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Experimental pain in the groin may refer into the lower abdomen: implications to clinical assessments

M. K. Drew^{b,c}, T.S. Palsson^a, R. P. Hirata^a, M. Izumi^{a,d},
G. Lovell^e, M. Welvaert^f, P. Chiarelli^b, P.G. Osmotherly^b, T. Graven-Nielsen^{a,*}

^a Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark.

^b School of Health Sciences, Faculty of Health and Medicine, University of Newcastle, Australia

^c Department of Physical Therapies, Australian Institute of Sport, Canberra, Australia

^d Department of Orthopedic Surgery, Kochi University, Japan

^e Department of Sports Medicine, Australian Institute of Sport, Canberra, Australia

^f UCRISE, University of Canberra, Canberra, Australia

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***Corresponding author:**

Professor Thomas Graven-Nielsen, DMSc, Ph.D.

Center for Neuroplasticity and Pain (CNAP)
SMI, Department of Health Science and Technology
Faculty of Medicine
Aalborg University

Fredrik Bajers Vej 7D-3
9220 Aalborg E, Denmark
Phone: +45 9940 9832
Fax: +45 9815 4008
<http://www.cnap.hst.aau.dk/>
E-mail: tgn@hst.aau.dk

1 **ABSTRACT**

2 **Objectives:** To investigate the effects of experimental **adductor** pain on the pain referral pattern,
3 mechanical sensitivity **and** muscle activity during common clinical tests.

4
5 **Design:** Repeated-measures design

6
7 **Methods:** In two **separate** sessions, 15 healthy males received a hypertonic (painful) and isotonic
8 (control) saline injection **to either the** adductor longus (AL) tendon to produce experimental groin
9 pain **or** into the rectus femoris (RF) tendon as a painful control. Pain intensity was recorded on a
10 visual analogue scale (VAS) with pain distribution indicated on body maps. Pressure pain thresholds
11 (PPT) were assessed bilaterally in the groin area. Electromyography (EMG) of relevant muscles was
12 recorded during six provocation tests. PPT and EMG assessment were measured before, during and
13 after experimental pain.

14
15 **Results:** Hypertonic saline induced higher VAS scores than isotonic saline ($p < 0.001$), and a local pain
16 distribution in **80%** of participants. **A proximal pain** referral to the lower abdominal region in 33%
17 (AL) and 7% (RF) of participants. Experimental pain (AL and RF) did not significantly alter PPT
18 values or the EMG amplitude in groin or trunk muscles during provocation tests when forces were
19 matched with baseline.

20
21 **Conclusions:** This study demonstrates that AL tendon pain was distributed locally in the majority of
22 participants but may refer to the lower abdomen. **Experimental adductor pain did not significantly**
23 **alter the mechanical sensitivity or muscle activity patterns.**

24
25 **Key Words:** athlete; EMG; pressure pain sensitivity; adductor longus tendon; rectus femoris tendon

26 **Introduction**

27 The prevalence of hip and groin pain in athletes is generally high with a career prevalence of 45%
28 reported in professional Australian football players¹ and a high incidence in sports such as football²
29 and ice hockey.³ Adductor-related groin pain is characterised as pain on resisted adduction and pain
30 on palpation of the adductor longus muscle.⁴ In contrast, abdominal symptoms present with pain on
31 resisted trunk flexion and pain on palpation of the rectus abdominis distal enthesis.⁵ Yet
32 characteristics of groin pain per se are poorly understood with few reports of pain referral patterns and
33 clinical symptomatology. Pain referral patterns are typically semi- (referring distally) or bi-directional
34 (referring both distally and proximally) with referred pain distributions extending to neighbouring
35 vertebral segments that are supplying the painful muscle or tendon.⁶ Clinically, pain in both the
36 adductor and abdominal area is associated with longer recovery times compared to a single site.⁷ The
37 role of pain referral patterns has not previously been examined and may present a plausible alternate
38 hypothesis to co-existing pain locations^{5, 8-10} in this region. That is, abdominal pain may present
39 clinically as a result of referred pain from the adductor region. If this is true, it challenges using pain
40 location alone as diagnostic criteria in either classifying patients into entities or to specific
41 pathoanatomical tissue diagnoses.

42

43 Electromyographic (EMG) muscle activity has been shown to be significantly reduced in m. adductor
44 longus, m. pectineus, and m. gracilis, in patients with a history of groin pain during clinical tests when
45 compared to healthy activity-matched-controls.¹¹ Such changes occur soon after the initiating painful
46 event.¹² Given the complex relationship between muscle and fascial structures in the groin and
47 abdominal region, this possible reduction in muscle activity could shift the balance of the forces
48 between the adductor and abdominal muscles thus influencing performance during diagnostic testing.
49 If muscle activation patterns change, it may be possible to maintain the same force output despite the
50 existence of a painful condition as shown in other pain states.^{13, 14} This may have clinical implications
51 with regards to the interpretation of clinical diagnostic tests due to alterations in muscle activity and
52 also the transition from acute into long-standing groin pain.¹⁵

53

54 Experimental pain caused by injection of hypertonic saline into tendons in healthy participants has
55 been shown to cause increased trunk muscle activity,^{16, 17} large pain referral patterns,^{16, 18} regional
56 hyperalgesia,^{16, 18, 19} and facilitated response to clinical orthopaedic tests for the hips and pelvic girdle.
57 ^{16, 18, 19} Therefore, a hypertonic saline model may provide insights into the effect of pain in the groin
58 region on the muscle activity, mechanical sensitivity, and referral patterns.

59

60 While many studies have focused on the diagnosis of groin pain in athletes, little is understood about
61 the effect of pain itself on the muscle activation during the diagnostic tests, pain referral patterns, and
62 mechanical sensitivity, all of which are recommended diagnostic criteria.⁴ This study aimed to
63 examine three hypotheses surrounding experimental pain at the proximal insertion of the adductor
64 longus: 1. The pain experienced can radiate superior to the pubic crest. 2. The pain experienced causes
65 alteration of EMG muscle activity patterns. 3. The pain experienced produces local deep tissue
66 hyperalgesia.

67 **Methods**

68 Fifteen healthy male participants were included for this study (mean \pm SD; age, 26.9 ± 3.4 years;
69 height, 183.9 ± 5.4 cm; weight, 81.5 ± 7.1 kg). Inclusion criteria were 1) no current or previous hip,
70 groin, or lumbar region injuries; 2) no signs of neurological disorders or rheumatologic diseases
71 which could affect the outcome of the experimental procedure; 3) no reported medication use either
72 on enrolment or on a regular basis; 4) currently participating in regular exercise or sport of total
73 duration of greater than or equal to 2.5 hours a week. Exclusion criteria were current injury, any
74 history of pain or injury in the hip, groin, lower abdominal or lumbar regions, a history of lower limb
75 injury in the previous 2 years, usage of cannabis, opioids or other drugs, current use of pain
76 medication, previous neurologic, musculoskeletal or mental illnesses, or lack of ability to cooperate.
77 Participants were given a detailed verbal and written explanation of the experimental procedure. All
78 participants provided written informed consent. The study was approved by the Danish Regional
79 Ethics Committee (N-20130036) and conducted in accordance with the Helsinki Declaration.

80

81 The experiment had a randomized, single-blinded, balanced-crossover, repeated-measures design
82 conducted in two sessions within one week. Randomisation was achieved through the selection of one
83 of 16 identical envelopes by an experimenter (blinded to the injector and experimenters) containing
84 one of all 16 possible order combinations of injection site, side, and injection site. Blinding was
85 achieved through unlabelled, identical pre-prepared syringes prior to the experimenters entering the
86 room. The participants were not advised of the order of injections at any stage throughout the
87 procedure.²⁰ Experimental groin pain and a painful control condition outside the groin area were
88 evaluated. Clinical provocation tests with recordings of the muscle activity and assessment of the
89 pressure pain sensitivity were administered at baseline, during and after (post-pain) experimental pain
90 with participant lying supine on a plinth. Prior to baseline testing, all participants were familiarised
91 with the experimental procedure and confirmed to be pain-free prior to commencing the study. The
92 post-pain state was defined as five minutes after the cessation of experimental pain.

93
94 The participants participated in two sessions and received one hypertonic and one isotonic saline
95 injection each session, one in each side of the same site (AL or RF) during each session. The alternate
96 site was injected in the following session. The order of the saline type (hypertonic or isotonic) and
97 site (AL or RF) and side (left or right) was randomised in a balanced way. Groin pain was induced by
98 injecting sterile hypertonic saline (1 ml, 5.8%) into the adductor longus (AL) tendon with isotonic
99 saline (1 ml, 0.9%) injected as a non-painful control into the same anatomical site on the contralateral
100 side within the same session. As a positive (painful) control injection outside the groin area, the
101 proximal tendon of the long head of the rectus femoris (RF) muscle was injected in a separate session.
102 The same volume of hypertonic or isotonic saline was injected into the control site as designated by
103 the randomisation. Participants and injector were blinded to saline type administered. All injections
104 were given by an orthopaedic surgeon (MI). After a standard disinfection protocol, the injections were
105 given over the duration of approximately 10 seconds using a 2-ml plastic syringe with a disposable
106 needle (27G). Pre-defined anatomical landmarks for injection sites for AL and RF tendons were
107 utilised. The location, depth and alignment of all injection sites were confirmed by real time

108 ultrasound (US) imaging (*Acuson 128XP10, NativeTM*). The AL tendon was identified using a
109 method previously described.¹⁸ Both the AL and RF injections positions followed a previously
110 published protocol (Supplement 1).²⁰

111
112 The pain intensity produced by hypertonic saline injections was assessed on a 10 cm electronic visual
113 analogue scale (VAS) which could be adjusted by using an external handheld slider. The VAS was
114 anchored with ‘no pain’ and ‘maximum pain’, 0 cm and 10 cm, respectively. A continuous recording
115 (sample frequency of 20 Hz) of the VAS signal was made after each injection until all pain had
116 subsided. For analysis, the area under VAS-time curve (VAS area) and VAS-peak were extracted.

