A NEW EXPERIMENTAL MODEL OF MUSCLE PAIN IN HUMANS BASED ON SHORT-WAVE DIATHERMY

C. A. Mista[a,b,*], S. Laugero[c], J. Adur[a,c], O. K. Andersen[d], J. A. Biurrun Manresa[a,b,d]

[a] Institute for Research and Development on Bioengineering and Bioinformatics (IBB), CONICET-UNER, Oro Verde, Argentina

[b] Laboratory for Rehabilitation Engineering and Neuromuscular and Sensory Research (LIRINS), National University of Entre Ríos, Oro Verde, Argentina

[c] Department of Bioengineering, National University of Entre Ríos, Oro Verde, Argentina

[d] Center for Neuroplasticity and Pain (CNAP), SMI®, Faculty of Medicine, Aalborg University, Aalborg, Denmark

***Corresponding Author:**

José Biurrun Manresa, Ph.D.

Institute for Research and Development on Bioengineering and Bioinformatics (IBB), CONICET-UNER, Oro Verde, Argentina

Tel: +54 0343 4975100

E-mail: jbiurrun@ingenieria.uner.edu.ar

ABSTRACT

Experimental models of pain in humans are crucial for understanding pain mechanisms. The most often used muscle pain models involve the injection of algesic substances, such as hypertonic saline solution or nerve growth factor, or exercise-induced delayed onset muscle soreness (DOMS). However, these models are either invasive or take substantial time to develop, and the elicited level of pain/soreness is difficult to control. To overcome these shortcomings, we propose to elicit muscle pain by a localized application of short-wave diathermy (SWD). In this crossover study, SWD was administered to eighteen healthy volunteers to the wrist extensor muscle group, with a constant stimulation intensity and up to 4 minutes. We measured pressure pain threshold (PPT) and pinprick sensitivity (PPS) and performed a psychophysical evaluation of muscle soreness at baseline and at 0, 30 and 60 minutes. SWD evoked localized muscle pain/soreness in the wrist extensor muscle group and a decrease of PPT in the treated arm compared with the control arm that lasted for at least 60 minutes, reflecting ongoing hyperalgesia after SWD application. PPS was not significantly altered 30 to 60 min following SWD, suggesting a minimal contribution from skin tissue to sustained hyperalgesia.

Trial registration

The study was preregistered in ClinicalTrials.gov (identifier: NCT03573219).

Perspective

This is the first study to show that SWD constitutes a viable alternative as an experimental acute muscle pain model in humans.

Key words (5 maximum): Short-wave diathermy, Experimental pain model, Hyperalgesia, Musculoskeletal pain

INTRODUCTION

Pain-related pathologies are associated with many concurrent physiological and psychophysical processes in patients, resulting in a large number of confounding factors in the evaluation of specific mechanisms behind pain [33]. To address this issue, researchers have developed surrogate experimental pain models that are tested on healthy volunteers, facilitating the assessment of pain effects on the sensory-motor system in a more focused way [4,12]. Furthermore, testing on healthy volunteers implies that pain patients do not have to undergo additional and possibly painful testing, reducing their overall burden. In general, experimental pain models should have the following desirable features: the model should be established in a reasonably short time, the stimuli that elicit pain should be reasonably controlled and the effects of the experiments should be fully reversible, short lasting and homogeneous across volunteers. However, to the best of our knowledge, there are no experimental pain models to date that fulfill all these conditions.

Experimental pain models use different stimulus modalities to elicit pain, including mechanical [29,37,41], chemical [3,42], thermal [23,24] or electrical stimuli [8,20,27], among others. For acute muscle pain, one of the most widely used models involves the administration of intramuscular injection of algesic substances into the muscle itself or its surrounding areas. To this end, the most commonly used substances are hypertonic saline solutions and nerve growth factor (NGF) [1,5,20,25,43]. However, both models require invasive procedures, present an uneven distribution of pain intensity and the time frames for the development of pain present distinct shortcomings in each case. For the hypertonic saline, the duration of pain is confined to a few minutes, and the short duration of pain prevents the assessment of relevant neuromodulation and pharmacological modulation of different interventions. On the other hand, NGF produces a long-lasting sensitization and pain is evoked only during a movement, but it requires 24 to 48 h to show measurable effects. Another commonly used model is delayed-onset muscle soreness (DOMS), which is a non-invasive alternative based on unaccustomed eccentric exercise routine. However, it also has a slow development (24 to 48 h), the resulting level of pain/soreness is hard to control and depends on the subject's training status [32]. Therefore, although these models are widely used, their limitations uphold the development of new alternatives.

In this regard, an unexplored possibility is to elicit muscle pain by a localized application of short-wave diathermy (SWD). High frequency oscillations of non-ionizing electromagnetic fields

77 in the radiofrequency (RF) range can heat deep tissues in a well-localized region [11,39]. In
78 particular, the RF electromagnetic fields can be produced in the range of 3 kHz to 300 GHz, but
79 frequencies around 27 MHz (short-wave) are preferred to heat deep tissues [10,16]. Deep tissue
80 heating by means of RF is a safe and extensively used technique in palliative treatment of pain
81 and as a healing agent in soft tissues [16,54]. However, SWD has not been previously explored with
82 intensities suitable for inducing acute muscle pain.

83 The aim of the present study was to investigate whether SWD can elicit localized muscle
84 pain/soreness on the wrist extensor muscle group. To this end, we performed a quantitative
85 evaluation of the model effects through the assessment of pressure pain thresholds (PPT) and
86 pinprick sensitivity (PPS) over extensor carpi radialis brevis (ECRB) muscles in both the
87 dominant and the non-dominant arm (acting as control). These assessments were conducted
88 before SWD conditioning stimulation and at 0, 30, and 60 minutes after SWD, in order to test for
89 long lasting effects.

