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*an experimental pain study*

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## Resisted adduction in hip neutral is a superior test to assess adductor longus pain: an experimental pain study

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**Resisted adduction in hip neutral is a superior provocation test to assess adductor longus pain: an experimental pain study**

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PROOF

**ABSTRACT**

The criterion of long-standing groin pain diagnoses in athletes usually relies on palpation and clinical tests. An experimental pain model was developed to examine the clinical tests under standardised conditions. Pain was induced by hypertonic saline injected into the proximal adductor longus tendon (AL) or rectus femoris tendon (RF) in 15 healthy male participants. Isotonic saline was injected contralaterally as a control. Pain intensity was assessed on a visual analogue scale (VAS). Resisted hip adduction at three different angles and trunk flexion were completed before, during and after injections. Pain provocation in the presence of experimental pain was recorded as a true positive compared with pain provocation in the non-pain conditions. Similar peak VAS scores were found after hypertonic saline injections into the AL and RF and both induced higher VAS scores than isotonic saline ( $P<0.01$ ). Adduction at  $0^\circ$  had the greatest positive likelihood ratio (+LR=2.8, 95%CI: 1.09-7.32) with  $45^\circ$  (-LR=0.0, 95%CI: 0.00-1.90) and  $90^\circ$  (-LR=0.0, 95%CI: 0.00-0.94) having the lowest negative LR. This study indicates that the  $0^\circ$  hip adduction test resisted at the ankles optimizes the diagnostic procedure without compromising diagnostic capacity to identify experimental groin pain. Validation in clinical populations is warranted.

**Key Words:** groin, athlete, pain, experimental pain, diagnostic test

## INTRODUCTION

The diagnosis of long-standing groin pain in athletes has been evaluated with many approaches ranging from very specific patho-anatomical diagnoses (Brennan et al. 2005; Cunningham et al. 2007; Schilders et al. 2007; Verrall et al. 2008) to sub-classification based on clinical assessments (Falvey et al. 2008; Holmich 2007). None of these approaches have achieved consensus in relation to which structures are actually responsible for producing pain. A recent systematic review of symphyseal and adductor-related groin pain (Branci et al. 2013) synthesised the results of imaging studies to four main findings: adductor longus enthesopathy, the secondary cleft sign, bone marrow oedema, and pubic symphysis-related degenerative changes. Clinically, it is difficult to differentiate between these findings without imaging. Furthermore, there are limited studies showing how these imaging findings change management as they commonly coexist. The prevalence of abnormal imaging findings is also high in asymptomatic populations (Branci et al. 2014; Branci et al. 2013) with poor association between abnormal imaging findings and risk of future injury (Robinson et al. 2014). The terminology and definitions have been recently improved following the 'Doha agreement meeting on terminology and definitions in groin pain in athletes' which adopted clinically-based taxonomy in three categories; "clinical entities" for groin pain, hip-related groin pain and other causes of groin pain in athletes (Weir et al. 2015).

Although movement and strength impairments exist in groin pain patients (Thorborg et al. 2014), how groin pain per se affects hip movement remains uncertain. Models using experimental pain in pre-determined anatomical structures are commonly used to investigate the mechanisms underlying various myofascial pain conditions (Graven-Nielsen 2006). Transient experimental pain commonly disturbs the motor performance during a given

task(Salomoni et al. 2013; Salomoni & Graven-Nielsen 2012) and case-control studies of sport-related chronic groin pain have shown strength deficits in bilateral squeeze (hip adduction) tests at 0°, 30° and 45° of hip flexion in various sporting populations (Malliaras et al. 2009; Mens et al. 2006; Nevin & Delahunt 2014). Studies evaluating the activation of the hip adductor muscles (Delahunt et al. 2011; Lovell et al. 2012) highlight that the m. adductor longus (AL), m. adductor brevis, m. adductor magus, m. gracilis, and m. pectineus are active during adduction in 0°, 45° and 90° of hip flexion (Lovell et al. 2012). Although the activation level of these muscles varies depending on test position, it is not possible to activate each adductor muscle separately during testing. Given this information, it is clinically difficult to differentiate which adductor muscle is responsible for the pain provoked. This is further confounded by clinical populations commonly presenting with more than one pathology (Holmich 2007) including **musculotendinous pathology of the abdominal region**. It is unknown how abdominal pathology affects diagnostic tests for the adductor and vice versa. Palpation together with various adduction tests are commonly used clinically as this is considered useful in diagnosing bone marrow oedema (Verrall et al. 2005), adductor-related pain (adductor enthesopathy) (Holmich et al. 2004), and pubic symphysis degeneration (Falvey et al. 2008; Holmich et al. 2004) with the most common tests being resisted bilateral adduction in 0° or 45° of hip flexion (Holmich 2007; Verrall et al. 2005). However, these tests have not been evaluated against a consistent reference standard and therefore it remains unclear to what extent the outcome of the tests is related to pain and sensitivity of the structures in the region. By using experimental pain models, it has been demonstrated how pain in the sacroiliac (Palsson & Graven-Nielsen 2012; Palsson et al.



2014) and hip joint (Izumi et al. 2014) affects the outcome of clinical diagnostic tests. Whether the same applies for the adductor muscles is however unknown.

This study was intended to evaluate (i) which of the commonly used tests are able to provoke experimental pain originating in the adductor longus proximal tendon, and (ii) whether abdominal manoeuvres are capable of provoking pain originating in the adductor longus proximal tendon.

## MATERIALS AND METHODS

Sixteen healthy, physically active male subjects were recruited through university advertisements and websites (mean  $\pm$  SD; age:  $27 \pm 3.4$  years, body mass index:  $23.85 \pm 2.15$ ). Inclusion criteria were males without current or previous hip, groin, or lumbar injuries who were physically active for at least 2.5 hours of regular, vigorous weekly exercise. Exclusion criteria included signs of neurological disorders or rheumatologic diseases which could affect the outcome of the experimental procedure. Participants who reported medication use either on enrolment or on a regular basis were also excluded. At baseline testing, the participant was asked whether any pain was experienced in the hip/groin when performing each test and a positive response was used for exclusion purposes. Subjects were given a detailed written and verbal explanation of the experimental procedure prior to giving their written informed consent. The study was conducted in accordance with the Helsinki Declaration and was approved by the Danish Regional Ethics Committee (N-20130036).

### *Study design*

The experiment was a randomized, single-blinded, balanced-crossover design, and was conducted in two sessions within one week. Randomisation was achieved through the selection of one of 16 identical envelopes by an experimenter (blinded to the injector and experimenters) with boxed randomisation with one of all 16 possible order combinations of injection site, side, and injection site. Blinding was achieved through unlabelled, identical pre-prepared syringes prior to the experimenters entering the room. The participants were not advised of the order of injections at any stage throughout the procedure. All assessments were performed with subjects lying on a plinth in supine. At baseline, subjects were familiarized with the experimental procedure where pain provocation tests were performed before (baseline), during, and after (post-pain) induction of experimental pain. The post-pain state was defined as five minutes after the cessation of pain indicated by a score of “0 cm” on VAS. The sequence of provocation tests was consistent throughout each session but the sides and order of injections (saline type and injection sites) were randomized. In each session a painful trial and a non-painful trial were completed in each side, respectively.

