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Modulation of exercise-induced hypoalgesia following an exercise intervention in healthy subjects

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Abstract

Background. Exercise is recommended to promote and maintain health and as treatment for more than 25 diseases and pain conditions. Exercise-induced hypoalgesia (EIH), a measure of descending pain inhibitory control, has been found impaired in some chronic pain conditions but it is currently unclear if EIH is modifiable. This study investigated if a long-term exercise intervention could modulate EIH in healthy subjects.

Methods. In 38 healthy subjects, EIH was assessed as change in pressure pain threshold (PPT) after a 3-minute isometric wall squat within the first week and after approx. seven weeks of military training (MT). Further, temporal summation of pain (TSP) and Knee injury and Osteoarthritis Outcome Score (KOOS) were assessed. Physical performance capacity was assessed using the Endurance 20-m shuttle run fitness test (20MSR). A *hypoalgesic* (EIH>0.0 kPa) and a *hyperalgesic* (EIH≤0.0 kPa) subgroup was defined based on baseline EIH. Change in EIH following MT was used as primary outcome.

Results. Increased EIH (P=0.008), PPT (P<0.003) and 20MSR (P<0.001) were found following MT, with no changes in TSP and KOOS (P>0.05). Subjects with a hyperalgesic EIH response at baseline (26% of the participants) presented significantly improved EIH following MT (P=0.010). Finally, an association between 20MRS change and EIH change was found (r=0.369; P=0.023).

Conclusions. MT increased EIH, especially in subjects who demonstrated a hyperalgesic response at baseline. Improvement in physical performance capacity was associated with an improvement in EIH, indicating that improvement in physical performance capacity may improve central pain mechanisms.

Keywords. Exercise-Induced Hypoalgesia; Mechanistic Pain Profiling; Exercise; Physical Activity; Pain Modulation.

Introduction

Exercise is recommended to promote and maintain health [1,2] and as treatment for more than 25 chronic diseases and pain conditions [3].

The mechanisms underlying the pain relieving effect of exercise are largely unknown but might include a possible decreased pro-inflammatory cytokine response [4], improvement in psychological parameters [5] and modulation of central descending pain inhibitory pathways after acute exercise bouts [6–8].

Mechanistic pain profiling aims to assess the pain mechanisms in healthy subjects and patients with chronic pain [9]. Peripheral hyperalgesia can be assessed as decreased pressure pain threshold (PPT) at a local painful site, whereas widespread hyperalgesia, argued to be part of central sensitization, can be assessed as decreased PPT remotely to a painful site [9,10]. Temporal summation of pain (TSP) is the increase in pain intensity following repeated painful stimulation and facilitated TSP is believed to be due to increased excitability of dorsal horn neurons [9,11]. Exercise-induced hypoalgesia (EIH) is typically assessed as the temporary change in pressure pain thresholds after an acute bout of exercise, and EIH is believed to represent a measure of descending pain inhibitory control [7,12]. In general, EIH seems functional (hypoalgesic) in asymptomatic subjects [7,13–15]. An impaired (hyperalgesic) EIH response has been reported in different pain populations [16–19], although a functional EIH response also has been reported in subgroups of knee osteoarthritis patients [6,18,19]. Furthermore, EIH has been utilized as a predictive factor for pain progression following total knee replacement [18] and exercise therapy [20] in knee osteoarthritis patients, and lower EIH has been reported in physically inactive individuals compared with physically active people [21,22]. This indicates EIH subgroup differences in relation to physical performance capacity and that exercise or an active lifestyle (enhanced physical performance capacity) may improve EIH although cross-sectional studies have found conflicting evidence in healthy subjects [14,23–26].

Previous longitudinal studies on exercise therapy in knee pain populations were unable to demonstrate improved EIH after pain relieving treatment [20,27,28] suggesting that EIH magnitude is not purely driven by clinical pain. Currently, no longitudinal studies have investigated the effect of an exercise program on EIH in healthy subjects. Basic military training (MT) represents a significant physical challenge [29,30], and new recruits must complete an extensive physical exercise program shown to improve physical fitness ratings [31,32]. The aims of this exploratory study were to investigate 1) if basic MT modulates mechanistic pain profiling measures (EIH, PPT, TSP), 2) if EIH response subgroups exist, and 3) if improvement in physical performance capacity was associated with improvement in EIH following MT.

The hypothesis for this study was that 1) mechanistic pain profiling measures can be modulated with MT, 2) EIH subgroups might exits, and 3) an association exist between change in physical performance capacity and change in EIH in healthy subjects.

Methods

Subjects

A review with a meta-analysis on cross-sectional studies found an average EIH effect size of 0.7 after short-lasting isometric exercise in healthy subjects [12]. To our knowledge, the change in EIH response magnitude has not previously been investigated in follow-up studies in healthy subjects. With an expected moderate effect size of 0.6, an estimated variation (standard deviation, SD) of 0.9, a statistical power of 80% and a significance level of 0.05, the sample size calculation yielded 36 subjects to be included in this project. To take larger variation and dropouts into account, 40 healthy recruits were included in this project and dropouts were not replaced.

New recruits from the 2nd Battalion of Danish Army Logistics, Aalborg, Denmark, were invited to participate in the study. Prior to military enrollment, all recruit candidates must complete an intensive physical examination, including check for any chronic musculoskeletal pain conditions, as

well as a mental examination by a medical doctor to establish if the candidates are fit for military training. No additional exclusion criteria were applied.

Procedure

Within the first week and after approx. seven weeks of basic MT, Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire, PPTs, TSP and EIH measures along with physical performance capacity testing using the Endurance 20-m Shuttle Run Fitness Test (20MSR) were obtained (figure 1).

Consumption of analgesics (categorized into 'yes' or 'no' to taking paracetamol [PCM], nonsteroidal anti-inflammatory drugs [NSAID] or opioids before the experiments on the days of participation in the test procedures) was recorded. Self-reported days (in ½ days) absent from MT and reasons for being absent were obtained at follow-up to describe adherence.

