



# Hypoalgesia after bicycling at lactate threshold is reliable between sessions

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## Abstract

**Purpose** Exercise decreases pain sensitivity known as exercise-induced hypoalgesia (EIH). However, the consistency of EIH after an acute exercise protocol based on subjective ratings of perceived exertion has been questioned. Objectives were to compare the effect on pressure pain thresholds (PPTs) after bicycling with work-rate at the lactate threshold compared with quiet rest, and investigate between-session reliability of EIH.

**Methods** Thirty-four healthy subjects completed three sessions with 7 days in-between. In session 1, the lactate threshold was determined via blood samples (finger-tip prick, > 2 mmol/l increase from warm-up) during a graded bicycling task. In session 2 and 3, all subjects performed (1) 15 min quiet-rest, and (2) 15 min bicycling (work-rate corresponding to the lactate threshold) in the two identical sessions. PPTs at the quadriceps and trapezius muscles were assessed before and after both conditions. Reliability was assessed by intraclass correlations (ICCs).

**Results** Bicycling increased quadriceps PPT compared with quiet-rest in both sessions [mean difference: 45 kPa (95% CI 19–72 kPa),  $P=0.002$ ]; however, the increase in trapezius PPT was not significant after exercise. The EIH responses demonstrated fair between-session test–retest reliability (quadriceps: ICC = 0.45; trapezius: ICC = 0.57,  $P < 0.05$ ), and agreement in EIH responders and non-responders between sessions was significant (quadriceps:  $\kappa=0.46$  and trapezius:  $\kappa=0.43$ ,  $P < 0.05$ ).

**Conclusions** In conclusion, bicycling at the lactate threshold increased PPT at the exercising muscle with fair reliability of the local EIH response. The results have implications for future EIH studies in subjects with and without pain and for clinicians who design exercise programs for pain relief.

**Keywords** Exercise · Exercise-induced hypoalgesia · Reliability · Pressure pain thresholds · Pain sensitivity

## Abbreviations

BMI Body mass index  
CI Confidence interval

CPM Conditioned pain modulation  
EIH Exercise-induced hypoalgesia  
ICC Intraclass correlation coefficient  
kPa Kilopascal  
[La-]<sub>b</sub> Blood-lactate concentration  
NRS Numerical rating scale  
PPT Pressure pain threshold  
RM-ANOVA Repeated-measures analysis of variance  
RPE Rating of perceived exertion  
RPM Rounds per minute  
SEM Standard error of measurement  
VO<sub>2max</sub> Maximum rate of oxygen consumption

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## Introduction

A decrease in the pain sensitivity following an acute bout of exercise also known as exercise-induced hypoalgesia (EIH) has been demonstrated in healthy subjects (Vaegter et al. 2014). EIH is often demonstrated as an increase in pressure pain threshold (PPT) at exercising or non-exercising/less active muscles, and has been suggested to be an indicator of endogenous pain inhibitory capacity (Lannersten and Kosek 2010). Reduced EIH responses or even the opposite (i.e., hyperalgesic) has been demonstrated in subjects with chronic pain compared with pain-free controls (Kosek et al. 1996; Lannersten and Kosek 2010). In addition to the discriminative value of EIH between subjects with chronic pain compared with pain-free controls, the EIH response may be related to treatment outcome after exercises (Fingleton et al. 2017) and surgery (Vaegter et al. 2017) indicating clinical utility. Ensuring clinical applicability, adequate validity and test–retest reliability is an essential prerequisite, but such knowledge on EIH is sparse.

Only few studies have previously investigated test–retest reliability of the EIH response after exercises. In two different studies, Vaegter and colleagues (Vaegter et al. 2018a, b) found that an isometric wall squat exercise and an aerobic incremental aerobic bicycling exercise, with a work-rate based on subjective ratings of perceived exertion, increased PPTs at exercising and non-exercising/less active muscles in two sessions separated by 1 week compared with a quiet-rest control condition, however the EIH responses demonstrated only fair between-sessions test–retest reliability (based on intraclass correlations [ICCs]), and the agreement in classification of EIH responders and non-responders between sessions was not significant. As subjective ratings of perceived exertion during aerobic exercise varies significantly over identical bicycling trials (Micalos et al. 2004; Vaegter et al. 2018a), it could be hypothesized that an exercise protocol based on an objectively defined exercise work-rate would improve the reliability of the EIH responses.

Several objective measures have been related to aerobic exercise performance (Jacobs et al. 2011) including lactate threshold. The lactate threshold is defined as the exercise work-rate beyond which the blood-lactate concentration  $[La-]_b$  increases more rapidly (Wasserman et al. 1973), often occurring at exercise intensities above 50%  $VO_{2max}$  (Buono and Yeager 1986), and lactate threshold can be used to accurately prescribe exercise work-rate. In healthy subjects as well as patient populations, the lactate threshold appears to be a better predictor of exercise capacity than  $VO_{2max}$  (Bentley et al. 2007; Coyle et al. 1983). Moreover, as determination of  $VO_{2max}$  requires intense

exhaustion of the investigated subject it may be difficult to attain in subjects with chronic pain. In addition, whether exercise at lactate threshold induces hypoalgesia has not previously been investigated.

This study investigated (1) the hypoalgesic response at the quadriceps femoris (exercising muscle) and the upper trapezius muscle (non-exercising/less active muscle) after a 15 min bicycling exercise with a work-rate corresponding to the lactate threshold compared with a 15 min resting condition in healthy subjects, (2) the between-session test–retest reliability of the test stimulus (PPT) and the EIH responses at exercising and non-exercising/less active muscles based on ICC values, and (3) the absolute between-session test–retest reliability based on agreement in classification of EIH responders and non-responders between sessions. It was hypothesized that (1) the exercise protocol increase PPTs at exercising and non-exercising/less active muscles compared with the resting condition, (2) PPTs and EIH demonstrate acceptable relative test–retest reliability, and (3) the classification of EIH responders and non-responders show significant agreement between sessions.

## Materials and methods

### Subjects

In total, 37 healthy subjects were recruited by advertisement at the local university college, and through social media. This study was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee (S-20170129), and all subjects provided written informed consent. None of the included subjects suffering from neurological, psychological, cardiovascular diseases, had any pain or used any pain medication during the weeks prior to participation. Three subjects had scheduling conflicts during the study period and did not attend all sessions. Thus, 34 healthy subjects [ $25.3 \pm 3.2$  (standard deviation) years old; (range 22–40 years); average body mass index (BMI)  $25.3 \pm 4.0$  kg/m<sup>2</sup> (range 19.9–34.7); 9 left-handed; 16 women] were included. All subjects were asked to refrain from physical exercises, coffee, and alcohol on the days of participation.