117
118 The quality of pain was assessed once the pain had subsided. Participants were allowed to answer
119 using either the Danish²¹ or English²² version of the McGill Pain Questionnaire based upon their
120 **language preference**. The Danish results were converted to the English equivalent for analysis.
121 Participants were asked to mark their pain distribution by filling in a standard body chart. Body areas
122 were divided into groin regions by using the “Groin Triangle”.²³ **The groin triangle is defined as the**
123 **triangle created by the three landmarks: the anterior superior iliac spine (ASIS), pubic tubercle and the**
124 **median point between the ASIS and the superior pole of the patella in the anterior coronal plane (‘3G**
125 **point’).**²⁴ Local pain was defined as pain experienced only at the injection site and related “Groin
126 Triangle” segment while referred pain was defined as any pain felt outside the segment containing the
127 injection site. The body regions were analysed by registering the frequency of pain experienced in the
128 region for all four injections.

129
130 Pressure pain thresholds (PPTs) were assessed at regional and distant sites using a handheld pressure
131 algometer (*Somedic, Sweden*) with a 1 cm² probe and using a 30 kPa/s ramp. The four bilateral
132 assessment sites were the AL tendon injection site, the RF tendon injection site, the anterior surface of
133 the superior pubic rami (PB), and the tibialis anterior (TA) muscle, measured as the proximal site 1/3
134 the distance from the lateral joint line of the knee to the inferior aspect of the lateral malleolus. Each

135 measurement was recorded three times at baseline with two measurements recorded during pain and
136 post-pain to ensure all testing could be completed within the short-lasting window of saline-induced
137 pain. The average of the measurements was used for statistical analysis. PPT measurement was ceased
138 at 1200 kPa to avoid sensitisation after repeated assessments.

139

140 A battery of six pain provocation tests (Supplement 2) was employed with all tests performed by a
141 single clinically-trained experimenter (MD). All participants were confirmed to be pain-free on all
142 tests prior commencing the study. The tests administered were as previously published:²⁰ 1) Bilateral
143 adduction (squeeze) test with hips at 0° resisted at the ankles²⁵ 2) A bilateral squeeze test¹¹ with hips
144 flexed at 45° 3) A bilateral squeeze test¹¹ with hips flexed to 90° 4) Resisted abdominal crunch²⁵ 5)
145 Resisted oblique crunch, one side at a time.²⁵ The force of contraction was measured using a hand-
146 held dynamometer (*MicroFET2, Hoggan Health Industries, USA*) at baseline, during-pain and post-
147 pain. The reliability of the 0° adduction test is high (ICC = 0.97, minimal detectable change (%) =
148 6.6).²⁶ Verbal encouragement by the assessor was given to ensure force output remained constant for
149 each repetition (within 10% of baseline measures).

150

151 The skin at each assessment site was shaved, abraded and cleaned with alcohol in accordance with the
152 SENIAM guidelines.²⁷ Disposable electrodes (*Ambu®*, *Neuroline 720, Denmark*) were mounted
153 bilaterally with an inter-electrode distance of 20 mm in a bipolar configuration at the m. tensor fascia
154 latae (TFL), the m. adductor longus (AL), m. rectus abdominis (RA), and m. external obliques (EO).¹¹
155 ²⁸ A ground electrode was placed on the right wrist. The EMG signal from the AL muscle was used as
156 reference to determine the time window for the amplitude analysis (from onset to offset)²⁹ where the
157 root-mean-square (RMS) value was extracted for all muscles during all six tests for the middle epoch
158 defined as middle third of the period between onset and offset (see Supplement 1 for extended
159 methodology). The RMS value represents the muscle activity of the muscle. The onsets and offsets
160 were automatically detected based on the AL muscle EMG data as previously described in detail by
161 Santello et al.²⁹ All onset/offset detections were confirmed by visual inspection at each time point. No

162 manual correction of the data was required. Onsets and offsets were not analysed as the research
163 question investigated related to maximal muscle activity pre-, during and post-experimental pain
164 conditions rather than changes in the order of activation as a result of pain. Filtered EMG data was
165 utilised for analysis however filter and normalised data to baseline measures is reported in the
166 supplements for the ease of interpretation clinically.

167

168 All data was assessed for normality using the Kolmogorov–Smirnov test. Means and standard
169 deviations (SD) are presented for parametric data. All statistical analyses were performed using Stata
170 13 IC unless indicated (*StataCorp, USA*). An *a priori* estimate of group size indicated 15 participants
171 were required (estimated 20% difference in effect parameters; $\alpha=5\%$; $\beta=20\%$; coefficient of
172 variance=25%). The VAS area was analysed with an analysis of variance (ANOVA) with muscle (*AL*
173 *and RF*) and injection (*hypertonic and isotonic*) as independent factors. To assess the relationship of
174 PPTs and the injection site, side and injection type, a linear mixed-effect model (restricted maximum
175 likelihood [REML] regression) was fitted with PPT site (*AL, pubic bone, RF, and tibialis anterior*),
176 injection type (*hypertonic and isotonic*), side (ipsi- or contralateral) and injection site (*RF and AL*)
177 and time (*baseline, during or post*) and their interactions as fixed-effects. For analysis, filtered EMG
178 data was utilised to assess the relationship between mean RMS-EMG of each clinical test and the
179 effects of injection type (*isotonic and hypertonic*), time point (*baseline, during, post-pain*), each
180 muscle (*AL, TFL, EO, RA*), injection site (*AL and RF*) and side (*ipsilateral and contralateral*) and
181 their interactions with a random effect for participant in a General Linear Mixed Model using the R
182 package lme4 (R Core Team, 2016).³⁰ This approach can handle missing data which created an
183 unbalanced design.³¹ Means were analysed *post-hoc* to explain significant effects. Bonferroni
184 correction was applied where multiple *post-hoc* analyses were undertaken. Significance was set at
185 $p<0.05$ for all statistical tests.

186

187 **Results**

188 The VAS area after hypertonic saline injected into the AL (13112 ± 11147 mm·s) and RF ($12110 \pm$
189 8829 mm·s) tendons were higher compared with isotonic saline (AL: 206 ± 405 mm·s; RF: $815 \pm$
190 2037 mm·s; ANOVA: $F(2,53)=20.05$, $p<0.001$). The VAS-peaks reported for each test condition
191 were AL isotonic (2 ± 4 mm), AL hypertonic (22 ± 12 mm), RF isotonic (4 ± 7 mm), and RF
192 hypertonic (22 ± 12 mm). The three most common words to describe the sensation after the AL tendon
193 hypertonic injections were “annoying” (33% of participants), “tugging” (27%) and “pressing” (27%)
194 whereas the three most common descriptions after the RF tendon hypertonic injections “tight” (47%),
195 “pressing” (33%), “annoying” (27%) for RF tendon.

196

197 Hypertonic saline-induced pain in the AL tendon primarily demonstrated a local pattern of pain where
198 it was mainly perceived within and medial to the “Groin Triangle” but also in the lower abdominal
199 region (Figure 1, Table 1). Injections of hypertonic saline into the RF tendon primarily caused pain
200 experienced within the triangle and the anterior and lateral thigh indicating a larger pain referral
201 pattern. During isotonic saline injections into the RF tendon, 11 participants drew the pain on the
202 anterior thigh. Pain in the contralateral side to the injection was also reported in one participant in
203 three areas (Figure 1) after the hypertonic injection into the RF tendon. No participants reported pain
204 on the contralateral side with an absence of pain in the ipsilateral injection side. Therefore, these
205 reports should be considered as bilateral pain distributions.

206

207 PPT values did not significantly change across time periods under any conditions. Significant fixed
208 effects were observed for the RF (REML: Coeff=362.5, 95%CI 265.8-564.2, $p<0.001$) and TA sites
209 (REML: Coeff=469.8, 95%CI 374.8-561.8, $p<0.001$) indicating that the TA and RF sites were
210 generally higher than the adductor and pubic sites. However, no significant fixed effects or
211 interactions were observed with the inclusion of time ($p=0.27-0.99$). As time was not a significant
212 fixed effect, this can be interpreted as the PPT values were not significantly influenced by
213 experimental pain conditions. The distributions of PPT values across the experimental conditions and
214 time points are presented in Figure 2.

215

216 The magnitude of the muscle activity did not change significantly across time periods under any
217 conditions when compared to baseline conditions. Normalised RMS-EMG for the “during” and “post”
218 conditions are presented in Supplementary Tables 1-4. A five-way interaction between clinical test,
219 injection type, muscle, injection site and side was observed ($F(15,7771)=8.68, p<0.001$) however time
220 was not a significant fixed effect in the model or any interactions. As time was not a significant fixed
221 effect it can be interpreted as the muscle activation patterns of the four muscles varied across the
222 clinical tests, injection type and site, and side when compared to each other yet were not significantly
223 uninfluenced by the experimental pain. Therefore, no *post-hoc* analyses were performed.

