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Sensory findings after stimulation of the thoracolumbar fascia with hypertonic saline suggest its contribution to low back pain

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ABSTRACT

Injection of hypertonic saline into deep tissues of the back (subcutis, muscle, or the surrounding fascia) can induce acute low back pain (LBP). So far, no study has analyzed differences in temporal, qualitative, and spatial pain characteristics originating from these tissues. The current study aimed to investigate the role of the thoracolumbar fascia as a potential source of LBP. In separate sessions, 12 healthy subjects received ultrasound-guided bolus injections of isotonic saline (0.9%) or hypertonic saline (5.8%) into the erector spinae muscle, the thoracolumbar fascia (posterior layer), and the overlying subcutis. Subjects were asked to rate pain intensity, duration, quality, and spatial extent. Pressure pain thresholds were determined pre and post injection. Injections of hypertonic saline into the fascia resulted in significantly larger area under the curve of pain intensity over time than injections into subcutis ($P < 0.01$) or muscle ($P < 0.001$), primarily based on longer pain durations and, to a lesser extent, on higher peak pain ratings. Pressure hyperalgesia was only induced by injection of hypertonic saline into muscle, but not fascia or subcutis. Pain radiation and pain affect evoked by fascia injection exceeded those of the muscle ($P < 0.01$) and the subcutis significantly ($P < 0.05$). Pain descriptors after fascia injection (burning, throbbing, and stinging) suggested innervation by both A- and C-fiber nociceptors. These findings show that the thoracolumbar fascia is the deep tissue of the back that is most sensitive to chemical stimulation, making it a prime candidate to contribute to nonspecific LBP but not to localized pressure hyperalgesia.

1. Introduction

Although spinal structures (vertebrae, intervertebral discs, annulus fibrosus, facet joints, and spinal ligaments) are recognized as common causes of low back pain (LBP) [15], the role of muscles, fasciae, and other soft tissues as a potential source of LBP is often underappreciated [63]. However, there is increasing evidence that muscles and fasciae are involved in the development of LBP [8,47,60,68,69]. Immunohistochemical studies showed that the thoracolumbar fascia is innervated by nociceptive free nerve endings [13,65]. Furthermore, it has been shown that lumbar dorsal horn neurons receive nociceptive input from the fascia [27], suggesting a potential role of the thoracolumbar fascia in LBP.

Injections of hypertonic saline are frequently used to excite nociceptors in deep tissues, resulting in an activation of the nociceptive system by depolarizing small-diameter nociceptive afferent neurons [23,36], while blocking the generation of action potentials in large-diameter nonnociceptive fibers [50]. An injection of hypertonic saline into the abductor digiti minimi muscle and the overlying subcutis elicited similar pain intensities [40], and injection into the infrapatellar fat pad led to substantial pain radiation [5], indicating that many soft tissues display pain sensitivity to chemical stimulation. In the lower limb (tibialis anterior muscle), hypertonic saline injection into the tendon induced higher pain scores and larger referred pain areas than injection into the muscle itself [20], and the overlying crural fascia also showed a higher pain sensitivity to hypertonic saline than the underlying muscle [21], suggesting that connective tissue may generally be more sensitive than muscle. In the lumbar region, injection of hypertonic saline into the paraspinal muscles can evoke acute LBP [1,26,31,41,52], with similar effects on posture as in LBP patients [70], but very few data are available about the thoracolumbar fascia as a potential source of LBP.

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Since there is no comparative study distinguishing the muscle fascia of the low back from other tissue types, this study aimed at investigating the relative contribution of the thoracolumbar fascia, the erector spinae muscle, and the overlying subcutis to pain intensity, pain duration, pain quality, pain distribution, and changes in pressure pain thresholds after isotonic and hypertonic saline injections as human surrogate models of acute LBP. We hypothesized that an injection of hypertonic saline into the thoracolumbar fascia causes the highest pain intensity, the largest pain radiation, and the most pronounced sensitization to blunt pressure.

2. Material and methods

2.1. Participants

Twelve healthy volunteers (6 female, 6 male; mean age: 24.0 ± 1.5 years, mean ± SD) with no history of back pain participated in this study. All volunteers had sufficient command of the German language. The criteria for exclusion were any medication, or recent surgeries to abdomen, legs, or back. None of the participants withdrew from the study prematurely. The local Ethics Committee of the Medical Faculty Mannheim, University Heidelberg, approved the experimental protocol on human volunteers (2010-274N-MA) according to the current version of the Declaration of Helsinki.

2.2. Experimental protocol

After signing a written consent form, all participants attended 3 study sessions separated by at least 5 days. In each session, pain intensity, pain quality, and pain distribution in response to isotonic/hypertonic saline, as well as pressure pain thresholds (PPT) before and after saline injections were determined (see below). Subjects were advised to lie on a bench face down, minimizing back muscle contraction. The PPT baseline was determined before any saline injection. After hypertonic or isotonic saline injection, the volunteers were asked to rate the magnitude of perceived pain at 10-second intervals for the first 2 minutes, and thereafter at 30-second intervals for the following 23 minutes (ie, total time of pain assessment was 25 minutes) on a numerical rating scale with the end points 0 (=no pain) and 100 (=most intense pain imaginable). The subjects marked the distribution of the experimentally induced pain on a standard human body scheme while they were perceiving it, that is, without depending on episodic pain memory.

After pain sensation had subsided, PPT was determined at 25 minutes after injection and pain qualities were inquired. Within each session, the experimental protocol was performed twice, with hypertonic saline on one side and isotonic saline on the contralateral side. A second determination of PPT was done at 50 minutes after saline injection.

2.3. Saline administration

Bolus injections (400 μL) of hypertonic saline (5.8%) or isotonic saline (0.9%) as a control were made into the posterior layer of the thoracolumbar fascia, the erector spinae muscle, and the overlying subcutis at lumbar level (L3/L4), about 4 cm lateral to the spinous processes. For all injections, the position of the injection needle was guided by ultrasound (Acuson X150; Siemens, Munich, Germany). Owing to the low echo contrast in subcutis and muscle, the saline distribution could only be assessed in the fascia by ultrasound imaging. Fig. 1 shows a typical example of an ultrasound image before (A) and after (B) hypertonic saline injection into the middle portion of the thoracolumbar fascia. Examples of ultrasound images after hypertonic saline injection for 5 additional volunteers are shown in Fig. 1C–G. The time course of resolution of hypertonic saline injection into the fascia was measured in 3 healthy volunteers (Fig. 1H). The hypoechoic area marking the injection volume was determined every 10 seconds after bolus injection of hypertonic saline as horizontal and vertical spread.

