assess current knowledge regarding nociceptive innervation of the TLF to better inform future mechanistic and clinical TLF research targeting LBP treatment. The results observed here, from a collective compilation and evaluation of studies on TLF nociceptive innervation, demonstrate that rodents and humans have an important nociceptive afferent TLF innervation and that the stimulation of this structure can lead to a central sensitization process in the long term. In this sense, these findings support the idea, through neurophysiological substrates related to nociceptive innervation, that TLF may be, at least in part, the origin of LBP. Although we recognize that the sensory innervation of the TLF encompasses proprioceptive, autonomic and nociceptive components, here we focus on nociception as a direct cause of pain. In that regard, articles for this review were restricted to preclinical and clinical including *ex vivo* ($n = 5$) and *in vivo* ($n = 5$) studies related to nociceptive investigation of TLF.

4.1 *Ex vivo* studies

Ex vivo studies were constituted primarily of specific immunohistochemistry assays to neuropeptides [32,41,46-48]. Mice [47], rats [32,41,46-48] and humans [41,48] presented positive immunoreactivity to SP and CGRP in the TLF, a nonspecialized connective tissue in the low back [51]. Furthermore, functional evidence confirmed the hypothesis of nociceptive innervation of TLF, since the chemical irritation of the fascia by local injection of CFA significantly increased SP- and CGRP-immunopositive fiber in the inner layer of TLF. In addition, electrical stimulation of the dorsal lumbar roots at an intensity for unmyelinated fibers increased the permeability of capillaries close to the endings causing neurogenic plasma extravasation, a phenomenon known to be caused by SP and CGRP [48]. DRG neurons traced from collagen matrix of connective tissue in the low back support the finding that populations of CGRP-immunoreactive fibers innervate the TLF [46,47]. Innervation density was higher in the thoracolumbar fascia compared to muscles of the back, supporting the view that the thoracolumbar fascia may make an under recognized contribution to chronic back pain. It has been demonstrated that CGRP and SP can act synergistically to promote inflammation and nociceptor sensitization since they are released by the peripheral or central projections of primary afferent neurons [52,53]. In the periphery, CGRP and SP induce the production and release of inflammatory cytokines, thus contributing to the peripheral sensitization of nociceptive neurons [54]. Centrally, when released into the spinal cord, CGRP and SP also induce central sensitization by acting on dorsal horn neurons [55]. Interestingly, it has been shown that CGRP can inhibit the degradation of SP and thus prolong its action [56]. Finally, CGRP may contribute to mechanical hyperalgesia or allodynia by enhancing the response of wide dynamic range neurons to cutaneous stimulation [57,58].

4.2 *In vivo* studies

In vivo and functional studies were constituted primarily of articles that evaluated nociceptive outcomes after chemical [35] and electrical [36,37] stimulation of the TLF relating to its contribution to LBP. In humans, the pain evoked by ultrasound-guided bolus injection of hypertonic saline [35] or stimulation electrical [36] in TLF tissue exceeded those of muscle. Furthermore, the pain after chemical stimulation of TLF, was described by the patients in rather extreme terms like cutting, tearing and stinging, which suggests innervation by both A- and C-fiber nociceptors [35,59,60]. In contrast, muscle-derived pain was predominantly attributed to sensory qualities like throbbing and pounding [37]. It is understood that C- and A-cutaneous afferents correlate with group IV and III muscle afferents [59]. However, the fact that muscle-associated pain differs from cutaneous-associated pain strongly suggests that the neurophysiological mechanisms are not similar. This is evident when electrical stimulation of the muscle nerves produces only a single pain, as opposed to electrical stimulation of the cutaneous nerves which present