224

225 Discussion

226 This is the first study to report the muscle activation pattern involved in commonly used clinical tests
227 for groin pain and mechanical sensitivity of the lower limb in an experimental pain model. This study
228 aimed to examine three hypotheses surrounding experimental pain at the proximal insertion of the
229 adductor longus. The results of this study support the hypothesis that experimental pain in the
230 proximal adductor longus can proximally refer to the lower abdomen and may explain why pain can
231 be experienced in both locations clinically. This study fails to provide evidence that experimental pain
232 in the AL alters the muscle activity and produces local or widespread deep tissue hyperalgesia. These
233 findings have implications for clinical assessment particularly related to diagnostic or classification
234 criteria which rely on pain referral patterns as they can be influenced by region structures.

235

236 The AL tendon produced a local pain distribution contained mainly medial to and within the “Groin
237 Triangle”. Moreover, in 33% of participants the tendon of adductor longus was capable of provoking
238 proximal referral into the lower abdominal region. This has clinical relevance as it is commonly
239 reported in the literature that multiple pathologies or clinical entities exist in athletes with groin pain.⁵
240 Experimentally-induced AL tendon pain is capable of producing false positive test results with
241 abdominal manoeuvres.²⁰ Therefore, comprehensive clinical assessment is required to rule out

242 involvement of AL tendon when pain in the lower abdomen is present particularly when coexisting
243 with pain in the upper inner thigh. **The results of experimental pain models²⁰ indicate that 45° and 90°**
244 **adduction tests have the best negative likelihood ratio, suggesting their utility to rule out adductor**
245 **longus as a potential source of nociception.** The positive control condition (experimental RF tendon
246 pain) produced a greater distribution of pain covering the regions within, lateral to and superior to the
247 groin triangle although no pain was reported medial to the triangle. Bilateral leg pain distribution was
248 produced in one participant under the RF tendon hypertonic and isotonic saline conditions. This
249 represents an unusual pain referral pattern that is not typically observed clinically and may be related
250 to individual characteristics of the participant.

251
252 In the present study, pain induced in **adductor** and thigh regions was unable to alter the mechanical
253 sensitivity. Primary mechanical hyperalgesia of the adductor longus tendon has been reported in
254 Australian football players currently experiencing groin pain.¹ **This** indicates the hypertonic saline
255 tendon pain model may not replicate the clinical pain presentations of groin region. Proximal
256 (secondary) hyperalgesia has been hypothesised to be explained by amplification of central pain
257 mechanisms.³² No change was observed at the pubic bone or distally on either sides which concurs
258 with clinical pain studies of the groin region.¹ The diagnostic criteria for adductor-related groin pain
259 are pain on resisted adduction tests with tenderness (**mechanical sensitivity**) on palpation.⁴ In acute
260 groin injuries, palpation (**mechanical sensitivity**) has the greatest diagnostic capacity to predict MRI
261 findings.³³ In the present study, no changes **were observed at the site of the injection or on** the pubic
262 bone PTTs under the AL or RF ipsilateral hypertonic saline-induced pain indicating **secondary**
263 **mechanical** hyperalgesia is less of a concern for this site. **Therefore,** hyperalgesia of the pubic bone
264 may represent local mechanical hyperalgesia rather than regional/widespread pain and as such may be
265 implicated as a nociceptive driver. **Clinically, mechanical sensitivity (tenderness on palpation) at the**
266 **pubic enthesis may represent local nociception rather than a consequence of adductor tendon pain (as**
267 **in the case of secondary hyperalgesia). Confirmation in the clinical setting is warranted however.**

268

269 The magnitude of muscle activity in the region during the painful condition was not statistically
270 significantly different from the baseline condition. This is hypothesised to be due to the study design
271 in which force was maintained equal to baseline measures. This indicates that irrespective of pain in
272 the region, the motor cortex may allow for the task to be completed with equal force production. The
273 0° adduction test has been suggested to be diagnostically superior to identify experimentally-induced,
274 adductor-related pain.²⁰ However, the results of this paper indicate that changes in muscle activation
275 less likely to be associated with the diagnostic capabilities reported. Again, this hypothesis should be
276 tested in clinical populations.

277
278 This study allowed the evaluation of the outcome measures under controlled conditions. This removes
279 the complications of multiple pathologies detected on clinical assessment⁵ and imaging⁸ in athletes
280 with groin pain. Nonetheless, pain generated from experimental models differs from clinical pain¹⁸
281 and replication of the results in clinical populations is warranted as previously indicated. In the
282 analysis of PPT and EMG data, a unified linear mixed model was chosen given its ability to account
283 for the characteristics of the data and to reduce the Type I error associated with multiple sub-grouping
284 analyses. The lack of positive findings observed may be potentially explained by lower power
285 however this is offset by the degrees of freedom created by every participant undertaking each
286 component of the study. Significant variability in the data was observed in the PPT and the level of
287 pain (VAS) measures across participants. This variability reduced the ability to obtain significant
288 effects; an increase in sample size is unlikely to alter the results and are likely to represent the
289 individual nature of the response to pain. *Post-hoc* power analyses are therefore not indicated.³⁴

290

291 Conclusion

292 This study has shown that pain arising from the adductor longus tendon is locally distributed in the
293 majority (80%) but capable of producing pain superior to the pubic crest in 33% of participants. PPTs
294 were not altered by experimental pain induced by hypertonic saline. An alteration of the magnitude of
295 EMG activity of the adductor longus, tensor fascia latae, rectus abdominis and external obliques was

296 not detected under experimental pain conditions when force was matched to baseline measures.
297 Therefore, diagnostic criteria based on pain distribution alone may be influenced by pain itself in the
298 region and may not represent tissue pathology or multiple clinical entities of groin pain.

299

300

301

302 **Practical Implications**

- 303 • The adductor longus tendon has a local pattern of pain distribution however can refer
304 proximally to the lower abdominal region.
- 305 • Diagnostic criteria based on pain distribution are potentially influenced by pain itself in the
306 region and may not represent tissue pathology.

307

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312

313 **Conflict of interest**

314 There are no conflicts of interest of the authors.

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397

398

TABLES

Table 1 Frequency of pain relative to the “Groin Triangle” following injections of hypertonic and isotonic saline into the adductor longus and rectus femoris tendons.

	Adductor Longus Tendon				Rectus Femoris Tendon			
	Isotonic saline		Hypertonic saline		Isotonic saline		Hypertonic saline	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
“Groin Triangle”								
Within the triangle	3 (20)	0	12 (80 %)	0	4 (27%)	1 (7%)	15 (100%)	2 (13%)
Lateral to the triangle	0	0	1 (7 %)	0	0	0	2 (13%)	0
Medial to the triangle	7 (47)	0	12 (80%)	0	0	0	0	0
Superior to the triangle	0	0	5 (33 %)	0	0	0	1 (7%)	0
<i>Other areas</i>								
Greater Trochanter	0	0	0	0	0	0	0	2 (13%)
Anterior Thigh	0	0	1 (7 %)	0	2 (13%)	0	5 (33%)	2 (13%)
Lateral Thigh	0	0	0	0	0	0	4 (27%)	1 (7%)
Knee	0	0	0	0	0	0	0	1 (7%)
Lower Leg	0	0	0	0	0	0	1 (7%)	2 (13%)
Foot	0	0	0	0	0	0	0	0

Contralateral/Ipsilateral relative to the side of injection; frequencies reported as number of responses (percentage)

FIGURE LEGENDS

Figure 1 Pain distributions of the adductor longus are indicated on the body chart's right side.

Figure 2 Distribution of the pressure pain thresholds at baseline, during pain and post-pain across injection types and sites represented as a box-plot.

1 **ABSTRACT**

2 **Objectives:** To investigate the effects of experimental adductor pain on the pain referral pattern,
3 mechanical sensitivity and muscle activity during common clinical tests.

4
5 **Design:** Repeated-measures design

6
7 **Methods:** In two separate sessions, 15 healthy males received a hypertonic (painful) and isotonic
8 (control) saline injection to either the adductor longus (AL) tendon to produce experimental groin
9 pain or into the rectus femoris (RF) tendon as a painful control. Pain intensity was recorded on a
10 visual analogue scale (VAS) with pain distribution indicated on body maps. Pressure pain thresholds
11 (PPT) were assessed bilaterally in the groin area. Electromyography (EMG) of relevant muscles was
12 recorded during six provocation tests. PPT and EMG assessment were measured before, during and
13 after experimental pain.

14
15 **Results:** Hypertonic saline induced higher VAS scores than isotonic saline ($p < 0.001$), and a local pain
16 distribution in 80% of participants. A proximal pain referral to the lower abdominal region in 33%
17 (AL) and 7% (RF) of participants. Experimental pain (AL and RF) did not significantly alter PPT
18 values or the EMG amplitude in groin or trunk muscles during provocation tests when forces were
19 matched with baseline.

20
21 **Conclusions:** This study demonstrates that AL tendon pain was distributed locally in the majority of
22 participants but may refer to the lower abdomen. Experimental adductor pain did not significantly
23 alter the mechanical sensitivity or muscle activity patterns.