The study was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee (N-20170070) and all subjects gave oral and written informed consent prior to the experiments. Trial registration number at clinicaltrials.gov: NCT03718663.

All subjects were included in February 2019. All data collection and testing were performed by the same investigators (SH, RCD, MBS). To ensure validity of the data, the oral information during the experiments was standardized. Paper versions of KOOS were used, and all other data were collected on written case-report forms.

Military training

Basic MT is the first phase of a recruit's introduction to the army and new recruits must complete an exercise program shown to improve physical fitness ratings [31,32]. The aim of the physical part of basic MT is to improve the physical function enabling the recruits to complete basic MT and to continue MT training afterwards effectively. Basic MT consists of one to three 45-60 minute sessions per week and in addition 10-15 micro sessions (10-15 minutes each) per week focusing on

strength, endurance and agility/mobility. The content of the sessions has previously been described and shown to improve fitness ratings in a military setting [33]. In short, the strength training consisted of the same exercises throughout the period (deadlifts, lunges, step-ups, and 1 leg squats for the lower body and pull-ups, dips, weighted push-ups and 1 arm rows for the upper body) with progressive total workload preferably performed with the recruit's own body weight as resistance. The endurance training consisted of cardiovascular training, e.g. running and muscle endurance training with different "workouts of the day" and "fight games" to create variation in the training. The agility/mobility training was focusing on flexibility/stretching and agility, e.g. obstacle courses. The intensity of the training was supervised and adjusted by educated military staff based on the capacity of the individual recruit.

An attendance score (%) was calculated for each subject by dividing the number of days attended/completed by the number of days scheduled [34] (five days per week) as previously conducted in similar studies on knee osteoarthritis patients and adherence to exercise therapy [20,35]. An attendance score of 100% describes subjects who attended/completed all days of MT. Legal leave of absence during all weekends (a total of 14 days) was preplanned. Hence, these days were not included in the calculation of the attendance score.

Physical performance capacity

Physical performance capacity was assessed using the 20MSR which is a test widely used to measure the maximum performance to predict the maximal aerobic capacity (VO_2-max) [36,37]. The 20MSR has been found valid in healthy adults [37] and reliable in military personnel [38]. 20MSR is a standard endurance test used by the 2nd Battalion of Danish Army Logistics, Aalborg, Denmark. The test consists of 1-minute stages of continuous running with incremental running speed starting at 8.5 km/h and increased by 0.5 km/h each minute. The individual subject is required to run between two lines 20-m apart with pace dictated by an audio signal played from a pre-recorded audio file. The test

ends when the subject fails reaching the end lines synchronously with the audio signals in two successive occasions [39]. All subjects performed the test simultaneously, as applied commonly, and the test was performed in an indoor gymnasium to reduce outdoor challenges [37].

Assessment of self-reported pain and function

KOOS was utilized at baseline and follow-up to obtain a subjective measure of knee pain and function. KOOS is a well-established questionnaire that can be used for short-term and long-term follow-up of several types of knee injury containing five subscales: Pain, other symptoms (Symptoms), function in activities of daily living (ADL), function in sport and recreation (Sport) and knee related quality of life (QoL). Each item in KOOS is scored from 0-4 on a Likert scale. Subscale scores are given separately (see www.koos.nu for user guide and scoring) ranging from 0 (worst) to 100 (best). KOOS has been found responsive, reliable and valid in several types of knee pain patients [40–42], and it has been used in previous studies to categorize healthy subjects [43,44]. KOOS was used in the current study to identify any knee pain and function limitations since knee pain and function limitations have been found associated with a hyperalgesic EIH in subgroups of knee pain patients [6,18,19].

Mechanistic pain profiling

PPTs and TSP were obtained with the subject sitting upright in a chair with a straight backrest, the knees in approximately 50 degrees of flexion with feet relaxed on the floor and the dominant shoulder relaxed against a wall.

The following sites were marked and used for pain sensitivity measurements: one site at the dominant musculus quadriceps femoris (QF, at the middle part of the muscle between the top of patella and spina iliaca anterior superior), one site at the dominant musculus tibialis anterior (TA, 5 cm distal to the tibial tuberosity) and one site at the contralateral musculus deltoideus (DE, at the middle part of the muscle belly of the middle deltoid).

PPTs were assessed at QF and DE using a handheld pressure algometer (Somedic AB Type II, Sweden) which has been found reliable in healthy subjects within and between sessions [24,45]. The probe (1 cm^2) was placed perpendicularly to the skin and an increasing pressure was applied (30 kPa/s) until the subject characterized the pressure as pain and pressed a button. PPTs were measured twice at each site with 20-sec intervals between assessments and the average for each site was used for statistical analysis.

The EIH response magnitude is typically calculated as the absolute or relative difference in the test stimulus (e.g. PPTs) after an acute exercise [7,12]. In the current study, the absolute change in PPT_{QF} and PPT_{DE} after a 3-minute isometric wall squat exercise was used to assess EIH.

Local EIH was assessed at QF (a muscle primarily involved in the acute exercise), while *remote* EIH was assessed at DE (a muscle remote to the exercising body region). The assessment sites QF [8,18,27] and DE [27,46] have previously been used in similar studies.

All subjects performed a 3-minute isometric wall squat exercise as the acute exercise to elicit EIH. Subjects were instructed to stand upright with their back against the wall, feet parallel and shoulder-width apart and hands by their sides. The subjects were instructed to lower their back down the wall until a knee flexion angle of approximately 100 degrees was reached and to maintain this position for a maximum of three minutes or until fatigue. Just after the acute exercise, the subjects were instructed to rate the pain intensity in the legs from 0–10 on the numeric rating scale (NRS) with 0 defined as “no pain” and 10 as “worst imaginable pain”. Further, they were asked to rate the perceived exertion (RPE) on a scale from 6-20 with 6 defined as “no exertion at all” and 20 as “maximal exertion”. RPE has previously been used in similar studies [7,13,15,47].