### Procedure

The experiment comprised three sessions. In session 1, subjects were (1) verbally introduced to the procedures and familiarized with the assessment of pressure pain threshold (PPT) at 2 body sites including the sites used in session 2 and session 3, and (2) the lactate threshold was determined sequentially via blood samples from finger-tip pinprick during a graded bicycling exercise task. In session 2 and 3, PPTs

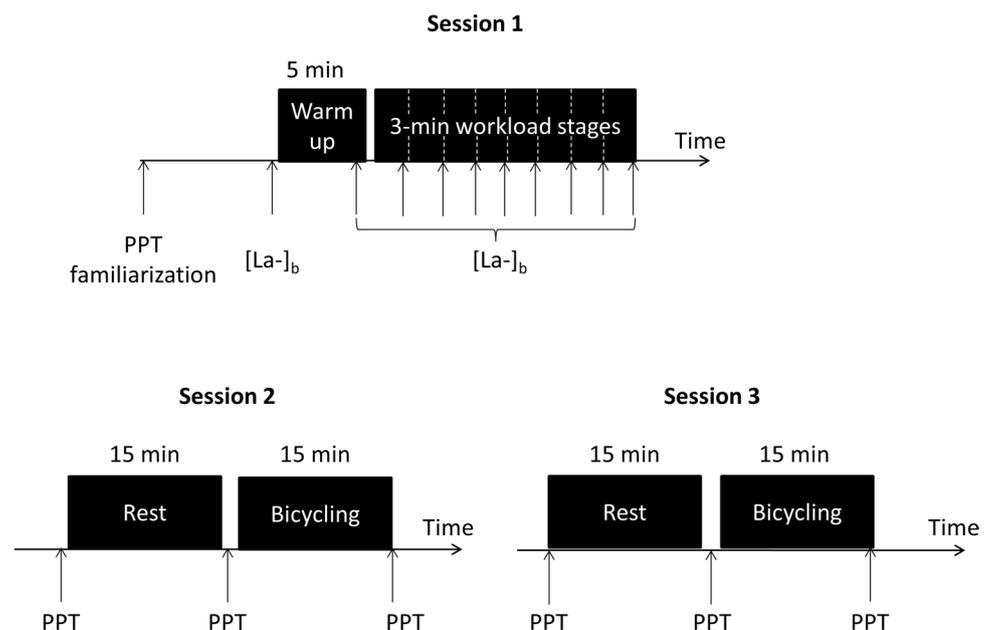
were initially recorded from the dominant thigh and the non-dominant shoulder. In addition, all subjects performed a 15 min quiet-rest condition, and a 15 min bicycling exercise in each of the two identical sessions. PPTs were assessed before and immediately after the quiet-rest and exercise conditions. Sessions were performed at the same time of the day and separated by 1 week (Fig. 1). This time frame was chosen to minimize potential carry-over effects from the pain sensitivity assessments and exertion after physical exercise between sessions as well as avoiding extensive changes in physical fitness level within subjects.

### Determination of lactate threshold

The blood-lactate response to a submaximal bicycling exercise was assessed while the subject was seated on an electronically braked standard laboratory bicycle ergometer (Ergomedic 928E, Monark Exercise AB, Vansbro, Sweden). Heart rate (Monark Heart Rate Monitor, Polar Electro, Lake Success, NY, USA) was monitored continuously throughout the test and subjects rated the perceived exertion (RPE) on a Borg's 6–20 scale, with 6 defined as 'no exertion at all' and 20 as 'maximal exertion'. The lactate threshold test was performed continuously in 3-min stages preceded by 5 min of light warm-up. The initial warm-up power output performed at eighty rounds per min (RPM) was selected on the basis of the subject's gender and reported usual training workload (Women, light-to-moderate usual workload: 60 W. Women, high usual workload: 80 W. Men, light-to-moderate usual workload: 80 W. Men, high usual workload: 100 W). This warm-up power

output represented a "very light" (Borg scale rating of perceived exertion (RPE)  $\leq 9$ ) workload for 23 of the subjects and "light" (Borg scale rating of perceived exertion (RPE)  $\leq 11$ ) for 11 of the subjects. Increments in power output were also selected based on gender and reported usual training workload (Women, light-to-moderate usual workload: 15 W. Women, high usual workload: 20 W. Men, light-to-moderate usual workload: 20 W. Men, high usual workload: 25 W), such that the test concluded after a maximum of 10 workload stages (median number of stages 6). Blood samples were taken via finger-tip pinprick at rest as well as during the last 30 s of the warm-up and each 3-min workloads. Whole  $[La^-]_b$  was measured with a portable  $[La^-]_b$  analyzer (Lactate Pro2, Arkay KDK, Kyoto, Japan) which has shown acceptable accuracy and reliability compared with a criterion blood analyzer (Bonaventura et al. 2015). The lactate threshold test was terminated when  $[La^-]_b$  began to rise exponentially ( $> 2$  mmol/l from the value at rest followed by an additional increase  $> 2$  mmol/l in the subsequent recording) ensuring that all subjects reached 4 mmol/l, which is known as the onset of blood-lactate accumulation (Kinderman et al. 1979; Sjodin et al. 1981; Heck et al. 1985). Since the  $[La^-]_b$  value at lactate threshold may vary between subjects (Stegman et al. 1981) an increase in  $[La^-]_b > 2$  mmol/l from the concentration after warm-up was used as lactate threshold instead of using a fixed  $[La^-]_b$  value. Prior to the lactate threshold test, subjects were instructed not to do any intense or long-duration exhausting exercises in the preceding 24 h, not to change their normal dietary program throughout the study period, and to arrive 10 min ahead of their scheduled test time.

**Fig. 1** Illustration of the experimental procedure performed on the three testing days. The pressure pain thresholds (PPT) familiarization procedure and determination of lactate threshold was performed in session 1. PPTs were assessed on two assessment sites (quadriceps and upper trapezius) before and immediately after rest and bicycling in session 2 and session 3



## Pressure pain threshold assessment

PPTs were assessed with a handheld pressure algometer (Somedic Sales AB, Sweden) with a stimulation area of 1 cm<sup>2</sup>, and a pressure rate increase at approximately 30 kPa/s at the middle of the exercising dominant quadriceps muscle (15 cm proximal to the base of patella), and at the non-exercising/less active non-dominant upper trapezius muscle (10 cm from the acromion in direct line with the neck). Subjects were instructed to press a button when the pressure was perceived as the first sensation of minimal pain, and the pressure intensity defined the PPT. Two PPT assessments with 20 s intervals between assessments were completed for each site and the average was used for analysis. The first PPT assessment at the quadriceps muscle was always performed first followed by the first PPT assessment at the trapezius. The order was then reversed for the second assessment. Subjects were seated on a plinth without foot support and with both arms resting on the thighs during assessments.

