Hypoalgesia after bicycling at lactate threshold is reliable between sessions

Vaegter, Henrik Bjarke; Bjerregaard, Louise Kathrine; Redin, Mia-Maja; Rasmussen, Sara Hartung; Graven-Nielsen, Thomas

Published in:
European Journal of Applied Physiology

DOI (link to publication from Publisher):
10.1007/s00421-018-4002-0

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

European Journal of Applied Physiology

HYPOALGESIA AFTER BICYCLING AT LACTATE THRESHOLD IS RELIABLE BETWEEN SESSIONS

--Manuscript Draft--

Manuscript Number:	EJAP-D-18-00433R1
Full Title:	HYPOALGESIA AFTER BICYCLING AT LACTATE THRESHOLD IS RELIABLE BETWEEN SESSIONS
Article Type:	Original Article
Keywords:	Exercise; Exercise-induced hypoalgesia; Reliability; lactate; pressure pain thresholds; pain sensitivity
Corresponding Author:	Henrik Bjarke Vaegter, Ph.D. Pain Research Group, Pain Center, University Hospital Odense, Denmark DENMARK
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Pain Research Group, Pain Center, University Hospital Odense, Denmark
Corresponding Author's Secondary Institution:	
First Author:	Henrik Bjarke Vaegter, Ph.D.
First Author Secondary Information:	
Order of Authors:	Henrik Bjarke Vaegter, Ph.D.
	Louise Kathrine Bjerregaard
	Mia-Maja Redin
	Sara Hartung Rasmussen
	Thomas Graven-Nielsen
Order of Authors Secondary Information:	
Funding Information:	TGN is a part of Center for Neuroplasticity and Pain (CNAP) supported by the Danish National Research Foundation (DNRF121) — Prof Thomas Graven-Nielsen
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 pinprick, > 2 mmol/l increase from warm-up) during a graded bicycling task. In session 2 and 3, all subjects performed i) 15 min quiet-rest, and ii) 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: κ=0.46 and trapezius: κ=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 designs exercise programs for pain relief.
Response to Reviewers:	Reviewer #1: This manuscript outlines experiments aimed at assessing the reliability of the EIH response following exercise at lactate threshold. The manuscript is generally well written, the question is novel and interesting, and the experimental methods are adequate to answer the hypotheses posed by the researchers. RESPONSE: Thank you Please see my comments and questions below for ways to strengthen the manuscript. Major Comments 1. There is a lack of reliability data on the EIH response and this should be highlighted to a greater extent in the introduction. Additionally, the fact that exercise at lactate threshold, rather than a fixed percentage of VO2 peak or heart-rate reserve is novel and potentially accounts for differences in fitness levels among participants. This should also be highlighted to a greater extent. RESPONSE: This has been further highlighted in the 2nd and 3rd paragraph of the introduction in the revised manuscript. How and why was the intensity of lactate threshold chosen (why not slightly above or slightly below)? What are the common perceptual responses at lactate threshold (RPE, muscle pain?) are they actually more similar among participants than a fixed percentage of VO2 peak? RESPONSE: Based on our previous EIH dose-response study (Vaegter et al., Pain 2014), we chose the intensity of the exact lactate threshold to 1) ensure that the exercise intensity was high enough to induce hypoalgesia, and 2) not so high that the influence of a higher peak muscle pain due to lactic acid build up could potentially influence the hypoalgesic response due to CPM effects. In hindsight, the intensity chosen may have been too low to induce hypoalgesia at the non-exercising trapezius muscle, and future studies might want to investigate test-retest reliability of EIH at e.g. 120% of lactate threshold. We have more clearly clarified the choices made about intensity in the 2nd paragraph of section 4.1 in the discussion. To our knowledge ratings of perceived exertion and muscle pain has not been compared between exercise protocols based on lactate threshold and percentage of VO2 peak. 2. The authors report their EIH responses as absolute changes (in kPa) from pre to post exercise. I would recommend calculating a percent change in PPT and reporting EIH in this manner as well. The percent change allows for differences among participants with different resting PPT values to be compared. It also allows for easier comparisons among testing sites where PPTs vary as happens in this study between the quad and trap muscles. If the authors chose to also report the absolute changes and/or absolute values from pre to post I would encourage them to run an ANCOVA on the data and co-vary for resting PPT. RESPONSE: Thank you for this suggestion. In the revised manuscript we also investigated EIH based on relative change in PPTs in addition to the analysis on absolute change in PPT. This has been added to the statistics section 2.6 and the results section 3.3. The effect was larger for the quadriceps muscle than the trapezius, however it did not change the results or interpretation of the findings. 3. There appears to have been a clear "learning" or time effect as it relates to the RPE and muscle pain responses to the cycling exercise with values being lower during the second session. Do the authors think this might have impacted the EIH response and account for some the lower EIH response during the second bout? This should be addressed in the discussion and perhaps mentioned as a limitation. RESPONSE: It is definitely possible, that the learning effect in relation to RPE and muscle pain may play in role on the EIH response. However, as previous studies have demonstrated a link between EIH after aerobic exercise and the 'pain inhibits pain' mechanisms with greater EIH after painful exercise than non-painful exercise we expected that the EIH response would be larger in session 2. Interestingly, no systematic errors in EIH at the quadriceps and trapezius muscles between sessions were found indicating that the reduced RPE and muscle pain in session 3 did not significantly influence the EIH response 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. We have addressed this more thoroughly in the