A modified von Frey pinprick stimulator with a weighted load (Aalborg University, Aalborg, Denmark) was used to assess TSP. A force of 25.6 g was applied once at TA, and the subject was asked to rate the pain intensity on the NRS. Then, 10 consecutive stimulations were applied (1-

second inter-stimulus intervals) to the same site and the subject was asked to rate the pain intensity of the last stimulation on the NRS. TSP was calculated as the absolute NRS difference between the last and first stimulation. This method has previously been used in similar studies [48–50].

Statistics

All data are presented as mean and standard error of the mean (SEM) if not otherwise stated. Assumptions of normality of the distribution for all variables were investigated using histograms and QQ plots and confirmed with the Shapiro–Wilks normality test. Normally distributed data (Shapiro-Wilks, $P > 0.05$) were analyzed with parametric statistics; otherwise, non-parametric analysis was applied. For all analyses, $P < 0.05$ was considered significant.

For single comparisons between baseline and follow-up data (20MSR, TSP, NRS, RPE), paired t-tests or Wilcoxon tests were applied. Additionally, single comparisons for differences in NRS and RPE between subjects completing and not completing the acute exercise at baseline were utilized using Mann-Whitney U-tests for independent samples.

For paired samples analysis, individual repeated-measures analysis of variance (RM-ANOVA) or related-samples Friedman's 2-way ANOVA by ranks was utilized for normally and non-normally distributed data, respectively. To investigate if MT influenced KOOS subscale scores, the factors *subscale* (Pain, Symptoms, ADL, Sport and QoL) and *intervention* (baseline and follow-up) were applied. To investigate if the acute exercise induced EIH at baseline, the absolute change in PPTs was analyzed with the factors *time* (before, after) and *site* (QF, DE). A similar analysis was made on the corresponding follow-up data. For the effect of MT on EIH, the factors *site* (QF, DE) and *intervention* (baseline, follow-up) were applied. To analyze the effect of MT on PPTs a similar analysis was made using PPTs before the wall squat exercise at baseline and follow-up.

Two subgroups were defined based on the baseline local EIH response: *Hypoalgesic* subgroup (EIH > 0.0 kPa) and *hyperalgesic* subgroup (EIH ≤ 0.0 kPa). Single comparison analyses for differences in

local and remote EIH at baseline between the subgroups was utilized using Mann-Whitney U tests for independent samples. Further, a single comparison analysis for difference in 20MSR improvement between EIH subgroups was utilized using an independent t-test.

To investigate the wall squat exercise-induced EIH at baseline in each subgroup, the absolute change in PPTs was analyzed with the factors *time* (before, after) and *site* (QF, DE) for each subgroup individually. Similar analyses were made on corresponding follow-up data. To investigate the effect of MT on EIH in the hypoalgesic subgroup, the factors *site* (QF, DE) and *intervention* (baseline, follow-up) were applied. A similar analysis was made on the corresponding hyperalgesic subgroup data. Subgroup differences in TSP were investigated using Wilcoxon tests before and after MT, respectively.

Bonferroni post hoc corrections for multiple comparisons were applied where relevant.

Correlation analyses using Pearson product-moment correlation (*r*) were applied to identify possible associations between change in physical performance capacity and change in EIH following MT.

Results

Forty subjects were asked and recruited for the project. Missing data due to terminated MT (n=1) and not participating in follow-up 20MSR because of knee injury (n=1) left 38 subjects for analysis with complete data at baseline and follow-up. All possible subjects fulfilled the eligibility criteria.

The included subjects were 20.5 ± 0.3 years old (range 18 - 24) with a BMI of 22.5 ± 0.3 kg/m^2 (range 17.6 – 27.2) at baseline. Of the included subjects, 24 (63.2%) were males and 31 (81.6%) had right dominant leg.

All baseline measurements were conducted on the third day of the basic MT; except the 20MSR, which was conducted on the following day as part of the preplanned MT. Corresponding follow-up measurements were made 47 days (6.7 weeks) after baseline measurements.

At baseline, three of the included subjects had taken PCM on the testing day before the experiments. At follow-up, two and three subjects had taken PCM and NSAIDs, respectively, on the testing day before the experiments.

The average self-reported days absent from MT were 1.0 ± 0.3 days (range 0 - 7) for the included subjects with a subsequent attendance score of 97.0 ± 0.8% (range 79 - 100).

Reasons for being absent were cold/influenza (n=5), musculoskeletal pain problems (n=4), testicle inflammation (n=1), scabies (n=1), kidney problem (n=1), throat infection (n=1), job interview (n=1) and private unspecified reason (n=1).

Physical performance capacity test

20MSR results were 1553.7 ± 65.0 m (range: 820 – 2320) and 1976.8 ± 57.8 m (range: 1260 – 2680) at baseline and follow-up, respectively, with a significant change (t-test; $P < 0.001$) of 423 ± 36.3 m (range: -240 – 820) corresponding to 31.0 ± 3.4% (range: -12.2 – 79.1), indicating that the MT intervention significantly improved VO_2-max of the subjects.

Self-reported pain and function

Friedman's ANOVA with the factors subscale (Pain, Symptoms, ADL, Sport and QoL) and intervention (baseline and follow-up) showed a significant main effect (Friedman's, X^2 (9) = 27.05; $P = 0.001$). Post hoc tests adjusted for multiple comparisons showed no significant change in any of the KOOS subscale scores following MT ($P > 0.1$, table 1) indicating that MT did not influence any of these measures.

Mechanistic pain profiling

Pressure pain thresholds before military training

At baseline, for the total group, Friedman's ANOVA with the factors site (QF, DE) and time (before, after acute exercise) showed a significant main effect (Friedman's, X^2 (3) = 37.67; $P < 0.001$). Post

hoc tests showed a significant change in PPT_{QF} after the acute exercise (Wilcoxon; adjusted P = 0.016), but no significant change in PPT_{DE} (Wilcoxon; P = 1.000) (Table 2).