90

METHODS

Participants

Nineteen healthy volunteers (7 females, age: 29 ± 5 years, weight: 69.3 ± 13.8 kg, height: 171.1 ± 10.4 cm, mean ± standard deviation) were recruited for the study. One volunteer was excluded after reporting a previous surgery in the non-dominant arm. Volunteers had no history of pain or neuromuscular disorders affecting the upper limb region. All volunteers received written and verbal description of the procedures and gave written inform consent. The study was approved by the Central Bioethics Committee for Biomedical Practice and Research, dependent from the Ministry of Health of Entre Rios (identifier: IS001890). Study preregistration, including original hypothesis, description of primary and secondary outcomes, and initial sample size consideration, was done at ClinicalTrials.gov (identifier: NCT03573219) and the Declaration of Helsinki was respected.

Short-wave diathermy

SWD was administered using a CEC M-8 short-wave thermotherapy unit (CEC Electrónica S.R.L., Argentina) that delivers RF at a frequency of 27.12 MHz. The device has two rectangular capacitive applicators (18 x 12 cm), that were positioned below and above the dominant forearm. Coplanar application was performed using the continuous wave mode. SWD application has two main parameters that can be controlled: application time and stimulation intensity. It is clear that at least one of these parameters must be fixed to reduce the degrees of freedom of the model. During pilot experiments, we tested both possible configurations: fixed application time with variable stimulation intensity, and fixed stimulation intensity with variable application time. The most consistent results in terms of pain/soreness elicited after stimulation were obtained using a fixed stimulation intensity at a constant value (12 out of 20 on the thermotherapy unit scale) and applying SWD for as long as subjects could tolerate the stimulation (i.e. until subjects reached tolerance threshold for thermal pain), at which moment stimulation was immediately interrupted. In this way, we found that the selected intensity was sufficiently low as to develop sustained pain/soreness within a reasonable time frame, and high enough to accumulate heat and prevent dissipation by the blood flow system.

Response profiles during SWD application

A computerized, custom-made Visual Analog Scale (VAS) was used to continuously track the response profile to thermal stimuli across subjects during the application of SWD. The scale range was from 0 to 100, where 0 represents no perception, 30 represents the pain threshold (defined here as the time to reach a painful sensation at the predefined stimulation intensity) and 100 represents the tolerance threshold (defined here as the time at which the pain sensation becomes intolerable). The scale was anchored according to response profiles observed during pilot experiments, in which the early parts of the response profiles were reported as clearly non-painful thermal sensations. Additionally, McGill questionnaires and pain drawing areas were used to describe the sensation when tolerance threshold was reached.

Pressure pain threshold assessment

Pressure pain thresholds (PPT) were assessed using a digital algometer (Somedic SenseLab AB, Sweden), directly over the extensor carpi radialis brevis (ECRB), using a 1 cm^2 round tip. Pressure was gradually increased from 0 kPA at a rate of approximately 30 kPa/s. PPT was defined as the pressure at which the mechanical sensation becomes painful. The assessment was repeated three time for each arm, alternating sides between measurements. The median value of the three assessments was used for further analysis [5,28]. Changes in PPT are indicative of the development of mechanical hyperalgesia in the muscle.

Pinprick assessment

Pinprick stimuli were applied perpendicularly on the skin over the ECRB muscle using a pinprick stimulator, consisting on a needle with a 0.25 mm^2 tip calibrated to a weight of 50 g. The stimulus was repeated three times for each arm, randomizing the order of assessment for each trial. Volunteers scored pinprick sensation on a Numerical Rating Scale (NRS), where 0 represents no perception, 30 represents the pain threshold, and 100 represents the tolerance threshold. Pinprick stimuli were assessed to differentiate deep-tissue from cutaneous hyperalgesia.

Self-reported muscle pain/soreness

A modified self-report Likert scale was used to follow the temporal progression of muscle pain/soreness at 0, 30, and 60 minutes after SWD, with 0 defining a complete absence of soreness and 6 indicating severe soreness (Table 1). This scale was selected based on previous studies reporting the effects of experimental muscle pain models [1,5]. Additionally, self-

assessment was repeated 24 h after the experiment, in order to check for potential longer lasting effects of SWD.

Experimental protocol

Volunteers participated in a single experimental session. They were instructed to sit down comfortably with the arms extended and the palm in prone position. Baseline measures (PPT, PPS, and self-reported muscle pain/soreness) were performed at the beginning of the experiment. SWD was then applied to the wrist extensor muscle group of the dominant arm, due to its incidence and prevalence of dominance in clinical muscle pain [49] (Fig. 1). Initially, low intensity SWD (4 out of 20 on the thermotherapy unit scale) was applied in order to localize the region to be treated without warming unwanted areas (such as wrist flexor muscles), according to verbal reports from the subjects. Afterwards, stimulation was stopped and restarted using the prefixed stimulation intensity (12 out of 20). Subjects were instructed to report the ongoing thermal sensation using the VAS scale during stimulation, until tolerance threshold for thermal pain was reached, stopping immediately after. PPT, PPS, and self-reported muscle pain/soreness were quantified immediately after SWD application, as well as 30 and 60 min later. The assessment was performed only three time points in order to avoid substantial habituation to mechanical stimulation.

Data analysis and statistics

Statistical analysis was performed using R v. 3.5.1 [46]. A two-way repeated measures ANOVA with within-subject factors *time* (0, 30, 60 minutes after SWD) and *arm* (treated and control) was used to evaluate differences in PPT and PPS due to the application of SWD, calculated as percentage of change from baseline. Mauchly's test was carried out to verify the assumption of sphericity, and the Greenhouse-Geisser correction was applied for PPS data. A non-parametric Friedman test was employed to quantify the self-reported muscle pain/soreness after SWD with within-subject factor *time* (baseline and 0, 30, 60 minutes after SWD). Tukey's post hoc tests (in its parametric and non-parametric versions) were carried out when appropriate. Values are reported as mean ± standard deviation or median [interquartile range] depending on whether the underlying data was normally distributed or not. *P* values smaller than 0.05 were regarded as statistically significant.