*Experimental groin and thigh pain*

Groin pain was induced by injecting sterile hypertonic saline (1 ml, 5.8%) into the adductor longus (AL) tendon. As a painful control injection outside the groin area, hypertonic saline (1 ml, 5.8%) was injected into the proximal tendon of the long head of the m. rectus femoris (RF) on a separate day. Isotonic saline (1 ml, 0.9%) was injected as a non-painful control at the same injection site however, on the contralateral side. The subjects received one hypertonic and one isotonic saline injection in each side during each session and the order of the saline type and site was randomized in a balanced way (left or right). The saline type was

1  
2  
3  
4 blinded to the subject and injector. Injections were given over the duration of approximately  
5  
6 10 seconds using a 2-ml plastic syringe with a disposable needle (27G). All injections were  
7  
8 given by an orthopaedic surgeon (MI) after a standard disinfection protocol. *Illustration of the*  
9  
10 *study design and an example of the experimental procedure is provided in Figure 1.*

11  
12  
13 Injection sites for AL and RF were localized using pre-defined anatomical landmarks  
14  
15 in the following manner; The AL was identified using a method previously described(Izumi  
16  
17 et al. 2014). The location, depth and alignment of the injection site were confirmed by real  
18  
19 time ultrasound (US) imaging (*Acuson 128XP10, NativeTM*). The subject lay in a supine  
20  
21 position with the leg to be injected supported in a slightly abducted, flexed position (figure  
22  
23 four position). The injection site was along the midline of the muscle, 1 cm from the pubic  
24  
25 bone. The skin was marked to designate the injection site, its depth and alignment of the AL  
26  
27 was confirmed by resisting hip adduction under ultrasound imaging. The AL tendon was  
28  
29 stabilised with two fingers and the needle penetrated the skin perpendicular to the tendon.  
30  
31

32  
33 To identify the RF injection site, the tendon of RF was found by manual palpation  
34  
35 below its attachment to the anterior inferior iliac spine and was followed distally towards the  
36  
37 musculotendinous junction. The injection site was defined as the centre of the tendon of the  
38  
39 long head of the rectus femoris and its location marked on the skin. The location and depth of  
40  
41 the injection was confirmed by the performance of hip flexion against resistance while under  
42  
43 ultrasound observation.  
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45

#### 46 47 48 *Assessment of pain and disability*

49  
50 The pain intensity induced by each saline injection was assessed on a 10 cm electronic visual  
51  
52 analogue scale (VAS) which could be adjusted by using an external handheld slider. The  
53  
54

VAS was anchored with ‘no pain’ and ‘maximum pain’, 0 cm and 10 cm, respectively. A continuous recording of the VAS signal was made after each injection until all pain had subsided (sample frequency of 20 Hz). For analysis, the peak pain (VAS peak) was extracted. After the pain had resolved, a validated questionnaire for groin pain (the Copenhagen Hip and Groin Outcome Score (HAGOS))(Thorborg et al. 2014) was administered in an English (Thorborg et al. 2011) or **Danish (HAGOS, [www.koos.nu](http://www.koos.nu) (2013)) version**. Median HAGOS subscale scores have been shown to approximate the maximum score (100 points) in healthy, injury free soccer players with the 95% reference ranges for subscales of: pain: 80.1–100, symptoms: 64.3–100, activities of daily living: 80.3–100, sport and recreational activities: 71.9–100, participation in physical activity: 75–100, and quality of living: 75–100 (Thorborg et al. 2014).

*Adductor pain provocation tests and force measurements*

A battery of 6 pain provocation tests was used with all tests performed by a single clinically-trained experimenter (MD). A single hand-held dynamometer (MicroFET2, Hoggan Scientific, USA) was used to measure maximum force (N) produced during the isometric contraction at baseline. Verbal feedback was given during the remainder of the session (during and post-pain) to ensure forces were consistent. When the tests were performed whilst experimentally-induced pain was present, the subject was asked whether the tests increased the experimental pain. A positive pain provocation test was defined as an increase in pain in the groin region during or after the administration of the clinical test. For all tests, the participant was supine on a plinth with hands comfortably resting on the lower ribcage. Instructions were given to perform a maximal contraction against the examiner’s hand for approximately 5 seconds, and encouragement was given to ensure matching of forces to

baseline measures. The tests administered (Supplement 1) were: 1) Bilateral adduction (squeeze) test with hips at 0° (Holmich et al. 2004) resisted at the ankles 2) A bilateral squeeze test with hips flexed at 45° (Lovell et al. 2012) 3) A bilateral squeeze test with hips flexed to 90° (Lovell et al. 2012) 4) Resisted abdominal crunch (Holmich et al. 2004) 5) Resisted oblique crunch, one side at a time. (Holmich et al. 2004)

### *Diagnostic capabilities*

Individual tests and combinations of tests were analysed to evaluate the diagnostic value of the groin pain provocation tests. Combinations of the three adduction tests (0°, 45° or 90° tests) and all six tests were analysed using the sum of the number of positive pain provocation tests for each participant. The combination battery of three adduction tests and the 0° test were used as the reference tests as indicated.

The following defined true positive, true negative, false positive and false negative conditions: (i) “True positive” was an increase in resting pain during the provocation test following hypertonic saline injection to the AL (experimental AL pain), and (ii) “false positives” were when an increase in resting pain occurred during testing in the isotonic AL control condition, or either of the RF conditions (hypertonic or isotonic). (iii) “True negatives” were recorded when no increase in resting pain occurred during testing following RF injections and isotonic AL control conditions. (iv) “False negatives” were recorded when no increase in resting pain was observed in the hypertonic saline injection into the AL during the tests. Two-by-two tables were created to calculate the sensitivity, specificity and likelihood ratios for each test procedure.

Receiver Operating Characteristic (ROC) curves allow selection of the optimal diagnostic conditions through evaluation of the performance of a diagnostic test (Zweig & Campbell 1993). ROC curves with Bonferroni correction for multiple comparisons were created using the painful control (RF hypertonic injection) and non-painful control (AL isotonic injection). Separate analyses for both control injections were undertaken. The number of positive pain provocations was utilised in the analysis of ROC curves. Comparisons of the area under the curve for each test or test battery were undertaken using the “jackknife method” (DeLong et al. 1988), a nonparametric estimate for variance comparisons (Efron & Stein 1981).

*Statistics*

All data was assessed for normality. Results are presented as mean ± standard deviation (SD) for parametric data and median and range for non-parametric data. All statistical analyses were undertaken in Stata 13 (*Stata 13 IC, StataCorp, USA*). To test the difference between strength measures between test positions, one-way ANOVA with Bonferroni correction for multiple post-hoc analyses was utilised using baseline results of the first session. To compare the VAS peak across the four injections (AL hypertonic, AL isotonic, RF hypertonic and RF isotonic), a linear mixed model with peak VAS as the fixed effect and participant as the random effect was utilised. Significance was set at  $P < 0.05$  for all statistical tests.

**RESULTS**

*Participants*

One subject was excluded at inclusion due to a history of adductor-related pain lasting for greater than six weeks in the previous year. The remaining 15 subjects completed a median of 6 hours of exercise per week (range 3-20 hours) with running (n=8) and swimming (n=6) being the primary modes of exercise each week. The HAGOS subsections responses were: Pain (median=100; IQR, 100-100); Quality of Life (100; 100-100); Physical Activity (100; 100-100); Symptoms (96; 93-100); Sport/Recreation (100; 100-100); Activities of Daily Living (100; 100-100).

### *MVC strength*

The absolute strength values are presented in Table 1. Adduction in 45° was the strongest test position (greatest force) but there was no significant difference between strength measurements at 45° and 90° positions. The force generated at the 0° position was significantly lower than in the 45° (F=96.93, df=5, P<0.001) and 90° (F=67.8, df=5, P<0.001) positions.

### *Experimental Pain*

A significant random effect for participant was found (Coeff=0.25, 95%CI 0.07 to 0.92) indicating that each participant responded differently. The hypertonic saline produced significantly higher peak VAS scores when comparing isotonic to hypertonic injections (AL, VAS peak, Coeff=-1.98, 95%CI -2.60 to -1.36; RF, VAS peak, Coeff=-1.82, 95%CI -2.44 to -1.19).

### *Diagnostic capabilities*

The sensitivity, specificity and likelihood ratios of the six clinical tests and combinations of tests are presented in Table 2. Resisted adduction in 0° of hip flexion showed the highest positive likelihood ratio when examined using both control conditions (hypertonic RF or isotonic AL injections). The negative likelihood ratio yielding the most conclusive reduction in likelihood of the disorder (LR=0.00) was achieved in 45° and 90° adduction tests and all abdominal manoeuvres in the non-painful AL tendon condition and only the 90° adduction test in the painful RF condition.