For the hypoalgesic subgroup (n=28, 73.7%), the corresponding results showed a significant main effect (Friedman's, X^2 (3) = 36.77; P < 0.001) with post hoc tests showing a significant change in PPT_{QF} (Wilcoxon; adjusted P < 0.001) but no significant change in PPT_{DE} (Wilcoxon; adjusted P = 0.272). In the hyperalgesic subgroup (n=10, 26.3%), Friedman's ANOVA with the factors site (QF, DE) and time (before, after acute exercise) showed a significant main effect (Friedman's, X^2 (3) = 22.68; P < 0.001). Post hoc tests showed a significant change in PPT_{QF} (Wilcoxon; adjusted P < 0.010) but no significant change in PPT_{DE} (Wilcoxon; adjusted P = 0.056) after the acute exercise. Collectively, this indicates that a local hypoalgesic EIH response but no remote EIH were induced in the total cohort and in the hypoalgesic subgroup, while a local hyperalgesic EIH response and no remote EIH response were induced in the hyperalgesic subgroup at baseline (Table 2).

Pressure pain thresholds after military training

At follow-up, for the total group, Friedman's ANOVA with the factors site (QF, DE) and time (before, after acute exercise) showed a significant main effect (Friedman's, X^2 (3) = 45.41; P < 0.001) with post hoc tests showing significant changes both in PPT_{QF} (Wilcoxon; adjusted P = 0.020) and PPT_{DE} (Wilcoxon; adjusted P = 0.008) after the acute exercise (table 2).

For the baseline hypoalgesic subgroup, the corresponding results showed a significant main effect (Friedman's, X^2 (3) = 30.66; P < 0.001) with post hoc tests showing a significant change in PPT_{QF} (Wilcoxon; adjusted P < 0.008) and PPT_{DE} (Wilcoxon; adjusted P = 0.002). For the baseline hyperalgesic subgroup, the corresponding results showed a significant main effect (Friedman's, X^2 (3) = 15.00; P < 0.002) with post hoc tests showing no significant change in PPT_{QF} (Wilcoxon; adjusted P < 0.074) and PPT_{DE} (Wilcoxon; adjusted P = 0.278). Collectively, this indicates that both local and remote EIH were induced following MT in the total group and in the baseline hypoalgesic

subgroup, but no EIH responses (neither hyperalgesic nor hypoalgesic) were induced in the baseline hyperalgesic subgroup (table 2).

Pressure pain threshold changes after military training

Using PPTs before the wall squat exercise at baseline and follow-up to test the effect of MT on PPTs in the total group, Friedman's ANOVA with the factors site (QF, DE) and intervention (baseline, follow-up) showed a significant main effect (Friedman's, X^2 (3) = 50.59; $P < 0.001$) with post hoc tests showing significant changes in PPT_{QF} (Wilcoxon; adjusted $P < 0.001$) and PPT_{DE} (Wilcoxon; adjusted $P < 0.001$).

For the baseline hypoalgesic subgroup similar analyses showed a significant main effect (Friedman's, X^2 (3) = 38.70; $P < 0.001$) with post hoc tests showing significant changes in PPT_{QF} (Wilcoxon; $P < 0.001$) and PPT_{DE} (Wilcoxon; adjusted $P = 0.002$). For the baseline hyperalgesic subgroup the corresponding results were a significant main effect (Friedman's, X^2 (3) = 16.20; $P < 0.001$) with post hoc tests showing no significant changes in PPT_{QF} (Wilcoxon; adjusted $P = 1.000$) and PPT_{DE} (Wilcoxon; adjusted $P = 0.228$). Collectively, this indicates that MT decreased pressure pain sensitivity globally in the total cohort and the baseline hypoalgesic subgroup, while the hyperalgesic subgroup was unchanged following MT.

Comparison of parameters in relation to the wall squat test

Five subjects (13.2%) did not complete the 3-minute wall squat exercise (seconds completed: 93 ± 19 [range: 42-142]) at baseline. No significant difference was found in NRS (6.3 ± 0.3 [range 2-9] vs 7.4 ± 0.8 [range 5 - 9]; Mann-Whitney U; $P = 0.235$) and RPE (14.5 ± 0.4 [10 - 19] vs 16.2 ± 0.8 [15-19]; Mann-Whitney U; $P = 0.146$) comparing subjects who completed and subjects who did not complete the 3-minute wall squat. All subjects completed the 3-minute wall squat exercise at follow-up.

RPE during the acute exercise at follow-up was significantly lower compared with baseline (14.7 ± 0.3 [range: 10 - 19] vs. 13.6 ± 0.4 [range: 7 - 18]; Wilcoxon, $P = 0.024$), while no difference in the corresponding NRS was found (6.5 ± 0.3 [range: 2 - 9] vs. 6.1 ± 0.3 [range: 3 - 9]; Wilcoxon, $P = 0.237$). This indicates that the acute exercise was perceived less exhaustive at follow-up although peak pain was unchanged.

Exercise-induced hypoalgesia before and after military training

EIH responses for the total group before and after MT are shown in figure 2. Friedman's ANOVA with the factors site (QF, DE) and intervention (baseline and follow-up) showed a significant main effect (Friedman's, X^2 (3) = 9.89; $P < 0.020$). Post hoc tests showed a significant change in remote EIH (Wilcoxon; adjusted $P = 0.008$), but no change in local EIH (Wilcoxon; $P = 1.000$) following MT, indicating that basic MT normalized remote EIH while a functional local EIH at baseline was unchanged.

No significant differences in local EIH (independent t-test; adjusted $P = 0.096$) and remote EIH (independent t-test; adjusted $P = 0.334$) were found, adjusted for multiple comparisons, comparing subjects completing and subjects not completing the 3-minute wall squat at baseline.

The baseline hypoalgesic subgroup showed significantly higher local EIH (70.6 ± 10.3 kPa vs. -65.3 ± 13.1 kPa; independent Mann-Whitney U; adjusted $P < 0.001$) and higher remote EIH (20.1 ± 14.1 kPa vs. -43.4 ± 17.2 kPa; independent Mann-Whitney U; adjusted $P = 0.022$) compared to the hyperalgesic subgroup at baseline.