discussion (4th paragraph section 4.2) in the revised manuscript.

4. There was also an effect, although not always statistically significant, of the rest period. This should be discussed more fully in the discussion. Why did this happen?
RESPONSE: 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. We have addressed this more thoroughly in the discussion (1st paragraph section 4.2) in the revised manuscript.

5. The finding of a lack of EIH in the trap muscles is interesting and unexpected to me. There is clear evidence in the literature of systemic or extra-segmental changes in PPT following exercise. The authors should consider correlating the EIH response at both sites to RPE and pain experienced during cycling. This might provide some insight as to whether a CPM-like mechanism was at work.
RESPONSE: We agree that the lack of hypoalgesia in the trapezius muscle was unexpected. As more thoroughly discussed in the revised manuscript, the intensity chosen in this study may have been too low to induce hypoalgesia at the non-exercising trapezius muscle. However, similar findings after moderate intensity aerobic exercise have been presented by Naugle et al. 2014 (Med Sci Sports Exerc). As included in section 3.4 of the revised results and the correlation table (Table 1), 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. 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). This suggests that the hypoalgesia produced was not significantly related to systemic CPM-like mechanisms in this exercise protocol.

I would also suggest correlating the percent EIH in the quad to the percent EIH in the trap. It would be interesting to know if those with the largest local response also exhibited the largest systemic response (i.e. if they are a responder at one site are they a responder at both).
RESPONSE: Thanks for this suggestion. We included this analysis in the revised manuscript. As illustrated in Table 1 and in the results section 3.4, 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. 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 turned insignificant (Table 1).

Minor Comments
1. pp4, ln 38 - change "on" to one
RESPONSE: Thank you. This has been corrected.

2. Throughout the manuscript the authors confuse performance, intensity, and work rate during exercise. Intensity commonly refers to a metabolic rate, often expressed as a percentage of VO2 peak. Performance commonly refers to time taken to complete a bout of work or a race. Work rate often refers to the measured power output during a bout of exercise. On pp. 12, ln 13 for example the authors state that men had a higher exercise intensity than women. The referenced figure shows work rate in watts rather than intensity in VO2. Please make appropriate changes to wording throughout the manuscript.
RESPONSE: Thanks a lot for bringing this to our attention. Throughout the revised manuscript and in Fig. 3A, intensity has been replaced by work rate.

3. Was testing performed in the same menstrual cycle phase for women?
RESPONSE: Unfortunately data were not collected in this study on the use of contraceptives, status of menopause, or menstrual cycle, which may affect the pain perception in the female participants (Riley et al. 1999). Howwever, different phases of the menstrual cycle do not appear to influence the magnitude of the EIH responsein

women (Hoeger Bement et al. 2009). This has been clarified in the 3rd paragraph of section 4.1 in the revised manuscript.

4. The suggestion that the shoulders are not exercising during cycling is curious to me. They are clearly used for maintaining posture. The authors might want to consider changing how they refer to the shoulders; perhaps as a "less active" muscle compared to the quad.
RESPONSE: Thanks for this suggestion. We agree that muscle activity in the upper trapezius muscle is likely part of the posture maintenance during bicycling with hands on the handlebar. We have changed non-exercising muscle to non-exercising/less active muscle in the revised manuscript.

5. In the discussion (pp. 16, ln 52) the authors refer to the fact that their data indicate that a familiarization session improved their reliability. There are multiple studies that have established this fact--I would recommend the authors citing those studies.
RESPONSE: We agree and have added reference for this in the revised manuscript.