*Receiver Operating Characteristic (ROC) curves*

The results of the ROC curve analysis is presented in Table 3. The 0° adduction test showed the best diagnostic performance as indicated by the area under the curve (AUC). A battery of three adduction tests did not significantly improve the diagnostic performance compared to the 0° or 90° indicating that a battery of three adduction tests does not significantly improve the diagnostic capability.

A significant difference was observed between a battery of three tests and the 45° adduction test (AL isotonic,  $P<0.00$ ; RF hypertonic,  $P=0.01$ ) indicating that a battery of three adduction test is diagnostically superior to the 45° test alone.

**DISCUSSION**

The results of this study highlight that pain provocation tests have a varied response across subjects and that if adductor-related pain is considered, the positive and negative likelihood ratios vary depending upon which position subjects are tested in. This study indicates that the 0° test showed the highest the positive likelihood ratio to detect experimental adductor longus pain without compromising diagnostic capacity as indicated by the AUC on the ROC curve.



*Provocation tests to assess groin pain*

This consistent with previous findings in clinical populations (Lovell et al. 2012). It is hypothesised that this may be related to the elongation of the adductor tendon and the cam-effect of the anatomical relationship between the rectus abdominis and adductor longus (Clark et al. 2010; Norton-Old et al. 2013) and unlikely to be related to higher EMG activation in this position as the EMG activation of the adductor longus has been shown to be maximised in the 45° position (Lovell et al. 2012). The 0° test being weaker than the 45° and 90° tests however, when considering lever length, is unlikely to be a cause. The abdominal manoeuvres did not improve the ability to diagnose experimental pain relating to m. adductor longus.

*Diagnostic capabilities of single provocation tests*

The results show that the 0° adduction test has the highest positive likelihood ratio indicating its utility to detect the presence of adductor longus-related pain and the 45° or 90° positions are best to rule out this disorder in the presence of a negative test finding (Table 2). The abdominal crunch or oblique crunches, both ipsi- and contralaterally, had poor specificity and as a result the AUC results were significantly lower on the ROC curves indicating lesser ability to discriminate between true positives or negatives. While the adductor longus and rectus abdominis are known to be intimately linked at the pubic bone through a common enthesis and aponeurosis (Robinson et al. 2007) which is likely to transmit forces between structures across the pubic symphysis (Norton-Old et al. 2013), it appears their response to pain provocation tests are separate under experimental groin pain conditions. It was hypothesised that the 0° test would be diagnostically superior to identify/reproduce/elicit groin pain due to its inclusion in a study sub-grouping clinical populations (Holmich 2007;

Holmich et al. 2004). This supports the experimental model representing the clinical situation well.

*Diagnostic capabilities of multiple test batteries*

The present study demonstrated the adduction tests had different diagnostic capabilities at different degrees of hip flexion and gives support to the need to further investigate each test in clinical populations. Positive pain provocation on any two out of three adduction tests (0°, 45° or 90°) showed the highest positive likelihood ratio in the analysis of combinations however, this did not exceed the diagnostic value of the 0° adduction test alone. Therefore little advantage is gained by the addition of the 45° and 90° adduction tests. A possible reason is that adductor longus is active during adduction in all angles of hip flexion (Lovell et al. 2012). As a consequence, the tests are not mutually exclusive with respect to activation (Lovell et al. 2012).

Using a battery of six tests did not improve the diagnostic performance to identify painful AL compared to using three tests indicating that there is little advantage obtained with the inclusion of the abdominal manoeuvres in clinical examination to diagnose adductor longus-related pain. Clinically however, patients present with multiple pathologies including rectus abdominis tendinopathies (Holmich 2007) and in such cases the abdominal tests may possess clinical worth. In this regard, an investigation of the sensitivity of the abdominal tests to true abdominal pain as the adductor tests are to AL pain may be informative.

*Limitations*

When interpreting the results of the study, two considerations must be accounted for: (i) pain generated from an experimental model differs from clinical pain (Izumi et al. 2014; Palsson

& Graven-Nielsen 2012) and (ii) the negative likelihood ratio of zero is likely to be artificially low and unlikely to be seen clinically. In our criteria, for a positive response to be recorded, the subject had to report a change in their resting pain and they had to indicate pain in the groin region. Typically patients do not report resting pain but rather pain on activity and therefore this study must be considered in the context of experimentally-induced pain when interpreting the results.

The three adduction test positions were chosen as they represent what is commonly used in clinical practice (Holmich 2007; Verrall et al. 2005). However, it is not a comprehensive test battery. Further inclusion of diagnostic tests were piloted (Hogan & Lovell 1997; Holmich et al. 2004; Verrall et al. 2005) and were removed due to practical limitations of excessive movement of the participant and time constraints of the tests. Dynamometry, as described by Thorborg and colleagues (Thorborg et al. 2013; Thorborg et al. 2011; Thorborg et al. 2010), was incompatible with the present study design due to time constraints of the experimental pain. The clinical tests and dynamometry were prioritised to replicate common diagnostic tests over strength values. As such the results of this study replicate the diagnostic procedure rather than strength assessments aimed at guiding rehabilitation. All strength measures were taken by a single examiner to minimise any measurement error associated with inter-tester conditions (Thorborg et al. 2011).

The clinical tests utilised represent common tests for the diagnosis of long-standing groin pain in athletes and as such the mechanisms of pain in the clinical population are likely to be different. This study indicates that the 0° test optimizes the diagnostic procedure without compromising diagnostic capacity to identify experimental adductor longus tendon pain.

Clinical tests for the groin area can be affected by pain alone in the absence of pathology

which is consistent with findings from other studies (Izumi et al. 2014; Palsson & Graven-Nielsen 2012; Palsson et al. 2014) highlighting that positive provocation tests may be induced by the present of pain in the absence of pathology *per se*. Explorations beyond pathological anatomical diagnoses of groin patients are warranted.

**PERSPECTIVES**

In experimentally-induced groin pain, the 0° adduction test has the best positive likelihood ratio to detect m. adductor longus-related groin pain. The 45° and 90° adduction test have the best negative likelihood ratio suggesting its utility to rule adductor longus-related groin pain out in experimentally-induced pain. A battery of three adduction tests does not have a superior diagnostic performance to the 0° adduction test, indicating the diagnostic procedure can be rationalised to a single test without losing diagnostic capacity. Abdominal manoeuvres in addition to the adduction tests do not increase the diagnostic performance of a test battery to identify experimental adductor longus-related pain.

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Nil

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**Table 1** – Mean ( $\pm$  SD, N = 15) baseline contraction forces measured in clinical provocation tests. A significant difference between 0° and both 45° and 90° adduction tests is illustrated (\*,  $P < 0.05$ ). A significant difference between the obliques crunch tests and the crunch test is illustrated ( $^{\dagger}$ ,  $P = 0.01$ ).

Clinical Test	Dynamometry Measurement (N)
0°	199 $\pm$ 40*
45°	296 $\pm$ 47
90°	267 $\pm$ 46
Crunch	251 $\pm$ 30
Left Oblique Crunch	204 $\pm$ 25 $^{\dagger}$
Right Oblique Crunch	216 $\pm$ 30



*Provocation tests to assess groin pain***Table 2** Diagnostic performance of the clinical tests and combinations of tests in parallel with respect to a true positive condition of experimentally-induced (hypertonic) adductor longus pain

