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EXERCISE-INDUCED HYPOALGESIA AFTER ISOMETRIC WALL SQUAT EXERCISE: A TEST-RETEST RELIABILTY STUDY

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ABSTRACT

Background: Isometric exercises decrease pressure pain sensitivity at exercising and non-exercising muscles known as exercise-induced hypoalgesia (EIH). No studies have assessed test-retest-reliability of EIH after isometric exercise. This study investigated the EIH on pressure pain thresholds (PPTs) after an isometric wall squat exercise. The relative and absolute test-retest reliability of the PPT as test stimulus and the EIH response at exercising and non-exercising muscles were calculated.

Methods: In two identical sessions, PPTs at the thigh and shoulder were assessed before and after 3 min quiet rest and 3 min wall squat exercise, respectively in 35 healthy subjects. Relative test-retest reliability of PPT and EIH was determined using analysis of variance models, Persons r, and intraclass correlations (ICCs). Absolute test-retest reliability of EIH was determined based on PPT standard error of measurements (SEM) and Cohens kappa for agreement between sessions.

Results: Squat increased PPTs at exercising and non-exercising muscles by 16.8±16.9% and 6.7±12.9%, respectively ($P<0.001$) with no significant differences between sessions. PPTs within and between sessions showed moderately-strong correlations ($r\geq0.74$), and excellent ($ICC\geq0.84$) within-session (rest) and between-sessions test-retest reliability. EIH responses at exercising and non-exercising muscles showed no systematic errors between sessions, however relative test-retest reliability was low (ICCs: 0.03-0.43), and agreement in EIH responders and non-responders between sessions was not significant ($\kappa<0.13$, $P>0.43$).

Conclusion: A wall squat exercise increased PPTs compared with quiet rest; however, the relative and absolute reliability of the EIH response was poor. Future research is warranted to investigate the reliability of EIH in clinical pain populations.

Key words: exercise, exercise-induced hypoalgesia, reliability, pressure pain thresholds

1. INTRODUCTION

Exercise decreases the pain sensitivity at exercising and non-exercising muscles known as exercise-induced hypoalgesia (EIH) [1, 2]. In healthy subjects, hypoalgesia after aerobic exercises (e.g. bicycling or running) has been demonstrated at moderate to high exercise intensities [3, 4] whereas hypoalgesia after isometric exercise (i.e. a muscle contraction without joint movement) has been demonstrated after both low and high intensity exercises [5, 6].

The magnitude of the EIH response is typically calculated as the absolute or relative difference in the test stimulus (e.g. pressure pain thresholds (PPT)) after the exercise condition compared with the test stimulus before the exercise condition. In patients with chronic musculoskeletal pain the acute effect of exercise is still controversial, since both hypoalgesia [7, 8] and hyperalgesia [9, 10] have been reported.

Despite reliable pressure methodology for assessment of pain sensitivity [11, 12], and an increasing number of studies investigating the effect of exercise, no studies have considered the measurement error of the test stimulus (e.g. normal variation in repeated assessments of PPT) as some of the change in e.g. PPT indicating a EIH response may be due to measurement error. Moreover, studies investigating the test-retest reliability of EIH are almost non-existing. Since exercise is an important part of many treatment programs for chronic pain [13] further knowledge on the between-session reliability is important. In addition to the investigation of the relative test-retest reliability (based on intraclass correlation coefficient (ICC) values), which reflects the ability of the EIH paradigm to differentiate between different subjects, quantification of the EIH within-subject reliability based on responders and non-responders (absolute test-retest reliability) could further advance the understanding of the EIH response of an individual subject which may be a step towards individualized pain rehabilitation.

So far, no studies have investigated the between session test-retest reliability of EIH after an isometric leg muscle contraction as the exercise condition, although this exercise condition seems to provide relevant reductions in pain sensitivity in patients with chronic pain [14]. Moreover, EIH after isometric exercises appear to be less dependent on exercise intensity [4] than aerobic exercises which may increase its applicability in subjects with chronic pain. In addition, isometric exercises may reduce temporal summation of pain in healthy subjects [15, 16], a central pain mechanism which is often facilitated in patients with chronic pain [17, 18] further enhancing its potential in pain rehabilitation.

The aims of this study in healthy subjects were to 1) compare the effect on PPT at exercising and non-exercising muscles after an isometric leg muscle exercise compared with quiet rest, and 2) investigate test-retest reliability of the test stimulus as PPT and the EIH response. It was hypothesised that 1) the isometric exercise would produce an increase in PPTs at exercising and non-exercising muscles compared with quiet rest, 2) PPTs and EIH would demonstrate fair to good relative test-retest reliability (based on ICC values), and 3) EIH responders and non-responders (based on normal variation in PPTs) would show significant agreement between sessions.

2. MATERIALS AND METHODS

2.1 Participants

Thirty-five healthy subjects (mean age of 23.1 ± 2.2 years; [range 20–30 years]; average body mass index (BMI) 23.1 ± 1.7 kg/m^2 [range 20.0-27.5]; 2 left-handed; 17 women) were included in this study that was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee (S-20160189) and all subjects provided written informed consent. Subjects were recruited by advertisement at the local university college in Northern Denmark, and through social media. None of the included subjects suffered from neurological, psychological, cardiovascular diseases, had any pain or used any pain medication during the weeks prior to and during participation. All subjects were asked to refrain from physical exercises, coffee and nicotine on the days of participation.