RESULTS

Reported pain intensity and quality during SWD

The induced muscle pain increased with different profiles for each subject, reaching the tolerance threshold in 1.59 [1.47] minutes (Fig. 2A). The resulting spatial extension of pain matched the treated forearm region (Fig. 2B). At the peak of the induced thermal muscle pain (i.e. at tolerance level), 22% of the participants described the pain as hot, 61% as burning, 11% as scalding, and the remaining 6% as searing.

Self-reported muscle pain/soreness

Subjects reported no muscle pain/soreness at baseline. A main effect of time was found for the self-reported muscle pain/soreness scores ($\chi^2_3 = 19.441$, $P < 0.001$). Post hoc tests showed that scores were significantly higher after SWD compared to baseline for all time points (P values ranging from < 0.001 to 0.043), but they were not significantly different among them (P values ranging from 0.065 to 0.945). None of the subjects reported muscle pain/soreness 24 h after the experiment (Fig. 3).

Pressure pain thresholds

A main effect of *arm* was found for the PPT change scores ($F_{1,17} = 8.897$, $\eta_p^2 = 0.34$, $P = 0.008$). The treated arm showed a significant decrease of PPT values compared to the control arm at all time points ($P = 0.008$; Fig. 4), presenting an average difference of 13% between arms. No significant differences were neither found for *time* ($F_{2,34} = 1.593$, $\eta_p^2 = 0.086$, $P = 0.218$) nor for the interaction ($F_{2,34} = 0.042$, $\eta_p^2 = 0.002$, $P = 0.958$).

Pinprick sensitivity

Data from one subject was excluded from the pinprick sensitivity analysis as an outlier (the reported value was over three times larger than the standard deviation). No significant differences were found for *arm* ($F_{1,17} = 1.069$, $\eta_p^2 = 0.059$, $P = 0.315$) or *time* ($F_{1.14,19.45} = 0.400$, $\eta_p^2 = 0.031$, $P = 0.487$). However, a significant interaction was found ($F_{2,32} = 3.802$, $\eta_p^2 = 0.192$, $P = 0.033$). The post-hoc analysis revealed that the treated arm showed a significant increase of the PPS compared with the control arm only immediately after SWD ($P = 0.019$).

DISCUSSION

In this study, we applied SWD to the wrist extensor muscle group of healthy volunteers in order to induce acute muscle pain. Stimulation was fixed to a constant intensity, and SWD was applied until the tolerance threshold was reached. We then assessed changes in PPT and PPS self-reported muscle pain/soreness up to 60 minutes after SWD application. SWD evoked localized muscle pain/soreness and a decrease of PPT in the treated arm compared with the control arm that lasted for at least 60 minutes, reflecting ongoing hyperalgesia during the period of experimentation. PPS was not significantly altered between 30 to 60 min following SWD, suggesting a minimal contribution from skin tissue to sustained hyperalgesia. To our knowledge, this is the first study to show that SWD constitutes a viable alternative as an experimental acute muscle pain model in humans.

Response profile during SWD application

Volunteers reported a rapid increase in thermal pain during SWD application, reaching the heat tolerance threshold in a couple of minutes in most cases. This behavior is likely associated with responses from polymodal afferent fibers that act as heat-sensitive receptors. The transduction of the thermal stimulus is performed by a subset of channel receptors within the muscle afferents that sense and signal within specific temperature ranges. These receptors, including the temperature-activated transient receptors (TRPV), not only detect temperature in innocuous range but also in the nociceptive range [30]. Once the thermal stimulus is transduced, group IV and, in less proportion, group III afferent fibers are associated with the transmission of the thermal stimulus from the muscle to the central nervous system [13,34]. Although the temperature inside the muscle was not measured during the SWD, it is known that temperatures over 43 °C are very uncomfortable for humans [9]. Therefore, even though it is hypothesized that the temperature inside the muscle was above 43 °C before the stimulation was stopped, application timespans of a few minutes (max. 4 min for this experiment) are not enough to induce permanent thermal damage on the muscle tissue [18,53].

Mechanisms associated with hyperalgesia induced by SWD

Subjects reported muscle soreness and a decrease in PPT values immediately after SWD application compared to the control arm, that lasted for at least 60 min after the intervention, reflecting hyperalgesia in the treated region. It should be noted that five subjects showed a slight

238 increase in PPT after SWD application. In all cases, however, the increase in PPT in the control
239 arm was larger, probably reflecting habituation to mechanical stimulation, so the net difference
240 between arms still showed an overall effect of SWD. Furthermore, four subjects did not report
241 muscle soreness at any time point after SWD, although cross-referencing the data showed that all
242 subjects but one showed a decrease in PPT.

243 The observed effect is most likely due to an inflammatory response of the neuromuscular system
244 triggered by a fast temperature increase in the muscle, coupled with an incapability of the
245 musculoskeletal tissue to dissipate heat at the same speed, resulting in the release of algesic
246 substances and/or local tissue damage. However, further studies assessing inflammatory markers
247 are required in order to confirm an ongoing inflammatory process and to gain deeper
248 understanding of the model effects in the muscle, since most inflammatory pain models are
249 related to skin [19,31,36,48]. In this regard, it is worth mentioning that although hyperalgesia has been
250 observed when inducing thermal pain in skin tissue [31], PPS after SWD only hinted at short-
251 lasting changes after application in the present study, suggesting that the contribution of the skin
252 to ongoing hyperalgesia after a few minutes is minimal.