Control Injection	Test	Sensitivity (95% CI)	Specificity (95% CI)	Positive Likelihood Ratio (95%CI)	Negative Likelihood Ratio (95%CI)
<b>Isotonic Adductor Longus Injection</b>	0°	0.93 (0.84-1.02)	0.67 (0.49-0.83)	2.82 (1.09-7.32)	0.10 (0.02-0.53)
	45°	1.00 (1.00-1.00)	0.20 (0.06-0.34)	1.25 (0.90-1.80)	0.00 (0.00-1.90)
	90°	1.00 (1.00-1.00)	0.40 (0.22-0.58)	1.67 (0.98-2.81)	0.00 (0.00-0.94)
	Crunch	1.00 (1.00-1.00)	0.07 (-0.02-0.16)	1.08(0.87-1.40)	0.00 (0.00-0.93)
	Ipsilateral oblique crunch	1.00 (1.00-1.00)	0.13 (0.02-0.26)	1.15 (0.88-1.57)	0.00 (0.01-2.89)
	Contralateral oblique crunch	1.00 (1.00-1.00)	0.13 (0.02-0.26)	1.15 (0.88-1.57)	0.00 (0.01-2.89)
<b>Hypertonic Rectus Femoris Injection</b>	0°	0.80 (0.66-0.94)	0.67 (0.50-0.84)	2.42 (0.92-6.40)	0.30 (0.11-0.79)
	45°	0.93 (0.84-1.02)	0.20 (0.06-0.34)	1.16 (0.82-1.65)	0.35 (0.05-2.69)
	90°	1.00 (1.00-1.00)	0.40 (0.22-0.58)	1.67 (0.98-2.81)	0.00 (0.00-0.94)
	Crunch	0.73 (0.57-0.89)	0.07 (-0.02-0.16)	0.78 (0.57-1.08)	3.86 (0.31-0.48)
	Ipsilateral oblique crunch	0.73 (0.57-0.89)	0.13 (0.02-0.26)	0.84 (0.58-1.21)	2.08 (0.32-0.13)
	Contralateral oblique crunch	0.80 (0.66-0.94)	0.13 (0.02-0.26)	0.92 (0.66-1.28)	1.54 (0.23-0.10)
<b>Isotonic Adductor Longus Injection</b>	Pain provocation on one adduction test	0.66 (0.49-0.84)	0.54 (0.36-0.72)	1.45 (0.71-2.97)	0.62 (0.19-2.02)
	Pain provocation on two adduction tests	0.87 (0.75-0.99)	0.63 (0.46-0.81)	2.41 (1.31-4.44)	0.20 (0.03-1.26)
	Pain provocation on three adduction tests	1.00 (1.00-1.00)	0.52 (0.34-0.70)	2.07 (0.64-3.75)	0.00 (0.04-5.47)
<b>Hypertonic Rectus Femoris Injection</b>	Pain provocation on one adduction test	0.67 (0.50-0.84)	0.54 (0.36-0.72)	1.45 (0.71-2.97)	0.62 (0.19-2.02)
	Pain provocation on two adduction tests	0.86 (0.73-0.98)	0.61 (0.43-0.78)	2.19 (1.21-3.96)	0.23 (0.04-1.48)
	Pain provocation on three adduction tests	1.00 (1.00-1.00)	0.52 (0.34-0.70)	2.07 (0.64-3.75)	0.00 (0.04-5.47)

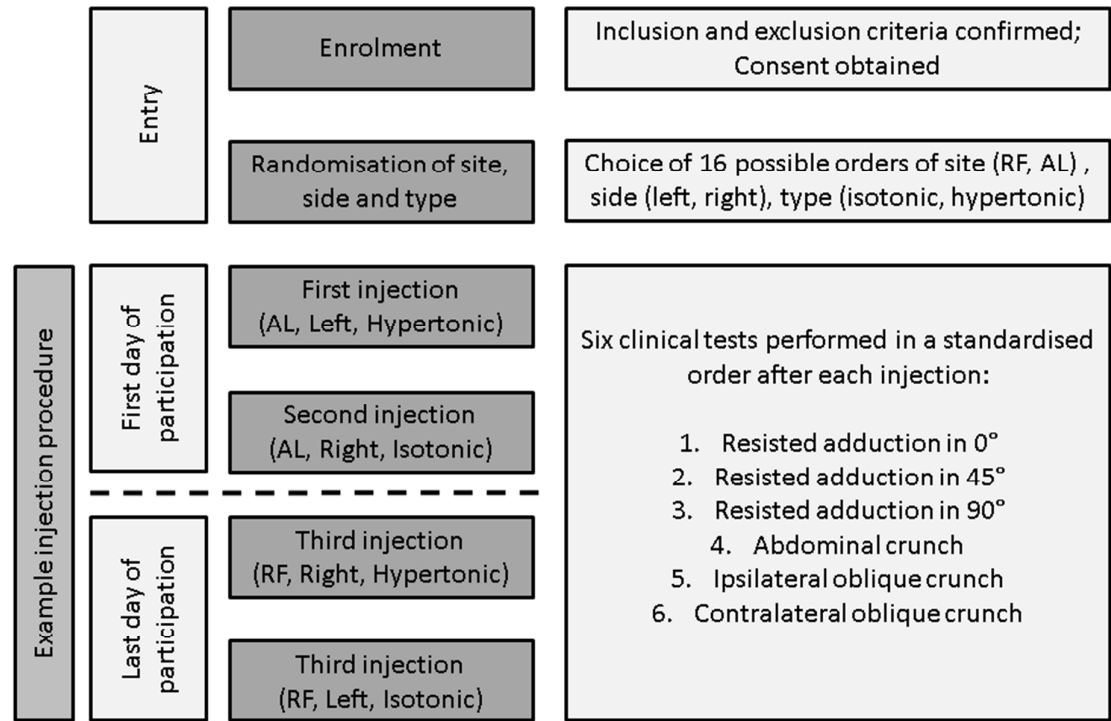
Test= provocation test; 95% CI= 95% confidence intervals

**Table 3** Receiver Operating Characteristic (ROC) curves and comparisons of the area under the curve (AUC) of individual and combinations of tests. Test: provocation test; AUC: area under the curve; Std. Err.: standard error; 95% CI: 95% confidence intervals; †p-values with Bonferroni correction for multiple analyses; \* indicates statistical significance (P<0.05)

Control Injection	Test	AUC	Std. Err.	95% CI		p-value†
Rectus Femoris Hypertonic Saline	0°	0.73	0.08	0.57	- 0.90	Reference
	45°	0.57	0.06	0.44	- 0.69	0.15
	90°	0.70	0.07	0.57	- 0.83	1.00
Adductor Longus Isotonic Saline	0°	0.80	0.07	0.66	- 0.94	Reference
	45°	0.60	0.05	0.50	- 0.70	0.04*
	90°	0.70	0.07	0.57	- 0.83	0.25
Rectus Femoris Hypertonic Saline	0°	0.73	0.08	0.57	- 0.90	Reference
	45°	0.57	0.06	0.44	- 0.69	0.39
	90°	0.70	0.07	0.57	- 0.83	1.00
	Crunch	0.40	0.07	0.27	- 0.53	0.00*
	Ipsi	0.43	0.07	0.29	- 0.58	0.02*
	Contra	0.47	0.07	0.33	- 0.6	0.07
Adductor Longus Isotonic Saline	0°	0.80	0.07	0.66	- 0.94	Reference
	45°	0.60	0.05	0.5	- 0.70	0.11
	90°	0.70	0.07	0.57	- 0.83	0.63
	Crunch	0.53	0.03	0.47	- 0.60	0.00*
	Ipsi	0.57	0.05	0.48	- 0.66	0.01*
	Contra	0.57	0.05	0.48	- 0.66	0.01*
Rectus Femoris Hypertonic Saline	Sum of 3 adduction tests	0.79	0.08	0.73	- 0.97	Reference
	0°	0.73	0.08	0.66	- 0.94	0.41
	45°	0.57	0.06	0.50	- 0.7	0.01*
	90°	0.70	0.07	0.57	- 0.83	0.62
Adductor Longus Isotonic Saline	Sum of 3 adduction tests	0.85	0.06	0.64	- 0.94	Reference
	0°	0.80	0.07	0.57	- 0.90	0.54
	45°	0.60	0.05	0.44	- 0.69	0.00*
	90°	0.70	0.07	0.57	- 0.83	0.06
Rectus Femoris Hypertonic Saline	Sum of 3 adduction tests	0.79	0.08	0.64	- 0.94	Reference
	Battery of all tests	0.79	0.08	0.63	- 0.95	0.98
Adductor Longus Isotonic Saline	Sum of 3 adduction tests	0.85	0.06	0.72	- 0.97	Reference
	Battery of all tests	0.85	0.06	0.72	- 0.97	1