2.2 Procedure

Subjects participated in two sessions at the same time of the day and separated by 1 week (Fig. 1) to avoid potential carry-over effects from the pain sensitivity assessments, and exertion after physical exercise between sessions, as well as to avoid extensive changes in physical fitness level within subjects.

All subjects were verbally introduced to the procedures and familiarized to assessment of PPT on the non-dominant thigh, which was not used for further assessments. In each session, PPTs were initially recorded from the dominant thigh and the non-dominant shoulder. In addition, all subjects had a 3 min quiet rest condition and performed a 3 min isometric wall squat exercise in each session. PPTs were assessed before and immediately after quiet rest and exercise.

2.3 Assessment of PPTs

PPTs were assessed using a handheld pressure algometer (Somedic Sales AB, Sweden) with a stimulation area of 1 cm^2. The rate of pressure increase was kept at approximately 30 kPa/s and the first time the pressure was perceived as minimal pain, the subject pressed a button and the pressure intensity defined the PPT. PPT measurements were conducted with the subject seated on a plinth without foot support and with both arms resting on the thighs. Two assessment sites were located and marked. Site one was located in the middle of the dominant quadriceps muscle, fifteen centimetres proximal to the base of patella. Site two was located in the non-dominant upper trapezius muscle, ten centimetres from the acromion in direct line with the 7^{th} cervical vertebra.

Three PPT assessments were completed for each site and the average was used for statistical analysis. Twenty-second intervals between assessments were kept.

2.4 Quiet rest

Subjects were instructed to relax in a seated position in a comfortable armchair for 3 min in a temperate and undisturbed room. PPT assessments were performed as described, before and immediately after 3 min of quiet rest.

2.5 Isometric wall squat exercise

Three min isometric wall squat exercises were performed by all subjects. Subjects were instructed to stand upright with their back against the wall, feet parallel and shoulder-width apart, and hands by their sides. A goniometer was aligned with the lateral epicondyle of the right femur and subjects were instructed to lower their back down the wall until a knee joint angle of 100° flexion was reached. All subjects were asked to maintain this position for a maximum of 3 min or until fatigue. Just before the exercise condition the subject was instructed to rate pain intensity in the legs on a 0-10 numerical rating scale (NRS), with 0 defined as "no pain" and 10 "as worst imaginable pain", and rating of perceived exertion (RPE) on Borg's 6-20 scale, with 6 defined as "no exertion at all" and 20 as "maximal exertion". Pain intensity in the legs and RPE were assessed after 1, 2, and 3 min. Immediately after 3 min of wall squat, PPT assessments were performed as described.

2.6 Statistics

Results are presented as mean and standard deviation (SD) in the text and as mean and standard error of the mean (SEM) in figures. The effect of sessions and gender on baseline PPTs was analysed with a repeated-measures analysis of variance (RM-ANOVA) with *session* (session 1 and session 2) and *assessment sit*e (quadriceps and trapezius) as within subject factor and *gende*r as between subject factor. The effects of exercise and rest on PPTs were analysed with a mixed-model ANOVA with *session* (session 1 and session 2), *condition* (exercise and rest), *assessment site* (quadriceps and trapezius), and *time* (before and after) as within subject factors and *gende*r as between subject factor. Furthermore, relative (percentage increases of the PPT-after versus PPT-before) differences in PPTs after wall squat were calculated. Changes in NRS scores and RPE during wall squat exercises were analysed with RM-ANOVAs with *session* (session 1 and session 2), and *time* (0, 1, 2, and 3 min) as within subject factors and *gende*r as between subject factor. *P*-values less than 0.05 were considered significant. In case of significant main effects or interactions in ANOVAs, Bonferroni corrected t-tests were used for post-hoc comparisons incorporating correction for the multiple comparisons. Spearman's rho was used to investigate associations between the EIH responses and peak leg pain as well as rating of exertion during wall squat.

Test-retest reliability of PPTs and the EIH responses, the systematic error between sets of PPT assessments (intra: before and after rest; inter: baseline first and second session) and absolute (PPT after exercise minus PPT before exercise) and relative (PPT after exercise minus PPT before exercise divided by PPT before exercise multiplied by 100%) change in PPTs after exercise (i.e. EIH responses at first and second session) were determined using RM-ANOVAs. Persons r and intraclass correlation coefficients (ICCs) based on a single rating, consistency, 2-way mixed effect model ($ICC_{3,1}$) were used reflecting the ability of the PPTs and EIH responses to differentiate between individuals. An ICC above 0.75 was taken as excellent reliability, 0.40–0.75 was fair to good reliability, and less than 0.40 defined poor reliability [19]. To assess the test-retest reliability within individual subjects, the standard error of measurements (SEM) of PPTs were estimated as the square root of the mean square error term in the RM-ANOVA [20] to investigate the frequency of subjects who had an increase in PPTs after exercise equal to or larger than the SEM and the agreement between sessions was compared with Cohen's kappa coefficient. Data were analysed using SPSS Statistics, version 24 (IBM, Armonk, NY, USA).