In contrast to subcutis or fascia injection, saline injection into the muscle was performed vertically about 1 cm beyond the fascia after pulling the skin sideways in order to prevent capillary effects after needle withdrawing, which may lead to fluid reflow. The solution was administered using a 1-mL syringe (Becton Dickinson, Madrid, Spain) and a 27G cannula.

The volunteers were informed that they would receive 2 injections per session into either muscle, fascia, or subcutis. The experimental design of the study was a fully balanced right-left crossover design comprising the order of hypertonic or isotonic saline injection and tissue type selection. All participants were blinded with regard to the injected solution and tissue.

2.4. Pressure pain threshold (PPT)

A pressure algometer (Wagner Instruments, Greenwich, CT, USA) with a round rubber tip (contact area 1 cm^2) was pressed on the skin overlying the erector spinae muscle. With the tip size used for stimulation, mainly nociceptors from deep tissues were activated, while the contribution of cutaneous nociceptors to the overall pain was small [35]. The PPT was determined at 4 different locations, including the point of injection (central) and 3 other areas approximately 5 cm cranial, caudal, and lateral to the injection site. The PPTs were determined with 3 series of ascending stimulus intensities, each with a ramp rate of approximately 50 kPa/s (≈0.5 kg/cm^2).

2.5. Pain distribution

All volunteers were asked to mark the distribution of their acute pain on a standardized 2-dimensional paper form body image while they perceived the experimentally induced LBP. The scheme showed the back, the abdominal, and leg region, of a drawn standardized body and was presented during the entire 25-minute postinjection period. One slim subject developed a slight compression nerve block of the lateral femoral cutaneous nerve due to the long duration of the face-down position, which led to paresthesia that was, however, clearly distinguished from saline-induced pain, and was thus disregarded in the analysis and therefore not plotted in the respective figures.

2.6. Pain quality

The assessment of pain qualities elicited by saline injection consisted of a list of verbal descriptors (Pain Perception Scale, "Schmerzempfindungs-Skala" [SES]) comprising 14 affective and 10 sensory items [19]. Descriptors were rated on a 4-level ordinal scale (0 = no match, 1 = light match, 2 = largely match, 3 = total match).

2.7. Statistics

Statistical analysis was performed using SigmaPlot software, version 12.0 (Systat Software, Inc, Chicago, IL, USA). Significant differences (at P-values < 0.05) were determined by 2-way repeated-measures analysis of variance (ANOVA; factors: tissue and saline concentration) followed by Holm-Sidak post hoc test correcting for multilevel comparison. A comprehensive overview of ANOVA analyses is given in Table 1. All values given in this study are depicted as mean ± SEM, unless stated otherwise (SD for biological variability of tissue thickness).