Figures

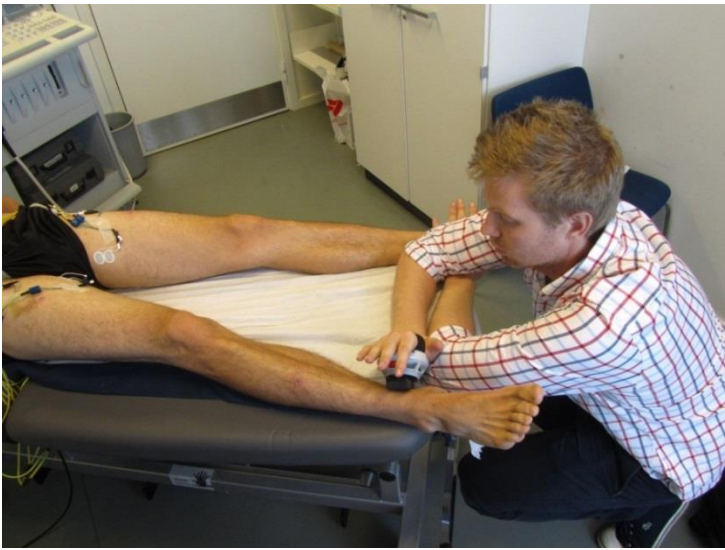
Figure 1 - The study design and an example of the experimental procedure illustrating order of injection on each day of participation.



RF: Rectus femoris injection site; Right: side of injection; Left; side of injection; Hypertonic: hypertonic saline injection; Isotonic: isotonic saline injection; site: injection site (adductor longus tendon or rectus femoris tendon); type: type of injection (hypertonic or isotonic)

Supplement 1. Clinical tests

Figure 1 - The 0° adduction test



The 0° adduction test: The subject lay with legs straight and the examiner placed the forearm between the medial malleoli with the dynamometer fixed to one end, the subject was instructed to adduct maximally

Figure 2 - The 45° adduction test



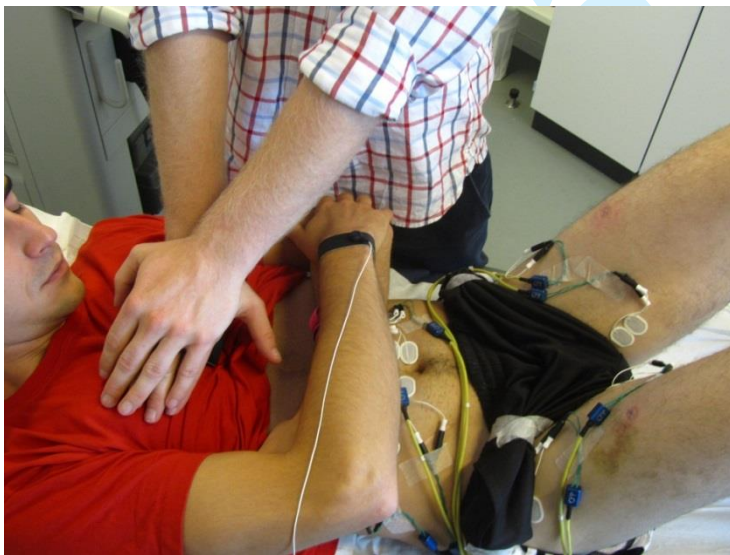
The 45° adduction test: The subject lay with the feet flat on the plinth with hips flexed to 45° and the knees at 90° of flexion and performed maximal adduction with the dynamometer held in place by the examiner between the medial condyles of the knees

Figure 3 - The 90° adduction test



The 90° adduction test: the subject lay with the hips and knees at 90° of flexion and performed maximal adduction with the dynamometer between the medial femoral condyles

Figure 4 - The abdominal crunch manoeuvre



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.

Figure 5 - The Oblique crunch manoeuvre





The oblique crunch manoeuvre: the dynamometer was positioned on the shoulder contralateral to the examiner who applied resistance while the subject performed an abdominal crunch towards the contralateral knee (Holmich et al. 2004).

## Supplement 2 – Example 2x2 table for diagnostic statistics

	Condition positive (hypertonic AL injection)	Condition negative (isotonic AL injection)
Clinical test positive (increased resting pain)	9	1
Clinical test negative (no increased resting pain)	6	14

Example is the results of the comparison of hypertonic and isotonic adductor longus injections for the 0° adduction test

Prof. Stephen Harridge  
Editor-in-chief The Scandinavian Journal of Medicine & Science in Sports

Aalborg, 6.5.2015

Dear Prof. Harridge,

Thank you for the positive and constructive comments/suggestions to our manuscript. Please find below our point-by-point response to the reviewer comments which we hope will meet the requirements for acceptance. We have taken all the comments and suggestions into close consideration. The reviewer comments are found below in bold below with replies to comments initiated by an asterisks (\*\*). Modified sections in the paper are identified in red. We feel the reviewer comments have strengthened the results and readability of this paper.

Kind Regards,

Thomas

**Reviewer: 1**

**GENERAL**

Nice work – you can be proud of this article and I would definitely recommend publication.  
In present form – some changes could help improve the piece:

**Main issues:**

Methods section in current form doesn't do justice to the good methods used – see below  
Results – the “basic data” not as well presented as the more complex calculations –see below  
Discussion – you need to help me and the reader better understand what some of the more complex stats actually mean – see below.

**TITLE:**

1. I am not sure what Scandinavian Journal policy is on this but consider more catchy title:  
Adduction testing in hip neutral best: an experimental pain study – or something like this??  
See what you think – key is to mention the key finding in the title to draw people to want to look at the paper.

\*\*

Response: The title has now been modified to: **Resisted adduction in hip neutral is a superior provocation test to assess adductor longus pain: an experimental pain study**

**1. INTRODUCTION:**

Sets the scene very well and comes to the point quickly. Justifies need for the paper.  
The Doha agreement meeting on terminology and definitions on groin pain in athletes also places



palpation and resistance testing as being central in the system – you can quote that here too (publication pending – Greg has the paper as one of the co –authors). Will help you justify your study.

\*\*

Response: Thank you for this suggestion. As this is not published, we prefer not to quote it as yet.

Page 5 line 19 – I agree multiple entities common and the combination of adductor and inguinal related is frequent. Consider an alternative term to “Abdominal pathology” to me this would suggest appendicitis etc. In new consensus paper we are calling it “inguinal-related groin pain”. Or something else that specifies its MSK pain?

\*\*

Response: Thank you, this has been modified in the following manner: *This is further confounded by clinical populations commonly presenting with more than one pathology (Holmich 2007) including musculotendinous pathology of the abdominal region.*

## 2. METHODS:

See general comments – you miss out on the opportunity to score better here when people evaluate your study with quality scores in the future. I would recommend going through the paper with the Downs and Black score to see where you would now drop points.

I would say you can add:

\*\*

Response: We appreciate these points being raised as this of course adds clarity to the methodology we used. Below are found our responses to each point

### 1. How participants were recruited.

\*\*

Response: *Sixteen healthy, physically active (at least 2.5 hours of regular, vigorous weekly exercise) male subjects without current or previous hip, groin, or lumbar injuries, were recruited through university advertisements and websites*

### 2. Use explicit terminology – Inclusion and exclusion criteria – now it is in there but not obvious.

\*\*

*Response: Sixteen healthy, physically active male subjects were recruited through university advertisements and websites (mean  $\pm$  SD; age:  $27 \pm 3.4$  years, body mass index:  $23.85 \pm 2.15$ ). Inclusion criteria were males without current or previous hip, groin, or lumbar injuries who physically active for at least 2.5 hours of regular, vigorous weekly exercise. Exclusion criteria included signs of neurological disorders or rheumatologic diseases which could affect the outcome of the experimental procedure. Participants who reported medication use either on enrolment or on a regular basis were also excluded. At baseline testing, the participant was asked whether any pain was experienced in the hip/groin when performing each test and a positive response was used for exclusion purposes.*

### 3. Now you mention the fact that they underwent physical exam on the top of page 9 – put this bit in inclusion/exclusion section.