In the hyperalgesic subgroup, Friedman's ANOVA with the factors site (QF, DE) and intervention (baseline and follow-up) showed a significant main effect (Friedman's, X^2 (3) = 21.60; $P < 0.001$). Post hoc showed significant changes in local EIH (Wilcoxon; adjusted $P = 0.010$) and remote EIH (Wilcoxon; adjusted $P = 0.010$), indicating that the intervention normalized local and remote EIH in the baseline hyperalgesic subgroup (figure 3a).

In the hypoalgesic subgroup, Friedman's ANOVA with the factors site (QF, DE) and intervention (baseline and follow-up) showed a non-significant main effect (Friedman's, X^2 (3) = 7.36; $P <$ 0.061), indicating that the intervention did not improve the baseline hypoalgesic EIH (figure 3b).

When assessing categorical individual EIH data, 28 (73.7%) and 10 (26.3%) subjects were in the hypoalgesic and hyperalgesic subgroup, respectively, both at baseline and following MT. 20 (52.6%) and two (5.3%) subjects were hypoalgesic and hyperalgesic, respectively, both at baseline and follow-up. Eight subjects (21.1%) changed from EIH hypoalgesic to hyperalgesic, while eight subjects (21.1%) changed the opposite way.

Temporal summation of pain

For the total group, TSP was 1.7 ± 0.2 (range: -1 – 5) and 1.4 ± 0.3 (range: -3 - 5) at baseline and follow-up, respectively, with no change following MT (Wilcoxon; $P = 0.398$). For the hypoalgesic subgroup, the corresponding results were 1.5 ± 0.2 (range: 1 - 3) and 1.0 ± 0.5 (range: -2 – 3) with no change following MT (Wilcoxon; $P = 0.471$). For the hyperalgesic subgroup, the corresponding results were 1.8 ± 0.6 (range: -1 - 5) and 1.4 ± 0.7 (range: -3 – 5) with no change following MT (Wilcoxon; $P = 0.572$). Collectively, this indicates that MT did not modulate TSP in any group.

Correlation between change in physical performance capacity and change in EIH

In the total group, absolute change in 20MSR was significantly associated with absolute change in remote EIH ($r = 0.369$; $P = 0.023$) but not with change in local EIH ($r = -0.212$; $P = 0.201$), indicating a positive association between systemic adaptive descending pain inhibitory control and physical performance capacity. No significant difference was found in 20MRS improvement between EIH subgroups (independent t-test; $P = 0.592$).

Discussion

The present study is the first to report a modulation of EIH following a long-term physical exercise intervention in healthy subjects. This study also found a subgroup (26%) of healthy subjects demonstrating a hyperalgesic EIH response at baseline that was normalized following the long-term physical exercise intervention. Additionally, a positive correlation between change in physical performance capacity and change in EIH following the intervention was demonstrated which suggests an association between improvement in physical performance capacity and improvement in descending pain inhibitory control.

Improved physical performance capacity following military training

Previous studies report improved running test results following MT [32,51,52] which is in agreement with the findings of the current study. On average, 20MRS reliability studies show that participants run 40 - 100 m longer at short term re-test indicating a learning effect [38,53]. The current study found an increased running distance of 423 ± 36.3 m following MT indicating increased physical performance capacity and not only a learning effect as the main reason for the improvement.

Modulation of exercise-induced hypoalgesia

Hansen et al. (2020) found that remote EIH was not elicited neither at baseline nor at follow-up to exercise therapy treatment in patients with knee osteoarthritis [20] while the current study reports a modulation of EIH following a long-term exercise intervention, indicating differences in pain mechanism adaptability between healthy subjects and chronic pain patients. In addition, previous follow-up studies on exercise therapy in rheumatoid arthritis [28] and knee osteoarthritis patients [20,27] showed that a normally functioning (hypoalgesic) EIH was not further improved following a long-term exercise intervention. The current study found that remote EIH in the pooled subject data and both local and remote EIH in the baseline hyperalgesic subgroup were improved following MT.

This is the first time, to our knowledge, that a modulation of EIH following a long-term exercise intervention has been shown in healthy subjects. This modulation could be due to increased concentration of anti-inflammatory cytokines, decreased concentration of pro-inflammatory cytokines and reduce glial cell activation, as shown in pre-clinical trials, leading to reduced nociceptor activity [54,55] which has been speculated to increase EIH [7]. Furthermore, changes in brainstem areas such as the periaqueductal gray and rostral ventromedial medulla after vigorous exercise programs [56,57] may be involved this EIH modulation.

Neither local nor remote EIH was modulated following the intervention in the baseline hypoalgesic subgroup indicating that the intervention was insufficient to further improve EIH in this subgroup or that the EIH response magnitude has a maximum capacity for its hypoalgesic response. An alternative explanation for the lack of improvement in the baseline hypoalgesic group may be that eight subjects (out of 28) presented as hyperalgesic at follow-up, although replaced with eight other subjects from the baseline hyperalgesic subgroup as shown by the individual data, giving a zero net effect. Limitations of the isometric wall squat test to elicit EIH consistently [15] or other unknown factors such as sleep deprivation [58] or cognitive stress [59] may explain why some subjects apparently become hyperalgesic following long-term exercise, since these factors have been found to impact central pain mechanisms and these were not accounted for in the current study. Further studies are needed to investigate these topics

Finally, psychological parameters such as mood states, fear of pain and pain catastrophizing have been shown to influence EIH inversely in healthy subjects [7], and factors such as muscle soreness [60], poor sleep quality [61], bad coping strategies [62], fear of pain [59] and stress symptoms [63] are also all known to increase pain sensitivity. The subjects in the current study were assessed shortly after starting basic MT and therefore may have been prone to stress, sleep deprivation and mental fatigue [29] which may be another explanation for the remote EIH modulation. This may also

explain why remote EIH was not elicited at baseline but only at follow-up where psychological parameters may have decreased because of habituation.