## Quiet-rest and bicycling conditions

In the quiet-rest conditions, subjects were instructed to relax in a seated position in a comfortable armchair for 15 min in a temperate and quiet room. Subjects performed two 15-min bicycling exercise conditions, in session 2 and session 3, respectively. The seat post of the stationary cycle (Ergo-medec 928E, Monark Exercise AB, Vansbro, Sweden) was adjusted so that the subject had approximately five-degree bend at the knee during the bottom phase of the pedal stroke. A heart rate monitor (Monark Heart Rate Monitor, Polar Electro, Lake Success, NY, USA) was strapped around the subject's chest. 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'. In session 2 and session 3, the bicycling work-rate was initially set to 50% of the lactate threshold. After 2.5 min of bicycling the resistance was increased to 75% of the lactate threshold, and after 5 min of bicycling the resistance was set to 100% of the lactate threshold whereafter the subject continued bicycling at that work-rate for the remaining time. Subjects were instructed to maintain a pedal rate as close to 75 rounds per min (RPM) as possible throughout the 15-min bicycling exercise. Heart rate, pain intensity in the legs, and RPE were assessed after 2, 4, 6, 9, 12, and 15 min.

## 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 distribution of PPTs across sessions did not deviate significantly from normality (Shapiro–Wilks test:  $P > 0.16$ ). The effect of sessions and gender on baseline PPTs was analyzed with a mixed-model analysis of variances (ANOVAs) with session (session 2 and session 3) and assessment site (quadriceps and trapezius) as within subject factor and sex as between-subject factor.

Changes in PPTs after bicycling and rest were compared in a repeated-measures ANOVA (RM-ANOVA) with session (session 2 and session 3), condition (exercise and rest), and assessment site (quadriceps and trapezius) as within subject factors, and sex as between-subject factor. Analyses were performed on absolute change (PPT-after minus PPT-before) and relative change ((PPT-after minus PPT-before)/PPT-before  $\times$  100%) in PPTs. Comparison of exercise parameters between session 2 and session 3 (NRS scores, RPE, and heart rate) were analyzed with RM-ANOVAs with session (session 2 and session 3), and time (0, 2, 4, 6, 9, 12, and 15 min) as within subject factors and sex as between-subject factor.  $P$  values less than 0.05 were considered significant. In case of significant factors or interactions in RM-ANOVAs, Bonferroni correction post-hoc test (paired t-tests) were used to correct for multiple comparisons. Pearson's correlational analyses were performed to determine possible associations between the EIH responses and the peak NRS pain scores, heart rate, and RPE during exercise. Due to multiple correlational analyses,  $P$  values equal to or less than 0.002 (0.05 / 28) were considered significant for the correlations.

For assessment of test–retest reliability of PPTs (within-session and between-session) and EIH (between-session), the systematic error between sets of PPT assessments (within-session: before and after rest in second and third session; between-session: baseline second and third session) and EIH responses (between-session: absolute change in PPTs after exercise in second and third session) were determined using RM-ANOVAs. Pearson's  $r$  and intraclass correlations (ICCs) based on a single rating, consistency, two-way mixed effect model were used reflecting the ability of the PPTs and EIH responses to differentiate values 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 (Shrout and Fleiss 1979).

Agreement in classification of EIH responders and EIH non-responders between session 2 and session 3, were assessed by the standard error of PPT measurement used as classifier as some of the changes in PPT in response to exercise may be due to measurement error. The SEM for repeated PPTs assessment (before and after rest) in session 2 and session 3 were estimated as the square root of the mean square error term in the RM-ANOVA output (Weir 2005). Subjects who had an increase in PPTs after exercise, which was larger than 1 $\times$ SEM of the PPTs, were classified as EIH responders and subjects who did not have an

increase in PPTs after exercise larger than  $1 \times \text{SEM}$  of the PPTs were classified as EIH non-responders. Agreement in the frequency of EIH responders and EIH non-responders between sessions was compared with Cohen's kappa coefficient. Data were analyzed using SPSS Statistics, version 24 (IBM, Armonk, NY, USA).

## Results

### Pain thresholds at baseline

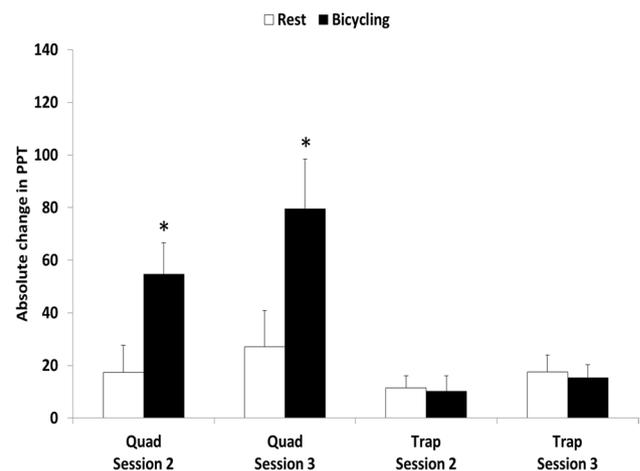
In sessions 2 and 3, baseline PPTs were higher in men (quadriceps:  $498 \pm 120$  kPa,  $572 \pm 162$  kPa and trapezius:  $297 \pm 76$  kPa,  $310 \pm 86$  kPa) compared with women [quadriceps:  $429 \pm 145$  kPa,  $448 \pm 168$  kPa and trapezius:  $223 \pm 77$  kPa,  $243 \pm 91$  kPa; ANOVA:  $F(1,32) = 6.29$ ,  $P = 0.017$ ]. Moreover, a significant main effect of assessment site was found [ANOVA:  $F(1,32) = 183.13$ ,  $P < 0.001$ ] with post-hoc test showing that PPT at the quadriceps muscle was significantly higher compared with PPT at the trapezius muscle ( $P < 0.001$ ).

### Lactate threshold

The  $[\text{La-}]_b$  was increased after warm-up ( $1.8 \pm 0.7$  mmol/l) compared with rest ( $1.4 \pm 0.4$  mmol/l; ANOVA:  $F(1,33) = 10.50$ ,  $P = 0.003$ ), but not significantly different between women and men ( $P = 0.95$ ). The median number of 3-min workload stages during lactate-threshold determination was 6 (range: 4–9), and the corresponding  $[\text{La-}]_b$  when the test was terminated was not significantly different between women ( $6.3 \pm 1.1$  mmol/l) and men ( $6.2 \pm 1.2$  mmol/l;  $P = 0.81$ ). Mean work-rate at the lactate threshold was  $151 \pm 39$  W. The exercise work-rate determined at the lactate threshold was larger in men ( $166 \pm 43$  W) compared with women ( $135 \pm 29$  W;  $P = 0.02$ ).