24
25 **Key Words:** athlete; EMG; pressure pain sensitivity; adductor longus tendon; rectus femoris tendon

26 **Introduction**

27 The prevalence of hip and groin pain in athletes is generally high with a career prevalence of 45%
28 reported in professional Australian football players¹ and a high incidence in sports such as football²
29 and ice hockey.³ Adductor-related groin pain is characterised as pain on resisted adduction and pain
30 on palpation of the adductor longus muscle.⁴ In contrast, abdominal symptoms present with pain on
31 resisted trunk flexion and pain on palpation of the rectus abdominis distal enthesis.⁵ Yet
32 characteristics of groin pain per se are poorly understood with few reports of pain referral patterns and
33 clinical symptomatology. Pain referral patterns are typically semi- (referring distally) or bi-directional
34 (referring both distally and proximally) with referred pain distributions extending to neighbouring
35 vertebral segments that are supplying the painful muscle or tendon.⁶ Clinically, pain in both the
36 adductor and abdominal area is associated with longer recovery times compared to a single site.⁷ The
37 role of pain referral patterns has not previously been examined and may present a plausible alternate
38 hypothesis to co-existing pain locations^{5, 8-10} in this region. That is, abdominal pain may present
39 clinically as a result of referred pain from the adductor region. If this is true, it challenges using pain
40 location alone as diagnostic criteria in either classifying patients into entities or to specific
41 pathoanatomical tissue diagnoses.

42

43 Electromyographic (EMG) muscle activity has been shown to be significantly reduced in m. adductor
44 longus, m. pectineus, and m. gracilis, in patients with a history of groin pain during clinical tests when
45 compared to healthy activity-matched-controls.¹¹ Such changes occur soon after the initiating painful
46 event.¹² Given the complex relationship between muscle and fascial structures in the groin and
47 abdominal region, this possible reduction in muscle activity could shift the balance of the forces
48 between the adductor and abdominal muscles thus influencing performance during diagnostic testing.
49 If muscle activation patterns change, it may be possible to maintain the same force output despite the
50 existence of a painful condition as shown in other pain states.^{13, 14} This may have clinical implications
51 with regards to the interpretation of clinical diagnostic tests due to alterations in muscle activity and
52 also the transition from acute into long-standing groin pain.¹⁵

53

54 Experimental pain caused by injection of hypertonic saline into tendons in healthy participants has
55 been shown to cause increased trunk muscle activity,^{16, 17} large pain referral patterns,^{16, 18} regional
56 hyperalgesia,^{16, 18, 19} and facilitated response to clinical orthopaedic tests for the hips and pelvic girdle.
57 ^{16, 18, 19} Therefore, a hypertonic saline model may provide insights into the effect of pain in the groin
58 region on the muscle activity, mechanical sensitivity, and referral patterns.

59

60 While many studies have focused on the diagnosis of groin pain in athletes, little is understood about
61 the effect of pain itself on the muscle activation during the diagnostic tests, pain referral patterns, and
62 mechanical sensitivity, all of which are recommended diagnostic criteria.⁴ This study aimed to
63 examine three hypotheses surrounding experimental pain at the proximal insertion of the adductor
64 longus: 1. The pain experienced can radiate superior to the pubic crest. 2. The pain experienced causes
65 alteration of EMG muscle activity patterns. 3. The pain experienced produces local deep tissue
66 hyperalgesia.

67 **Methods**

68 Fifteen healthy male participants were included for this study (mean \pm SD; age, 26.9 ± 3.4 years;
69 height, 183.9 ± 5.4 cm; weight, 81.5 ± 7.1 kg). Inclusion criteria were 1) no current or previous hip,
70 groin, or lumbar region injuries; 2) no signs of neurological disorders or rheumatologic diseases
71 which could affect the outcome of the experimental procedure; 3) no reported medication use either
72 on enrolment or on a regular basis; 4) currently participating in regular exercise or sport of total
73 duration of greater than or equal to 2.5 hours a week. Exclusion criteria were current injury, any
74 history of pain or injury in the hip, groin, lower abdominal or lumbar regions, a history of lower limb
75 injury in the previous 2 years, usage of cannabis, opioids or other drugs, current use of pain
76 medication, previous neurologic, musculoskeletal or mental illnesses, or lack of ability to cooperate.
77 Participants were given a detailed verbal and written explanation of the experimental procedure. All
78 participants provided written informed consent. The study was approved by the Danish Regional
79 Ethics Committee (N-20130036) and conducted in accordance with the Helsinki Declaration.

80

81 The experiment had a randomized, single-blinded, balanced-crossover, repeated-measures design
82 conducted in two sessions within one week. Randomisation was achieved through the selection of one
83 of 16 identical envelopes by an experimenter (blinded to the injector and experimenters) containing
84 one of all 16 possible order combinations of injection site, side, and injection site. Blinding was
85 achieved through unlabelled, identical pre-prepared syringes prior to the experimenters entering the
86 room. The participants were not advised of the order of injections at any stage throughout the
87 procedure.²⁰ Experimental groin pain and a painful control condition outside the groin area were
88 evaluated. Clinical provocation tests with recordings of the muscle activity and assessment of the
89 pressure pain sensitivity were administered at baseline, during and after (post-pain) experimental pain
90 with participant lying supine on a plinth. Prior to baseline testing, all participants were familiarised
91 with the experimental procedure and confirmed to be pain-free prior to commencing the study. The
92 post-pain state was defined as five minutes after the cessation of experimental pain.

93

94 The participants participated in two sessions and received one hypertonic and one isotonic saline
95 injection each session, one in each side of the same site (AL or RF) during each session. The alternate
96 site was injected in the following session. The order of the saline type (hypertonic or isotonic) and
97 site (AL or RF) and side (left or right) was randomised in a balanced way. Groin pain was induced by
98 injecting sterile hypertonic saline (1 ml, 5.8%) into the adductor longus (AL) tendon with isotonic
99 saline (1 ml, 0.9%) injected as a non-painful control into the same anatomical site on the contralateral
100 side within the same session. As a positive (painful) control injection outside the groin area, the
101 proximal tendon of the long head of the rectus femoris (RF) muscle was injected in a separate session.
102 The same volume of hypertonic or isotonic saline was injected into the control site as designated by
103 the randomisation. Participants and injector were blinded to saline type administered. All injections
104 were given by an orthopaedic surgeon (MI). After a standard disinfection protocol, the injections were
105 given over the duration of approximately 10 seconds using a 2-ml plastic syringe with a disposable
106 needle (27G). Pre-defined anatomical landmarks for injection sites for AL and RF tendons were
107 utilised. The location, depth and alignment of all injection sites were confirmed by real time

108 ultrasound (US) imaging (*Acuson 128XP10, NativeTM*). The AL tendon was identified using a
109 method previously described.¹⁸ Both the AL and RF injections positions followed a previously
110 published protocol (Supplement 1).²⁰

111
112 The pain intensity produced by hypertonic saline injections was assessed on a 10 cm electronic visual
113 analogue scale (VAS) which could be adjusted by using an external handheld slider. The VAS was
114 anchored with ‘no pain’ and ‘maximum pain’, 0 cm and 10 cm, respectively. A continuous recording
115 (sample frequency of 20 Hz) of the VAS signal was made after each injection until all pain had
116 subsided. For analysis, the area under VAS-time curve (VAS area) and VAS-peak were extracted.

117
118 The quality of pain was assessed once the pain had subsided. Participants were allowed to answer
119 using either the Danish²¹ or English²² version of the McGill Pain Questionnaire based upon their
120 language preference. The Danish results were converted to the English equivalent for analysis.
121 Participants were asked to mark their pain distribution by filling in a standard body chart. Body areas
122 were divided into groin regions by using the “Groin Triangle”.²³ The groin triangle is defined as the
123 triangle created by the three landmarks: the anterior superior iliac spine (ASIS), pubic tubercle and the
124 median point between the ASIS and the superior pole of the patella in the anterior coronal plane (‘3G
125 point’).²⁴ Local pain was defined as pain experienced only at the injection site and related “Groin
126 Triangle” segment while referred pain was defined as any pain felt outside the segment containing the
127 injection site. The body regions were analysed by registering the frequency of pain experienced in the
128 region for all four injections.

129
130 Pressure pain thresholds (PPTs) were assessed at regional and distant sites using a handheld pressure
131 algometer (*Somedic, Sweden*) with a 1 cm² probe and using a 30 kPa/s ramp. The four bilateral
132 assessment sites were the AL tendon injection site, the RF tendon injection site, the anterior surface of
133 the superior pubic rami (PB), and the tibialis anterior (TA) muscle, measured as the proximal site 1/3
134 the distance from the lateral joint line of the knee to the inferior aspect of the lateral malleolus. Each

135 measurement was recorded three times at baseline with two measurements recorded during pain and
136 post-pain to ensure all testing could be completed within the short-lasting window of saline-induced
137 pain. The average of the measurements was used for statistical analysis. PPT measurement was ceased
138 at 1200 kPa to avoid sensitisation after repeated assessments.

139

140 A battery of six pain provocation tests (Supplement 2) was employed with all tests performed by a
141 single clinically-trained experimenter (MD). All participants were confirmed to be pain-free on all
142 tests prior commencing the study. The tests administered were as previously published:²⁰ 1) Bilateral
143 adduction (squeeze) test with hips at 0° resisted at the ankles²⁵ 2) A bilateral squeeze test¹¹ with hips
144 flexed at 45° 3) A bilateral squeeze test¹¹ with hips flexed to 90° 4) Resisted abdominal crunch²⁵ 5)
145 Resisted oblique crunch, one side at a time.²⁵ The force of contraction was measured using a hand-
146 held dynamometer (*MicroFET2, Hoggan Health Industries, USA*) at baseline, during-pain and post-
147 pain. The reliability of the 0° adduction test is high (ICC = 0.97, minimal detectable change (%) =
148 6.6).²⁶ Verbal encouragement by the assessor was given to ensure force output remained constant for
149 each repetition (within 10% of baseline measures).