Reviewer #2: Overall, I thought this was a well written and designed study. While many studies have been conducted on EIH, there is limited data on the reliability of EIH. Thus, studies like this are needed in the field of pain and exercise. I have just a few minor comments.
RESPONSE: Thank you

Methods 2.3: Is there a reference for the lactate threshold determination procedure? Is this a common method used to determine lactate threshold?
RESPONSE: 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 in different studies. Bentley et al. 2007 (Sports Med) showed that modification of these parameters might influence the physiological variables tested especially submaximal and maximal variables like peak power output, but also lactate threshold. We have clarified this in the discussion section 4.1.

Methods 2.4: What was the order of the PPT assessments (quadriceps v trapezius)? Was the order counterbalanced among participants?
RESPONSE: The first PPT assessment at the quadriceps muscle was always assessed first followed by the first PPT assessment at the trapezius. The order was then reversed for the second assessment. This has been clarified in the methods section 2.4 in the revised manuscript.

Discussion - EIH after bicycling: I think it would be useful to describe the intensity of the exercise in terms of moderate or vigorous exercise. What intensity of aerobic exercise did the lactate threshold equate to for participants? Prior research shows that moderate intensity aerobic exercise does not decrease PPT's at a remote site, while vigorous aerobic exercise does (Naugle KM et al., 2014). Another study has demonstrated duration thresholds for aerobic EIH (Hoffman MD 2004). Is it possible that the 15 minute duration was not long enough to induce systemic pain inhibitory mechanisms?
RESPONSE: As described in the revised manuscript section 3.2, the mean intensity at the lactate threshold was 151±39 W. Based on subjects' RPE and HR during exercise the exercise equate moderate intensity which likely is the reason why remote EIH was not observed as this intensity might have been too low to elicit systemic EIH. This has been further discussed in section 4.1. Moreover, the study by Naugle et al. 2014 has been included as reference for this discussion. Regarding the exercise duration, previous studies (e.g. Vaegter et al. 2014; Vaegter et al. 2015; Vaegter et al. 2018) have demonstrated a robust EIH response based on pressure pain thresholds after 10-15 min aerobic exercise. However, it is possible that with aerobic exercise at moderate intensity a longer duration might have increased the EIH response as it has been demonstrated with isometric exercise.

HYPOALGESIA AFTER BICYCLING AT LACTATE THRESHOLD IS RELIABLE BETWEEN SESSIONS

Henrik Bjarke Vaegter[1,2], Louise Kathrine Bjerregaard[3], Mia-Maja Redin[3], Sara Hartung Rasmussen[3], Thomas Graven-Nielsen[4]

[1] Pain Research Group, Pain Center, Odense University Hospital, Odense, Denmark

[2] Institute of Clinical Research, Faculty of Health Sciences, University of Southern Denmark

[3] School of Physiotherapy, University College Lillebaelt, Odense, Denmark

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

Original paper for Eur J Appl Physiol

Running title: Reliability of aerobic exercise hypoalgesia

Conflict of Interest and Source of Funding: There are no actual or potential conflicts of interest for any of the authors. No funding was received for this study. TGN is a part of Center for Neuroplasticity and Pain (CNAP) supported by the Danish National Research Foundation (DNRF121).

Corresponding author:

Henrik Bjarke Vaegter Ph.D.
Pain Research Group, Pain Center, University Hospital Odense
Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark
Heden 7-9, Indgang 200
DK – 5000 Odense C
Tel.: +45 65413869; fax: +45 65415064.
E-mail address: henrik.bjarke.vaegter@rsyd.dk
ORCID ID: 0000-0002-7707-9947

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 pinprick, > 2 mmol/l increase from warm-up) during a graded bicycling task. In session 2 and 3, all subjects performed i) 15 min quiet-rest, and ii) 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 designs exercise programs for pain relief.

Key words: 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
$[La\text{-}]_b$	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_{2max}	maximum rate of oxygen consumption

1. 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 & 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 & 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; Vaegter et al. 2018b) 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 one 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 & 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.

2. MATERIALS AND METHODS

2.1 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 suffered 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 ± 3.2 (standard deviation) years old; [range 22–40 years]; average body mass index (BMI) 25.3 ± 4.0 kg/m^2 [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.

2.2 Procedure

The experiment comprised three sessions. In session 1, subjects were i) 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 ii) the lactate threshold were 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.