### Comparison of bicycling and rest

The ANOVAs of absolute and relative change in PPTs after bicycling and rest demonstrated a significant interaction between conditions and assessment sites (Fig. 2; ANOVA:  $F(1,33) = 8.30$ ,  $P = 0.007$ ) with post-hoc test showing a larger increase in PPT at the exercising quadriceps muscle (session 2: 13.5%; session 3: 15.7%) after bicycling in both sessions compared with the resting condition [mean difference: 45 kPa (95% CI 19–72 kPa),  $P = 0.002$ ]. No significant difference between bicycling (session 2: 4.6%; session 3: 5.8%) and rest (session 2: 5.9%; session 3: 7.4%) was found at the non-exercising/less active trapezius muscle ( $P = 0.81$ ). No significant absolute or relative changes in PPTs after



**Fig. 2** Mean (+SEM,  $n = 34$ ) change in pressure pain threshold (PPT, kilopascal (kPa)) recorded at the two assessment sites (quadriceps and upper trapezius muscles) after 15 min quiet-rest and 15 min bicycling in session 2 and session 3. Significantly different compared with rest condition (\* $P < 0.05$ ). *Quad* m. quadriceps dominant side, *Trap* upper trapezius muscle non-dominant side

bicycling [ $F(1,32) < 1.28$ ,  $P > 0.27$ ] were found between women and men.

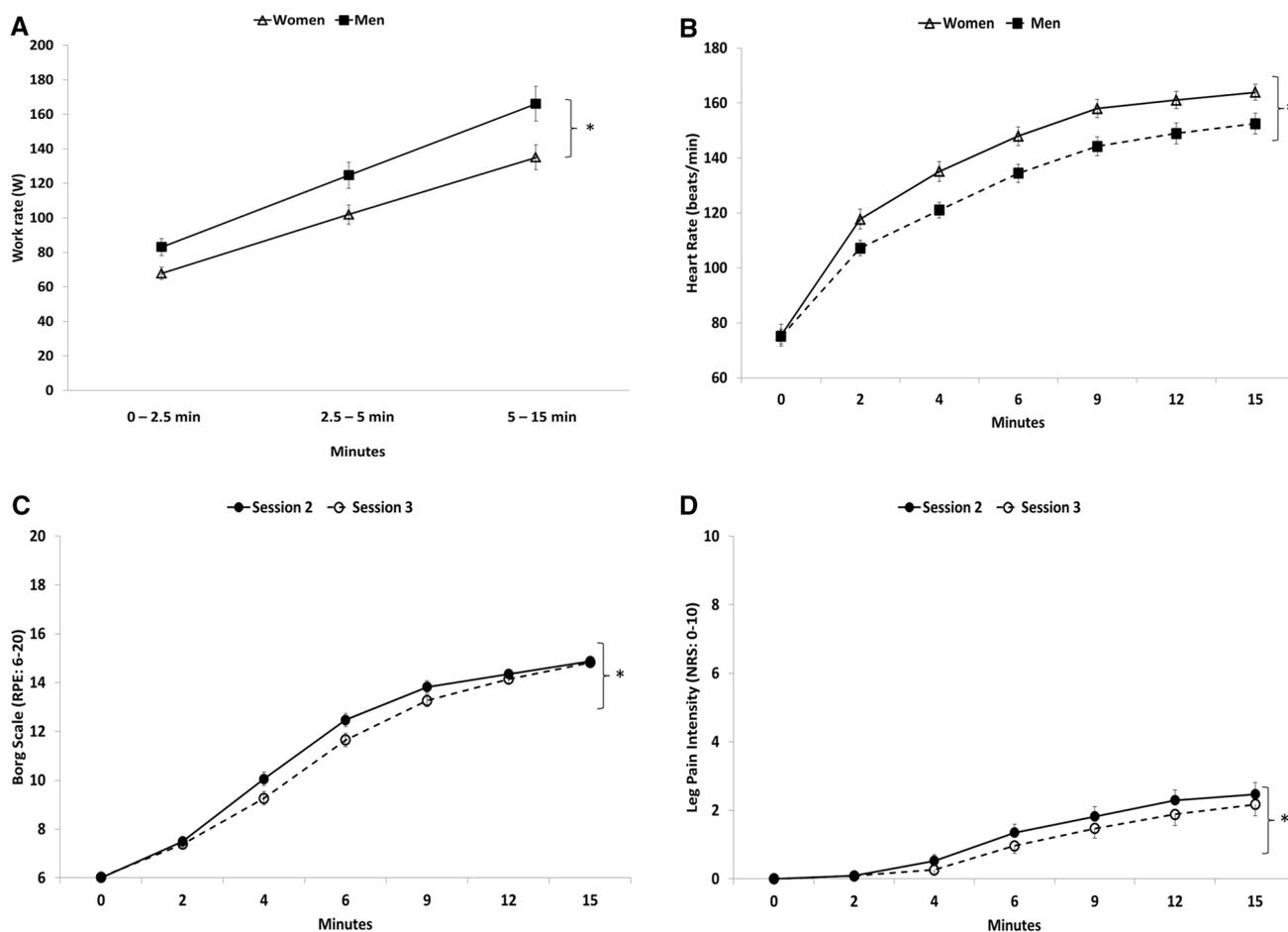
### Comparison of exercise parameters between sessions

The work-rate of bicycling in the two sessions did not differ as identical protocols were used; however, a significant effect of gender was found with higher exercise work-rate in men compared with women [mean difference: 23.1 W (95% CI 3.7–42.5); Fig. 3a, ANOVA:  $F(1,32) = 5.86$ ,  $P = 0.021$ ].

Heart rate during exercise increased over time [Fig. 3b,  $F(6,192) = 908.25$ ,  $P < 0.001$ ] with post-hoc test showing progressively increasing higher heart rate among all time points ( $P < 0.001$ ). Moreover, heart rate during exercise was higher in women compared with men [mean difference: 11.5 beats/min (95% CI 3.7–19.2); ANOVA:  $F(1,32) = 9.01$ ,  $P = 0.005$ ]. No significant difference in heart rate between sessions were found [ANOVA:  $F(1,32) = 0.88$ ,  $P = 0.36$ ].

Rating of perceived exertion was increased over time [Fig. 3c; ANOVA:  $F(6,192) = 712.34$ ,  $P < 0.001$ ] with post-hoc test showing increasing RPE over time (all time points,  $P < 0.001$ ). Moreover, a difference was found between session 2 and session 3 [ $F(1,32) = 6.05$ ,  $P = 0.019$ ] with higher RPE during exercise in session 2 compared with session 3 [mean RPE difference: 0.35 (95% CI 0.06–0.64);  $P = 0.019$ ]. No significant difference in RPE between women and men were found [ANOVA:  $F(1,32) = 0.25$ ,  $P = 0.62$ ].