253 Several mechanisms may be involved in the initiation and prolongation of the inflammation in
254 response to SWD. Among these mechanisms, neurogenic inflammation could account for the
255 rapid development of hyperalgesia. This inflammation process is triggered by the axon reflex,
256 which causes a release of pro-inflammatory neuropeptides from the afferent fibers [7,22]. Different
257 substances are involved in the process, including substance P and calcitonin gene-related peptide
258 that are released both peripherally as well as in the dorsal spinal cord [35]. These substances
259 mediate neurogenic inflammation symptoms by interacting with muscular and connective tissue
260 cells [6]. Another pathway that may be associated with the observed hyperalgesia is the release of
261 pro-inflammatory substances by the musculoskeletal tissue itself [51]. For instance, interleukin-6
262 (IL-6) might be particularly relevant, since it has been previously demonstrated that it is highly
263 upregulated following a significant increase of heat in the muscle [51,52]. This pro-inflammatory
264 cytokine is synthesized in the initial stage of inflammation, and has also been suggested to play a
265 role in the process of pathological pain [45,55].

266 As mentioned before, mechanical hyperalgesia observed after SWD is presumably associated
267 with the described peripheral responses at the beginning of the process. Nevertheless, central

mechanisms cannot be excluded as a contributing modulatory factor. There is strong evidence that acute peripheral inflammation involves specific central mechanisms, for example, through hyperexcitability of dorsal horn nociceptive neurons [2,38,48] and through changes in the release of inflammatory mediators or vasodilation by the sympathetic and parasympathetic system [50]. In rats, subcutaneous inflammation causes an increase of spinal glia activity, constituting a direct evidence of a central change induced by an acute stimulation [44]. In human experimental inflammation of the skin, it has been suggested that central excitability remains increased once triggered even after a single stimulation, and it does not require an ongoing nociceptive input [19]. However, the contribution of central mechanisms to hyperalgesia remains unclear, and further studies are needed to elucidate the processes involved in muscle inflammation after SWD.

SWD as a muscle pain model

The experimental pain model presented in this study has several key features. First, it is based on an exogenous stimulation technique that does not require invasive procedures, unlike intramuscular injection of algesic substances that are invasive and demand additional precautions when used, including correct asepsis, the use of sterile and disposable substances, and experience in the injection technique to avoid damage to nerves or other structures [5,15,40]. In addition, the hyperalgesia observed after the stimulation presented similar profiles compared to those reported using NGF or DOMS, but without requiring a long development time [5,17]. Furthermore, in contrast with muscle exercise induced pain, as DOMS, it is unlikely that SWD causes substantial damage, since this would results in release of algesic substances, including cytokinin and NGF, and would cause hyperalgesia and movement evoked pain for at least 24-48 hours after the intervention [5,26,47]. This does not correspond with the findings presented here, where there was an absence of discomfort or movement evoked pain when assessed after 24 h. In terms of the scientific and ecological validity of the model, it is worth stressing that even though the stimulus used to induce pain is thermal, the duration and nature of the effects observed suggest that the physiological mechanisms responsible for maintaining pain/soreness are of inflammatory origin. Finally, it should be noted that our goal is not to replace or criticize existing muscle pain/soreness models, but to develop an alternative or complementary model that is non-invasive and can be easily applied using inexpensive equipment commonly used for physical therapy, in which the stimulation parameters can be readily controlled. Furthermore, the model has a rapid

development time and a reasonable duration, and was successfully established in almost all volunteers tested, which suggests good reliability.

Limitations and future work

Given the fact that there is no previous investigation about SWD as a pain model, the parameters of the stimulation were set after pilot experiments, and hence further work is required to fully describe the effects of changes in the parameters (e.g. SWD intensity, application time, type of applicator) in the model outcome. As described in the pre-registered protocol, we aimed at a 20% change in PPT compared to baseline, and the observed effect was slightly smaller. Thus, we hypothesize that a larger and more consistent effect can be achieved using different parameters, for example using a higher stimulation intensity or by rekindling. In addition, it might be argued whether PPT or PPS stimulate both skin and muscle; although it has been shown that the pinprick threshold is increased whereas PPT values are not affected when the skin in anesthetized [14]. Furthermore, muscle tissue subjected to a sustained temperature of 42 °C over 30 minutes showed not effect in the force exerted in rats, although it drastically affected the contractile properties of the muscle, reducing tetanic and peak twitch tension [21]. Therefore, studies assessing muscle control and function during movement or a force task may have to take the changes in contractile properties into consideration in their conclusions. Finally, further experiments are required in order to confirm the physiological mechanisms behind the model, in order to better associate it with mechanisms found in physiopathological conditions.

CONCLUSION

This new model based on SWD represents a promising tool for investigating muscle pain/soreness in humans. The main advantages of the model are its non-invasiveness, the ability to control stimulation parameters, and the convenience of the time frame in which pain and hyperalgesia are developed.

CONFLICT OF INTEREST

None declared

ACKNOWLEDGEMENT

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121).