2.3 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 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) ≤ 9) workload for 23 of the subjects and 'light" (Borg scale rating of perceived exertion (RPE) ≤ 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 seconds of the warm-up and each 3-min workloads. Whole $[La\text{-}]_b$ was measured with a portable $[La\text{-}]_b$ analyzer (Lactate Pro2, Arkray 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 $[La\text{-}]_b$ 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 $[La\text{-}]_b$ value at lactate threshold may vary between subjects (Stegman et al. 1981) an increase in $[La\text{-}]_b > 2$ mmol/l from the concentration after warm-up was used as lactate threshold instead of using a fixed $[La\text{-}]_b$ value. Prior to the lactate threshold test, subjects were instructed not to do any intense or long duration exhausting exercises in the preceding 24 hours, not to change their normal dietary program throughout the study period, and to arrive 10 min ahead of their scheduled test time.

2.4 Pressure pain threshold assessment

PPTs were assessed with a handheld pressure algometer (Somedic Sales AB, Sweden) with a stimulation area of 1 cm^2, and a pressure rate increase at approximately 30 kPa/s at the middle of the exercising dominant quadriceps muscle (fifteen centimeters proximal to the base of patella), and at the non-exercising/less active non-dominant upper trapezius muscle (ten centimeters 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.

2.5 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

(Ergomedic 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 where after the subject continued bicycling at that work-rate for the remaining time. Subjects were instructed to maintain a pedal rate as close to seventy-five 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.

2.6 Statistics

Results are presented as mean and standard deviation (SD) in the text and as mean and standard error of the mean (SEM) in figures. The 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 * 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 corrected 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. Persons r and intraclass correlations (ICCs) based on a single rating, consistency, 2-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 & 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 change 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 1xSEM of the PPTs was classified as EIH responders and subjects who did not have an increase in PPTs after exercise larger than 1xSEM of the PPTs was 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).

3. RESULTS

3.1 Pain thresholds at baseline

In sessions 2 and 3, baseline PPTs was higher in men (quadriceps: 498 ± 120 kPa, 572 ± 162 kPa and trapezius: 297 ± 76 kPa, 310 ± 86 kPa) compared with women (quadriceps: 429 ± 145 kPa, 448 ± 168 kPa and trapezius: 223 ± 77 kPa, 243 ± 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 ($P < 0.001$).

3.2 Lactate threshold

The $[La^-]_b$ was increased after warm-up (1.8 ± 0.7 mmol/l) compared with rest (1.4 ± 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 $[La^-]_b$ when the test was terminated was not significantly different between women (6.3 ± 1.1 mmol/l) and men (6.2 ± 1.2 mmol/l; $P = 0.81$). Mean work-rate at the lactate threshold was 151±39 W. The exercise work-rate determined at the lactate threshold was larger in men (166 ± 43 W) compared with women (135 ± 29 W; $P = 0.02$).

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

3.4 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 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 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 ± 2.0) and session 3 (2.2 ± 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).

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

3.6 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 10 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), $P = 0.012$).

4. 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 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.

4.1 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; Vaegter et al. 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 anti-nociceptive effects demonstrated for other aerobic exercise protocols (Ellingson et al. 2014; Vaegter et al. 2014; Vaegter et al. 2015; Vaegter et al. 2018a). Hypoalgesia at the exercising muscle could be related to the Gate Control

Theory (Melzack & 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 & 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). Howwever, different phases of the menstrual cycle do not appear to influence the magnitude of the EIH response in women (Hoeger Bement et al. 2009).

4.2 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; Vaegter et al. 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 re-test 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 (Balaguier 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 didn't 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.

4.3 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 designs exercise programs for pain relief. Future research is warranted to investigate the applicability of these findings in a clinical pain population.

REFERENCES

1. Balaguier R, Madeleine P, Vuillerme N. Is one trial sufficient to obtain excellent pressure pain threshold reliability in the low back of asymptomatic individuals? A test-retes study. PLoS One 2016;11(8):e0160866.

2. Bentley DJ, Newell J, Bishop D. Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. Sports Med 2007;37(7):575-86.

3. Bonaventura JM, Sharpe K, Knight E, Fuller KL, Tanner RK, Gore CJ. Reliability and accuracy of six hand-held blood lactate analysers. J Sports Sci Med 2015;14(1):203-14.

4. Buono MJ, Yeager JE. Intraerythrocyte and plasma lactate concentrations during exercise in humans. Eur J Appl Physiol Occup Physiol 1986;55(3):326-9.

5. Coyle EF, Martin WH, Ehsani AA, et al. Blood lactate threshold in some well-trained ischemic heart disease patients. J Appl Physiol Respir Environ Exerc Physiol 1983;54(1):18-23.

6. Ellingson LD, Koltyn KF, Kim JS, Cook DB. Does exercise induce hypoalgesia through conditioned pain modulation? Psychophysiology 2014;51(3):267-76.