The NRS ratings of leg-pain intensity during bicycling increased over time [Fig. 3d; ANOVA:  $F(6,192) = 48.48$ ,  $P < 0.001$ ] with post-hoc test showing increased NRS scores



**Fig. 3** Mean ( $\pm$  SEM,  $n=34$ ) exercise work-rate (**a**), heart rate (**b**), rating of perceived exertion (**c**), and numerical rating scale (NRS) scores of leg-pain intensity (**d**) assessed during exercise in session 2 and session 3. As exercise work-rate and heart rate was not significantly

different between session 2 and session 3, the mean of sessions are presented. Significantly different between women and men ( $*P < 0.05$ ). Significantly different compared with the other session ( $^{\dagger}P < 0.05$ )

over time (all time points,  $P < 0.001$ ) except between the first two assessments (0 min and 2 min). Moreover, a difference was found in reported NRS pain scores between session 2 and session 3 [ANOVA:  $F(1,32) = 8.64$ ,  $P = 0.006$ ] with higher pain NRS scores in session 2 compared with session 3 [mean NRS difference: 0.25 (95% CI 0.08–0.42);  $P = 0.006$ ]. No significant difference in peak NRS scores of pain intensity between session 2 ( $2.5 \pm 2.0$ ) and session 3 ( $2.2 \pm 2.0$ ;  $P = 0.08$ ), and between men and women were found ( $P = 0.48$ ).

Significant correlations were found between peak HR and the change in PPT at the trapezius muscle after exercise in both sessions, which could indicate a link between a baroreceptor mechanism and EIH. Moreover, both the absolute and relative change in PPT at the quadriceps muscle was significantly associated with the change in PPT at the trapezius muscle after exercise in session 2 suggesting that subjects with the largest local EIH response also exhibited the largest systemic EIH response. In session 3, this association

was not significant. However, after adjusting for multiple correlations, all associations between the EIH responses at the quadriceps or trapezius muscles and the peak NRS pain scores, heart rate, and RPE during exercise turned insignificant (Table 1).

### Test–retest reliability of PPTs and EIH

Within-session test–retest reliability of PPT at the quadriceps muscle showed no systematic errors between two repeated assessments separated by 15 min of quiet rest, although the difference in session 3 approached significance [ANOVA:  $F(1,33) < 3.80$ ,  $P > 0.06$ ]; assessments were strongly correlated ( $r \geq 0.91$ ), and ICCs were excellent with values  $\geq 0.95$  (Table 2). However, within-session test–retest reliability of PPT at the trapezius muscle showed higher PPT after rest compared with before rest in both sessions [ANOVA:  $F(1,33) > 6.21$ ,  $P > 0.02$ ]; assessments were

**Table 1** Pearson's correlations between absolute and relative change in PPTs after exercise, peak heart rate (HR), peak rating of perceived exertion (RPE), and peak muscle-pain intensity (NRS) during exercise in the two sessions

| Variables                | Correlation  | HR session 2  | RPE session 2 | NRS session 2 | Abs Δ PPT trap session 2 | Rel Δ PPT trap session 2 | HR session 3  | RPE session 3 | NRS session 3 | Abs Δ PPT trap session 3 | Rel Δ PPT trap session 3 |
|--------------------------|--------------|---------------|---------------|---------------|--------------------------|--------------------------|---------------|---------------|---------------|--------------------------|--------------------------|
| Abs Δ PPT Quad Session 2 | R<br>P value | 0.10<br>0.57  | 0.22<br>0.21  | 0.11<br>0.52  | 0.40<br>0.018            | -                        | -             | -             | -             | -                        | -                        |
| Rel Δ PPT Quad Session 2 | R<br>P value | 0.12<br>0.51  | 0.33<br>0.058 | 0.08<br>0.64  | -                        | 0.48<br>0.004            | -             | -             | -             | -                        | -                        |
| Abs Δ PPT Trap Session 2 | R<br>P value | 0.35<br>0.04  | 0.20<br>0.26  | -0.13<br>0.46 | -                        | -                        | -             | -             | -             | -                        | -                        |
| Rel Δ PPT Trap Session 2 | R<br>P value | 0.39<br>0.023 | 0.15<br>0.39  | -0.13<br>0.48 | -                        | -                        | -             | -             | -             | -                        | -                        |
| Abs Δ PPT Quad Session 3 | R<br>P value | -             | -             | -             | -                        | -                        | -0.2<br>0.91  | 0.07<br>0.71  | 0.16<br>0.375 | -0.04<br>0.81            | -                        |
| Rel Δ PPT Quad Session 3 | R<br>P value | -             | -             | -             | -                        | -                        | 0.01<br>0.94  | 0.20<br>0.25  | 0.28<br>0.11  | -                        | -0.06<br>0.75            |
| Abs Δ PPT Trap Session 3 | R<br>P value | -             | -             | -             | -                        | -                        | 0.39<br>0.023 | 0.10<br>0.585 | 0.12<br>0.51  | -                        | -                        |
| Rel Δ PPT Trap Session 3 | R<br>P value | -             | -             | -             | -                        | -                        | 0.33<br>0.06  | 0.17<br>0.35  | 0.05<br>0.79  | -                        | -                        |

Due to multiple correlational analyses, *P* values equal to or less than 0.002 (0.05/24) were considered significant  
*Abs Δ* absolute change, *Rel Δ* relative change, *PPT* pressure pain threshold, *Quad* quadriceps femoris muscle, *Trap* upper trapezius muscle

**Table 2** Within-session test–retest reliability for pressure pain threshold at the dominant quadriceps and non-dominant upper trapezius muscles before and after the quiet-rest condition

| Variable       | Before rest, mean $\pm$ SD (95% CI) | After rest, mean $\pm$ SD (95% CI) | Within-session difference, mean $\pm$ SD (95% CI) | <i>P</i> value | Pearson <i>r</i>         | ICC (95% CI)     | Standard error of measurement (kPa) |
|----------------|-------------------------------------|------------------------------------|---|----------------|--------------------------|------------------|-------------------------------------|
| Quad Session 2 | 466 $\pm$ 135 kPa (418–513)         | 483 $\pm$ 148 kPa (431–534)        | 17 $\pm$ 60 kPa (– 4 to 38)                       | 0.105          | 0.91<br><i>P</i> < 0.001 | 0.95 (0.90–0.98) | 43                                  |
| Quad Session 3 | 513 $\pm$ 174 kPa (453–574)         | 540 $\pm$ 204 kPa (469–612)        | 27 $\pm$ 81 kPa (– 1 to 55)                       | 0.059          | 0.92<br><i>P</i> < 0.001 | 0.95 (0.91–0.98) | 57                                  |
| Trap Session 2 | 263 $\pm$ 84 kPa (233–292)          | 274 $\pm$ 83 kPa (245–303)         | 11 $\pm$ 27 kPa (2–21)                            | 0.018          | 0.95<br><i>P</i> < 0.001 | 0.97 (0.95–0.99) | 19                                  |
| Trap Session 3 | 279 $\pm$ 94 kPa (246–311)          | 296 $\pm$ 96 kPa (263–330)         | 18 $\pm$ 37 kPa (4–31)                            | 0.010          | 0.92<br><i>P</i> < 0.001 | 0.96 (0.92–0.98) | 27                                  |

strongly correlated ( $r \geq 0.92$ ), and ICCs were excellent with values  $\geq 0.96$ .