pain in two phases [60]. Corroborating the findings of our scoping review, it has been previously described that fascial pain quality is different, as cutaneous pain is well localized and described as stabbing, burning or cutting, while muscle pain tends to be referred and described as tearing, cramping or cramping pressing [60]. Finally, electrophysiological recordings from dorsal horn neurons in spinal cord rats showed that the nociception evoked by the injection of a chemical agent irritating in the lumbar spinous process increased the proportion of neurons responsive to input from TLF and neurons in the L3 spinal segment that previously did not respond to input from the fascia in naive animals [49]. Interestingly, this study also showed that most dorsal horn neurons that receive nociceptive input from the TLF are convergent and have additional input from other tissues, such as skin and lumbar muscles, at least in rats [49]. Possibly the nociceptors located in the fascia are the beginning of the nociceptive pathway from the lumbar region soft tissue to the spinal dorsal horn and to other central locations from there. Because input from all soft tissues of the low back converge onto the same dorsal horn neurons, no separate pathway from fascia to higher centers appears to exist. This may be the explanation why a pain from the TLF is difficult to distinguish from the pain from other soft tissues of the low back [49,50]. Hoheisel et al. (2015) [50] made an intrafascial CFA injection in the TLF and registered dorsal horn neurons activity in the lumbar segment L3. They observed that the proportion of neurons having input from all deep somatic tissues rose from 10.8% to 33.3%, when compared with control. One of the most relevant findings in animals with fasciitis was the appearance of new receptive fields in deep somatic tissues beyond the fascia, located outside the lumbar region and extending to the distal hindlimb. The appearance of new receptive fields could be a possible explanation for the spread of pain in patients with LBP.

4.3 Limitations

Limitations associated with this scoping review include selecting publications in English only which could potentially reduce the number of studies being retrieved from the literature search.

4.4. Clinical perspectives and future directions

Published guidelines have proposed non-pharmacological approaches such as exercise and physical therapy (massage, acupuncture, spinal manipulative therapy) as first-line treatments for low back pain due primarily to concerns about the risk-benefit ratio of opioids and suboptimal opioid-related results in clinical trials [61-63]. A major gap in LBP research includes the contribution of musculoskeletal tissues (unspecialized muscles and connective tissues) in the lower back of the spine [64]. Although not well understood, the pathophysiology of non-specialized connective tissues, their relationship to LBP and other conditions of chronic musculoskeletal pain, can include fibrosis, chronic inflammation and neuroplastic changes, such as altered sensitivity [65-69]. Thus, the results of the present study highlight the role of connective tissue innervation in the development of low back pain by demonstrating the presence of sensory nerve fiber endings within the collagen matrix of non-specialized connective tissue and that structural innervation of these connective tissues is important for functional changes associated with neurogenic inflammation and persistent pain. Based on the results of this review, it is suggested that the treatment of TLF should also be consider in LBP therapeutic approaches.

Despite the recent increase in the number of basic science investigations describing the nociceptive innervation of TLF, this review has identified numerous areas that require further study to advance the field and deepen our understanding of the fascia approach. Although the peripheral evidence exists and provides strong evidence for neurophysiological substrates of the nociceptive innervation of the fascia, there is limited evidence related to the central (supra-spinal) mechanisms involved. Future preclinical studies related to techniques aimed towards treatment (i.e. manipulation/mobilization) of TLF mimicking the clinical setting could be useful to advance knowledge and help in the treatment of musculoskeletal pain. In this sense, preclinical studies that record the results

immediately after (or shortly thereafter) the delivery of fascial manipulation, long-term or longitudinal studies are needed, as well as studies that investigate the physiological impact of various of fascial manipulation dosage. As in the França et al., 2020 [70] study, preclinical studies are just beginning to recognize and demonstrate that analgesic modulation related to fascial manipulation involves complex mechanistic interactions. Thus, preclinical studies that investigate endogenous neurological systems, such as: endogenous opioids [71], endocannabinoids [72], adenosinergic system [73], and/or neuroimmune contributions [70,74] are needed, and these effects may require certain fascial manipulation dosage thresholds be achieved. Preclinical studies investigating the effects of fascial manipulation on: gene expression, neurotransmitter / neuropeptide / cytokine release, mechanosensitive ion channel activation, neuroimmune response, global cortical / spinal circuit connectivity and descending inhibition [73], are needed to advance the manual therapy field and deepen our understanding of fascial manipulation.

5. Conclusion

While more studies are clearly needed, findings from this review based on histological, immunohistochemical, electrophysiological, behavioral and clinical evidence, support the view that the TLF potentially plays a major role, or at least makes significant contribution to clinical LBP.

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