3. RESULTS

3.1 Baseline PPTs

PPTs was higher in men (quadriceps: 797 ± 141 kPa, trapezius: 496 ± 94 kPa) compared with women (quadriceps: 540 ± 220 kPa, trapezius: 312 ± 132 kPa; $F(1,33) = 13.50$, $P < 0.001$). A main effect of assessment site was found for baseline PPTs ($F(1,33) = 124.00$, $P < 0.001$) with post-hoc test showing that PPT at the quadriceps site was increased compared with PPTs at the trapezius site in both men and women ($P < 0.001$). No significant differences in baseline PPTs were found between sessions ($F(1,33) = 1.05$, $P = 0.31$).

3.2 Comparison of exercise parameters between sessions

All subjects completed the wall squat exercise for the entire three min during session 1 and session 2, respectively. Rating of perceived exertion was progressively increasing (Fig. 3A; $F(3,99) = 508.76$, $P < 0.001$) with post hoc test showing higher RPE at each time point compared with the previous time point ($P < 0.001$). Moreover, a difference was found between session 1 and session 2 ($F(1,33) = 21.70$, $P < 0.001$) with higher RPE during wall squat in session 1 compared with session 2 ($P < 0.001$). No significant difference in RPE between women and men were found ($F(1,33) = 1.71$, $P = 0.20$).

Pain intensity in the legs reported during wall squat increased over time (Fig. 3B; $F(3,99) = 106.49$, $P < 0.001$) with post hoc test showing higher pain intensity at each time point compared with the previous time point ($P < 0.001$). No significant differences in pain intensity were found between session 1 and session 2 ($F(1,33) = 0.09$, $P = 0.77$) or between men and women ($F(1,33) = 0.10$, $P = 0.75$).

3.3 Change in PPTs after isometric exercise and quiet rest

The ANOVA of the PPTs demonstrated an interaction between conditions, assessment sites and time (Fig 2; $F(1,33) = 22.28$, $P < 0.001$), with post-hoc test showing increased PPTs after wall squat in session 1 and session 2 compared with before wall squat ($P < 0.001$) with no significant differences in PPTs after quiet rest ($P > 0.12$). Squat increased PPTs at exercising and non-exercising muscles by 16.8±16.9% and 6.7±12.9%, and the increase in PPT at the quadriceps was larger compared with PPT at the trapezius ($P < 0.001$). Significant correlations were found between the EIH response at the trapezius muscle and the rating of perceived exertion in session 1 ($r = 0.42$, $P = 0.013$). No significant correlations were found between EIH responses and peak leg pain intensity during wall squat.

3.4 Test-retest reliability of PPTs and EIH

Within-sessions (rest) test-retest reliability of PPT at the quadriceps and trapezius muscles, respectively, showed no systematic errors between repeated assessments (Table 1; $F(1,34) < 2.20$, $P > 0.14$), assessments were strongly correlated ($r \geq 0.95$), and ICCs were excellent with values ≥ 0.97 for both sites.

Between-sessions test-retest reliability of baseline PPT at the quadriceps and trapezius muscles, respectively, showed no systematic errors (Table 2; $F(1,34) < 0.98$, $P > 0.33$), which was also reflected in the 95 % CI of the mean differences, where zero is within the interval. Moreover, between sessions assessments were moderately correlated ($r \geq 0.74$), and ICCs were excellent with values ≥ 0.84 for both sites.

Between-session test-retest reliability of the EIH responses at the quadriceps and trapezius muscles, respectively, showed no systematic errors (Table 2; $F(1,34) < 0.32$, $P > 0.57$). Between sessions EIH responses were however not significantly correlated and ICCs were between 0.03 and 0.43, respectively.

3.5 Difference in PPTs after wall squat considered to be a real EIH effect

The minimal differences needed between repeated PPT assessments on a subject for the difference in the PPT to be considered real were 56 kPa and 62 kPa for quadriceps and 34 kPa and 33 kPa on trapezius in session 1 and session 2, respectively (Table 1). Twenty-three and 25 subjects demonstrated increases in PPT at the quadriceps muscle after wall squat larger than PPT before wall squat plus the SEM in session 1 and session 2, respectively with 17 subjects demonstrating increases in PPT larger than PPT before wall squat plus SEM in both sessions (Table 3; $\kappa = 0.08$ (95% CI, -0.25 to 0.41), $P = 0.65$). Sixteen and 15 subjects demonstrated increases in PPT at the trapezius muscle larger than the PPT before wall squat plus the SEM in session 1 and session 2, respectively with 8 subjects demonstrating larger increases in PPT after wall squat in both sessions ($\kappa = 0.13$ (95% CI, -0.20 to 0.46), $P = 0.43$).

4. DISCUSSION

This study is the first to investigate relative and absolute between-sessions test-retest reliability of exercise-induced hypoalgesia after an isometric exercise condition in healthy subjects. As hypothesised, the 3 min wall squat exercise significantly increased PPTs at exercising and non-exercising muscles in both sessions, with larger and more frequent EIH at the exercising muscle. Assessment of PPTs showed excellent within-session and between-sessions test-retest reliability.