Between-session test–retest reliability of PPT at the quadriceps and trapezius muscles, respectively, showed no systematic errors, although the PPT quadriceps difference between sessions approached significance [ANOVA:  $F(1,33) < 4.04$ ,  $P > 0.05$ ], which was also reflected in the 95% CI of the mean differences, where zero lies within the interval. Moreover, between-sessions assessments were moderately correlated ( $r \geq 0.62$ ), and ICCs were excellent with values  $\geq 0.76$  for both sites (Table 3).

No systematic errors in EIH at the quadriceps and trapezius muscles between sessions were found [ANOVA:  $F(1,33) = 1.41$ ,  $P > 0.24$ ], and between-session test–retest reliability of EIH at the quadriceps (local EIH) and trapezius (remote EIH) muscles were fair-to-good with ICCs of 0.45 and 0.57, respectively (Table 3).

### Change in PPTs after exercise considered as an EIH responder

The minimal differences needed between two PPT assessments separated by 15 min on a subject for the difference

in the PPT for a subject to be considered an EIH responder were 43 kPa and 57 kPa for quadriceps and 19 kPa and 27 kPa on trapezius in session 2 and session 3, respectively (Table 2). Nineteen and 20 subjects demonstrated increases in PPT at the quadriceps muscle larger than 1xSEM in session 2 and session 3, respectively. Fifteen subjects were classified as local EIH responders in both sessions, and ten subjects were consistently classified as EIH non-responders [Table 4;  $\kappa = 0.46$  (95% CI 0.17 to 0.75),  $P = 0.007$ ]. Twelve and 13 subjects demonstrated increases in PPT at the trapezius muscle larger than 1xSEM at session 2 and session 3, respectively. Eight subjects were classified as EIH responders in both sessions, and seventeen subjects were consistently classified as EIH non-responders in both sessions [Table 4;  $\kappa = 0.43$  (95% CI 0.11 to 0.74),  $P = 0.012$ ].

## Discussion

As hypothesized, the bicycling exercise significantly increased PPT at the exercising quadriceps muscle in both sessions compared with a duration-matched quiet rest control condition; however, contrary to the hypothesis the

**Table 3** Between-session relative test–retest reliability for baseline pressure pain threshold (PPT) and exercise-induced hypoalgesia (EIH) assessed at the dominant quadriceps and non-dominant upper trapezius muscles as absolute change in PPT at session 2 and session 3

| Variable       | Session 2, mean $\pm$ SD (95% CI) | Session 3, mean $\pm$ SD (95% CI) | Between-session difference, mean $\pm$ SD (95% CI) | <i>P</i> value | Pearson <i>r</i>         | ICC (95% CI)     |
|----------------|-----------------------------------|-----------------------------------|--|----------------|--------------------------|------------------|
| Quadriceps     | 466 $\pm$ 135 kPa (418–513)       | 513 $\pm$ 174 kPa (453–574)       | 48 $\pm$ 139 kPa (– 1 to 96)                       | 0.052          | 0.62<br><i>P</i> < 0.001 | 0.76 (0.51–0.88) |
| Trapezius      | 263 $\pm$ 84 kPa (233–292)        | 278 $\pm$ 94 kPa (246–311)        | 16 $\pm$ 70 kPa (– 8 to 40)                        | 0.188          | 0.70<br><i>P</i> < 0.001 | 0.82 (0.64–0.91) |
| EIH Quadriceps | 55 $\pm$ 70 kPa (30–79)           | 80 $\pm$ 110 kPa (41–118)         | 25 $\pm$ 122 kPa (– 18 to 68)                      | 0.244          | 0.29<br><i>P</i> = 0.09  | 0.45 (0.1–0.72)  |
| EIH Trapezius  | 10 $\pm$ 35 kPa (– 2 to 22)       | 15 $\pm$ 29 kPa (5–25)            | 5 $\pm$ 36 kPa (– 7 to 18)                         | 0.396          | 0.42<br><i>P</i> = 0.015 | 0.57 (0.1–0.79)  |

**Table 4** Crosstabulations of the EIH responders and non-responders after 15 min of bicycling at session 2 and session 3 at the quadriceps muscle and the trapezius muscle

| EIH response at the exercising quadriceps muscle after bicycling    | EIH responders in session 2 |    |
|---|-----------------------------|----|
|   | Yes                         | No |
| EIH responders in session 1   |                             |    |
| Yes   | 15                          | 4  |
| No  | 5                           | 10 |
| EIH response at the non-exercising trapezius muscle after bicycling | EIH responders in session 2 |    |
|   | Yes                         | No |
| EIH responders in session 1   |                             |    |
| Yes   | 8                           | 4  |
| No  | 5                           | 17 |

Responders and non-responders are classified based on the standard error of measurement (SEM) for two repetitive pressure pain threshold (PPT) assessments (before and after 15 min rest). Responders are defined as an increase in PPT after bicycling larger than PPT before bicycling plus 1 SEM

increase in trapezius PPT was not significantly different after exercise and quiet-rest. The EIH response at exercising and non-exercising/less active muscles demonstrated fair-to-good between-session test–retest reliability. In addition, the agreement in EIH responders and non-responders between sessions was significant. Assessment of PPTs showed excellent within-session and between-session test–retest reliability.