7. Fingleton C, Smart K, Doody C. Exercise-induced Hypoalgesia in People with Knee Osteoarthritis with Normal and Abnormal Conditioned Pain Modulation. Clin J Pain 2017;33(5):395-404.

8. Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L. Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: A reliability study. Pain 2015;156(11):2193-202.

9. Heck H, Mader A, Hess G, Mücke S, Müller R, Hollman W. Justification of the 4-mmol/l lactate threshold. Int J Sports Med 1985;6:117-130.

10. Hoeger Bement MK, Rasiarmos RL, DiCapo JM. The role of the menstrual phase in pain perception before and after an isometric fatiguing contraction. Eur J Appl Physiol 2009;106(1):105-12.

11. Hoffman MD, Shepanski MA, Ruble SB, Valic Z, Buckwalter JB, Clifford PS. Intensity and duration threshold for aerobic exercise-induced analgesia to pressure pain. Arch Phys Med Rehabil 2004;85(7):1183-7.

12. Jacobs RA, Rasmussen P, Siebenmann C, et al. Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. J Appl Physiol 2011;111(5):1422-30.

13. Kindermann W, Simon G, Keul J. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. Eur J Appl Physiol Occup Physiol 1979;42(1):25-34.

14. Koltyn KF, Trine MR, Stegner AJ, Tobar DA. Effect of isometric exercise on pain perception and blood pressure in men and women. Med Sci Sports Exerc 2001;33(2):282-90.

15. Kosek E, Ekholm J, Hansson P. Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. Pain 1996;64(3):415-23.

16. Kosek E, Lundberg L. Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. Eur J Pain 2003;7(3):251-8.

17. Lannersten L, Kosek E. Dysfunction of endogenous pain inhibition during exercise with painful muscles in patients with shoulder myalgia and fibromyalgia. Pain 2010;151(1):77-86.

18. Melzack R, Wall PD. Pain mechanisms: a new theory. Science 1965;150(3699):971-9.

19. Micalos PS, Marino FE, Kay D. Reduced muscle pain intensity rating during repeated cycling trials. Journal of Sports Science & Medicine 2004;3(2):70-5.

20. Naugle KM, Naugle KE, Fillingim RB, Samuels B, Riley JL 3rd. Intensity thresholds for aerobic exercise-induced hypoalgesia. Med Sci Sports Exerc 2014;46(4):817-25.

21. Nielsen MM, Mortensen A, Sorensen JK, Simonsen O, Graven-Nielsen T. Reduction of experimental muscle pain by passive physiological movements. Man Ther 2009;14(1):101-9.

22. Riley JL, 3rd, Robinson ME, Wise EA, Price DD. A meta-analytic review of pain perception across the menstrual cycle. Pain 1999;81(3):225-35.

23. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull 1979;86(2):420-8.

24. Sjodin B, Jacobs I. Onset of blood lactate accumulation and marathon running performance. Int J Sports Med 1981;2(1):23-6.

25. Stegmann H, Kindermann W, Schnabel A. Lactate kinetics and individual anaerobic threshold. Int J Sports Med 1981;2(3):160-5.

26. Sternberg WF, Bokat C, Kass L, Alboyadjian A, Gracely RH. Sex-dependent components of the analgesia produced by athletic competition. J Pain 2001;2(1):65-74.

27. Vaegter HB, Handberg G, Graven-Nielsen T. Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. Pain 2014;155(1):158-67.

28. Vaegter HB, Handberg G, Jorgensen MN, Kinly A, Graven-Nielsen T. Aerobic exercise and cold pressor test induce hypoalgesia in active and inactive men and women. Pain Med 2015;16(5):923-33.

29. Vaegter HB, Handberg G, Graven-Nielsen T. Hypoalgesia After Exercise and the Cold Pressor Test is Reduced in Chronic Musculoskeletal Pain Patients With High Pain Sensitivity. Clin J Pain 2016;32(1):58-69.

30. Vaegter HB, Handberg G, Emmeluth C, Graven-Nielsen T. Preoperative Hypoalgesia after Cold Pressor Test and Aerobic Exercise is Associated with Pain Relief Six Months after Total Knee Replacement. Clin J Pain 2017;33(6):475-84.

31. Vaegter HB, Dorge DB, Schmidt KS, Jensen AH, Graven-Nielsen T. Test-Retest Reliabilty of Exercise-Induced Hypoalgesia After Aerobic Exercise. Pain Med 2018a. Epub ahead of print.