REFERENCES

1. Andersen H, Arendt-Nielsen L, Svensson P, Danneskiold-Samsøe B, Graven-Nielsen T: Spatial and temporal aspects of muscle hyperalgesia induced by nerve growth factor in humans. Exp brain Res 191:371–82, 2008.
2. Andrew D, Greenspan JD: Mechanical and Heat Sensitization of Cutaneous Nociceptors After Peripheral Inflammation in The Rat. J Neurophysiol 82:2649–56, 1999.
3. Babenko V, Graven-Nielsen T, Svensson P, Drewes AM, Jensen TS, Arendt-Nielsen L: Experimental human muscle pain and muscular hyperalgesia induced by combinations of serotonin and bradykinin. Pain 82:1–8, 1999.
4. Le Bars D, Gozariu M, Cadden SW: Animal models of nociception. Pharmacol Rev 53:597–652, 2001.
5. Bergin MJG, Hirata R, Mista C, Christensen SW, Tucker K, Vicenzino B, Hodges P, Graven-Nielsen T: Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain. Pain Med 16:2180–91, 2015.
6. Campos MM, Calixto JB: Neurokinin mediation of edema and inflammation. Neuropeptides 34:314–22, 2000.
7. Chiu IM, von Hehn CA, Woolf CJ: Neurogenic Inflammation – The Peripheral Nervous System's Role in Host Defense and Immunopathology. Nat Neurosci 15:1063–7, 2012.
8. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP: Central hypersensitivity in chronic pain after whiplash injury. Clin J Pain 17:306–15, 2001.
9. Draper DO, Castro JL, Feland B, Schulthies S, Eggett D: Shortwave Diathermy and Prolonged Stretching Increase Hamstring Flexibility More Than Prolonged Stretching Alone. J Orthop Sport Phys Ther 34:13–20, 2004.
10. Draper DO, Knight K, Fujiwara T, Castel JC, Caste C: Temperature Change in Human Muscle During and After Pulsed Short-Wave Diathermy. J Orthop Sports Phys Ther 29:13–22, 1999.
11. Goats GC: Continuous short-wave (radio-frequency) diathermy. Br J Sports Med 23:123–7, 1989.
12. Graven-Nielsen T: Fundamentals of muscle pain, referred pain, and deep tissue

hyperalgesia. Scand J Rheumatol Suppl 122:1–43, 2006.

13. Graven-Nielsen T, Arendt-Nielsen L, Mense S: Thermosensitivity of muscle: High-intensity thermal stimulation of muscle tissue induces muscle pain in humans. J Physiol 540:647–56, 2002.

14. Graven-Nielsen T, Mense S, Arendt-Nielsen L: Painful and non-painful pressure sensations from human skeletal muscle. Exp Brain Res 159:273–83, 2004.

15. Graven-Nielsen T, Sergerdahl M, Svensson P, Arendt-Nielsen L: Methods for induction and assessment of pain in humans with clinical and pharmacological examples. Methods pain Res CRC press Boca Raton, FL; :264–304, 2001.

16. Guo L, Kubat NJ, Nelson TR, Isenberg RA: Meta-Analysis of Clinical Efficacy of Pulsed Radio Frequency Energy Treatment. Ann Surg 255:457–67, 2012.

17. Hedayatpour N, Falla D, Arendt-Nielsen L, Farina D: Sensory and electromyographic mapping during delayed-onset muscle soreness. Med Sci Sports Exerc 40:326–34, 2008.

18. Ichinoseki-Sekine N, Naito H, Saga N, Ogura Y, Shiraishi M, Giombini A, Giovannini V, Katamoto S: Changes in muscle temperature induced by 434 MHz microwave hyperthermia. Br J Sports Med 41:425–9, 2007.

19. Kilo S, Schmelz M, Koltzenburg M, Handwerker HO: Different patterns of hyperalgesia induced by experimental inflammation in human skin. Brain 117 (Pt 2:385–96, 1994.

20. Laursen RJ, Graven-Nielsen T, Jenser TS, Arendt-Nielsen L: Quantification of local and referred pain in humans induced by intramuscular electrical stimulation. Eur J Pain 1:105–13, 1997.

21. Locke M, Celotti C: The effect of heat stress on skeletal muscle contractile properties. Cell Stress Chaperones 19:519–27, 2014.

22. Meggs WJ: Neurogenic inflammation and sensitivity to environmental chemicals. Environ Health Perspect 101:234–8, 1993.

23. Meh D, Denišlič M: Quantitative assessment of thermal and pain sensitivity. J Neurol Sci 127:164–9, 1994.

24. Miron D, Duncan GH, Bushnell MC: Effects of attention on the unpleasantness and intensity of thermal pain. Pain 39:345–352, 1989.

25. Mista CA, Christensen SW, Graven-Nielsen T: Modulation of motor variability related to experimental muscle pain during elbow-flexion contractions. Hum Mov Sci 39:222–35,

2015.

26. Murase S, Terazawa E, Queme F, Ota H, Matsuda T, Hirate K, Kozaki Y, Katanosaka K, Taguchi T, Urai H, Mizumura K: Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayed-onset muscle soreness). J Neurosci 30:3752–61, 2010.

27. Neziri AY, Curatolo M, Nüesch E, Scaramozzino P, Andersen OK, Arendt-Nielsen L, Jüni P: Factor analysis of responses to thermal, electrical, and mechanical painful stimuli supports the importance of multi-modal pain assessment. Pain 152:1146–55, 2011.

28. Neziri AY, Scaramozzino P, Andersen OK, Dickenson AH, Arendt-Nielsen L, Curatolo M: Reference values of mechanical and thermal pain tests in a pain-free population. Eur J Pain 15:376–83, 2011.

29. Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T: Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. J Pain 6:348–55, 2005.

30. Patapoutian A, Peier AM, Story GM, Viswanath V: Thermotrp channels and beyond: Mechanisms of temperature sensation. Nat Rev Neurosci 4:529–39, 2003.

31. Pedersen JL, Kehlet H: Hyperalgesia in a human model of acute inflammatory pain: a methodological study. Pain 74:139–51, 1998.

32. Proske U, Morgan DL: Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol 537:333–45, 2001.

33. Raffaeli W, Arnaudo E: Pain as a disease: an overview. J Pain Res Dove Press; 10:2003–8, 2017.

34. Raja SN, Meyer RA, Campbell JN: Mechanisms of Somatic Pain. Anesthesiology 68:571–90, 2018.

35. Richardson JD, Vasko MR: Cellular mechanisms of neurogenic inflammation. JPharmacolExpTher 302:839–45, 2002.

36. Sandkühler J: Models and mechanisms of hyperalgesia and allodynia. Physiol Rev 89:707–58, 2009.

37. Sarlani E, Greenspan JD: Gender differences in temporal summation of mechanically evoked pain. Pain 97:163–9, 2002.

38. Schaible H-G: Peripheral and central mechanisms of pain generation. Analgesia :3–28, 2007.

39. Shields N, Gormley J, O'Hare N: Short-wave diathermy: current clinical and safety practices. Physiother Res Int 7:191–202, 2002.