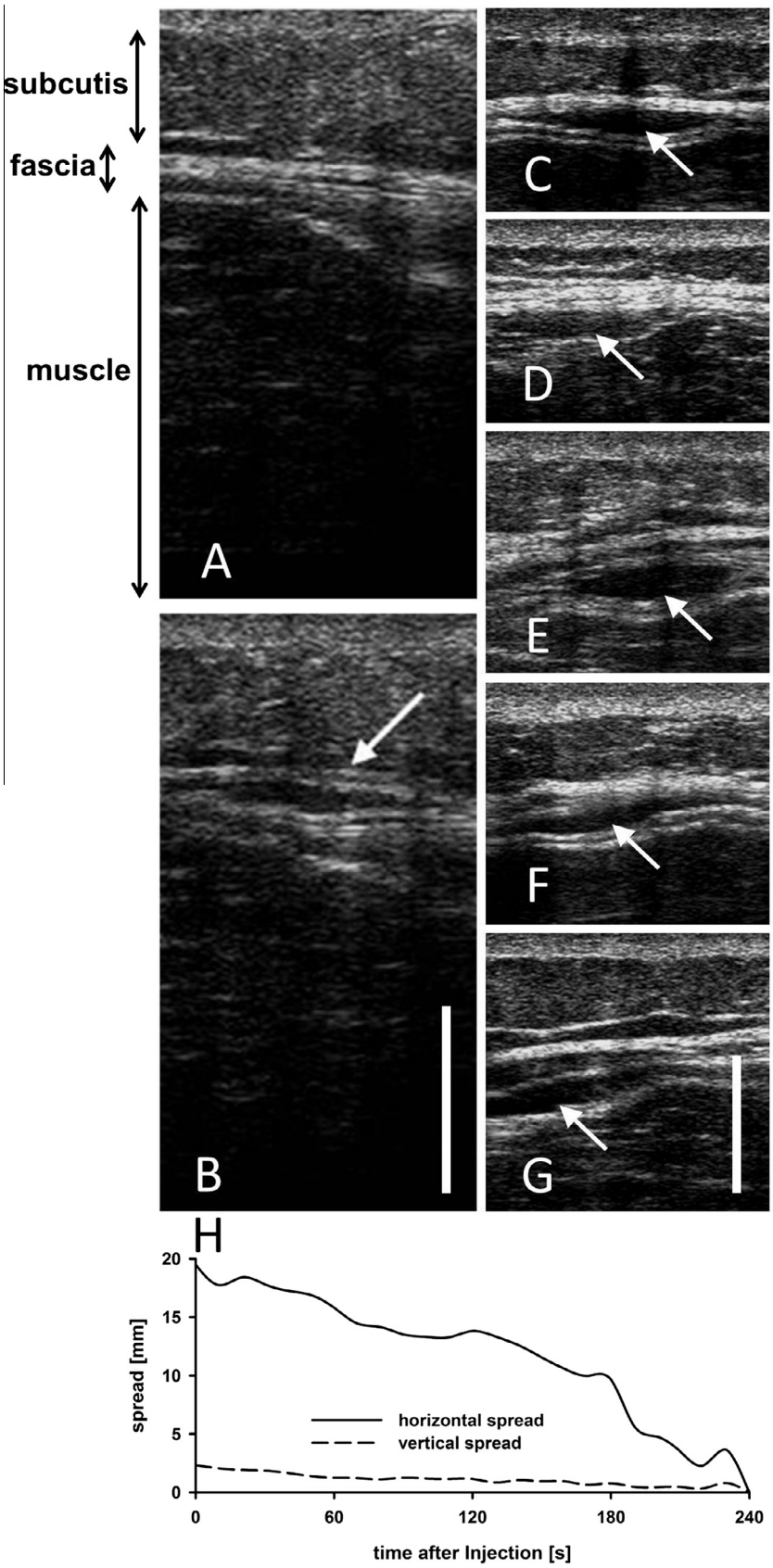

Fig. 1. Ultrasound images of the subcutis, the thoracolumbar fascia, and the erector spinae muscle before (A) and approximately 3 seconds after (B) the injection of hypertonic saline into the fascia (white arrows; scale bar: 1 cm). (C–G) Examples of ultrasound images of fascia injections in 5 additional subjects. (H) Average time course of volume spread in 3 subjects after fascia injection of hypertonic saline measured as horizontal (solid line) and vertical spread (dashed line).

Table 1
Summary of 2-way repeated-measures analysis of variance (ANOVA) on pain parameters.

	Factor: tissue	Factor: isotonic/hypertonic	Factor: tissue × isotonic/hypertonic
Area under the curve	F = 9.9; $P < 0.001^{***}$	F = 27.7; $P < 0.001^{***}$	F = 11.4; $P < 0.001^{***}$
Peak pain	F = 1.3; $P = 0.30$	F = 44.3; $P < 0.001^{***}$	F = 1.3; $P = 0.29$
Pain duration	F = 4.7; $P < 0.05^{*}$	F = 92.4; $P < 0.001^{***}$	F = 9.0; $P < 0.01^{***}$
Pain distribution	F = 3.5; $P < 0.05^{*}$	F = 18.7; $P < 0.01^{**}$	F = 4.7; $P < 0.05^{*}$
SES – affective	F = 4.9; $P < 0.05^{*}$	F = 11.3; $P < 0.01^{**}$	F = 7.7; $P < 0.01^{**}$
SES – sensory	F = 2.7; $P = 0.09$	F = 77.4; $P < 0.001^{***}$	F = 4.3; $P < 0.05^{*}$
PPT (after 25 minutes)	F = 1.1; $P = 0.34$	F = 0.01; $P = 0.91$	F = 5.3; $P < 0.05^{*}$
PPT (after 50 minutes)	F = 0.03; $P = 0.97$	F = 0.05; $P = 0.82$	F = 1.4; $P = 0.29$

SES, Pain Perception Scale, "Schmerzempfindungs-Skala"; PPT, pressure pain threshold; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Before calculations, the data of pain intensity ratings and PPT were transformed into decadic logarithms to achieve secondary normal distribution [3,46], since previous data obtained in larger cohorts provided solid evidence for log-normal distribution of PPT and other psychophysical data [58,59]. The log PPT values were then normalized to baseline (log value − log baseline value) for further analysis (equivalent to calculating percentage changes in PPT), whereas the area under the pain intensity curve was calculated with re-transformed log values. The change of PPT within each PPT site after hypertonic saline injection was tested by 2-tailed paired *t* test vs isotonic saline.

For analysis of pain qualities, the statistical significance was determined separately for every single descriptor item and tissue, as well as differences between isotonic and hypertonic saline by single-tailed paired *t* test vs "0 = no match" and vs isotonic saline. The descriptors were grouped into sensory and affective summary scores according to the original publication of the SES scale [19], which were then subjected to 2-way ANOVA.

Regarding the pain drawings of each subject, areas were digitized and transformed into a color-coded image using Adobe Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA, USA). In the group analysis, body areas with high or low occurrence of pain were illustrated in dark red or light yellow, respectively. Body areas without pain appeared white. The sizes of pain areas were computed as percentages of the whole body surface and analyzed by 2-way ANOVA.

3. Results

3.1. Fascia depth and visualization of injection volume after hypertonic saline injection

The results of fascia depth location in all volunteers revealed an average upper margin of 6.2 ± 1.9 mm and a lower margin of 8.3 ± 2.1 mm. Thus, the approximate thickness of the fascia in healthy volunteers was about 2.1 ± 0.5 mm (mean ± SD, n = 12).

In addition, the results of the temporal scans revealed that the visible hypoechoic area of the injected hypertonic solution within the fascia, recorded via ultrasound imaging, already decreased to a minimum after <4 minutes (Fig. 1H). Moreover, the fluid showed a maximal detectable spread of 19.5 ± 2.6 mm in length and a maximal height of 2.3 ± 0.6 mm (both mean ± SD, n = 3) around the injection site. Owing to the low echo contrast of saline vs subcutaneous or muscle tissue, the distribution of the injected saline volume could not be assessed by ultrasound imaging in the subcutis or muscle.

3.2. Pain intensity and duration after hypertonic/isotonic saline injection

Pain intensity ratings plotted against time, area under the curve for the first 15 minutes after injection, peak pain rating, and pain duration after isotonic and hypertonic saline injections are shown in Fig. 2. First, the injection of hypertonic saline induced higher and longer-lasting pain ratings in all tissues than stimulation by isotonic saline and peak pain ratings after hypertonic saline appeared considerably later than after isotonic saline (Fig. 2A). Second, an injection of hypertonic saline into the fascia evoked longer pain duration and higher pain intensities compared to subcutis and muscle injections within 25 minutes post injection (Fig. 2A).