150

151 The skin at each assessment site was shaved, abraded and cleaned with alcohol in accordance with the
152 SENIAM guidelines.²⁷ Disposable electrodes (*Ambu®*, *Neuroline 720, Denmark*) were mounted
153 bilaterally with an inter-electrode distance of 20 mm in a bipolar configuration at the m. tensor fascia
154 latae (TFL), the m. adductor longus (AL), m. rectus abdominis (RA), and m. external obliques (EO).¹¹
155 ²⁸ A ground electrode was placed on the right wrist. The EMG signal from the AL muscle was used as
156 reference to determine the time window for the amplitude analysis (from onset to offset)²⁹ where the
157 root-mean-square (RMS) value was extracted for all muscles during all six tests for the middle epoch
158 defined as middle third of the period between onset and offset (see Supplement 1 for extended
159 methodology). The RMS value represents the muscle activity of the muscle. The onsets and offsets
160 were automatically detected based on the AL muscle EMG data as previously described in detail by
161 Santello et al.²⁹ All onset/offset detections were confirmed by visual inspection at each time point. No

162 manual correction of the data was required. Onsets and offsets were not analysed as the research
163 question investigated related to maximal muscle activity pre-, during and post-experimental pain
164 conditions rather than changes in the order of activation as a result of pain. Filtered EMG data was
165 utilised for analysis however filter and normalised data to baseline measures is reported in the
166 supplements for the ease of interpretation clinically.

167

168 All data was assessed for normality using the Kolmogorov–Smirnov test. Means and standard
169 deviations (SD) are presented for parametric data. All statistical analyses were performed using Stata
170 13 IC unless indicated (*StataCorp, USA*). An *a priori* estimate of group size indicated 15 participants
171 were required (estimated 20% difference in effect parameters; $\alpha=5\%$; $\beta=20\%$; coefficient of
172 variance=25%). The VAS area was analysed with an analysis of variance (ANOVA) with muscle (*AL*
173 *and RF*) and injection (*hypertonic and isotonic*) as independent factors. To assess the relationship of
174 PPTs and the injection site, side and injection type, a linear mixed-effect model (restricted maximum
175 likelihood [REML] regression) was fitted with PPT site (*AL, pubic bone, RF, and tibialis anterior*),
176 injection type (*hypertonic and isotonic*), side (ipsi- or contralateral) and injection site (*RF and AL*)
177 and time (*baseline, during or post*) and their interactions as fixed-effects. For analysis, filtered EMG
178 data was utilised to assess the relationship between mean RMS-EMG of each clinical test and the
179 effects of injection type (*isotonic and hypertonic*), time point (*baseline, during, post-pain*), each
180 muscle (*AL, TFL, EO, RA*), injection site (*AL and RF*) and side (*ipsilateral and contralateral*) and
181 their interactions with a random effect for participant in a General Linear Mixed Model using the R
182 package *lme4* (R Core Team, 2016).³⁰ This approach can handle missing data which created an
183 unbalanced design.³¹ Means were analysed *post-hoc* to explain significant effects. Bonferroni
184 correction was applied where multiple *post-hoc* analyses were undertaken. Significance was set at
185 $p<0.05$ for all statistical tests.

186

187 **Results**

188 The VAS area after hypertonic saline injected into the AL (13112 ± 11147 mm·s) and RF ($12110 \pm$
189 8829 mm·s) tendons were higher compared with isotonic saline (AL: 206 ± 405 mm·s; RF: $815 \pm$
190 2037 mm·s; ANOVA: $F(2,53)=20.05$, $p<0.001$). The VAS-peaks reported for each test condition
191 were AL isotonic (2 ± 4 mm), AL hypertonic (22 ± 12 mm), RF isotonic (4 ± 7 mm), and RF
192 hypertonic (22 ± 12 mm).The three most common words to describe the sensation after the AL tendon
193 hypertonic injections were “annoying” (33% of participants), “tugging” (27%) and “pressing” (27%)
194 whereas the three most common descriptions after the RF tendon hypertonic injections “tight” (47%),
195 “pressing” (33%), “annoying” (27%) for RF tendon.

196

197 Hypertonic saline-induced pain in the AL tendon primarily demonstrated a local pattern of pain where
198 it was mainly perceived within and medial to the “Groin Triangle” but also in the lower abdominal
199 region (Figure 1, Table 1). Injections of hypertonic saline into the RF tendon primarily caused pain
200 experienced within the triangle and the anterior and lateral thigh indicating a larger pain referral
201 pattern. During isotonic saline injections into the RF tendon, 11 participants drew the pain on the
202 anterior thigh. Pain in the contralateral side to the injection was also reported in one participant in
203 three areas (Supplementary 3) after the hypertonic injection into the RF tendon. No participants
204 reported pain on the contralateral side with an absence of pain in the ipsilateral injection side.
205 Therefore, these reports should be considered as bilateral pain distributions.

206

207 PPT values did not significantly change across time periods under any conditions. Significant fixed
208 effects were observed for the RF (REML: Coeff=362.5, 95%CI 265.8-564.2, $p<0.001$) and TA sites
209 (REML: Coeff=469.8, 95%CI 374.8-561.8, $p<0.001$) indicating that the TA and RF sites were
210 generally higher than the adductor and pubic sites. However, no significant fixed effects or
211 interactions were observed with the inclusion of time ($p=0.27-0.99$). As time was not a significant
212 fixed effect, this can be interpreted as the PPT values were not significantly influenced by
213 experimental pain conditions. The distributions of PPT values across the experimental conditions and
214 time points are presented in Figure 2.

215

216 The magnitude of the muscle activity did not change significantly across time periods under any
217 conditions when compared to baseline conditions. Normalised RMS-EMG for the “during” and “post”
218 conditions are presented in Supplementary Tables 1-4. A five-way interaction between clinical test,
219 injection type, muscle, injection site and side was observed ($F(15,7771)=8.68, p<0.001$) however time
220 was not a significant fixed effect in the model or any interactions. As time was not a significant fixed
221 effect it can be interpreted as the muscle activation patterns of the four muscles varied across the
222 clinical tests, injection type and site, and side when compared to each other yet were not significantly
223 uninfluenced by the experimental pain. Therefore, no *post-hoc* analyses were performed.

224

225 **Discussion**

226 This is the first study to report the muscle activation pattern involved in commonly used clinical tests
227 for groin pain and mechanical sensitivity of the lower limb in an experimental pain model. This study
228 aimed to examine three hypotheses surrounding experimental pain at the proximal insertion of the
229 adductor longus. The results of this study support the hypothesis that experimental pain in the
230 proximal adductor longus can proximally refer to the lower abdomen and may explain why pain can
231 be experienced in both locations clinically. This study fails to provide evidence that experimental pain
232 in the AL alters the muscle activity and produces local or widespread deep tissue hyperalgesia. These
233 findings have implications for clinical assessment particularly related to diagnostic or classification
234 criteria which rely on pain referral patterns as they can be influenced by region structures.

235

236 The AL tendon produced a local pain distribution contained mainly medial to and within the “Groin
237 Triangle”. Moreover, in 33% of participants the tendon of adductor longus was capable of provoking
238 proximal referral into the lower abdominal region. This has clinical relevance as it is commonly
239 reported in the literature that multiple pathologies or clinical entities exist in athletes with groin pain.⁵
240 Experimentally-induced AL tendon pain is capable of producing false positive test results with
241 abdominal manoeuvres.²⁰ Therefore, comprehensive clinical assessment is required to rule out

242 involvement of AL tendon when pain in the lower abdomen is present particularly when coexisting
243 with pain in the upper inner thigh. The results of experimental pain models²⁰ indicate that 45° and 90°
244 adduction tests have the best negative likelihood ratio, suggesting their utility to rule out adductor
245 longus as a potential source of nociception. The positive control condition (experimental RF tendon
246 pain) produced a greater distribution of pain covering the regions within, lateral to and superior to the
247 groin triangle although no pain was reported medial to the triangle. Bilateral leg pain distribution was
248 produced in one participant under the RF tendon hypertonic and isotonic saline conditions. This
249 represents an unusual pain referral pattern that is not typically observed clinically and may be related
250 to individual characteristics of the participant.

251

252 In the present study, pain induced in adductor and thigh regions was unable to alter the mechanical
253 sensitivity. Primary mechanical hyperalgesia of the adductor longus tendon has been reported in
254 Australian football players currently experiencing groin pain.¹ This indicates the hypertonic saline
255 tendon pain model may not replicate the clinical pain presentations of groin region. Proximal
256 (secondary) hyperalgesia has been hypothesised to be explained by amplification of central pain
257 mechanisms.³² No change was observed at the pubic bone or distally on either sides which concurs
258 with clinical pain studies of the groin region.¹ The diagnostic criteria for adductor-related groin pain
259 are pain on resisted adduction tests with tenderness (mechanical sensitivity) on palpation.⁴ In acute
260 groin injuries, palpation (mechanical sensitivity) has the greatest diagnostic capacity to predict MRI
261 findings.³³ In the present study, no changes were observed at the site of the injection or on the pubic
262 bone PTTs under the AL or RF ipsilateral hypertonic saline-induced pain indicating secondary
263 mechanical hyperalgesia is less of a concern for this site. Therefore, hyperalgesia of the pubic bone
264 may represent local mechanical hyperalgesia rather than regional/widespread pain and as such may be
265 implicated as a nociceptive driver. Clinically, mechanical sensitivity (tenderness on palpation) at the
266 pubic enthesis may represent local nociception rather than a consequence of adductor tendon pain (as
267 in the case of secondary hyperalgesia). Confirmation in the clinical setting is warranted however.