\*\*

Response: This has now been corrected. *Exclusion criteria were signs of neurological disorders or rheumatologic diseases which could affect the outcome of the experimental procedure. Participants who*

*reported medication use either on enrolment or on a regular basis were also excluded. At baseline testing, the participant was asked whether any pain was experienced in the hip/groin when performing each test and a positive response was used for exclusion purposes.*

**4. You mention randomisation – expand on this – how was this done and by whom etc – you are now left guessing as a reader.**

**\*\***

Response: This has been expanded on page 5: *Randomisation was achieved through the selection of an envelope (blinded to the injector, participant and experimenters) with boxed randomisation with one the possible orders of injection site, side, and injection site.*

**5. You say saline type blinded – who prepared this – how was blinding assured – again now you have done blinding and probably allocation concealment – but I can’t tell how. At the current time you have done all the hard work and used a good design but readers won’t be able to see this from the paper.**

**\*\***

Response: This has been expanded on page 5: *Blinding was achieved through pre-prepared syringes prior to the experimenters entering the room. The participants were not advised of the order of injections at any stage throughout the procedure.*

**3. RESULTS**

**You have handled the more complex part of the results well but as a reader I would like to know more about the “basic data” than you now present. This gives you more of a feel for things. You will also drop points on future quality scoring at the moment For example –**

**1. For HAGOS you present median and range – add in an IQR here.**

**\*\***

Response: Thank you, this has now been corrected in the following manner on page 10:

*The HAGOS subsections responses were: Pain (median=100; IQR, 100-100); Quality of Life (100; 100-100); Physical Activity (100; 100-100); Symptoms (96; 93-100); Sport/Recreation (100; 100-100); Activities of Daily Living (100; 100-100).*

**2. For the pain scores – you present “significant random effect” – I am not very strong on this kind of stats and will admit I have no idea what this is. Can you explain this to me.**

**\*\***

Thanks for the opportunity to explain: In linear mixed modelling, there are fixed and random effects. Fixed effects are similar to factors in a repeated measure ANOVA. Random effects are known interactions. Using a mixed model, we can account for interactions which will otherwise be assumed as separate. ANOVA is a form of mixed model.

**Further I would advise presenting some “basic” data first – what were the mean/median scores (plus SD or IQR) for the different tests under the different conditions – for example) degrees adduction with saline of pain injection.**

**\*\***

There was no range of motion testing undertaken as stretching a muscle injected with saline will relieve the experimental pain. All force measures were matched to baseline values to ensure the observed provocation was not due to a change in force output. Therefore, we have not altered the manuscript.

**Now I can't see anywhere just how painful the painful injections were. You could add this into table 1.**

\*\*

We appreciate this valuable comment. However, in this paper, we were primarily interested in whether the tests could specifically 'provoke' pain when the origin on pain was known. Therefore, the pain intensity was of less relevance to the research question in this paper, although we focussed on this in a follow-up study currently under review.

**I am curious how long the pain from the injections lasts? Could you say something about this.**

\*\*

As indicated above, we did not focus on this in the paper. However, pain from hypertonic saline into tendons and muscles normally lies between 4-7/10 on a VAS scale and lasts for 7-10 minutes. (Loram et al.) This was also true for our study.

Loram L, Horwitz E, Bentley A. Gender and site of injection do not influence intensity of hypertonic saline-induced muscle pain in healthy volunteers. *Manual Therapy*. 14: 526-530.

#### 4. DISCUSSION

**The discussion is brief and well written – well done.**

**I would suggest looking at the second sentence again –**

**The 0 test “optimises diagnostic procedure” without compromising “diagnostic capacity”. Both the terms in speech marks are not well defined. Try and be as concrete as possible here when you describe your results. Keep it practical and related to your data. Something like:**

**The 0 degree test elicited the highest levels of pain after use of an experimental pain injection in the AL tendon.....**

**You don't need to take my wording – but try and just talk about what the data showed in this opening section.**

\*\*

Response: Thank you for this remark. We can see that this may be a bit broad and therefore difficult to interpret. Therefore, the following changes have been made in the text on page 12: *This study indicates that the 0° test showed the highest the positive likelihood ratio to detect experimental adductor longus pain without compromising diagnostic capacity as indicated by the AUC on the ROC curve.*

**In last 2 sentences of 1st paragraph you also say:**

**1. There was a difference in strength – state which ones stronger or weaker here.**

\*\*

Response: We had overlooked this in the manuscript but we agree that this needs to be clarified. Therefore the following changes have been made in the text on page 12: *The 0° test was weaker than the 45° and 90° tests however, when considering lever length, this is unlikely to be a cause.*

**2. “diagnostic procedure” in last line – again this is not an everyday term – see if you can make this more concrete.**

\*\*

Response: We acknowledge this and have therefore made the following changes in the text on page 14: *The abdominal manoeuvres did not improve the ability to diagnose experimental pain relating to m. adductor longus.*

Second paragraph onwards:

My main point here is that you have used a number of more complex techniques which I, and I would guess most of readership, will not be massively familiar with. Adding in concrete examples of what the stats show is going to really help me and the readers understand your data.

As an example:

Page 13 – line 42: abdo moves.... Had poor specificity and as a result AUC results were significantly lower on the ROC curves.

You can improve this by then adding a line

\*\*\*

Response: We can see that this might not be clear and thank you for raising the point. To address this we have made the following changes on page 12: *The abdominal crunch or oblique crunches, both ipsi- and contralaterally, had poor specificity and as a result the AUC results were significantly lower on the ROC curves indicating lesser ability to discriminate between true positives or negatives.*

This means.....

To help us interpret the data in an easy to understand clinical sense.

The way you do this in the 1st paragraph of the third section on diagnostic capabilities of multiple tests is perfect example of how to explain it simply.

Page 14 Line 38: No advantage of clinical exam abdo manoeuvres in painful groin.

I don't agree with this. In practice some athletes present with inguinal related groin pain or distal rectus problems – in these cases doing resisted abdominal testing may help clinicians. You mention this straight after the line yourself too. So to say you don't need to do them for all "groin pain" is not justified by your data. I would suggest changing to "adductor-related" or something similar.

\*\*\*

Response: We fully agree with this comment and have therefore changed the sentence on page 14: *Using a battery of six tests did not improve the diagnostic performance to identify painful AL compared to using three tests indicating that there is little advantage obtained with the inclusion of the abdominal manoeuvres in clinical examination to diagnose adductor longus-related pain.*

Page 16 last 2 paragraphs:

Here suddenly the introduction of central vs. peripheral crops up rather unexpectedly. I agree from my clinical experience that this may be quite common in practice but having these two whole paragraphs on it here rather disrupts the flow of the article. I would try and shorten this down to just a brief mention.

\*\*\*

Response: Thank you for this comment. We fully agree as this is not the focus of the paper. This has been removed without altering the topic of the paragraph. The paragraph now reads:

*The clinical tests utilised represent common tests for the diagnosis of long-standing groin pain in athletes and as such the mechanisms of pain in the clinical population are likely to be different. Future radiology studies should include clinical assessment data enabling clinicians and researchers to interpret the differing responses to pain provocation tests in the presence of the imaging findings. This will allow clinical validation of the experimental results presented in this paper.*

*Clinical tests for the groin area can be affected by pain alone in the absence of pathology which is consistent with findings from other studies (Izumi et al. 2014; Palsson & Graven-Nielsen 2012; Palsson et al. 2014) highlighting that positive provocation tests may be induced by the present of pain in the absence of pathology per se.*

**PERSPECTIVES:**

**First three lines – great and nicely concrete.**

**Last line – see above – only relates to adductor related issues – to much of a sweeping statement. Specify further.**

**Hope these comments help.**

**\*\***

Response: We agree and have implemented similar changes as we did previously in the text: *Abdominal manoeuvres in addition to the adduction tests do not increase the diagnostic performance of a test battery to identify experimental adductor longus-related pain.*

**Reviewer: 2****Comments to the Author****Regarding SJMSS-O-045-15**

**This is a very nice and very relevant study, examining a subject that has been debated for many years. The design is nice and the interpretation seems adequate.**

**I have however, following comments:**

**P4 L 18**

**I would include the recent papers by Branci et al as well as they are clearly questioning if imaging can help differentiating.**

**\*\***

Response: Thank you for the kind reminder. These citations have now been added.