ANOVA of the area under the pain intensity curve (Pain AUC) as a global measure of pain (B) revealed a significant main effect of "tissue" (F = 9.9, $P < 0.001$) and "saline concentration" (F = 27.7, $P < 0.001$) and showed a statistically significant interaction between both (F = 11.4, $P < 0.001$; 2-way repeated-measures ANOVA; Table 1). The Pain AUC after hypertonic saline into the fascia was significantly higher than after subcutis ($P < 0.01$) or muscle injections ($P < 0.001$, Fig. 2B). Additionally, subcutis injections were also more painful than muscle injections ($P < 0.01$). In the fascia and subcutis, the Pain AUC after hypertonic saline was significantly higher than after isotonic saline injections (both $P < 0.001$), while there was no significant difference in muscle ($P = 0.13$). Injection of isotonic saline only led to very weak pain ratings in all tissues, and the first minutes of the pain rating time course to isotonic saline matched well with the decay of injection volume calculated from Fig. 1H. The randomized order of saline injection had no effect on Pain AUC.

Although the interaction term of the 2-way ANOVA in peak pain was not significant, 1-way ANOVA revealed a significant role of tissue on pain after hypertonic saline (F = 8.1, $P < 0.01$), but not on pain evoked by isotonic saline (F = 0.004, $P > 0.99$). Injections into the fascia induced the highest perceived mean peak pain, which was significantly higher than that induced by injection into the muscle ($P < 0.01$). Although pain in the fascia was 39% more painful than in the subcutis, this difference failed to be significant ($P < 0.18$, Fig. 2C).

Differences in pain duration (Fig. 2D) were more prominent than in peak pain, and 2-way ANOVA of pain duration revealed a significant main effect of "tissue" (F = 4.7, $P < 0.05$) and "saline concentration" (F = 92.4, $P < 0.001$), as well as a statistically significant interaction between both (F = 9.0, $P < 0.01$). Pain duration after hypertonic saline injection into the muscle was significantly lower than after subcutis and fascia injections (both $P < 0.001$).

3.3. Pain distribution

Saline injection evoked pain at the point of injection and in adjacent areas. The spatial distribution of perceived pain is shown in Fig. 3A. Both hypertonic and isotonic saline injections into the different tissues induced LBP located unilaterally on the side of injection. Overall, hypertonic saline led to a more widespread distribution of pain. In addition, pain after fascia and muscle hypertonic stimulation radiated into the ipsilateral ventral area of the body. A more widespread pain radiation after fascia stimulation is also obvious. A 2-way ANOVA showed a significant main effect

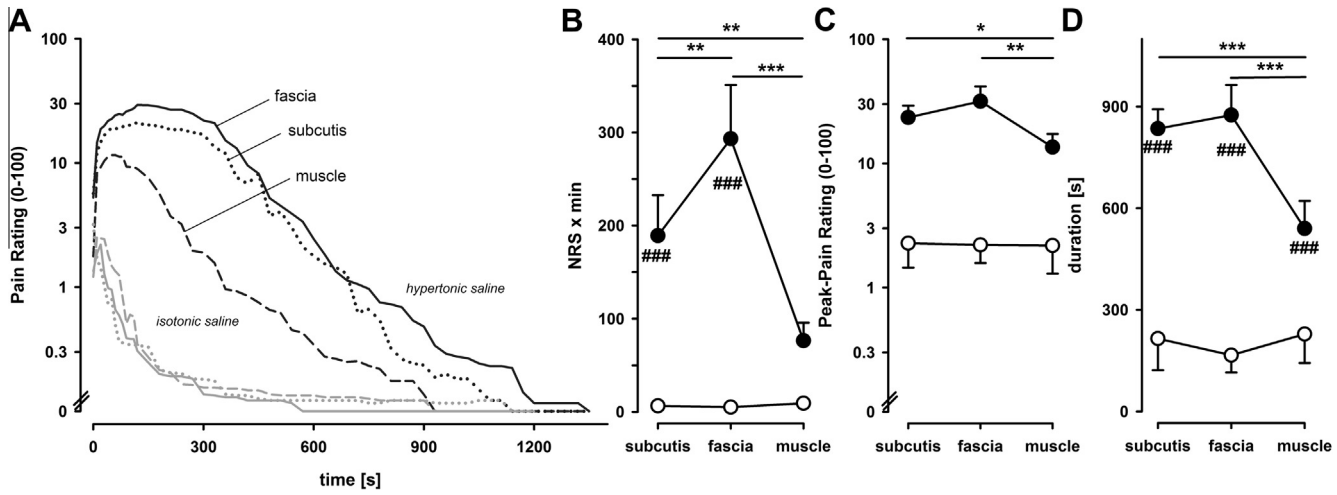

Fig. 2. Pain intensity after hypertonic (5.8%) and isotonic (0.9%) saline injection plotted against time (A). Pain area under the curve (B), peak pain (C), and pain duration (D) after hypertonic (filled circles) and isotonic (open circles) saline injections. An injection of hypertonic saline into the fascia led to a higher pain area under the curve compared to a hypertonic saline injection into the muscle and the subcutis (A). Differences in peak pain (C) were less pronounced than differences in pain duration, which was considerably shorter after muscle injection of hypertonic saline compared to subcutis and fascia injections. ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ between tissues after hypertonic injections; $^{\#\#\#}P < 0.001$ vs isotonic saline injection; n = 12). NRS, numeric rating scale.

of "tissue" (F = 3.5, $P < 0.05$) and "saline concentration" (F = 18.7, $P < 0.01$), as well as a significant "tissue × saline concentration" interaction (F = 4.7, $P < 0.05$; Table 1).

Post hoc analysis showed that after fascia injection of hypertonic saline, the area of evoked pain was 1.23 ± 0.33% of the standardized body image (dorsal and ventral aspect) and 2–3 times larger than after subcutis (0.65 ± 0.19%, $P < 0.05$) and muscle injections (0.45 ± 0.08%, $P < 0.01$; Fig. 3B). Isotonic saline injection in the fascia (0.07 ± 0.02%) and the subcutis (0.05 ± 0.02%) revealed a significantly smaller size of pain area compared to a hypertonic saline stimulation ($P < 0.001$ within fascia, $P < 0.05$ within subcutis). In contrast, injection of isotonic saline into the muscle resulted in pain distribution (0.18 ± 0.07%), which was not significantly different from the pain area after injection of hypertonic saline ($P = 0.25$).