268

269 The magnitude of muscle activity in the region during the painful condition was not statistically
270 significantly different from the baseline condition. This is hypothesised to be due to the study design
271 in which force was maintained equal to baseline measures. This indicates that irrespective of pain in
272 the region, the motor cortex may allow for the task to be completed with equal force production. The
273 0° adduction test has been suggested to be diagnostically superior to identify experimentally-induced,
274 adductor-related pain.²⁰ However, the results of this paper indicate that changes in muscle activation
275 less likely to be associated with the diagnostic capabilities reported. Again, this hypothesis should be
276 tested in clinical populations.

277
278 This study allowed the evaluation of the outcome measures under controlled conditions. This removes
279 the complications of multiple pathologies detected on clinical assessment⁵ and imaging⁸ in athletes
280 with groin pain. Nonetheless, pain generated from experimental models differs from clinical pain¹⁸
281 and replication of the results in clinical populations is warranted as previously indicated. In the
282 analysis of PPT and EMG data, a unified linear mixed model was chosen given its ability to account
283 for the characteristics of the data and to reduce the Type I error associated with multiple sub-grouping
284 analyses. The lack of positive findings observed may be potentially explained by lower power
285 however this is offset by the degrees of freedom created by every participant undertaking each
286 component of the study. Significant variability in the data was observed in the PPT and the level of
287 pain (VAS) measures across participants. This variability reduced the ability to obtain significant
288 effects; an increase in sample size is unlikely to alter the results and are likely to represent the
289 individual nature of the response to pain. *Post-hoc* power analyses are therefore not indicated.³⁴

290

291 **Conclusion**

292 This study has shown that pain arising from the adductor longus tendon is locally distributed in the
293 majority (80%) but capable of producing pain superior to the pubic crest in 33% of participants. PPTs
294 were not altered by experimental pain induced by hypertonic saline. An alteration of the magnitude of
295 EMG activity of the adductor longus, tensor fascia latae, rectus abdominis and external obliques was

296 not detected under experimental pain conditions when force was matched to baseline measures.
297 Therefore, diagnostic criteria based on pain distribution alone may be influenced by pain itself in the
298 region and may not represent tissue pathology or multiple clinical entities of groin pain.

299

300

301

302 **Practical Implications**

- 303 • The adductor longus tendon has a local pattern of pain distribution however can refer
304 proximally to the lower abdominal region.
- 305 • Diagnostic criteria based on pain distribution are potentially influenced by pain itself in the
306 region and may not represent tissue pathology.

307

308 **Acknowledgements**

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312

313 **Conflict of interest**

314 There are no conflicts of interest of the authors.

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398

TABLES

Table 1 Frequency of pain relative to the “Groin Triangle” following injections of hypertonic and isotonic saline into the adductor longus and rectus femoris tendons.

	Adductor Longus Tendon				Rectus Femoris Tendon			
	Isotonic saline		Hypertonic saline		Isotonic saline		Hypertonic saline	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
“Groin Triangle”								
Within the triangle	3 (20)	0	12 (80 %)	0	4 (27%)	1 (7%)	15 (100%)	2 (13%)
Lateral to the triangle	0	0	1 (7 %)	0	0	0	2 (13%)	0
Medial to the triangle	7 (47)	0	12 (80%)	0	0	0	0	0
Superior to the triangle	0	0	5 (33 %)	0	0	0	1 (7%)	0
<i>Other areas</i>								
Greater Trochanter	0	0	0	0	0	0	0	2 (13%)
Anterior Thigh	0	0	1 (7 %)	0	2 (13%)	0	5 (33%)	2 (13%)
Lateral Thigh	0	0	0	0	0	0	4 (27%)	1 (7%)
Knee	0	0	0	0	0	0	0	1 (7%)
Lower Leg	0	0	0	0	0	0	1 (7%)	2 (13%)
Foot	0	0	0	0	0	0	0	0

Contralateral/Ipsilateral relative to the side of injection; frequencies reported as number of responses (percentage)

FIGURE LEGENDS

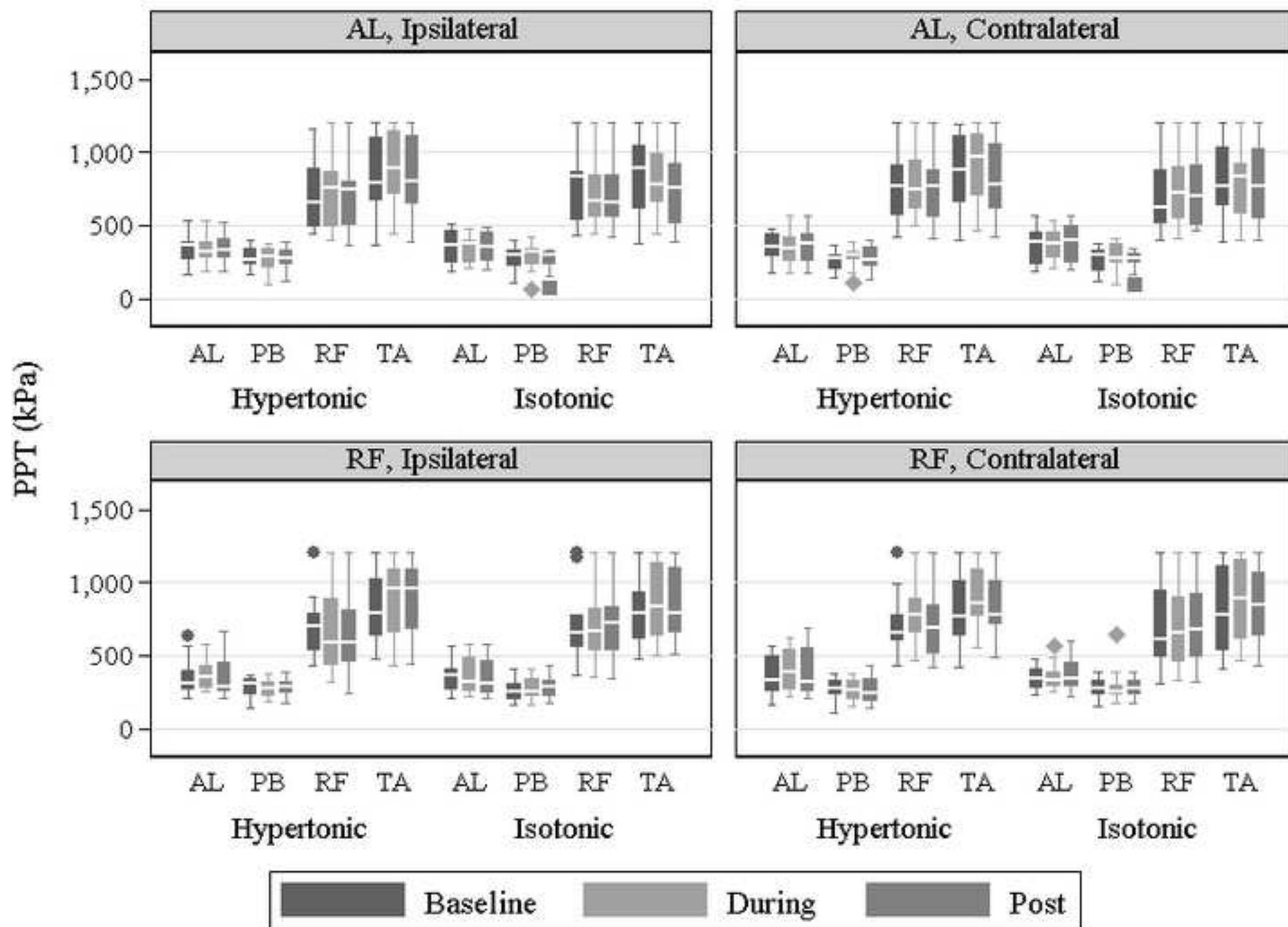
Figure 1 Pain distributions of the adductor longus are indicated on the body chart's right side.

Figure 2 Distribution of the pressure pain thresholds at baseline, during pain and post-pain across injection types and sites represented as a box-plot.

Figure 1
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Figure 2
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Supplement 1 – Extended methodology**Injection position**

The AL tendon was identified using a method previously described.¹³ The participant lay supine with the leg to be injected supported in a slightly abducted, flexed position (figure four position) with the heel resting on the knee on the contralateral side. The injection site was along the midline of the tendon, 1 cm from the pubic bone. The skin was marked and AL tendon position was confirmed by resisting hip adduction under ultrasound imaging.

The RF tendon was found by manual palpation below its attachment to the anterior inferior iliac spine and was followed distally towards the musculotendinous junction. The injection site was defined as the centre of the tendon of the long head of the rectus femoris muscle. The location and depth of the injection was confirmed by the performance of hip flexion against resistance while under ultrasound observation. The injection site was marked after confirmation.