A recent cross-sectional study reported no relationship between EIH (or conditioned pain modulation [CPM]), and functional performance capacity (VO_2-max) in endurance-trained athletes and normally active controls [26]. This is in contrast to the current study which found an association between improvement in physical performance capacity and improvement in EIH. Further studies are needed to investigate this association.

Assessment of exercise-induced hypoalgesia

Both local [15] and remote [15,64] EIH after the wall squat exercise have been demonstrated; however, conflicting results have been reported in relation to the EIH magnitude in working (local) muscles or resting (remote) muscles in healthy subjects; some studies report higher local EIH [13,15,24] while other studies report similar magnitude of local and remote EIH [23,65,66]. This is partially in agreement with the current findings eliciting only local EIH but not remote EIH at baseline. Additionally, the subgroup analysis revealed the baseline local hyperalgesic subgroup also demonstrated a significantly lower remote EIH at baseline.

In previous studies, all [15] or >85% [64] of healthy subjects and pain patients were able to complete the wall squat exercise, which is in line with the current findings where 86.8% managed to complete the wall squat exercise at baseline, while all subjects completed it at follow-up, which indicate that the methodology is comparable to previous literature.

The wall-squat test evokes pain and this can trigger a descending pain inhibitory response as often seen using the CPM paradigm. CPM is associated with EIH in healthy subjects [46,67] and in patients with knee osteoarthritis [19]; therefore, the EIH response may partially be due to a painful input triggering the descending pain inhibitory systems. However, the current study found similar peak pain intensities during the wall squat exercise at baseline and follow-up, although remote EIH

was increased at follow-up, suggesting that "pain-inhibits-pain" was not the only mechanism affecting EIH. A recent study found no significant association between the EIH response and pain intensity during the wall squat exercise in healthy subjects [15], which further supports that "pain-inhibits-pain" in not the only driver of EIH. In addition, another study in healthy subjects and chronic whiplash patients showed that CPM was not associated with EIH elicited using the wall squat exercise [64]. Another recent study reported a significant association between perceived exertion and remote EIH after the 3-minute wall squat exercise [15]. Conclusively, and in line with the current study, this could indicate that performance of this isometric task and the subsequent EIH response is more related to perceived exertion than peak pain.

It is noteworthy that a recent study found the reliability of EIH using the wall-squat test to be poor [15], which may explain some of the conflicting results in relation to EIH in the current study and in general. Additionally, the subgrouping in the current study was exploratory and based on the local EIH response. Subgrouping based on the remote EIH response, or other subgroupings, may have shown different results. Further research within optimizing EIH reliability and understanding EIH is encouraged.

Pressure pain sensitivity

A previous study found no changes in upper and lower body PPTs following 6 weeks of moderate-to-vigorous intensity aerobic exercise training in young healthy subjects [68] which is in contrast to the results demonstrated in the current study. The increased descending inhibitory drive alone or in combination with improved physical performance capacity reported in the current study, and/or e.g. enhanced anti-inflammatory cytokine response [4], may explain this global increase in pressure pain sensitivity following MT.

Temporal summation of pain

Facilitated TSP has been reported in chronic pain patients, e.g. whiplash [69], fibromyalgia [70] and knee osteoarthritis [48,49,71]. Further, lower TSP has been found in athletes compared with non-athletes [72], and a higher level of self-reported physical activity is associated with lower TSP [73]. The latter being in contrast to the current findings showing that TSP was unaffected following MT which indicate that MT did not modulate this central pain mechanism although physical performance capacity was enhanced significantly. However, the unaffected TSP following MT may also be explained by a floor effect since the TSP baseline measure was rather low, which is expected in healthy individuals [7,44,58,74].

Limitations

The current study is limited by the lack of a control group but strengthened by the longitudinal design. Further, the included population was rather homogenous, which may limit the generalizability since societal factors and other factors may influence pain responses [7]. Additionally, as MT (and exercise programs in general) represents both physical and psychological challenges [29,30] the current study is limited by a lack of psychosocial measures as these have been shown to influence the EIH response magnitude [59]. Finally, since EIH responses are more variable in pain populations compared with healthy pain free subjects [12], lack of assessment of acute and chronic pain conditions, other than knee pain, is a limitation to the current study.

Conclusion

This study is the first to report an improvement of EIH following a long-term physical exercise intervention in healthy subjects. In addition, this study reports that pain sensitivity facilitation rather than inhibition after the acute exercise occurred in 26% of healthy subjects, and that these pain hyperalgesic subjects report a hypoalgesic response following 7 weeks of training. Finally, a positive

association between improvement in physical performance capacity and improvement in EIH was found. Collectively, this adds new insight into the relationship between central pain sensitivity measures and physical function in healthy subjects.