The EIH response at exercising and non-exercising muscles demonstrated poor to fair between-sessions test-retest reliability, and the agreement in EIH responders and non-responders between sessions was not significant.

4.1 The effect of wall squat on PPTs

The current study demonstrated increases in PPTs at exercising and non-exercising muscles immediately after a short duration isometric exercise, which is in agreement with previous research [5, 21, 22]. Moreover, the increase in PPT was significantly larger and more frequent at the exercising muscles compared with non-exercising muscles, which is in accordance with previous results after isometric exercise [23, 24]. These findings indicate that hypoalgesia after isometric exercise is related to activation of systemic pain inhibitory mechanisms in combination with local or segmental pain inhibitory mechanisms.

Previous studies have indicated that the systemic hypoalgesic effect demonstrated after exercise could be related to conditioned pain modulation (CPM) and thus influenced by the experience of pain during the exercise condition. A previous study demonstrated that the conditioned pain modulatory response predicted the EIH response in 21 healthy subjects [25], and a study in subjects with chronic knee pain have demonstrated a relationship between EIH and CPM [26] indicating that subjects who demonstrate a greater ability to activate the descending inhibitory systems, report greater hypoalgesia following isometric exercise. However, the current findings showed no significant association between leg pain intensity during wall squat and the subsequent EIH response, suggesting that CPM was not a primary mechanism of EIH after the isometric wall squat exercise used in this study. This finding is in agreement with a recent study in patients with chronic whiplash and healthy subjects showing that the CPM response was not significantly associated with the EIH response after wall squat [14]. Interestingly, a significant association between rating of perceived exertion and the EIH response at the non-exercising trapezius muscle was found indicating that other aspects of the subjective experience of exercise than pain per se could influence the systemic EIH response. This finding should be further investigated in the future.

4.2 Test-retest reliability of PPT and EIH

For PPTs at the quadriceps and trapezius muscles, the within-session and between-session relative test-retest reliability demonstrated excellent ICC values (>0.8) confirming previous studies reporting ICCs above 0.7 [11, 27], suggesting that PPT is a reliable method to quantitatively assess pain sensitization mechanisms in humans. In addition, we reported the absolute test-retest reliability as the SEM and the smallest real difference for PPTs at the quadriceps and trapezius muscles within a re-test period of 3 min, an interval which might be more relevant in term of evaluation of pain modulatory capacity (e.g. EIH and CPM) in future studies.

Although multisegmental EIH was produced after the 3 min wall squat exercise condition in both sessions, and no significant difference in EIH response was found between the sessions, the between-session relative test-retest reliability for EIH was low when assessed at exercising and non-exercising muscles. Moreover, based on the SEM the agreement in EIH responders and non-responders between the two sessions was not significant indicating that although isometric exercise decreases pain sensitivity, considerable inter-individual difference in whether an individual has an EIH response or not between sessions exists. The poor reliability of the EIH responses could be due to the significant difference in rating of perceived exertion between sessions, as this measure was positively associated with the EIH response. In addition, it could be related to the reliability of the CPM mechanism that is associated with the EIH response after isometric exercise [25, 26]. The current between-session EIH reliability show similar ICCs to what have been demonstrated for between-session CPM responses assessed with manual algometry. Although a recent systematic review on the test–retest reliability of CPM concluded that the intra-session reliability was good to excellent only 50% of the included studies found good to excellent between-session reliability [28] and several studies have demonstrated poor to fair between-session reliability [29-31]. Improvement of EIH reliability in future protocols likely require 1) strict standardization procedures for the test stimulus which has improved the reliability of the assessment of similar pain modulatory mechanisms [32], and 2) a better understanding of the physiology of the phenomenon, so that potential intervening factors could be identified and controlled for between sessions.

In patients with chronic pain, studies have demonstrated impaired EIH after isometric exercise compared with asymptomatic controls [8, 33]; however the absolute and relative reliability of this reduced response is unknown. However, the response may be expected to be even more unreliable as pain patients has been shown to have higher variability in PPTs compared with healthy participants [34] further increasing the SEM.

The main limitations of this study were the non-randomized order between quiet rest and exercise.

4.3 Conclusions

A 3 min wall squat exercise increased PPTs at exercising and non-exercising muscles compared with quiet rest with larger and more frequent EIH at the exercising muscle. The relative and absolute reliability of the EIH responses was low making EIH results after isometric exercise less reliable for individually based assessment. These data have evident impact for future studies investigating EIH after isometric exercises in subjects with and without pain and potentially for the health care practitioner who designs exercise programs for pain relief. Future research is warranted to optimize reliability of EIH interventions and to investigate the reliability of EIH in clinical pain populations.

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Figure Legends

Fig. 1: Experimental procedure performed on both testing days. Pressure pain thresholds (PPTs) were assessed on two assessment sites (quadriceps and trapezius muscles) before and immediately after quiet rest and isometric exercise, respectively.