Electromyographic placement, sampling and data extraction

The skin at each assessment site was shaved, abraded and cleaned with alcohol in accordance with the SENIAM guidelines.²⁰ Disposable electrodes (*Ambu®*, *Neuroline 720*, *Denmark*) were mounted bilaterally with an inter-electrode distance of 20 mm in a bipolar configuration at the m. tensor fascia latae (TFL), the m. adductor longus (AL), m. rectus abdominis (RA), and m. external obliques (EO).^{6,21} A ground electrode was placed on the right wrist. All electrodes were secured by tape (*Micropore™ Surgical Tape*, *3M™*, *USA*). The electromyographic (EMG) signals were sampled at 2048 Hz with a gain of 2000 using a 128-channel surface EMG amplifier (*WS1 OT Bioelettronica*, *Italy*) and converted to digital form by a 12-bit analogue-to-digital converter (*LISiN-OT Bioelettronica*, *Torino*, *Italy*; *-3 dB bandwidth 10-500 Hz*). The digitized EMG signals were band-pass filtered (4th order, zero-phase-lag Butterworth, 25 to 450 Hz). The EMG signal from the AL muscle was used as reference to determine the time window for the amplitude analysis (from onset to offset)²² where the root-mean-square (RMS) value was extracted for all muscles for the middle epoch

defined as middle third of the period between onset and offset. The onsets and offsets were automatically detected based on the AL muscle EMG data as previously described in detail by Santello et al.²² In short, the accumulated integrated EMG (iEMG) was normalized to 1 in both amplitude and time and subtracted by a reference line with slope equal to 1. The time point where this difference was larger was defined as the muscle activity onset. Similar approach was used for the offset activity. All onset/offset detections were confirmed by visual inspection at each time point (baseline, during pain, post-pain). No manual correction of the data was required.

Supplement 2 – Clinical tests



(a) The 0° adduction test: The participant lay with legs straight and the examiner placed the forearm between the medial malleoli with the dynamometer fixed to one end, the participant was instructed to adduct maximally. (b) The 45° adduction test: The participant lay with the feet flat on the plinth with hips flexed to 45° and the knees at 90° of flexion and performed maximal adduction with the dynamometer held in place by the examiner between the medial condyles of the knees. (c) The 90° adduction test: the participant lay with the hips and knees at 90° of flexion and performed maximal adduction with the dynamometer between the medial femoral condyles. (d) The abdominal crunch manoeuvre: the participant performed maximal trunk flexion with the hips and knees in the same position as the 45° test. The dynamometer was placed on the sternum where the examiner applied resistance to the movement. (e) The oblique crunch manoeuvre: the dynamometer was positioned on the shoulder contralateral to the examiner who applied resistance while the participant performed an abdominal crunch towards the contralateral knee.

Supplement 3 – Pain distribution for the control injections



Adductor Longus - Isotonic



Rectus Femoris - Hypertonic



Rectus Femoris - Isotonic

Supplementary Table 1. Mean normalised RMG-EMG [95%CI] during the clinical tests after experimentally-induced groin (AL hypertonic injections) and thigh pain (RF hypertonic injections). The RMS-EMG is normalised to baseline (100%).

Injection	Test position	Adductor longus		Tensor fascia latae		Rectus abdominis		External oblique	
		Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
AL Hypertonic Injection	0° adduction	84.6 [72.4, 96.7]	90.3 [75.88,104.65]	99.1 [78.3,119.9]	102.8 [85.5,120.2]	111.7 [88.8,134.6]	121.8 [94.5,149.0]	116.2 [90.9,141.4]	126.2 [96.6,155.9]
	45° adduction	92.0 [80.3,103.8]	92.3 [81.1,102.6]	97.1 [65.3,128.8]	95.6 [70.1,121.1]	116.3 [97.1,135.5]	109.85 [81.9,137.8]	118.9 [95.8,142.0]	121.0 [103.3,138.7]
	90° adduction	104.6 [92.5,116.7]	116.7 [92.8,140.7]	108.3 [92.9,123.8]	126.5 [93.4,159.7]	119.3 [106.9, 131.7]	118.2 [106.0, 130.3]	113.4 [83.5,143.3]	125.3 [95.0,155.6]
	Resisted abdominal crunch	106.4 [92.8,120.0]	108.7 [91.7,125.7]	161.3 [104.7,217.8]	115.6 [99.8,131.3]	116.1 [101.1,131.1]	102.9 [93.2,112.6]	98.0 [77.6,118.4]	98.4 [82.7,114.1]
	Ipsilateral Resisted oblique crunch	95.6 [81.3,109.8]	98.8 [88.9,108.7]	105.3 [73.7,137.0]	103.1 [93.9,112.4]	115.9 [85.8,146.0]	107.4 [95.7,119.2]	99.9 [82.5,117.3]	102.8 [84.3,121.4]
	Contralateral Resisted oblique crunch	102.2 [92.0,112.5]	111.2 [81.8,140.6]	121.5 [82.9,160.2]	107.8 [87.0,128.7]	116.3 [83.7,148.8]	93.7 [78.0,109.4]	96.1 [84.3,107.8]	99.9 [86.6,113.2]
RF Hypertonic Injection	0° adduction	105.8 [92.5,119.0]	107.7 [95.6,119.7]	112.8 [94.3,131.3]	107.9 [94.6,121.2]	191.3 [31.4,351.2]	152.8 [71.1,234.5]	123.9 [104.3, 143.4]	120.6 [101.4, 139.8]
	45° adduction	94.4 [86.4,102.4]	96.4 [87.9,105.0]	84.1 [65.0,103.3]	100.6 [77.9,123.3]	111.1 [81.8,140.4]	110.9 [85.2,136.6]	104.6 [81.5,127.8]	105.0 [83.0,127.1]
	90° adduction	106.8 [91.3,122.3]	108.9 [95.8,122.1]	125.3 [102.7, 147.9]	111.7 [92.8,130.7]	108.7 [92.8,124.7]	111.1 [92.5,129.7]	109.6 [95.8,123.4]	104.8 [91.0,118.6]
	Resisted abdominal crunch	113.3 [103.1, 123.5]	111.3 [93.2,129.5]	114.3 [90.0,144.7]	108.7 [82.1,135.3]	105.3 [95.3,115.3]	108.0 [98.4,117.6]	102.6 [75.5,129.7]	101.5 [81.2, 121.8]
	Ipsilateral Resisted oblique crunch	105.3 [92.7,117.9]	105.9 [85.6,126.3]	140.0 [72.3,207.7]	103.4 [88.9,117.8]	111.0 [98.6,123.5]	113.5 [100.3, 126.7]	116.0 [98.5,133.5]	116.3 [100.4,132.2]
	Contralateral Resisted oblique crunch	91.2 [76.2,106.3]	97.4 [67.9,127.0]	89.2 [67.8,110.6]	83.4 [65.7,101.1]	102.4 [87.5,117.2]	106.2 [87.9,124.6]	96.7 [79.0,114.4]	99.9 [74.5,125.3]

Supplementary Table 2. Mean normalised RMG-EMG [95%CI] during the clinical tests after control injections into the groin (AL isotonic injections) and thigh (RF isotonic injections). The RMS-EMG is normalised to baseline (100%).

Injection	Test position	Adductor longus		Tensor fascia latae		Rectus abdominis		External oblique	
		Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
AL Isotonic Injection	0° adduction	96.5 [87.1,106.0]	95.3 [86.3,104.3]	137.6 [52.5,222.6]	113.5 [78.5,148.4]	122.3 [90.9,153.6]	142.9 [83.6,202.3]	108.8 [89.4,128.2]	106.0 [87.1,124.9]
	45° adduction	108.6 [96.8,120.4]	109.4 [95.3,123.6]	111.3 [92.2,130.4]	129.1 [97.1,161.1]	128.7 [89.4,167.9]	280.5 [-77.9,638.9]	113.4 [95.0,131.8]	128.5 [99.9,157.1]
	90° adduction	103.6 [90.0,117.3]	101.4 [88.1,114.6]	97.2 [77.7,116.6]	99.3 [84.2,114.5]	120.8 [56.7,185.0]	162.7 [120.0,313.3]	109.5 [93.5,125.4]	111.4 [91.2,131.6]
	Resisted abdominal crunch	116.9 [98.2,137.6]	100.5 [85.8,115.2]	100.0 [83.5,116.4]	97.9 [83.0,112.8]	105.2 [95.9,114.5]	106.3 [80.1,132.5]	91.3 [82.2,100.3]	96.8 [81.0,112.6]
	Ipsilateral Resisted oblique crunch	106.7 [88.8,124.6]	105.7 [94.8,116.6]	108.9 [90.4,127.4]	119.2 [88.2,150.1]	101.6 [86.3,116.9]	112.8 [86.5,139.0]	93.3 [82.1,104.5]	90.7 [78.8,102.6]
	Contralateral Resisted oblique crunch	106.9 [94.8,118.9]	110.5 [90.6,130.5]	101.7 [87.7,115.7]	117.1 [94.7,139.5]	114.8 [91.1,138.5]	139.6 [88.9,190.4]	103.1 [91.8,114.3]	100.0 [86.4,113.6]
RF Isotonic Injection	0° adduction	105.0 [91.8,118.2]	102.4 [90.7,114.2]	132.9 [64.2,201.5]	140.7 [60.0,221.4]	115.9 [91.1,140.8]	110.2 [85.7,134.6]	109.3 [85.0,133.7]	108.8 [84.8,132.8]
	45° adduction	102.1 [92.1,112.2]	103.6 [94.4,112.8]	104.8 [84.3,125.4]	132.1 [76.6,187.5]	98.4 [87.2,109.7]	102.7 [92.5,113.0]	110.9 [87.3,134.4]	110.1 [85.0,135.3]
	90° adduction	106.7 [92.7,120.6]	104.5 [91.1,117.9]	133.0 [81.3,184.6]	106.1 [87.6,124.7]	91.9 [67.7,116.2]	93.8 [65.9,121.6]	96.2 [78.4,114.1]	92.4 [78.5,106.4]
	Resisted abdominal crunch	102.3 [92.3,112.3]	107.3 [88.3,126.4]	101.8 [77.1,126.5]	111.2 [95.1,127.4]	89.1 [76.9,101.3]	90.0 [78.0,101.9]	93.4 [81.5,105.4]	88.8 [74.0,103.6]
	Ipsilateral Resisted oblique crunch	120.1 [98.3,141.8]	124.2 [93.7,154.7]	124.0 [84.2,163.8]	111.7 [86.8,136.7]	109.3 [82.6,136.0]	105.8 [81.6,130.1]	110.5 [90.5,130.5]	110.8 [86.8,134.9]
	Contralateral Resisted oblique crunch	110.5 [92.8,128.2]	108.4 [88.2,128.5]	105.4 [92.1,118.7]	102.0 [88.2,115.9]	100.6 [85.7,115.5]	99.3 [84.8,113.9]	100.7 [91.3,110.2]	99.8 [90.6,108.9]