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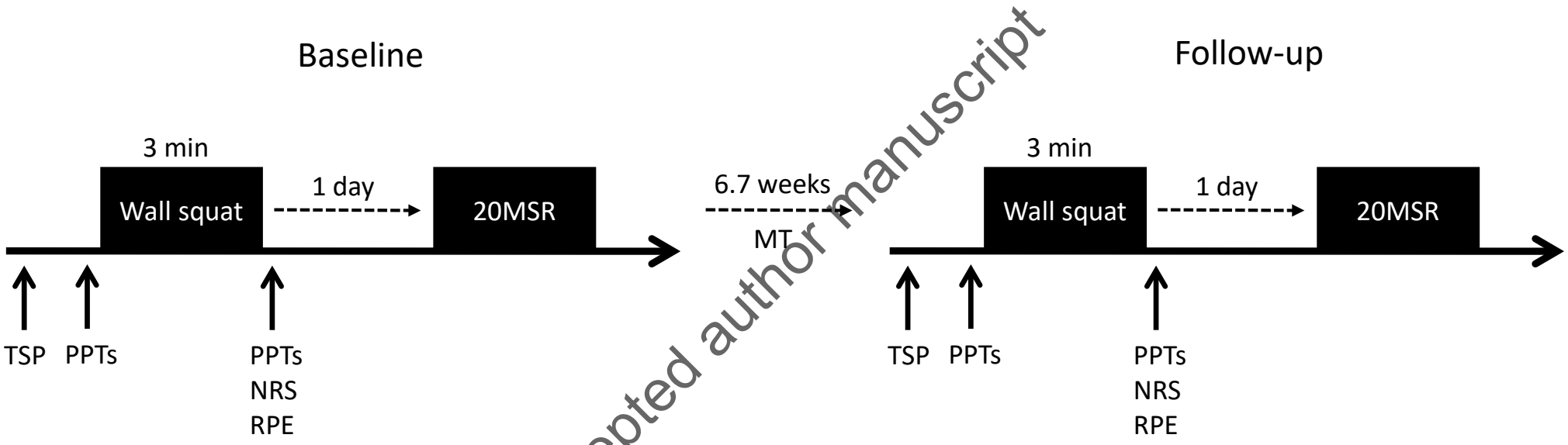
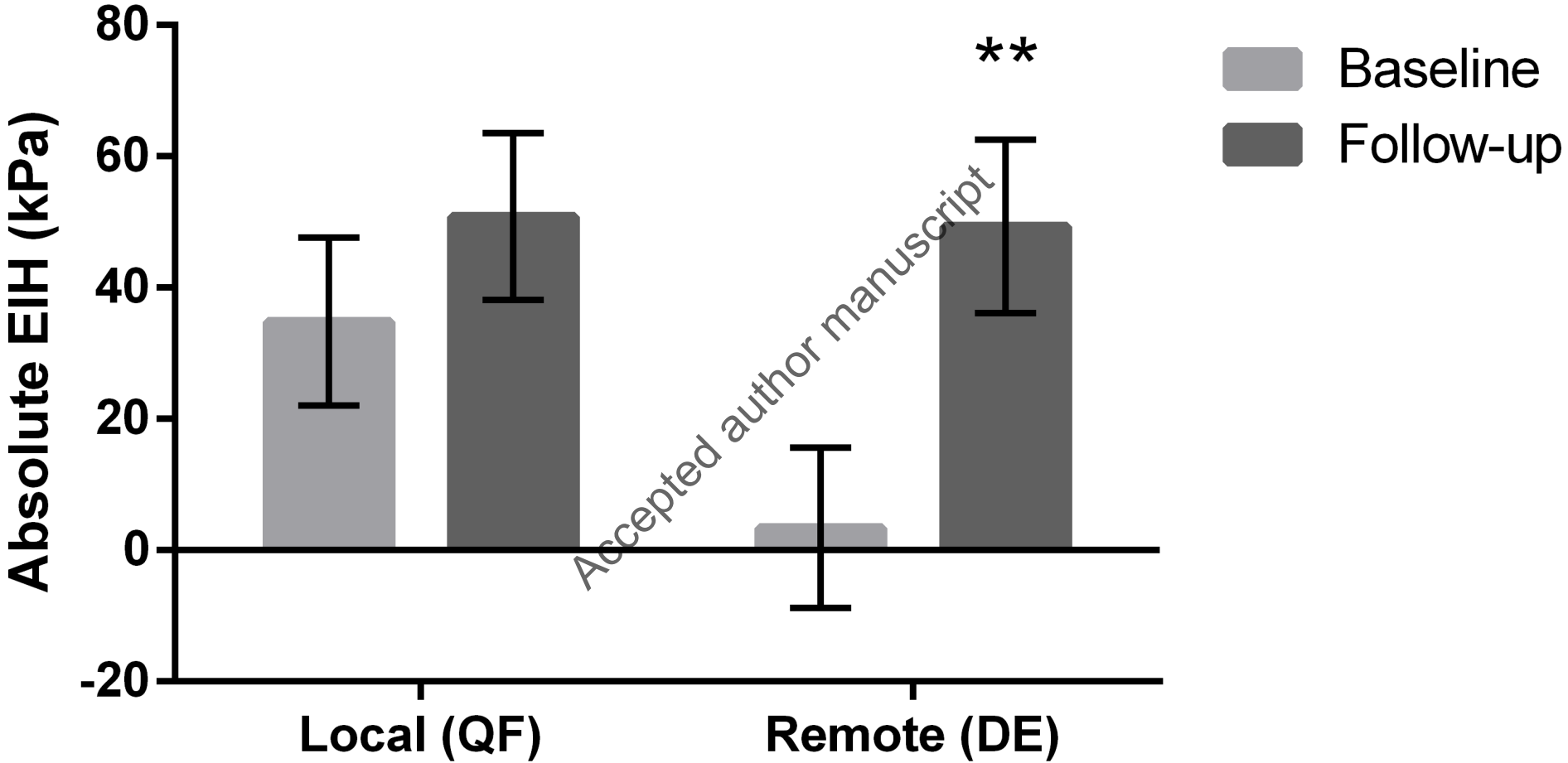
Figure legends

Figure 1. An illustration of the experimental procedures at baseline and follow-up. Pressure pain thresholds (PPTs) were assessed at the dominant quadriceps femoris and contralateral deltoideus muscles before and after the 3-minute isometric wall squat test. Temporal summation of pain (TSP) was assessed at the tibialis anterior muscle. Just after the wall squat, the subjects were instructed to rate the pain intensity in the legs from 0–10 on a numeric rating scale (NRS). Further, the subjects were asked to rate the perceived exertion (RPE) on a scale from 6-20. The Endurance 20-m Shuttle Run Fitness Test (20MSR) was pre-planned by the military one day after the other experimental procedures. MT, military training.

Figure 2. Absolute EIH at baseline and follow-up. QF, m. quadriceps femoris; DE, m. deltoideus; kPa, kilopascal. Error bars represent SEM. Significant difference between baseline and follow-up (n=38) is indicated by ** ($P < 0.01$).