Fig. 2: Mean (± SEM, N = 35) pressure pain threshold (PPT) recorded at two assessment sites (quadriceps and trapezius) before and immediately after 3 min quiet rest and 3 min wall squat. Significantly different compared with baseline (*, NK: $P < 0.05$) and significantly different response compared to other assessment site (†, $P < 0.05$). 'Quad': m. quadriceps dominant side. 'Trap': upper trapezius muscle non-dominant side.

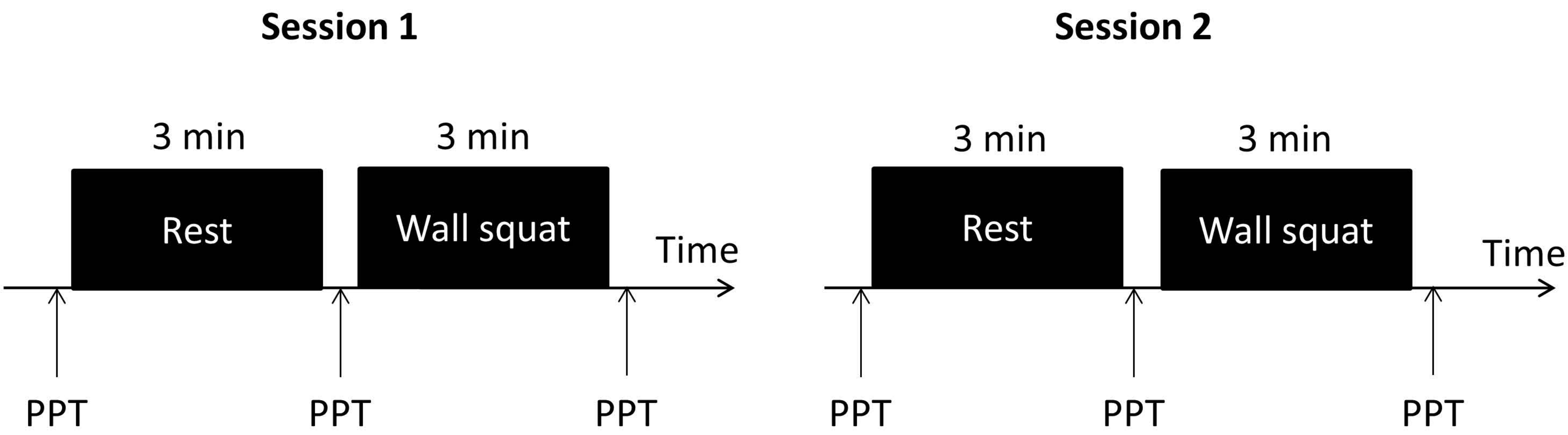
Fig. 3: Mean (± SEM, N = 35) rating of perceived exertion [A], and leg muscle pain intensity [B] assessed during wall squat exercise in session 1 and session 2 (*, $P < 0.05$).

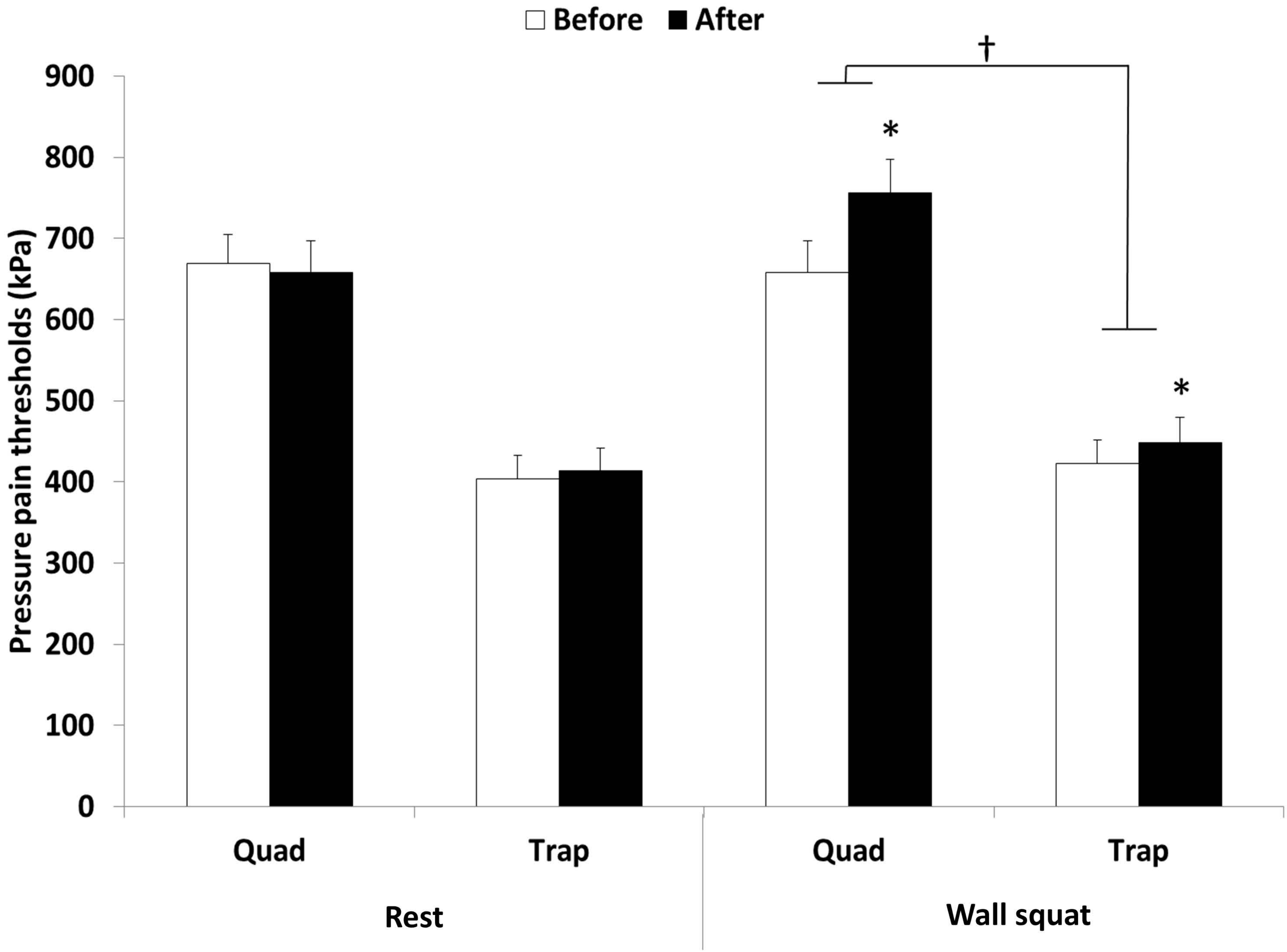
Table 1: Within-session (before and after rest) test-retest reliability for pressure pain threshold at the dominant quadriceps and non-dominant upper trapezius muscles.