40. Sjøogaard G, Lundberg U, Kadefors R: The role of muscle activity and mental load in the development of pain and degenerative processes at the muscle cell level during computer work. Eur J Appl Physiol 83:99–105, 2000.

41. Staud R, Cannon RC, Mauderli AP, Robinson ME, Price DD, Vierck CJ: Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome. Pain 102:87–95, 2003.

42. Svensson P, Cairns BE, Wang K, Hu JW, Graven-Nielsen T, Arendt-Nielsen L, Sessle BJ: Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. Pain 101:221–7, 2003.

43. Svensson P, Wang K, Arendt-Nielsen L, Cairns BE: Effects of NGF-induced muscle sensitization on proprioception and nociception. Exp brain Res 189:1–10, 2008.

44. Sweitzer SM, Colburn RW, Rutkowski M, DeLeo JA: Acute peripheral inflammation induces moderate glial activation and spinal IL-1β expression that correlates with pain behavior in the rat. Brain Res 829:209–21, 1999.

45. Tanaka T, Narazaki M, Kishimoto T: IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol 6:a016295, 2014.

46. Team RC: R: A Language and Environment for Statistical Computing. Vienna, Austria; 2018.

47. Tegeder L, Zimmermann J, Meller ST, Geisslinger G: Release of algesic substances in human experimental muscle pain. Inflamm Res 51:393–402, 2002.

48. Treede RD, Meyer RA, Raja SN, Campbell JN: Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 38:397–421, 1992.

49. Vicenzino B, Wright A: Lateral epicondylalgia I: epidemiology, pathophysiology, aetiology and natural history. Phys Ther Rev 1:23–34, 1996.

50. Waldburger JM, Firestein GS: Regulation of peripheral inflammation by the central nervous system. Curr Rheumatol Rep 12:370–8, 2010.

51. Welc SS, Clanton TL, Dineen SM, Leon LR: Heat stroke activates a stress-induced cytokine response in skeletal muscle. J Appl Physiol 115:1126–37, 2013.

52. Welc SS, Judge AR, Clanton TL: Skeletal muscle interleukin-6 regulation in

hyperthermia. AJP Cell Physiol 305:406–13, 2013.

53. Yarmolenko PS, Moon EJ, Landon C, Manzoor A, Hochman DW, Viglianti BL, Dewhirst MW: Thresholds for thermal damage to normal tissues: An update. Int J Hyperth 27:320–43, 2011.

54. Yu C, Peng R-Y: Biological effects and mechanisms of shortwave radiation: a review. Mil Med Res Military Medical Research; 4:24, 2017.

55. Zhang J-M, An J: Cytokines, Inflammation and Pain. Int Anesthesiol Clin 45:27–37, 2007.

FIGURE LEGENDS

Fig. 1. A) Short wave diathermy (SWD) was applied to the forearm until tolerance threshold was reached, while volunteers reported the response profile of thermal stimuli using an Analog Visual Scale (VAS). B) Experimental procedure. Pressure pain threshold (PPT) and pinprick (PPS) were first measured at baseline. Afterwards, SWD was administered to the dominant forearm. A self-reported muscle pain/soreness assessment of the model effects was carried out, then PPT and PPS assessment was repeated after 0, 30, and 60 min.

Fig. 2. A) Time course of the reported thermal induced muscle pain during SWD (0 represents the beginning of the stimulation), it can be noted that tolerance thermal threshold was reached with different speed across subjects. B) pain chart drawings of the painful area at the tolerance threshold in the treated arm.

Fig. 3. Self-reported muscle soreness scores of the treated arm at baseline, 0, 30, 60 min, and 24 hs.

Fig. 4. Individual (light line) and average (heavy line) PPT from treated and control arm at 0, 30, 60 minutes after intervention. Values are presented as percentage of change from baseline -i.e. before SWD. ** Treated arm showed a significant decrease in PPT compared with control arm for all time points ($P = 0.008$).

Fig. 5. Individual (light line) and average (heavy line) PPS scores from treated and control arm at 0, 30, 60 minutes after application of SWD. Values are presented as percentage of change from baseline -i.e. before SWD. * Treated arm showed a significant increase of the PPS compared with the control arm only immediately after SWD ($P = 0.019$).