3.4. Pain quality

Fig. 4 illustrates the analysis of SES pain descriptors. An injection of isotonic saline into any of the 3 tissues (A–C) led primarily to pain qualities of "burning" and "stinging" of mild intensity (below 1.0 on the 0–3 scale). Affective pain descriptors were usually not chosen at all. An injection of hypertonic saline led to a higher intensity of the "burning" and "stinging" pain qualities, which were significantly above the isotonic saline ratings for injection into the subcutis. Moreover, pain qualities that were significantly stronger after hypertonic than isotonic saline differed between tissues: "burning," "throbbing," "scalding," "stinging," and "hot" for subcutis (D), "beating," "throbbing," "scalding," and "hot" for fascia (E), and "beating" for muscle injections (F). In addition, affective pain descriptors such as "agonizing," "heavy," and "horrible" were chosen at a mild but significant level for subcutis and fascia.

SES pain descriptors were further analyzed as average scores for affective and sensory subscales (Fig. 5). Two-way ANOVA revealed a significant interaction in rating magnitude between tissues and saline concentrations within the affective descriptors (F = 7.7, $P < 0.01$) and the sensory descriptors (F = 4.3, $P < 0.05$; Table 1). After hypertonic saline injection into the fascia, subjects perceived higher affective pain than after subcutis ($P < 0.05$) or muscle stimulation ($P < 0.001$). Additionally, affective pain ratings from subcutis injections were also significantly higher than from muscle ($P < 0.05$; Fig. 5). Additionally, after hypertonic saline stimulation of the muscle, the sensory ratings were significantly lower than after fascia ($P < 0.05$) and subcutis stimulation ($P < 0.01$).

3.5. Pressure pain threshold

Fig. 6 depicts the changes in PPT at 25 minutes (A) and 50 minutes (B) after hypertonic or isotonic saline injection. Prior to saline injection, average PPT was approximately 400 kPa and differed by <±5% between the different experiments. The analysis of changes in PPTs of all investigated PPT sites showed a significant interaction between "tissue" and "saline concentration" (F = 5.3; $P < 0.05$; Table 1) 25 minutes post injection. Post hoc testing revealed hyperalgesia for blunt pressure at 25 minutes after hypertonic saline injection into the muscle, with a 10.5% reduction of PPT compared to isotonic saline injection ($P < 0.05$). In contrast, compared to isotonic saline injections, no significant pressure hyperalgesia was identified after hypertonic saline injection into the fascia or subcutis (change of PPT: +3.2% and +6.6%; n.s. vs isotonic saline, but $P < 0.05$ vs muscle). No significant differences were seen at 50 minutes post injection (Table 1). The randomized order of saline injection had no effect on the change in PPTs. Furthermore, Fig. 6C depicts the change of PPT of each PPT site 25 minutes after intramuscular injection. Two-tailed paired t test revealed hyperalgesic effects after hypertonic saline stimulation only at the injection site ($P < 0.05$ vs isotonic saline; central). The PPT sites cranial and caudal to the injection site tended to show effects ($P = 0.056$ and $P = 0.066$, respectively). The lateral PPT site ($P = 0.155$) was far from showing threshold changes.

4. Discussion

Almost all deep tissues are supplied with considerable densities of nociceptive innervation [9]. Accordingly, many osseous and soft tissues have been related to the development of LBP. Here, we show that the human thoracolumbar fascia is more sensitive to chemical stimulations by hypertonic saline than the underlying erector spinae muscle and overlying subcutis, according to various pain measures (Pain AUC, peak pain, pain duration, pain distribution, and affective pain descriptors). Contrary to our hypotheses, hyperalgesia to blunt pressure, a frequent sensory sign in both