32. Vaegter HB, Lyng KD, Yttereng FW, Christensen MH, Sørensen MB, Graven-Nielsen. Exercise-induced Hypoalgesia After Isometric Wall Squat Exercise: A Test-Retest Reliability Study. Pain Med 2018b. Epub ahead of print.

33. Wasserman K, Whipp BJ, Koyl SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. J Appl Physiol 1973;35(2):236-43.

34. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J Strength Cond Res 2005;19(1):231-40.

Figure Legends

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.

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.

Fig. 3: Mean (+/- 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 (†, $P < 0.05$).

Figure 1

Click here to download Figure Figure 1.tif

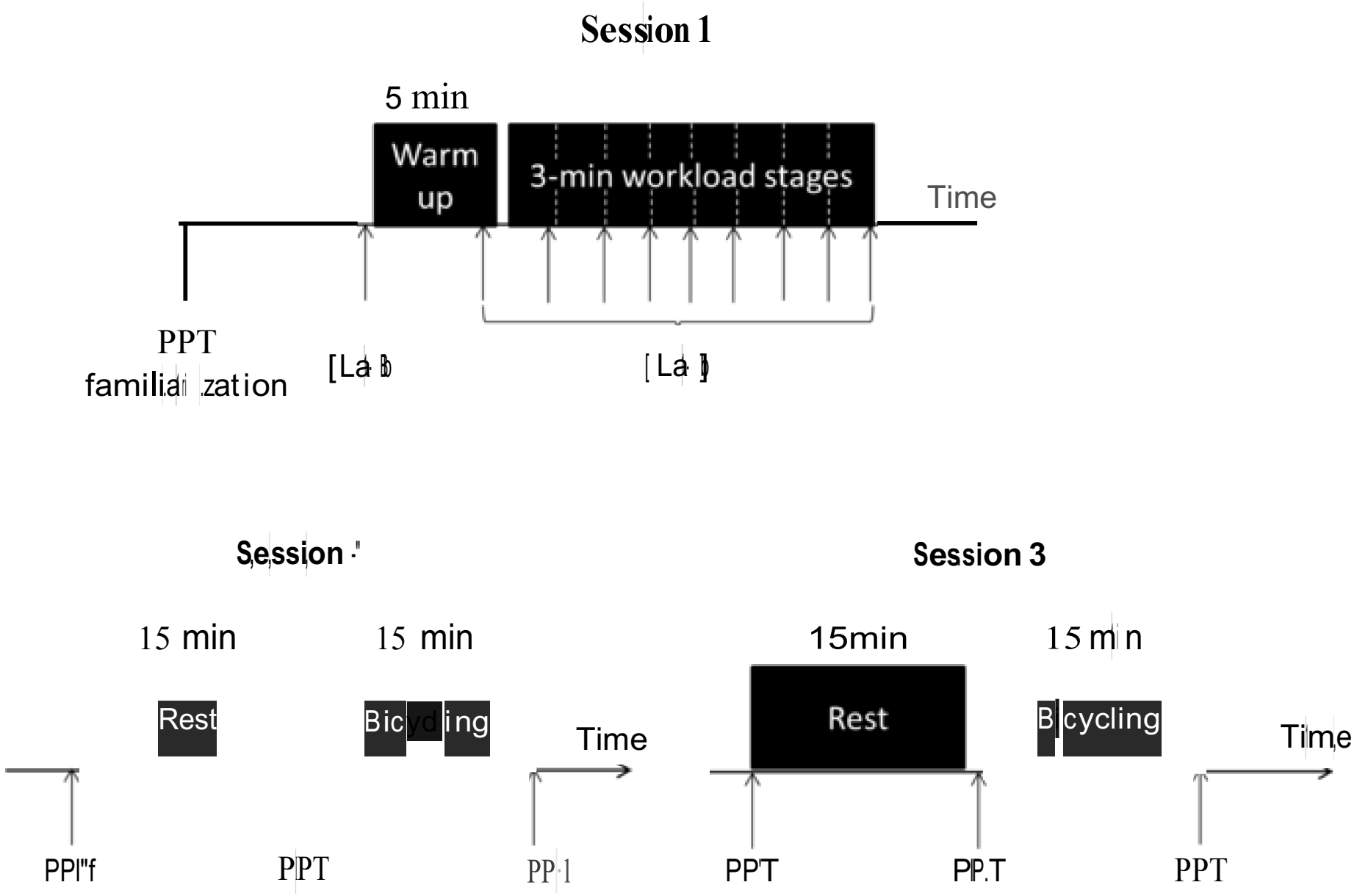

Figure 2

Click here to download Figure Figure 2.tif