Table 2: Between-session test-retest reliability for baseline pressure pain threshold (PPT) and exercise-induced hypoalgesia (EIH) after wall squat assessed at the dominant quadriceps and non-dominant upper trapezius muscles as absolute and relative change in PPT.

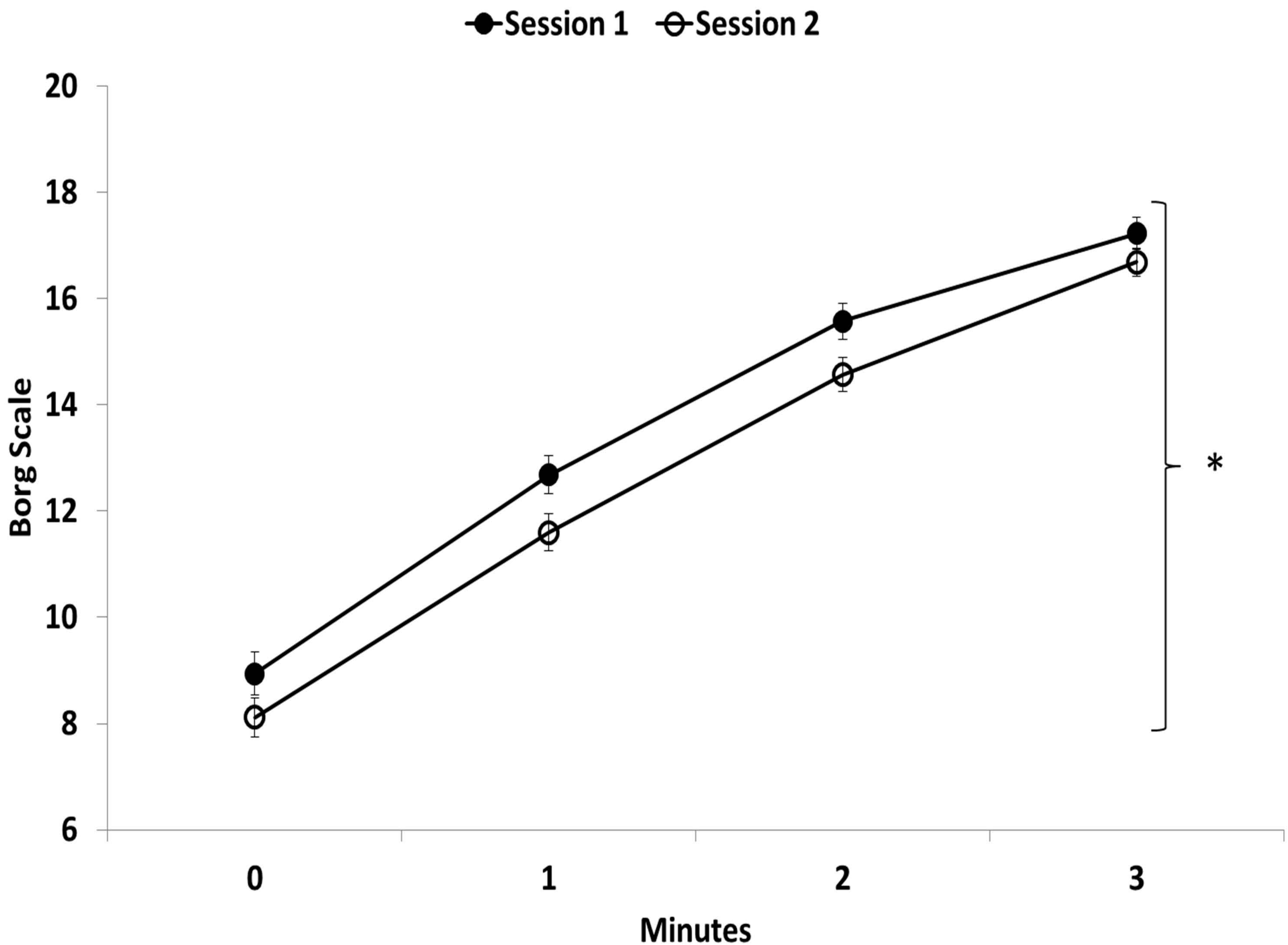
Table 3: Crosstabulations of the EIH responders and non-responders after wall squat at session 1 and session 2 at the quadriceps muscle and the trapezius muscle. Responders are based on increase