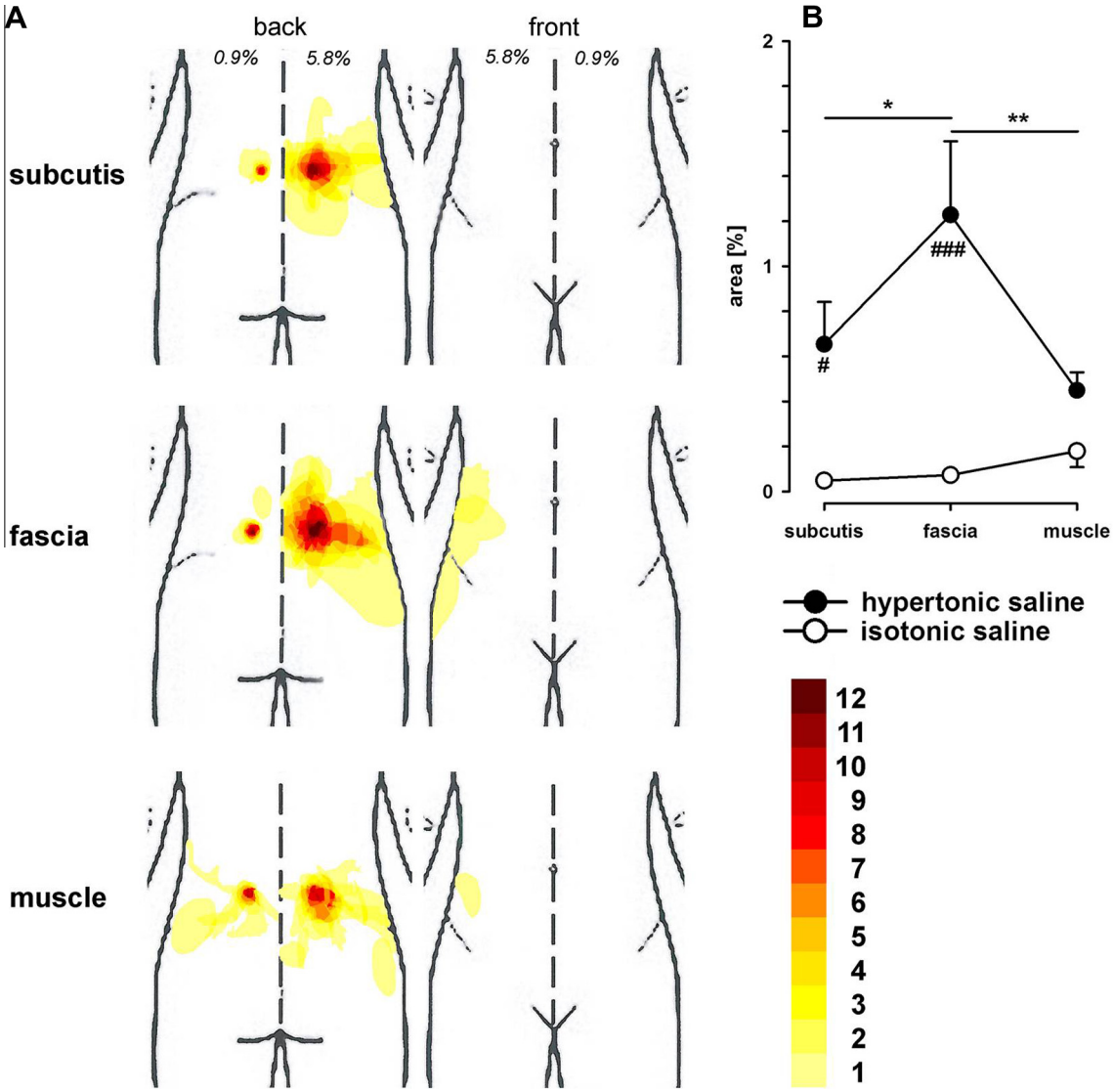

Fig. 3. Body chart (A) showing superimposed pain distributions after isotonic (0.9%) and hypertonic (5.8%) saline injections of all volunteers. The white areas mark body parts without pain in any subjects; the dark red areas and the light yellow areas mark body parts with high and low occurrence of overlapping pain distribution, respectively. The pain areas after hypertonic (filled circles) and isotonic (open circles) saline injections were calculated in percentage of the total standardized body image (B). Hypertonic saline injections into the fascia resulted in a higher pain radiation compared to pain areas of the subcutis and muscle evoked by the same stimulus. ($^{*}P < 0.05$; $^{**}P < 0.01$ between tissues after hypertonic injections; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs isotonic saline injection; n = 12).

localized acute and widespread chronic LBP [7,22,53], could only be induced by injections into the muscle, but not fascia or subcutis.

4.1. Pain intensity

Injections of hypertonic saline into deep somatic tissues have been used for decades to induce deep pain [31]. Hypertonic saline (5%) excites all group IV muscle afferents in rats [28]. Microneurography of muscle nerves demonstrated the presence of group III (Aδ) and IV (C-fiber) nociceptors in human muscle, and intraneural microstimulation elicited a cramp-like pain sensation [49,61]. Although there are no comparable studies on human fascia, the significantly higher Pain AUC, peak pain, pain duration, and affective pain rating after fascia injection of hypertonic saline compared to muscle suggest that nociceptive afferents within the thoracolumbar fascia are important for detection of chemical stimulation in the lower back.

Control injections of identical volumes of isotonic saline induced only weak and short-lived pain sensation, indicating that changes in tissue pressure induced by the bolus injections played a negligible role in pain induction in deep tissues investigated here. Moreover, pain to isotonic saline was not higher for fascia injections than for other deep tissues. Therefore, differences in tissue compliance are unlikely to explain the differences between fascia and adjacent deep tissues. However, clearance of hypertonic saline from the injection site may be slower for fascia than muscle due to its low vascularization.

Since our subjects did not move during pain perception, the low pain intensity after muscle injection is not due to active contraction or stretching of the paraspinal muscles shown to suppress pain to intramuscular hypertonic saline injection [67]. Rather, it may be related to the lower density of nociceptive endings in the low back muscle compared to the overlying fascia [65] or a less pronounced central representation [27].

A higher sensitivity to painful stimulation of fascia as compared to muscle has also been reported previously for the crural fascia and tibialis anterior muscle [21]. The difference in perceived pain between fascia and muscle of the back thus extends the results