**P4 L 35**

**Brooke 2012 is not available – cannot be on the ref list**

**\*\***

Response: Thank you for pointing this out. This is an Honours thesis and available through the University of Wollongong, Australia. We have removed this reference to reduce any confusion a reader may have.

**P 7**

**I would suggest a flow chart to describe how this study was conducted. The section from line 13 – 26 is not clear. Combined with the further description on the next page I get increasingly confused. This should be rewritten and a flow chart added.**

**\*\***

Response: Thank you for this comment, we prefer to maintain a description of the study process and following careful review we have settled on what we feel is an appropriate description of the injection procedure.

**P7**

**How can both isotonic and hypertonic saline have 5.8%?**

**\*\***

Response: Apologies, this was overseen when reading through the manuscript and has now been corrected.

Isotonic saline (1 ml, 0.9%) was injected...

**P7 L22**  
**The same site?? AL ...RF?? I do not follow?**

\*\*

Response: We appreciate the misunderstanding. Each participant received an injection in the same site on each side (AL or RF) on each day. The alternate site was injected on the subsequent day. By this statement, we are indicating that they did not get injected in the RF and AL on the same day.

This has been clarified in the Method section on page 6: *the same injection site however, on the contralateral side.*

**P8 L33-38**  
**Why was the HAGOS done after the injection and the pain had resolved?**

\*\*

Response: This is definitely a valid comment and we see how the procedure may cause confusion. The HAGOS was filled in at this time point due to practical reasons as it allowed streamlining of the experimental procedure. All patients were educated to report their responses relating to prior to the injections. Furthermore, all questions in the HAGOS refer to pain/symptoms etc in the past week prior to coming to the experimental session.

**What is meant by Danish 2013?**

\*\*

Response: This is the referencing style, provided for the journal through Endnote, for the website related to the first reference. We have altered the manuscript on page 7 to be more informative: *Danish (www.koos.nu (2013)) version.*

**Why is Nevin & Delahunt included as a reference for HAGOS?**

\*\*

Response: Nevin and Delahunt provided validation that the HAGOS can dichotomise athletes with and without longstanding groin pain. In table 1, in their paper they showed a significant difference in all subsections of the HAGOS between the cases and controls. To remove the confusion to the reader, we have removed this reference.

**P9 L35**  
**Oblique sit up – Reference Holmich et al 2004**

\*\*

Response: We value your kind suggestion. This has been added.

**P12 L 22**  
**What does “in both conditions” refer to?**

\*\*

Response: Thank you for the point of clarification. This has been altered to: *Resisted adduction in 0° of hip flexion showed the highest positive likelihood ratio when examined using both control conditions (hypertonic RF or isotonic AL injections).*

**P13 L 15-18**  
**I am not sure what you mean? Please explain further. Elongation? Cam-effect?**

\*\*

Response: Given the anatomy of the adductor longus, placing it in the flexed hip position will shorten the muscle. In neutral to extension position of the hip the adductor longus will have to be relatively elongated compared to a flexed hip position. It is well established that the RA and AL are continuous around the pubic crest. Anatomically, there has to be a cam for this to occur.

This has been clarified on page 12: *This consistent with previous findings in clinical populations (Lovell et al. 2012). It is hypothesised that this may be related to the elongation of the adductor tendon and the cam-effect of the anatomical relationship between the rectus abdominis and adductor longus (Clark et al. 2010; Norton-Old et al. 2013)*

**P13 L20**

**Clark et al is just an early abstract (and should not be referenced) of the Norton-Old et al 2012 – which is actually a 2013 paper.**

\*\*

Response: We fully agree and thank you for the clarification. We had referenced the e-ahead of print version but this has now been updated.

**P13 L 51-53**

**...which can transmit forces... I would put in a “probably” can transmit....Reading the paper and acknowledging its limitations I still think we need better proof.**

\*\*

Response: This is a valid point which we agree with. The section in the paper has therefore been changed in the following manner: *While the adductor longus and rectus abdominis are known to be intimately linked at the pubic bone through a common entheses and aponeurosis (Robinson et al. 2007) which is likely to transmit forces between structures across the pubic symphysis (Norton-Old, Schache 2013)*

**P14 L29-30**

**Strike little advantage and add nothing instead. The strength of the 0 degree test is diluted by adding the others.**

\*\*

Response: We thank you for your comment and see your point. However, given the limits of an experimental pain study; we feel this statement extends beyond our data. We would prefer to keep our wording.

**P14 L 43**

**Painful groin .... Change to painful adductor longus. You are supporting this yourself in the next sentences.**

\*\*

Response: A valid remark which has now been accounted for in the text:

*Using a battery of six tests did not improve the diagnostic performance to identify painful AL compared to using three tests indicating that there is little advantage obtained with the inclusion of the abdominal manoeuvres in clinical examination to diagnose adductor longus-related pain.*

**P16 L4-6**

**Why?**

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Response: Thanks, this part certainly deserves more clarifications. Given the inherent design of the study, hypertonic saline is not clinical pain induced through peripheral tissue damage. Hypertonic saline induces pain by causing a transient change in the chemical milieu surrounding the free nerve endings within the muscle. It is unknown which pain mechanisms are involved in longstanding adductor-related pain and such a discussion is beyond the scope of this paper.

**P16 L13-20**  
**This is a bit strange to me? Why? & How? Please take a look at the recent finding by Branci et al in BJSM.**

\*\*

Response: We acknowledge this remark. This statement has been removed based on advice from the first reviewer.

PROOF



Prof. Stephen Harridge

Editor-in-chief The Scandinavian Journal of Medicine & Science in Sports

Aalborg, 6.5.2015

Dear Prof. Harridge,

Thank you for the further positive and constructive comments/suggestions to our manuscript. Please find below our point-by-point response to the reviewer comments which we hope will meet the requirements for acceptance.

Again, we have taken all the comments and suggestions into close consideration. The reviewer comments are found below in bold below with replies to comments initiated by an asterisks (\*\*). Modified sections in the paper are identified in red (initial revision) and purple (second revision). We feel the reviewer comments have strengthened the results and readability of this paper.

We thank you for your continued support of this paper.

Kind Regards,

Thomas

**Comments from the Section Editor:**

**Authors have done a nice job in revising their paper and responding to reviewer comments. They are advised to revise the paper in line with the reviewers' final minor suggestions regarding clarification of randomization and injection procedures.**

\*\*

Thank you, we have addressed these in the below. The manuscript has been modified accordingly.

Reviewers Comments to Author:

Reviewer: 1

Comments to the Author

Dear Authors -

Thanks for the well written and structured, clear reply. It makes life as a reviewer much nicer!  
I think you have a great paper and would recommend publication for sure.  
My only comment at this stage is with regard to randomization/blinding and allocation concealment section. These are huge "plus points" of your methodology but the description now only extends to 3 lines:

"Randomisation was achieved through the selection of an envelope (blinded to the injector and experimenters) with boxed randomisation with one the possible orders of injection site, side, and injection site. Blinding was achieved through pre-prepared syringes prior to the experimenters entering the room. The participants were not advised of the order of injections at any stage throughout the procedure. "

While they are now described, i reckon if you go for next level of detail here it will add quality to the paper:

- 1. Who picked envelope/ how many were prepared / which orders were in there
- 2. I don't understand how the blinding worked from current description - were the syringes in a sheath?