Figure 3. Absolute EIH in the hyperalgesic (a) and hypoalgesic (b) subgroup at baseline and follow-up. Baseline hyperalgesic subgroup (n=10) and baseline hypoalgesic subgroup (n=28). QF, m. quadriceps femoris; DE, m. deltoideus; kPa, kilopascal. Error bars represent SEM. * Indicates significant differences ($P < 0.05$) between baseline and follow-up.





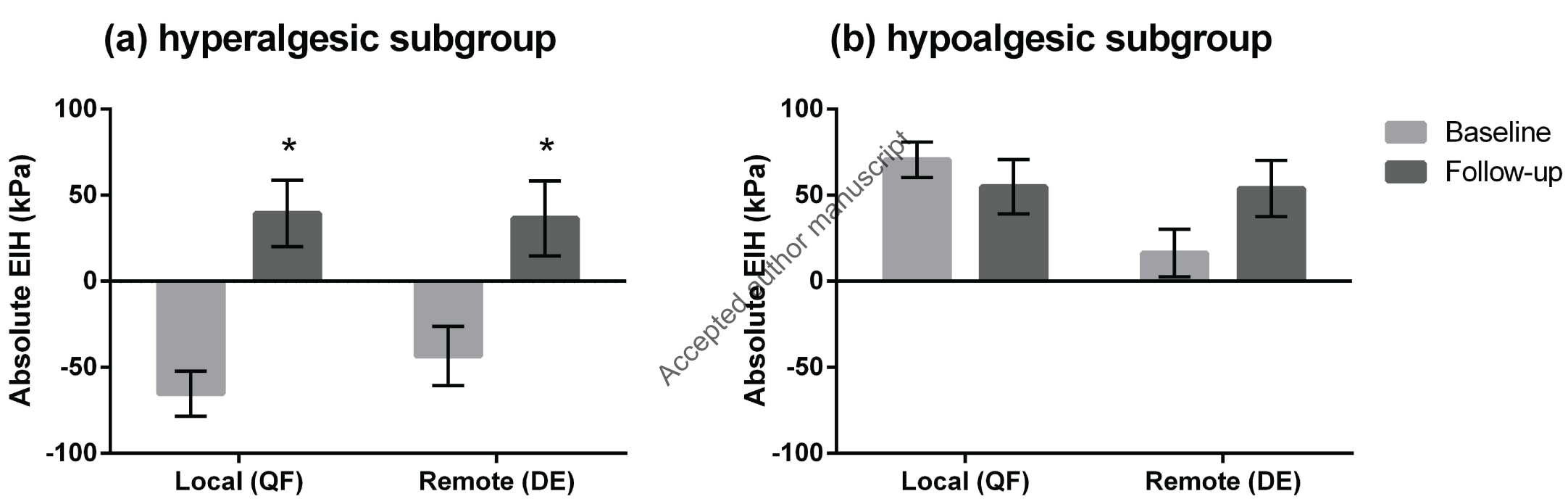


Table 1

KOOS subscales (0-100)	Baseline		Follow-up		Significance
	Mean ± SEM	Range	Mean ± SEM	Range	Adjusted *P*
Pain	92.0 ± 2.0	47 - 100	91.4 ± 2.2	50 – 100	0.982
Symptoms	88.0 ± 1.7	54 – 100	92.2 ± 1.4	71 – 100	0.105
ADL	93.5 ± 1.4	68 – 100	94.2 ± 1.5	69 – 100	0.658
Sport	90.0 ± 2.0	60 – 100	86.9 ± 2.8	30 – 100	0.394
Quality of Life	88.3 ± 2.6	44 – 100	89.1 ± 2.1	63 - 100	0.634

Table 1. KOOS subscale scores at baseline and follow-up (n=38). No significant change in any of the KOOS subscale scores were found comparing baseline to follow-up ($P > 0.1$).

Table 2

	Baseline values					Follow-up values				
Total group (n=38)	Before wall squat		After wall squat			Before wall squat		After wall squat		
PPT site	Mean ± SEM	Range	Mean ± SEM	Range	Adjusted *P*	Mean ± SEM	Range	Mean ± SEM	Range	Adjusted *P*
QF (kPa)	433.7 ± 23.4	122 – 662	468.5 ± 26.7	129 – 812	**0.016**	532.4 ± 27.3	238 – 952	583.2 ± 32.0	224 – 1118	**0.020**
DE (kPa)	360.4 ± 25.2	98 – 655	363.8 ± 24.8	118 – 770	1.000	453.5 ± 32.4	144 – 851	502.8 ± 37.3	160 – 1102	**0.008**
Baseline hypoalgesic subgroup (n=28)										
QF (kPa)	426.7 ± 29.4	122 - 540	497.2 ± 33.1	129 – 812	**< 0.001**	555.9 ± 34.4	238 – 952	610.8 ± 40.1	224 – 1118	**0.008**
DE (kPa)	366.4 ± 31.8	98 - 655	386.5 ± 30.8	118 - 770	0.272	472.2 ± 41.7	144 – 851	526.1 ± 47.4	160 – 1102	**0.002**
Baseline hyperalgesic subgroup (n=10)										
QF (kPa)	453.4 ± 35.7	284 - 600	388.2 ± 30.7	230 – 517	**0.010**	466.6 ± 32.9	337 – 685	506.0 ± 39.4	287 – 713	0.074
DE (kPa)	343.6 ± 36.7	197 - 541	300.2 ± 32.7	152 - 436	0.056	401.2 ± 37.0	246 - 648	437.7 ± 47.0	200 - 703	0.278

Table 2. Pressure pain thresholds (PPT) at baseline and follow-up, before and after the 3-minute wall squat exercise for the total group and the hypo- and hyperalgesic subgroups. QF, musculus quadriceps femoris; DE, musculus deltoideus; kPa, kilopascal; SEM, standard error of the mean. Values represent mean ± SEM and range. *P*-values in bold represent significant values.