491

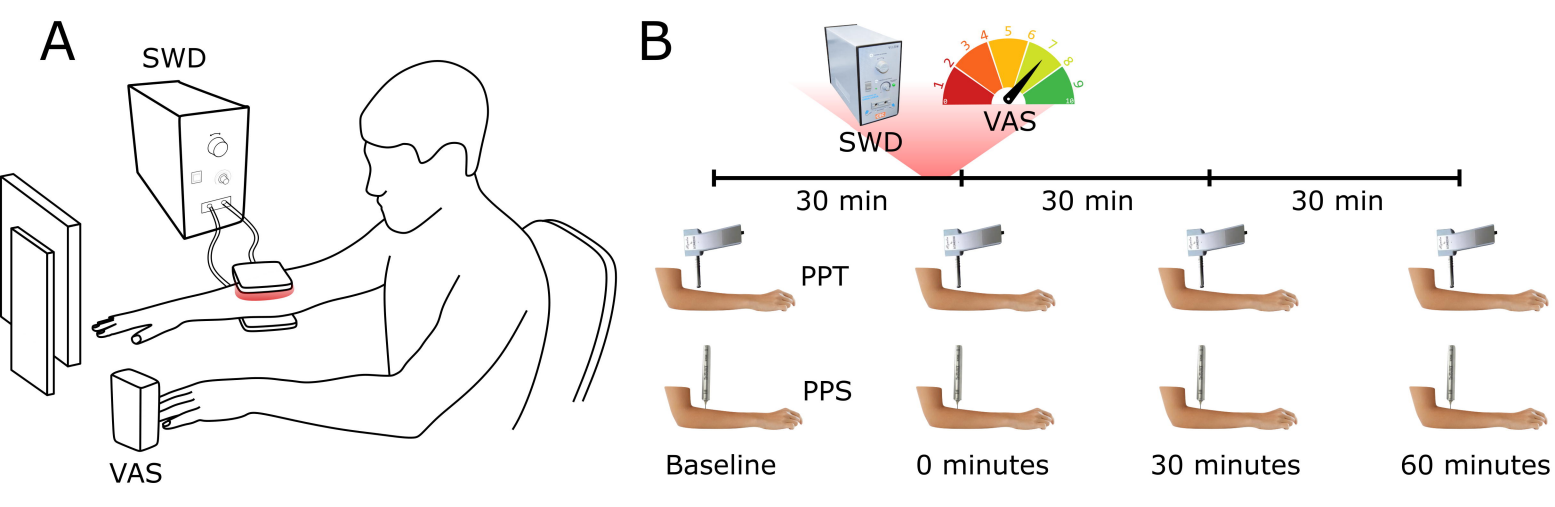
492 **TABLES**

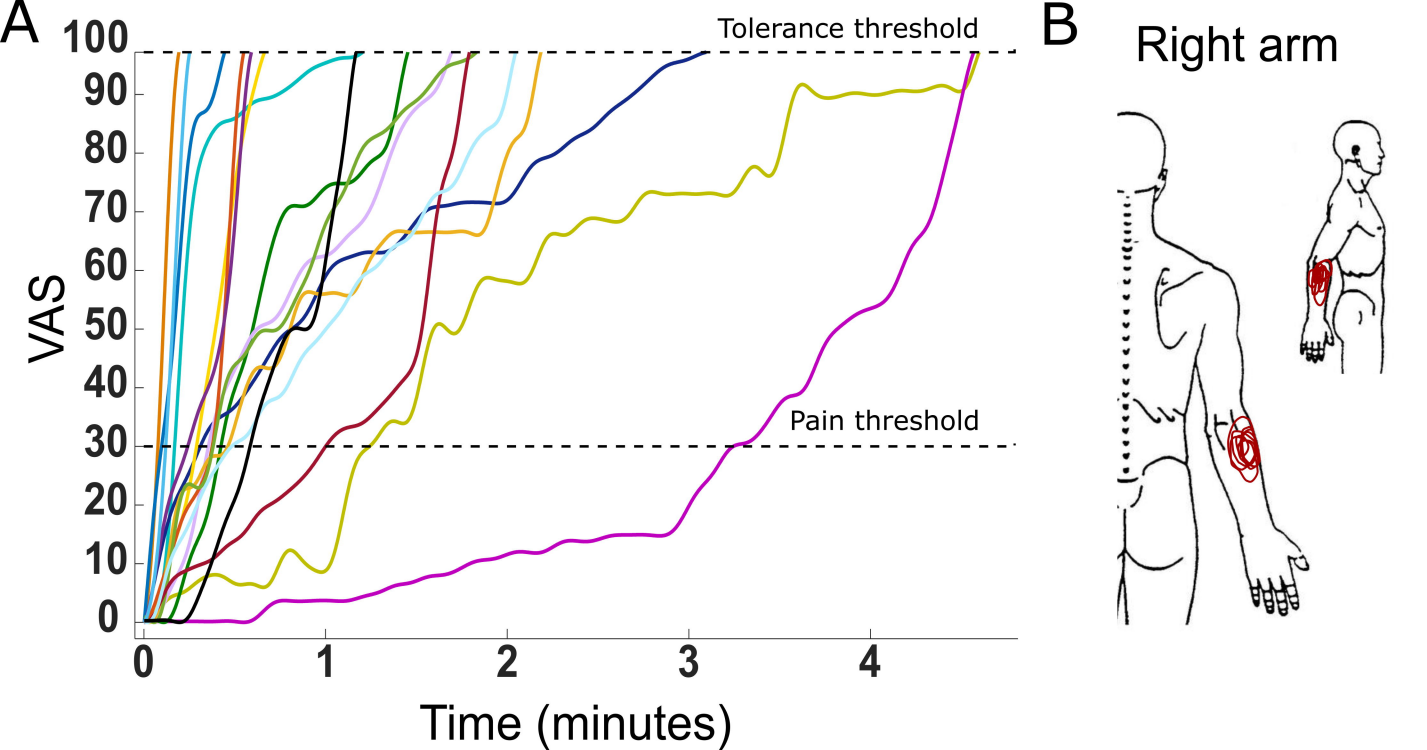
493 **Table 1.** Modified Likert scale of muscle pain/soreness.