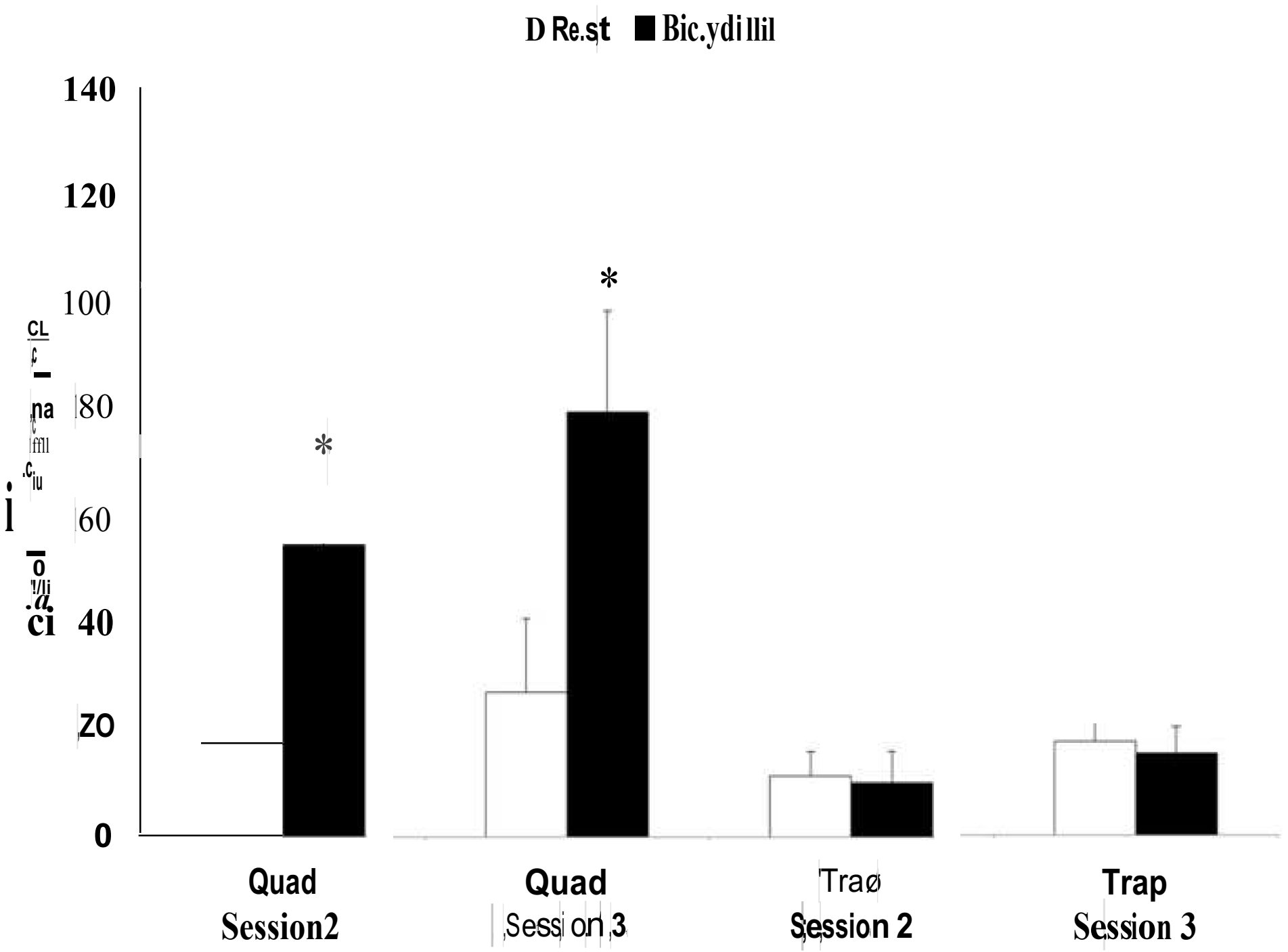

Figure 3A-3D

Click here to download Figure Figur 3A-3D.tif

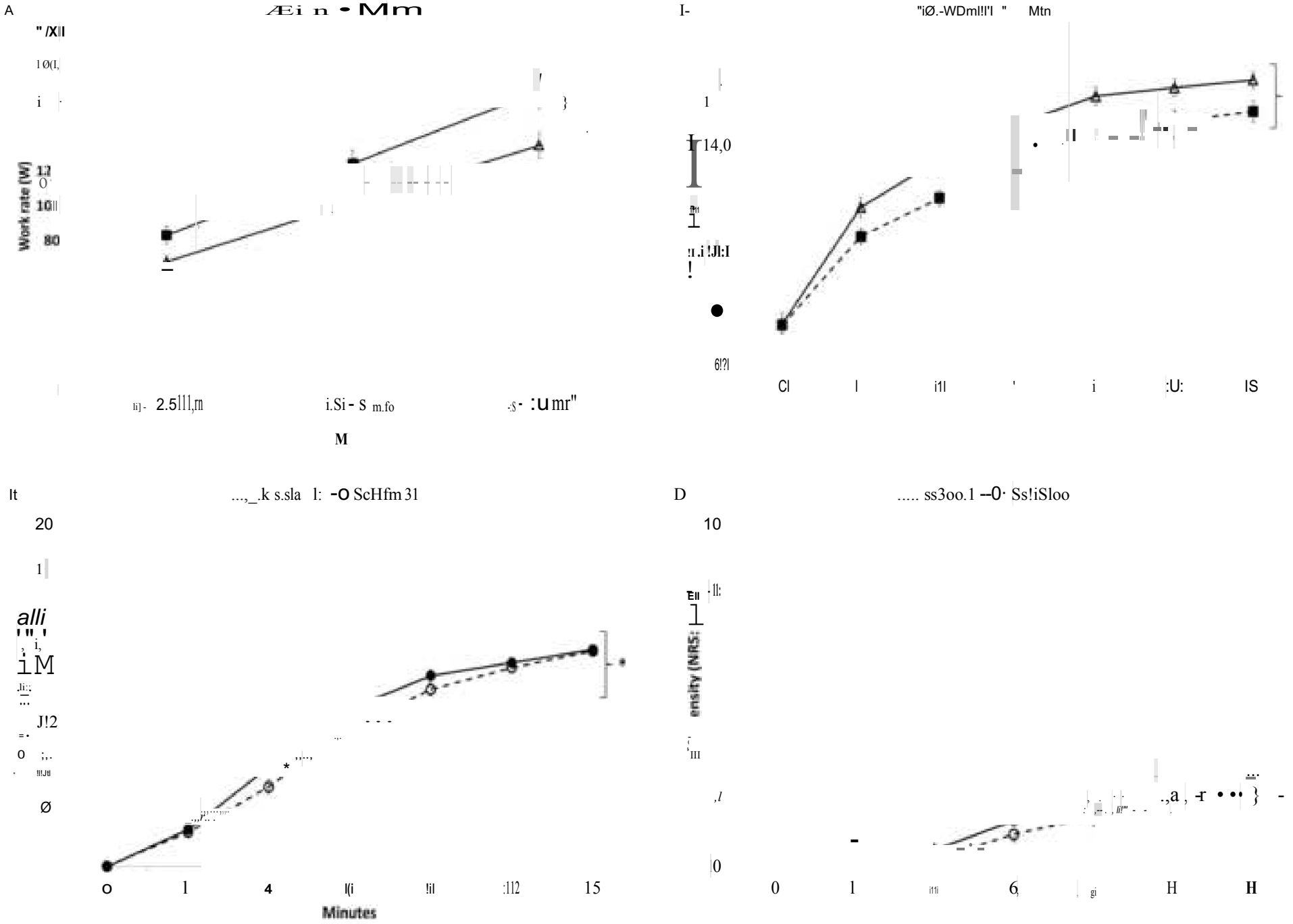

Table 1: Pearson's correlations between absolute and relative change in PPTs after exercise, peak heart rate (HR), peak rating of perceived exertion (RPE), and peak muscle pain intensity (NRS) during exercise in the two sessions. Due to multiple correlational analyses, P-values equal to or less than 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.

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

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

Table 3

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

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

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

Pain Research Group, Pain Center, University Hospital Odense
Department of Anesthesiology and Intensive Care Medicine
Heden 9, Entrance 201
DK - 5000 Odense C
Denmark
Phone: +45 6061 6864 (direct)
henrik.bjarke.vaegter@rsyd.dk

Editors in Chief H. Westerblad & K.R. Westerterp
European Journal of Applied Physiology

Odense, May 24th, 2018

Author contribution statement

Authors: Henrik Bjarke Vaegter, Louise Kathrine Bjerregaard, Mia-Maja Redin, Sara Hartung Rasmussen, and Thomas Graven-Nielsen.

All authors contributed to the design of the study, the analysis and interpretation of the data, as well as making intellectual contributions to its content. Henrik Bjarke Vaegter, Louise Kathrine Bjerregaard, Mia-Maja Redin, and Sara Hartung Rasmussen collected all data in the laboratory. All authors approved the final manuscript.