Supplementary Table 3. Mean normalised RMG-EMG [95%CI] during the clinical tests in the post-pain condition after experimentally-induced groin (AL hypertonic injections) and thigh pain (RF hypertonic injections) had resolved. The RMS-EMG is normalised to baseline (100%).

Injection	Test position	Adductor longus mean (%), [95% CI]		Tensor fascia latae mean (%), [95% CI]		Rectus abdominis mean (%), [95% CI]		External oblique mean (%), [95% CI]	
		Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
AL Hypertonic Injection	0° adduction	93.7 [79.1,108.4]	96.5 [86.6,106.5]	99.1 [78.3,119.9]	102.8 [85.5,120.2]	115.5 [78.6,12.5]	119.8 [87.0,152.7]	116.3 [84.1,148.4]	115.5 [87.9,143.1]
	45° adduction	98.9 [87.4,110.4]	98.5 [88.9,108.2]	120.9 [89.4,152.3]	121.5 [91.6,151.3]	116.5 [94.6,138.4]	106.2 [90.7,121.6]	116.2 [93.0,139.4]	117.2 [88.4,146.0]
	90° adduction	103.0 [90.0,116.0]	106.2 [93.2,119.3]	101.3 [90.7,111.8]	128.1 [88.7,167.5]	110.2 [92.8,127.6]	102.9 [93.2,112.6]	105.4 [87.4,123.5]	118.3 [97.1,139.5]
	Resisted abdominal crunch	101.7 [89.3,114.0]	102.3 [87.3,117.4]	138.6 [105.5,171.7]	103.0 [85.2,120.8]	98.0 [87.6,108.4]	106.1 [96.1,116.1]	104.8 [81.3,128.4]	94.9 [83.6,106.2]
	Ipsilateral Resisted oblique crunch	117.0 [87.1,147.0]	110.1 [98.3,121.8]	102.5 [83.8,121.3]	108.6 [82.3,135.0]	119.8 [85.6,153.9]	119.4 [93.2,145.5]	103.5 [82.5,124.5]	109.4 [82.0,136.8]
	Contralateral Resisted oblique crunch	125.8 [89.8,161.8]	108.6 [99.0,118.2]	121.5 [81.2,161.9]	129.7 [96.1,163.2]	105.3 [88.7,121.9]	99.1 [84.2,113.9]	100.1 [83.5,116.6]	100.0 [86.4,113.6]
RF Hypertonic Injection	0° adduction	107.3 [96.5,118.1]	111.2 [98.0,124.5]	110.6 [94.7,126.5]	107.9 [85.7,130.1]	133.0 [43.8,222.2]	108.0 [64.4,151.7]	105.1 [87.3,123.0]	101.4 [84.2,118.7]
	45° adduction	106.1 [97.2,115.0]	105.1 [97.5,112.6]	96.5 [75.2,117.9]	118.6 [86.5,150.6]	98.3 [90.0,106.6]	100.6 [91.7,109.5]	104.1 [88.7,119.5]	102.8 [90.1,115.5]
	90° adduction	106.3 [93.0,119.6]	106.2 [91.9,120.6]	128.9 [100.7,149.1]	116.8 [92.9,140.7]	111.6 [93.7,129.6]	116.0 [95.1,137.0]	109.0 [92.7,125.2]	105.5 [88.8,122.3]
	Resisted abdominal crunch	103.0 [90.1,116.0]	102.1 [94.4,109.7]	111.6 [83.3,140.0]	115.9 [95.6,136.2]	110.3 [101.0,119.5]	113.9 [102.8,125.0]	108.8 [83.6,134.0]	108.2 [85.5,130.9]
	Ipsilateral Resisted oblique crunch	108.0 [83.9,132.1]	100.5 [80.8,120.3]	97.0 [86.3,107.7]	104.1 [83.5,124.6]	111.8 [88.9,134.8]	113.3 [98.4,128.3]	112.2 [93.3,131.1]	111.1 [96.0,126.2]
	Contralateral Resisted oblique crunch	92.1 [72.5,111.7]	92.8 [77.4,108.1]	91.7 [73.2,110.2]	85.6 [67.7,103.5]	92.6 [78.0,107.1]	95.1 [79.4,110.8]	93.7 [76.5,110.9]	96.2 [72.7,119.6]

Supplementary Table 4. Mean normalised RMG-EMG [95%CI] during the clinical tests in the post-pain condition after control injections into the groin (AL isotonic injections) and thigh (RF isotonic injections). The RMS-EMG is normalised to baseline (100%).

Injection	Test position	Adductor longus		Tensor fascia latae		Rectus abdominis		External oblique	
		Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
AL Isotonic Injection	0° adduction	101.8 [88.5,115.1]	111.0 [94.3,127.8]	113.5 [80.1,146.9]	103.8 [71.2,136.4]	118.0 [93.4,142.6]	118.2 [89.3,147.1]	104.1 [84.4,123.8]	117.6 [91.0,144.1]
	45° adduction	110.6 [97.3,123.9]	109.4 [94.8,124.0]	129.9 [79.0,180.8]	114.2 [89.5,138.9]	125.3 [75.8,174.8]	125.6 [87.4,163.7]	125.8 [85.6,166.1]	117.7 [98.15,137.2]
	90° adduction	105.5 [86.7,124.3]	103.7 [92.2,115.1]	105.1 [78.5,131.7]	104.6 [82.0,127.2]	89.1 [70.8,107.4]	89.1 [74.5,107.4]	101.2 [85.1,105.4]	104.9 [86.2,123.7]
	Resisted abdominal crunch	104.8 [84.6,124.9]	113.9 [95.3,132.5]	95.9 [75.9,116.0]	87.4 [68.6,106.1]	106.4 [86.1,126.7]	110.6 [95.0,126.1]	104.3 [94.0,114.6]	99.4 [90.9,108.0]
	Ipsilateral Resisted oblique crunch	100.2 [83.3,117.2]	126.3 [96.6,155.9]	116.5 [83.4,149.7]	113.6 [79.6,147.7]	98.8 [83.6,114.0]	103.8 [87.7,119.9]	92.6 [76.0,109.3]	97.1 [80.9,113.2]
	Contralateral Resisted oblique crunch	117.1 [88.9,145.4]	97.5 [78.5,116.5]	127.7 [106.0,149.4]	107.2 [64.5,149.9]	129.3 [89.5,169.1]	134.0 [83.4,184.6]	97.3 [83.5,111.1]	116.9 [82.5,151.3]
RF Isotonic Injection	0° adduction	107.4 [93.1,121.8]	115.1 [101.4,128.8]	180.8 [54.3,307.2]	118.6 [85.7,151.6]	131.2 [95.1,167.3]	134.1 [93.7,174.5]	145.6 [104.1,187.2]	132.3 [96.5,168.1]
	45° adduction	101.5 [91.0,112.1]	100.2 [89.6,110.8]	133.3 [85.64,180.9]	150.9 [92.1,209.7]	91.0 [76.0,106.0]	86.7 [73.9,99.4]	91.6 [78.7,104.5]	94.4 [78.9,109.9]
	90° adduction	100.2 [89.4,110.9]	99.6 [89.4,109.8]	133.9 [88.4,179.5]	163.0 [87.9,238.2]	81.7 [67.8,95.6]	82.6 [70.9,94.3]	89.2 [75.1,108.9]	89.2 [73.8,104.6]
	Resisted abdominal crunch	112.9 [89.0,136.9]	103.1 [87.0,119.1]	102.1 [78.8,125.4]	122.5 [78.7,166.3]	102.1 [80.3,123.9]	103.4 [93.3,113.5]	99.2 [86.9,111.6]	106.6 [95.4,117.8]
	Ipsilateral Resisted oblique crunch	111.1 [82.1,140.1]	110.9 [99.2,122.6]	99.9 [77.7,122.1]	167.3 [39.3,295.4]	100.6 [84.1,117.1]	117.7 [95.7,139.7]	102.2 [88.3,116.1]	110.6 [88.0,133.1]
	Contralateral Resisted oblique crunch	101.2 [83.0,119.4]	97.5 [82.0,112.9]	128.2 [80.2,176.2]	97.9 [83.8,111.7]	98.8 [80.9,116.7]	103.9 [89.8,118.1]	96.7 [83.8,109.6]	100.5 [87.5,113.5]