\*\*

Thank you, we have addressed these points. The paragraph now reads:

*Study design*

The experiment was a randomized, single-blinded, balanced-crossover design, and was conducted in two sessions within one week. Randomisation was achieved through the selection of one of 16 envelopes by an experimenter (blinded to the injector and experimenters) with boxed randomisation with one of all 16 possible order combinations of injection site, side, and injection site. Blinding was achieved through unlabelled, identical pre-prepared syringes prior to the experimenters entering the room. The participants were not advised of the order of injections at any stage throughout the procedure. All assessments were performed with subjects lying on a plinth in supine. At baseline, subjects were familiarized with the experimental procedure where pain provocation tests were performed before (baseline), during, and after (post-pain) induction of experimental pain. The post-pain state was defined as five minutes after the cessation of pain indicated by a score of "0 cm" on

VAS. The sequence of provocation tests was consistent throughout each session but the sides and order of injections (saline type and injection sites) were randomized. In each session a painful trial and a non-painful trial were completed in each side, respectively.

**Reviewer: 2**

**Comments to the Author**

Thank you for the revised paper, I think it has improved and is now even better. I have however, a few comments that I think are important:

**Adding to Reviewer 1:** The Doha agreement paper will be out around June 1st so you can easily include it in the paper and I would certainly support that!

**1. INTRODUCTION:**

Sets the scene very well and comes to the point quickly. Justifies need for the paper. The Doha agreement meeting on terminology and definitions on groin pain in athletes also places palpation and resistance testing as being central in the system – you can quote that here too (publication pending – Greg has the paper as one of the co –authors). Will help you justify your study.

**\*\***

Response: Thank you for this suggestion. This study has been published in the time taken to revise this manuscript and is now included in the introduction.

A recent systematic review of symphyseal and adductor-related groin pain (Branci et al. 2013) synthesised the results of imaging studies to four main findings: adductor longus enthesopathy, the secondary cleft sign, bone marrow oedema, and pubic symphysis-related degenerative changes. Clinically, it is difficult to differentiate between these findings without imaging. Furthermore, there are limited studies showing how these imaging findings change management as they commonly coexist. The prevalence of abnormal imaging findings is also high in asymptomatic populations (Branci et al. 2014; Branci et al. 2013) with poor association between abnormal imaging findings and risk of future injury (Robinson et al. 2014). *The terminology and definitions of groin pain in athletes have been recently improved following the ‘Doha agreement meeting on terminology and definitions in groin pain in athletes’ which adopted clinically-based taxonomy in three categories; “clinical entities” for groin pain, hip-related groin pain and other causes of groin pain in athletes.*

**Page 7 line 20:**

I believe you are short of a “were” physically active....

**\*\***

Response: Thank you, this has been corrected.

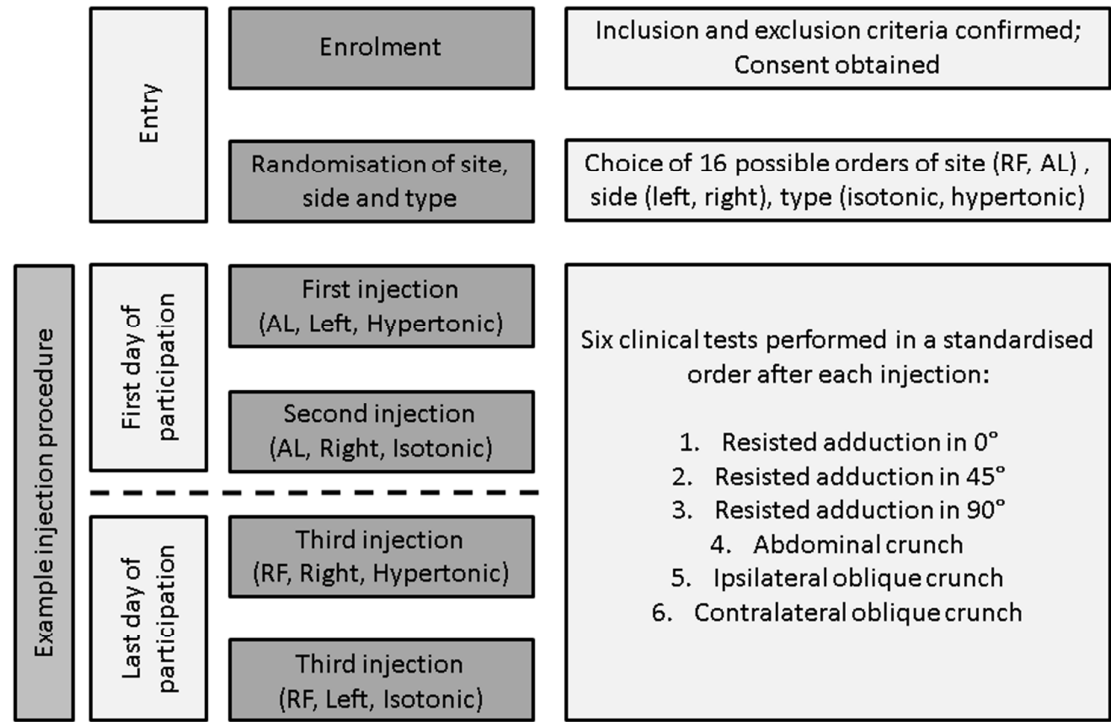
Inclusion criteria were males without current or previous hip, groin, or lumbar injuries who were physically active for at least 2.5 hours of regular, vigorous weekly exercise.

**Page 8:**  
Groin pain was induced by injecting sterile hypertonic saline (1 ml, 5.8%) into the adductor longus (AL) tendon. As a painful control injection outside the groin area, hypertonic saline (1 ml, 5.8%) was injected into the proximal tendon of the long head of the m. rectus femoris (RF) on a separate day. Isotonic saline (1 ml, 0.9%) was injected as a non-painful control at the same injection site however, on the contralateral side. The subjects received one hypertonic and one isotonic saline injection in each side during each session and the order of the saline type and site was randomized in a balanced way (left or right). The saline type was blinded to the subject and injector. Injections were given over the duration of approximately 10 seconds using a 2-ml plastic syringe with a disposable needle (27G). All injections were given by an orthopaedic surgeon (MI) after a standard disinfection protocol.

This is still not clear. I think it is important to remember that you often know and understand your own procedure by heart but the readers and perhaps even more the non-native English readers do not. I would really argue for a more clear and idiot-safe explanation @ I am not sure I could repeat the procedure from this explanation???

\*\*

Response: We acknowledge this concern and have added a supplementary figure to address this point. We hope that this assist the reviewer and readers to better understand the experimental procedure.



Is this sentence correct? Could it be “being” instead of “was”??

The 0° test was weaker than the 45° and 90° tests however, when considering lever length, is unlikely to be a cause

\*\*

Response: Thank you, this has been corrected.

The 0° test *being* weaker than the 45° and 90° tests however, when considering lever length, is unlikely to be a cause.

Page 17 line 32-39

This section has not been removed as stated in your reply??? It is still a bit strange to me? Why? & How? Please take a look at the recent finding by Branci et al in BJSM.

Future radiology studies should include clinical assessment data enabling clinicians and researchers to interpret the differing responses to pain provocation tests in the presence of the imaging findings. This will allow clinical validation of the experimental results presented in this paper.

\*\*

Response: The section was modified due to the comments (below in bold and italics). The comments pertained to the pain mechanism rather than the statement regarding radiology studies. We acknowledge the results of Branci et al, particularly relating to the high prevalence rates in asymptomatic athletes. This further section is now removed.

“Page 16 last 2 paragraphs:

Here suddenly the introduction of central vs. peripheral crops up rather unexpectedly. I agree from my clinical experience that this may be quite common in practice but having these two whole paragraphs on it here rather disrupts the flow of the article. I would try and shorten this down to just a brief mention.”

The manuscript now appears as:

The clinical tests utilised represent common tests for the diagnosis of long-standing groin pain in athletes and as such the mechanisms of pain in the clinical population are likely to be different. This study indicates that the 0° test optimizes the diagnostic procedure without compromising diagnostic capacity to identify experimental adductor longus tendon pain. Clinical tests for the groin area can be affected by pain alone in the absence of pathology which is consistent with findings from other studies (Izumi et al. 2014; Palsson & Graven-Nielsen 2012; Palsson et al. 2014) highlighting that positive provocation tests may be induced by the present of pain in the absence of pathology per se. Explorations beyond pathological anatomical diagnoses of groin patients are warranted.