### EIH after bicycling

Bicycling at individual-based lactate threshold work-rate increased PPT at the exercising quadriceps muscle, which is in accordance with previous research on local versus remote effects after aerobic exercise (Vaegter et al. 2014, 2018a). However, rather unexpected the bicycling exercise did not have specific exercise-related effects on the non-exercising/less active muscle compared with quiet rest. These findings indicate that hypoalgesia, after the exercise protocol used in this study, is primarily related to activation of local or segmental pain inhibitory mechanisms, and not systemic pain inhibitory mechanisms with widespread antinociceptive effects demonstrated for other aerobic exercise protocols (Ellingson et al. 2014; Vaegter et al. 2014, 2015, 2018a). Hypoalgesia at the exercising muscle could be related to the Gate Control Theory (Melzack and Wall 1965), where limb movement during exercise may excite large-diameter afferent nerve fibers inhibiting nociceptive. Interestingly, in healthy subjects, passive movements induced hypoalgesia compared with a control condition, indicating a potential role of joint movement or proprioception in EIH (Nielsen et al. 2009). However, if this was the main mechanism, aerobic exercise at a low work-rate would also be expected to produce hypoalgesia in the exercising body parts, which is often not the case in healthy subjects (Vaegter et al. 2014).

Lack of increase in PPT at a remote/non-exercising site after moderate work-rate aerobic exercise as used in this study are in agreement with a previous study demonstrating increases in PPT only after vigorous work-rate exercise (Naugle et al. 2014). The lack of a multisegmental hypoalgesic response after exercise in this study may be related to the relatively low pain intensity experienced during the exercise condition which was nearly half compared with pain intensity experienced during bicycling protocols used previously (Vaegter et al. 2018a). Based on a previous EIH dose–response study (Vaegter et al. 2014), the work-rate of the exact lactate threshold was chosen to ensure that the exercise work-rate was high enough to induce hypoalgesia, but not so high that the influence of a higher peak muscle pain due to lactic acid build up would potentially influence the hypoalgesic response due to CPM effects. Previous studies have demonstrated a link between EIH after aerobic exercise and the ‘pain inhibits pain’ mechanisms. Ellingson and colleagues showed that EIH after bicycling was greater following painful exercise than non-painful exercise (Ellingson et al. 2014), and this hypothesis is further supported by studies in chronic pain patients finding an association between reduced EIH and reduced CPM (Fingleton et al. 2017; Vaegter et al. 2016). In contrast to these findings, a recent EIH study showed a larger EIH response in a session when less pain was experienced during bicycling exercise compared to a session where more intense pain was reported during exercise (Vaegter et al. 2018a). Although the reduced muscle pain rating may be associated with the larger EIH response, thus supporting the hypothesis of shared mechanisms, further research on the mechanisms of EIH is warranted. In addition, as the work-rate chosen in this study may have been too low to induce hypoalgesia at the non-exercising/less active trapezius muscle, future studies might want to investigate test–retest reliability of EIH at e.g., 120% of lactate threshold. It should also be noted that several different

incremental cycling protocols that can be modified in terms of starting and subsequent work rates, increments, and duration of each stage have been described for determination of  $[La^-]_b$ , and it has previously been shown that modification of these parameters might influence the physiological variables tested including the lactate threshold (Bentley et al. 2007).

The EIH responses did not differ between women and men, which is in agreement with previous studies showing comparable EIH responses in men and women (Hoffman et al. 2004; Kosek and Lundberg 2003; Vaegter et al. 2015). However, other studies have shown larger effects in women (Koltyn et al. 2001; Sternberg et al. 2001), and limitations regarding the gender effects should be considered. First of all, this study was not powered to detect potential gender differences as it was not the primary aim. Moreover, no data were collected on menstrual cycle or the use of contraceptives which may affect pain perception in women (Riley et al. 1999). However, different phases of the menstrual cycle do not appear to influence the magnitude of the EIH response in women (Hoeger Bement et al. 2009).

### Reliability of PPT and EIH

Although the results showed some systematic bias for quadriceps in session 3 and for trapezius in both sessions, the within-session (before and after rest) and between-session test–retest reliability for PPTs at the quadriceps and trapezius muscles demonstrated excellent ICC values ( $> 0.75$ ) suggesting that PPT is a reliable quantitative method to assess muscle pain sensitivity in humans. This finding is in accordance with previous PPT reliability studies reporting good-to-excellent ICC values (Graven-Nielsen et al. 2015; Vaegter et al. 2016, 2018a). Moreover, the SEM reported for PPTs at the quadriceps and trapezius muscles in this study was smaller than previously shown at the same muscles within a similar retest period of 15 min (Vaegter et al. 2018a), indicating that the inclusion of an initial familiarization session reduces the measurement error, which are in agreement with previous findings (Balgulier et al. 2016). It should be noted that one of the main limitations of this study were the non-randomized order between quiet rest and exercise as that SEM for the PPTs were determined based on the quiet-rest control condition which was performed before the bicycling condition in session 2 and session 3. In case, the within-session variability in PPTs decreases over time due to a training effect of repeated assessments, the SEM could be overestimated. Finally, although not consistently significant, some variation was observed in PPT at the trapezius muscle with increasing PPT after rest. The fact that the increase in PPT after rest was only for the trapezius muscle and not the quadriceps muscle suggests that it was not related to contextual factors like anxiety due to the procedures, or

distraction during assessment. One potential reason could be related to a larger habituation effect at this muscle (compared with the quadriceps muscle) which further supports the inclusion of a control condition when investigating EIH but also the responder/non-responder approach.

The EIH response produced at the exercising quadriceps muscle after the bicycling exercise were comparable between the two sessions, and the between-session test–retest reliability for EIH showed a fair-to-good ICC value. Moreover, the agreement in classification of EIH responders and non-responders with 1xSEM of the PPTs as classifier between sessions was significant, indicating improved reliability of this exercise protocol compared to a previous aerobic exercise protocol (Vaegter et al. 2018a).

Although the increase in PPT at the non-exercising/less active muscle was comparable between bicycling and rest, the reliability of the systemic EIH response was fair-to-good ICC, and the agreement in classification of EIH responders and non-responders between sessions was significant. These findings could indicate that although the aerobic exercise protocol did not induce multisegmental hypoalgesia on a group level, approximately 30% of the included subjects consistently had a systemic EIH response across sessions.

Despite significant agreement in classification between EIH responders and non-responders between sessions, 25% of the subjects ( $n = 9$ ) had discrepancies in their EIH classification between sessions. This could be influenced by training effects (e.g., performing the first test serves as practice for subsequent tests) as more subjects were classified as EIH responders in session 3 than in session 2. Significant differences between session 2 and session 3 in leg-pain intensity and ratings of exertion during exercise may also influence the results. Interestingly, no systematic errors in EIH at the quadriceps and trapezius muscles between sessions were found although RPE and muscle-pain intensity was significantly reduced in session 3 compared with session 2, suggesting that pain during exercise did not play a significant role in the magnitude of the EIH with this exercise protocol. In support of this, no significant associations were found between the EIH responses and the RPE or muscle pain during exercise. Further standardization of these factors should be investigated in future EIH-reliability studies. Moreover, although increases in PPTs are observed rather consistently after exercises, other aspects of the pain experience (e.g., pain tolerance) may be modulated more reliably by exercise, and should be investigated in the future.