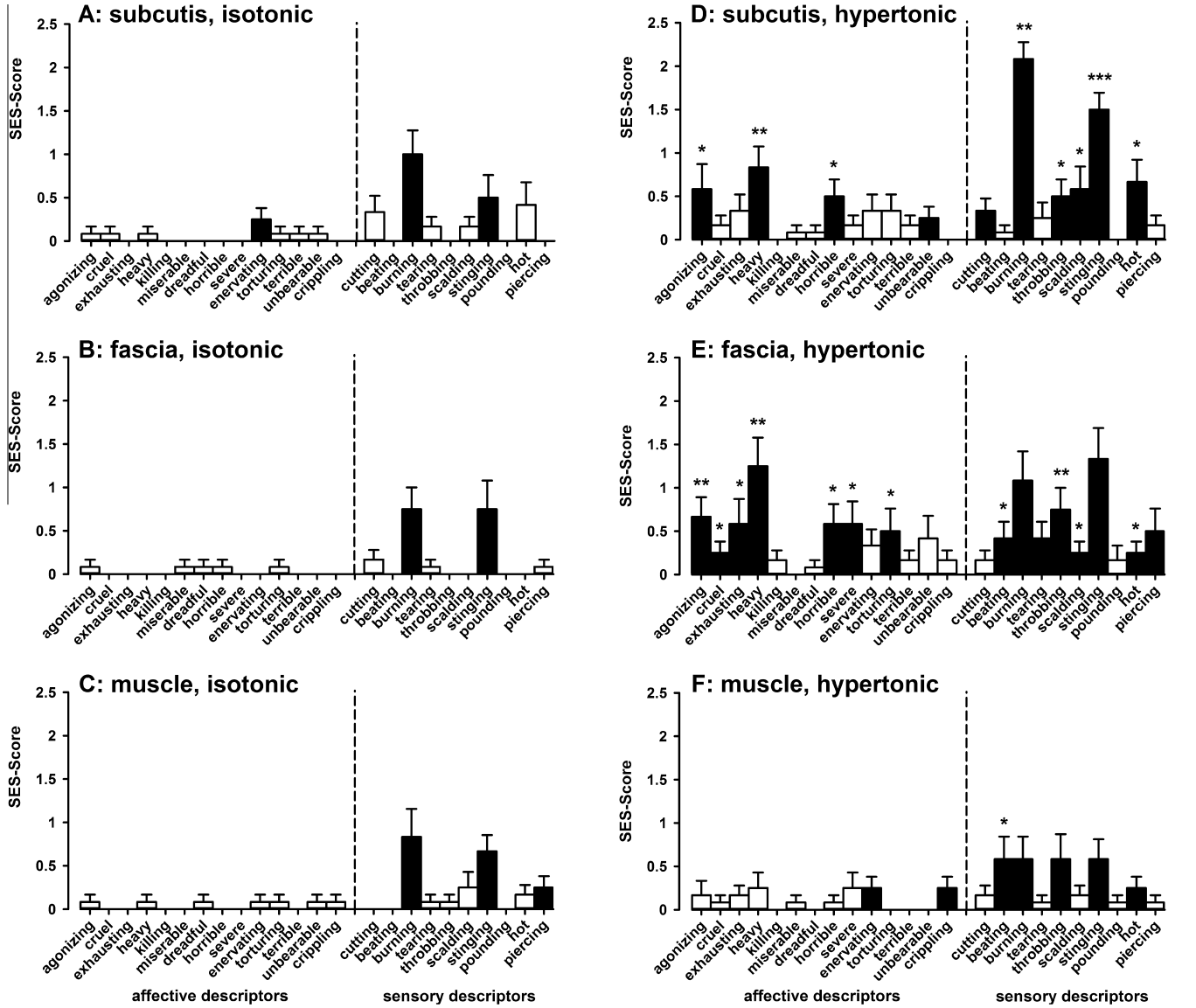

Fig. 4. Affective and sensory pain qualities of the "Schmerzempfindungs-Skala" (SES) induced by an isotonic (A–C) and hypertonic (D–F) saline injection into the subcutis (A, D), the fascia (B, E) and the muscle (C, F). Ratings are given on a 0–3 scale. The filled bars indicate a significant difference in rating magnitude of the descriptor ($P < 0.05$) vs "zero." ($^{*}P = 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs isotonic saline injection; n = 12).

from distal extremities, and suggests that the thoracolumbar fascia provides a higher afferent barrage and may play a more dominant role in the development and/or persistency of LBP than muscle.

4.2. Pain distribution

In all experiments, pain was confined to the ipsilateral side regardless of saline concentration or tissue type. Similarly, Palsson and Graven-Nielsen [54] did not find pain radiation to the contralateral side after saline injection into the posterior sacroiliac ligament.

The painful area after fascia injection exceeded those after subcutaneous or intramuscular injection by far, but stayed mostly within the injected dermatome. Since the purported association of "unusual" pain drawings with psychological factors in chronic pain was not valid in meta-analyses [11,48,55], pain radiation patterns in pain drawings may give useful information on biological factors such as neuronal signal processing. A stronger barrage of nociceptive input from fascia than muscle stimulation would explain both higher pain intensity and more pronounced pain radiation, which are often correlated.

The pain radiation after fascia injection was in the typical locations of "lumbago" in LBP patients. The notion that the thoracolumbar fascia may play a major role in LBP is supported by its increased thickness [39] and reduced shear strain in subjects with chronic LBP [38]. But pain radiation after fascia injection was also similar to that seen in pseudoradicular LBP patients [18], and even consistent with that given by patients with lumbar facet joint syndrome [51]. Future experiments should directly compare pain radiation of fascia activation with that of the clinical pain in patients with acute or chronic LBP.

4.3. Pain quality

Experimental pain models usually evoke a much lower affective pain component than clinical pain, even at similar pain intensity [12,25]. Given that affective pain qualities are the ones most commonly mentioned by LBP patients [42], it is remarkable that

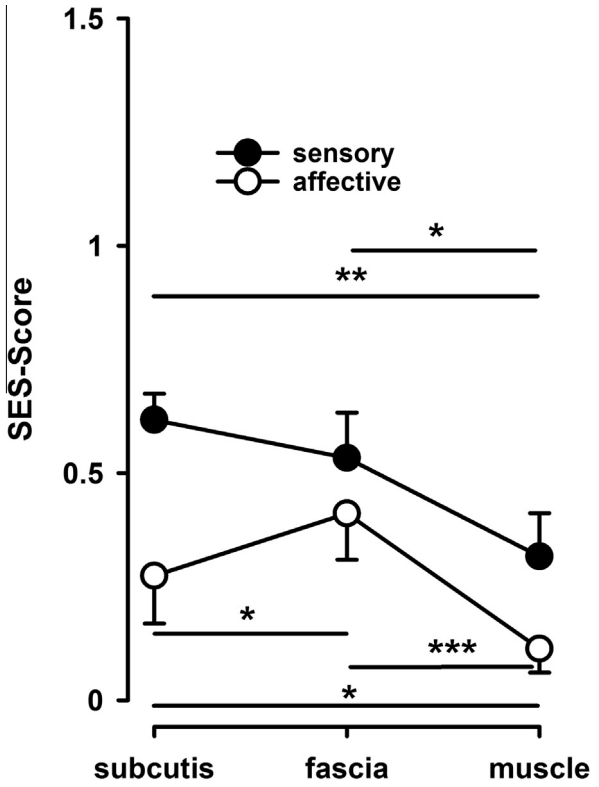

Fig. 5. Sensory and affective pain quality summary scores after hypertonic saline injections (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n = 12). SES, "Schmerzempfindungs-Skala".

hypertonic saline injection into the fascia, but neither muscle nor subcutis, yielded substantial affective pain ratings that were almost as high as sensory pain ratings. Thus, hypertonic saline injection into the thoracolumbar fascia, but not muscle or subcutis, promises to be a valuable experimental pain model, mimicking both the sensory and affective pain components in nonspecific acute LBP patients.

There is a long tradition using descriptors of pain quality to identify input from specific nociceptor subtypes. Cutaneous stimuli exciting Aδ fibers are more likely to elicit pricking and stinging pain sensations, whereas stimulation of nociceptive C fibers was described as "hot," "burning," "dull," and "pressing" [4,10,43,44,62]. As injection of both isotonic and hypertonic saline evoked pain qualities of "burning" and "stinging" in all tissues analyzed, both A- and C-fiber nociceptors are likely to contribute to the mechanically evoked pain.