in pressure pain thresholds (PPT) after wall squat larger than PPT before wall squat plus the standard error of measurement (SEM) for two repetitive PPT assessments.


Session 1
3 min
3 min
Rest
Wall squat
Time
PPT
PPT
PPT
Session 2
3 min
3 min
Rest
Wall squat
Time
PPT
PPT
PPT



Before
After
Pressure pain thresholds (kPa)
900
800
700
600
500
400
300
200
100
0
†
*
*
Quad
Trap
Quad
Trap
Rest
Wall squat


Session 1
Session 2
Borg Scale
20
18
16
14
12
10
8
6
0
1
2
3
Minutes
*

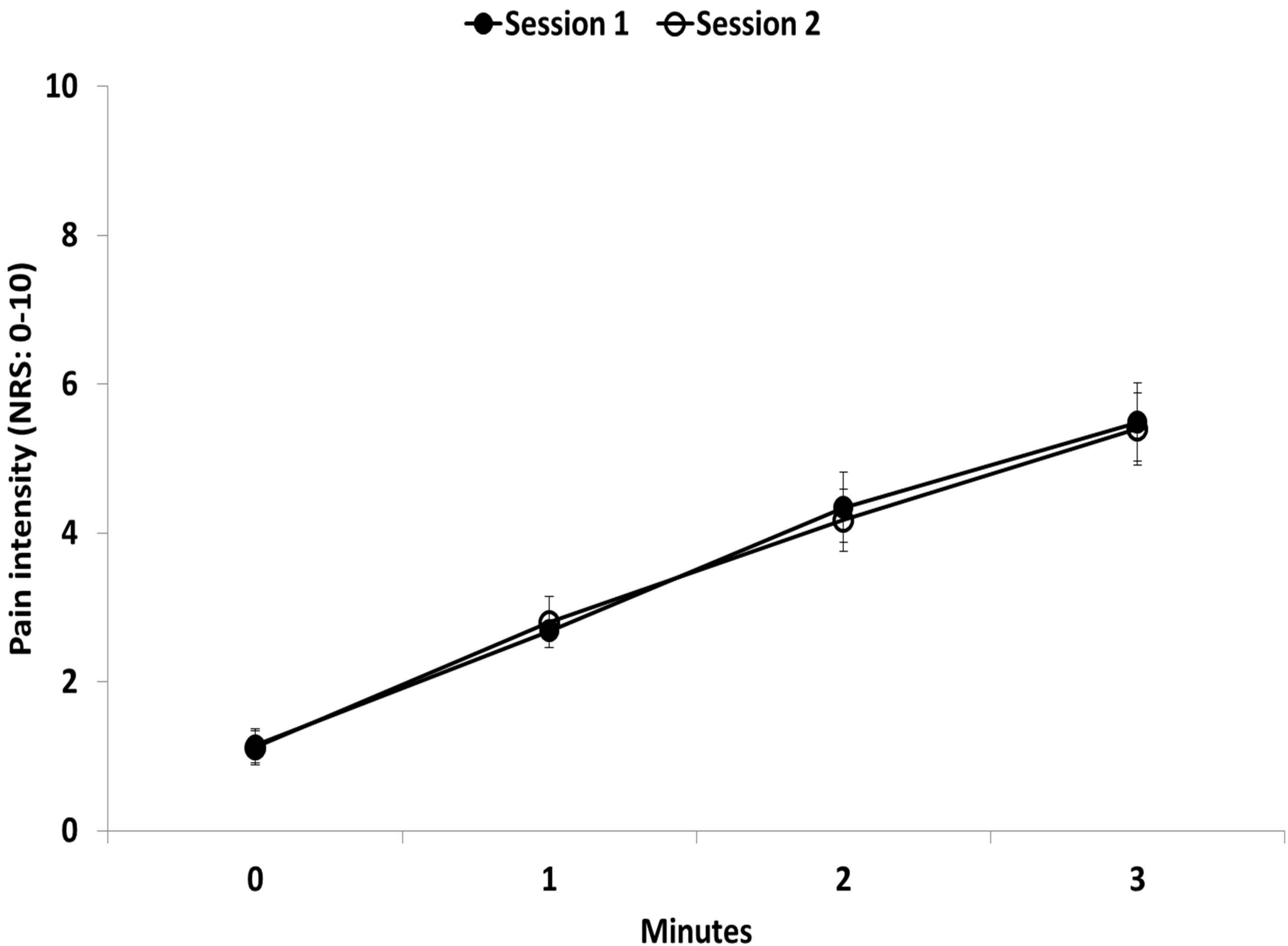

Session 1
Session 2
Pain intensity (NRS: 0-10)
10
8
6
4
2
0
0
1
2
3
Minutes

Table 1: Within-session test-retest reliability for pressure pain threshold at the dominant quadriceps and non-dominant upper trapezius muscles