### Conclusions

A short bicycling exercise at the individual-based lactate threshold increased PPT at the exercising muscle

compared with a duration-matched control condition. The EIH response showed fair-to-good between-session reliability, and the classification of EIH responders and non-responders between sessions were significant. Strict standardization procedures in relation to the exercise protocol seem to increase the reliability of the EIH response compared to previous studies (Vaegter et al. 2018a), and this should be considered in future EIH studies. The results have implications for future EIH studies in subjects with and without pain and for clinicians who design exercise programs for pain relief. Future research is warranted to investigate the applicability of these findings in a clinical pain-population.

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## Compliance with ethical standards

**Conflict of interest** There are no actual or potential conflicts of interest for any of the authors.

## References

- Balaguier R, Madeleine P, Vuillerme N (2016) Is one trial sufficient to obtain excellent pressure pain threshold reliability in the low back of asymptomatic individuals? A test-retest study. *PLoS One* 11(8):e0160866
- Bentley DJ, Newell J, Bishop D (2007) Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. *Sports Med* 37(7):575–586
- Bonaventura JM, Sharpe K, Knight E, Fuller KL, Tanner RK, Gore CJ (2015) Reliability and accuracy of six hand-held blood lactate analysers. *J Sports Sci Med* 14(1):203–214
- Buono MJ, Yeager JE (1986) Intraerythrocyte and plasma lactate concentrations during exercise in humans. *Eur J Appl Physiol Occup Physiol* 55(3):326–329
- Coyle EF, Martin WH, Ehsani AA et al (1983) Blood lactate threshold in some well-trained ischemic heart disease patients. *J Appl Physiol Respir Environ Exerc Physiol* 54(1):18–23
- Ellingson LD, Koltyn KF, Kim JS, Cook DB (2014) Does exercise induce hypoalgesia through conditioned pain modulation? *Psychophysiology* 51(3):267–76
- Fingleton C, Smart K, Doody C (2017) Exercise-induced hypoalgesia in people with knee osteoarthritis with normal and abnormal conditioned pain modulation. *Clin J Pain* 33(5):395–404
- Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L (2015) Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: A reliability study. *Pain* 156(11):2193–2202
- Heck H, Mader A, Hess G, Mücke S, Müller R, Hollman W (1985) Justification of the 4-mmol/l lactate threshold. *Int J Sports Med* 6:117–130
- Hoeger Bement MK, Rasiarimos RL, DiCapo JM (2009) The role of the menstrual phase in pain perception before and after an isometric fatiguing contraction. *Eur J Appl Physiol* 106(1):105–112
- Hoffman MD, Shepanski MA, Ruble SB, Valic Z, Buckwalter JB, Clifford PS (2004) Intensity and duration threshold for aerobic exercise-induced analgesia to pressure pain. *Arch Phys Med Rehabil* 85(7):1183–1187
- Jacobs RA, Rasmussen P, Siebenmann C et al (2011) Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol* 111(5):1422–1430
- Kindermann W, Simon G, Keul J (1979) The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Appl Physiol Occup Physiol* 42(1):25–34
- Koltyn KF, Trine MR, Stegner AJ, Tobar DA (2001) Effect of isometric exercise on pain perception and blood pressure in men and women. *Med Sci Sports Exerc* 33(2):282–290
- Kosek E, Lundberg L (2003) Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. *Eur J Pain* 7(3):251–258
- Kosek E, Ekholm J, Hansson P (1996) Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. *Pain* 64(3):415–423
- Lannersten L, Kosek E (2010) Dysfunction of endogenous pain inhibition during exercise with painful muscles in patients with shoulder myalgia and fibromyalgia. *Pain* 151(1):77–86
- Melzack R, Wall PD (1965) Pain mechanisms: a new theory. *Science* 150(3699):971–979
- Micalos PS, Marino FE, Kay D (2004) Reduced muscle pain intensity rating during repeated cycling trials. *J Sports Sci Med* 3(2):70–75
- Naugle KM, Naugle KE, Fillingim RB, Samuels B, Riley (2014) JL 3rd. Intensity thresholds for aerobic exercise-induced hypoalgesia. *Med Sci Sports Exerc* 46(4):817–825
- Nielsen MM, Mortensen A, Sorensen JK, Simonsen O, Graven-Nielsen T (2009) Reduction of experimental muscle pain by passive physiological movements. *Man Ther* 14(1):101–109
- Riley JL 3rd, Robinson ME, Wise EA, Price DD (1999) A meta-analytic review of pain perception across the menstrual cycle. *Pain* 81(3):225–235
- Shrout PE, Fleiss JL (1979) Intraclass correlations: uses in assessing rater reliability. *Psychol Bull* 86(2):420–428
- Sjodin B, Jacobs I (1981) Onset of blood lactate accumulation and marathon running performance. *Int J Sports Med* 2(1):23–26
- Stegmann H, Kindermann W, Schnabel A (1981) Lactate kinetics and individual anaerobic threshold. *Int J Sports Med* 2(3):160–165
- Sternberg WF, Bokar C, Kass L, Albojadjian A, Gracely RH (2001) Sex-dependent components of the analgesia produced by athletic competition. *J Pain* 2(1):65–74
- Vaegter HB, Handberg G, Graven-Nielsen T (2014) Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. *Pain* 155(1):158–167
- Vaegter HB, Handberg G, Jorgensen MN, Kinly A, Graven-Nielsen T (2015) Aerobic exercise and cold pressor test induce hypoalgesia in active and inactive men and women. *Pain Med* 16(5):923–933
- Vaegter HB, Handberg G, Graven-Nielsen T (2016) Hypoalgesia after exercise and the cold pressor test is reduced in chronic musculoskeletal pain patients with high pain sensitivity. *Clin J Pain* 32(1):58–69
- Vaegter HB, Handberg G, Emmeluth C, Graven-Nielsen T (2017) Preoperative hypoalgesia after cold pressor test and aerobic exercise is associated with pain relief six months after total knee replacement. *Clin J Pain* 33(6):475–484

- Vaegter HB, Dorge DB, Schmidt KS, Jensen AH, Graven-Nielsen T (2018a) Test–retest reliability of exercise-induced hypoalgesia after aerobic exercise. *Pain Med* (**Epub ahead of print**)
- Vaegter HB, Lyng KD, Yttereng FW, Christensen MH, Sørensen MB, Graven-Nielsen T (2018b). Exercise-induced hypoalgesia after isometric wall squat exercise: a test-retest reliability study. *Pain Med* (**Epub ahead of print**)
- Wasserman K, Whipp BJ, Koysl SN, Beaver WL (1973) Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 35(2):236–243
- Weir JP (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *J Strength Cond Res* 19(1):231–240