Although the descriptor "burning" was used for injections in all 3 tissues in our study, there was no significant difference between isotonic and hypertonic saline into fascia, suggesting that this descriptor is not typical for LBP related to chemical activation of fascia nociceptors. However, "burning" is one of the descriptors that suggest a neuropathic pain component to LBP, that is, when fascia input is probably less important [2,6,16–18].

4.4. Pressure pain threshold

This study compared for the first time the capacity of 3 deep tissue types to elicit hyperalgesia to blunt pressure [66]. Notably, although the pain elicited by hypertonic muscle injection was low, this was the only stimulation after which a hyperalgesia to blunt pressure occurred. Decreases in PPT after chemical stimulation of muscles have been described earlier [29,30]. In the lower limb, however, chemical stimulation of the tibialis anterior muscle tendon also led to increased pressure sensitivity [20]. Because it is known that cutaneous afferents play a minor role in pain evoked by blunt pressure [24,34,64] and that peripheral sensitization of peripheral nociceptive afferents, rather than central sensitization, contributes to blunt pressure hyperalgesia in human pain models [32,33,37], we propose that the hyperalgesia to blunt pressure in our data is likely due to peripheral sensitization of muscle nociceptors [28]. The nonsignificant spread of pressure hyperalgesia after intramuscular saline stimulation in this study was confined to the injected muscle, which is also consistent with peripheral sensitization.

Moreover, since the afferent barrage originating from fascia is stronger than from muscle, an injection of hypertonic saline into fascia should have caused a larger central sensitization [45]. The lack of changes in PPT after chemical stimulation of the subcutis and fascia in this study suggests no cross-interaction between tissues and that the localized decrease of PPT in nonspecific acute and

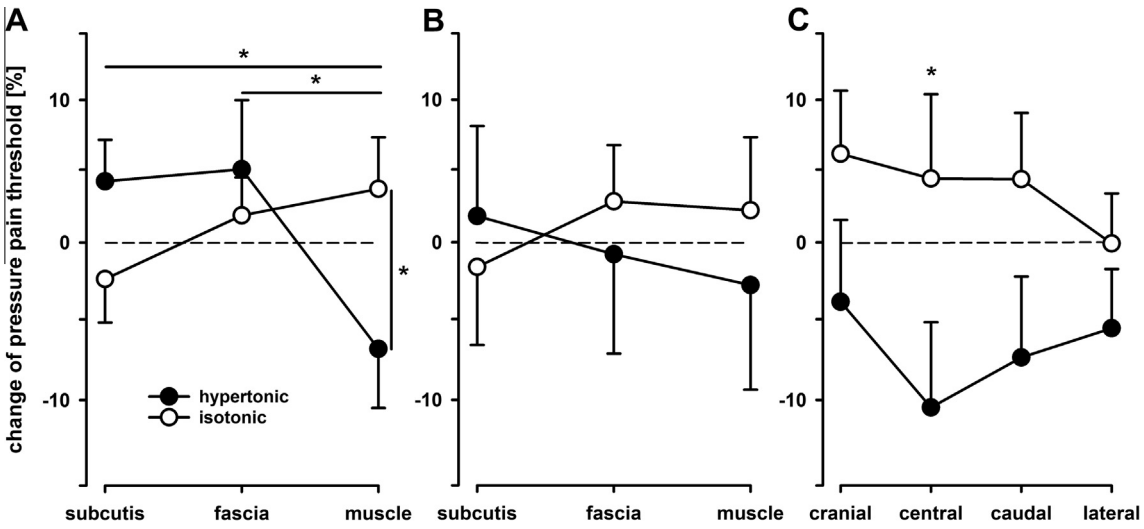

Fig. 6. Percentage changes in pressure pain thresholds (PPT) from baseline 25 minutes (A; n = 12) and 50 minutes post injection (B; n = 7) of hypertonic and isotonic saline into the subcutis, the fascia, and the muscle. The PPT after intramuscular injection of hypertonic saline showed a reduction compared to the PPT after isotonic saline injection and a significant difference compared with fascia or subcutis injections (*$P < 0.05$). (C) Changes in pressure pain threshold of the different PPT sites 25 minutes after intramuscular stimulation (n = 12). Only the injection site revealed a reduction of PPT, not the adjacent PPT sites.

chronic LBP patients [7,53] may be related to peripheral sensitization of muscle nociceptors, whereas widespread decreases in PPT are consistent with a contribution of central sensitization [22,56,57]. Although fascia stimulation led to a higher pain intensity, duration, and radiation, the muscle seems to be more prone to chemically induced hyperalgesia than the fascia or subcutis. However, measurement of hyperalgesia by PPT may have missed a hyperalgesia in subcutis or fascia for technical reasons, since PPT is also insensitive to identify hypoalgesic or hyperalgesic skin [33–35,37,64].

4.5. Technical considerations

Injections of 400 μL of hypertonic saline may activate nociceptors by mechanical distension or by chemical activation [28]. Although injections into the thoracolumbar fascia led to a visible separation of sheets in ultrasound imaging, distension is unlikely to play a major role for pain induction: first, the pain intensity evoked by isotonic saline decreased directly after injection and disappeared within 5 minutes, whereas pain due to hypertonic saline lasted for up to 20 minutes. Secondly, the hypoechoic area of the injected solution within the fascia, recorded via ultrasound, already disappeared within 4 minutes. Thus, ultrasound-guided injections into thoracolumbar fascia may be useful for testing its sensitivity and, possibly, also for sensitizing and desensitizing its nociceptors [14].

4.6. Conclusion

This study has shown that ultrasound-guided injection of hypertonic saline can specifically target the thoracolumbar fascia in the lower back and induces intense tonic pain with a strong affective component and a pain radiation similar to acute LBP. Pain in this model is not due to fascia distension. Consistent with previous animal experiments, the fascia is the most pain-sensitive deep tissue in the lower back, and its innervation may hence play a major role in acute localized LBP. Furthermore, an inflammation or disorganization of the thoracolumbar fascia may contribute to chronic LBP. This prediction may be tested by comparing the pain descriptor profile and pain radiation patterns of fascia injections with those of clinical LBP. In contrast, a "burning" pain quality suggests neuropathic LBP, and hyperalgesia to blunt pressure seems to require peripheral sensitization of muscle nociceptors.

Conflict of interest statement

The authors declare no conflicts of interest.

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