Variable	Before Rest Mean ± SD (95%CI)	After Rest Mean ± SD (95%CI)	Absolute within-session difference Mean ± SD (95%CI)	Relative within-session difference Mean ± SD (95%CI)	P-value	Effect size	Pearson r	ICC3,1 (95%CI)	Standard error of measurement
Quad Session 1	687±288 kPa (588 - 786)	667±298 kPa (565 – 770)	20±80 kPa (-8 – 47)	3.2±12.0% (-0.9 – 7.3)	0.154	0.059	0.96 P<0.001	0.98 (0.96 – 0.99)	56 kPa
Quad Session 2	658±237 kPa (576 - 740)	656±274 kPa (562 – 750)	2±88 kPa (-29 – 31)	1.0±13.5% (--3.6 – 5.7)	0.917	<0.000	0.95 P<0.001	0.97 (0.94 – 0.99)	62 kPa
Trap Session 1	418±219 kPa (342 - 494)	425±210 kPa (353 – 497)	-7±48 kPa (-24 – 9)	-2.8±12.7% (-7.2 – 1.5)	0.394	0.021	0.98 P<0.001	0.99 (0.98 – 0.99)	34 kPa
Trap Session 2	396±179 kPa (334 - 457)	408±178 kPa (346 – 469)	-12±47 kPa (-28 – 4)	-3.8±12.5% (-8.1 – 0.5)	0.148	0.061	0.97 P<0.001	0.98 (0.97 – 0.99)	33 kPa

Table 2: Between-session test-retest reliability for baseline pressure pain threshold (PPT) and exercise-induced hypoalgesia after wall squat assessed at the dominant quadriceps and non-dominant upper trapezius muscles as absolute and relative change in PPT.

Variable	Session 1 Mean ± SD (95%CI)	Session 2 Mean ± SD (95%CI)	Absolute between-session difference Mean ± SD (95%CI)	Relative between-session difference Mean ± SD (95%CI)	P-value	Effect size	Pearson r	ICC3,1 (95%CI)	Standard error of measure-ment
Quad Baseline	687±288 kPa (588 - 786)	658±238 kPa (576 - 740)	29±195 kPa (-38 – 96)	-1.9±24.5% (-10.3 – 6.5)	0.774	0.022	0.74 P<0.001	0.84 (0.69 – 0.92)	138 kPa
Trap Baseline	418±220 kPa (343 - 494)	396±179 kPa (334 - 458)	22±133 kPa (-23 – 68)	-1.0±29.1% (-10.9 – 9.0)	0.330	0.028	0.80 P<0.001	0.88 (0.76 – 0.94)	94 kPa
EIH Quad (absolute)	100±102 kPa (65 – 135)	96±104 kPa (61 – 132)	4±148 kPa (-47 – 54)	25.9±357.7% (-97.0 – 148.8)	0.888	0.001	0.04 P=0.836	0.08 (-0.71 – 0.46)	105 kPa
EIH Quad (relative)	16.8±16.9 kPa (10.9 – 22.6)	17.1±16.7 kPa (11.4 – 22.8)	-0.3±21.3 kPa (-7.6 – 7.0)	-39.1±398.0% (-175.8 – 97.6)	0.927	<0.000	0.20 P = 0.257	0.33 (-0.33 – 0.66)	15.0%
EIH Trap (absolute)	29±57 kPa (9 – 48)	22±50 kPa (4 – 39)	7±75 kPa (-18 – 33)	14.3±354.0% (-107.7 – 135.8)	0.577	0.009	0.02 P = 0.931	0.03 (-0.90 – 0.51)	53 kPa
EIH Trap (relative)	6.7±12.9 kPa (2.2 – 11.1)	5.7±11.5 kPa (1.7 – 9.7)	1.0±14.7 kPa (-4.1 – 6.0)	25.1±310.5% (-81.6 – 131.7)	0.700	0.004	0.28 P = 0.108	0.43 (-0.13 – 0.71)	10.4%

Table 3: Crosstabulations of the EIH responders and non-responders after wall squat at session 1 and session 2 at the quadriceps muscle and the trapezius muscle. Responders are based on increase in pressure pain thresholds (PPT) after wall squat larger than PPT before wall squat plus the standard error of measurement (SEM) for two repetitive PPT assessments.

EIH response at the exercising quadriceps muscle after wall squat		**EIH responders in session 2**	
		Yes	**No**
EIH responders in session 1	**Yes**	17	6
	No	8	4
EIH response at the non-exercising trapezius muscle after wall squat		**EIH responders in session 2**	
		Yes	**No**
EIH responders in session 1	**Yes**	8	8
	No	7	12