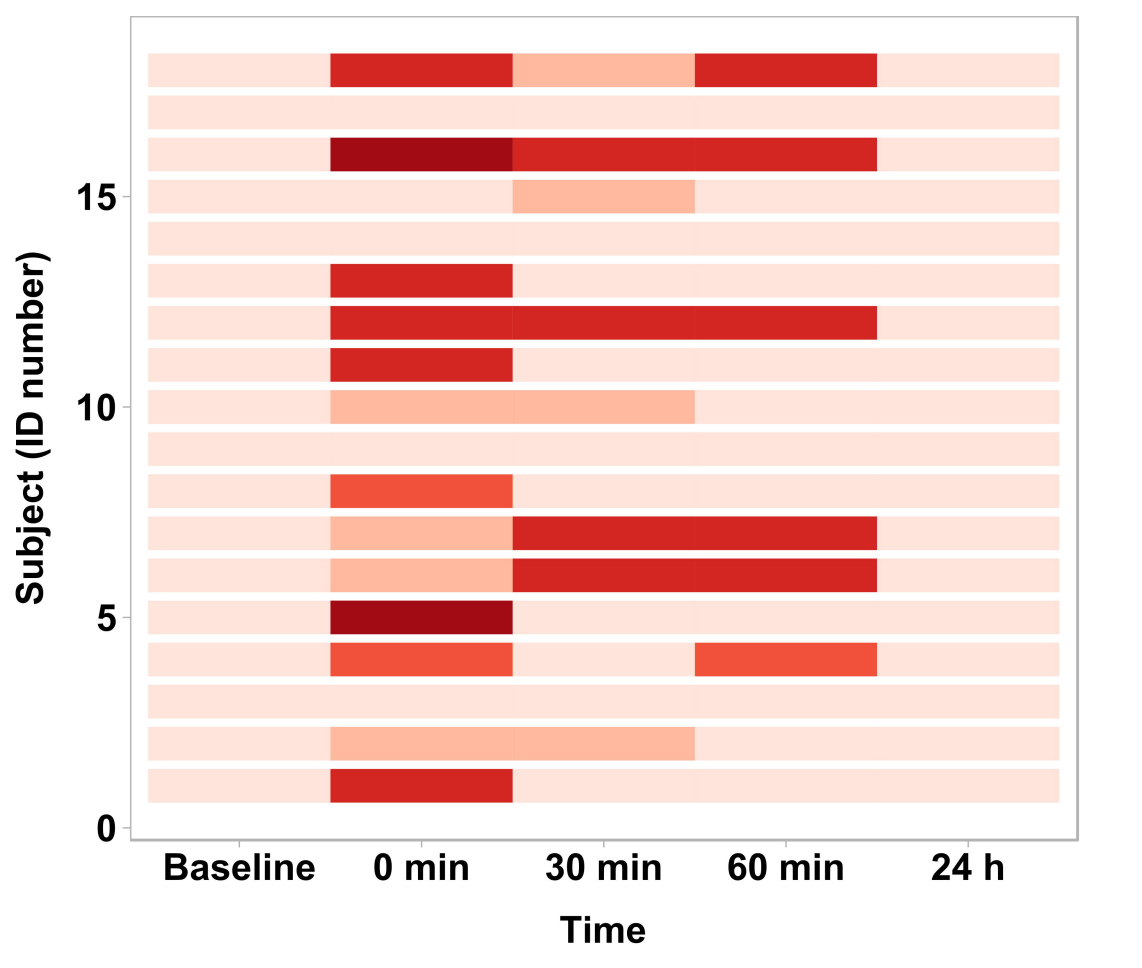
Score	Description
0	a complete absence of soreness
1	a light soreness in the muscle felt only when touched/a vague ache
2	a moderate soreness felt only when touched/a slight persistent ache
3	a light muscle soreness when lifting objects or carrying objects
4	a light muscle soreness, stiffness or weakness when moving the wrist without gripping an object
5	a moderate muscle soreness, stiffness or weakness when moving the wrist
6	a severe muscle soreness, stiffness or weakness that limits my ability to move

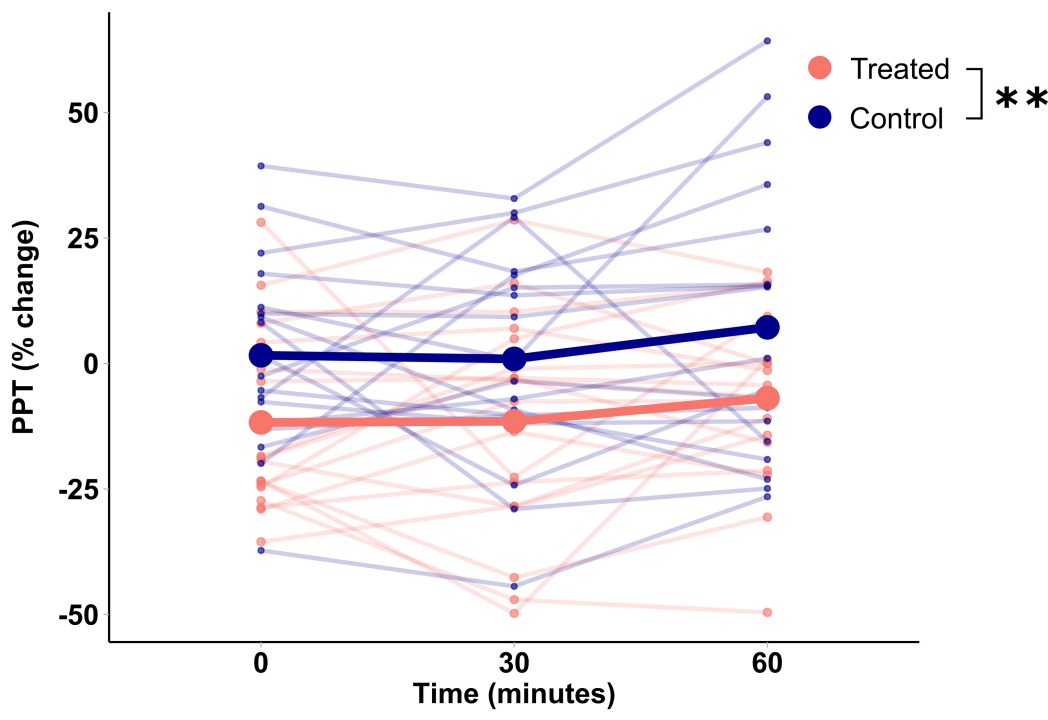
494

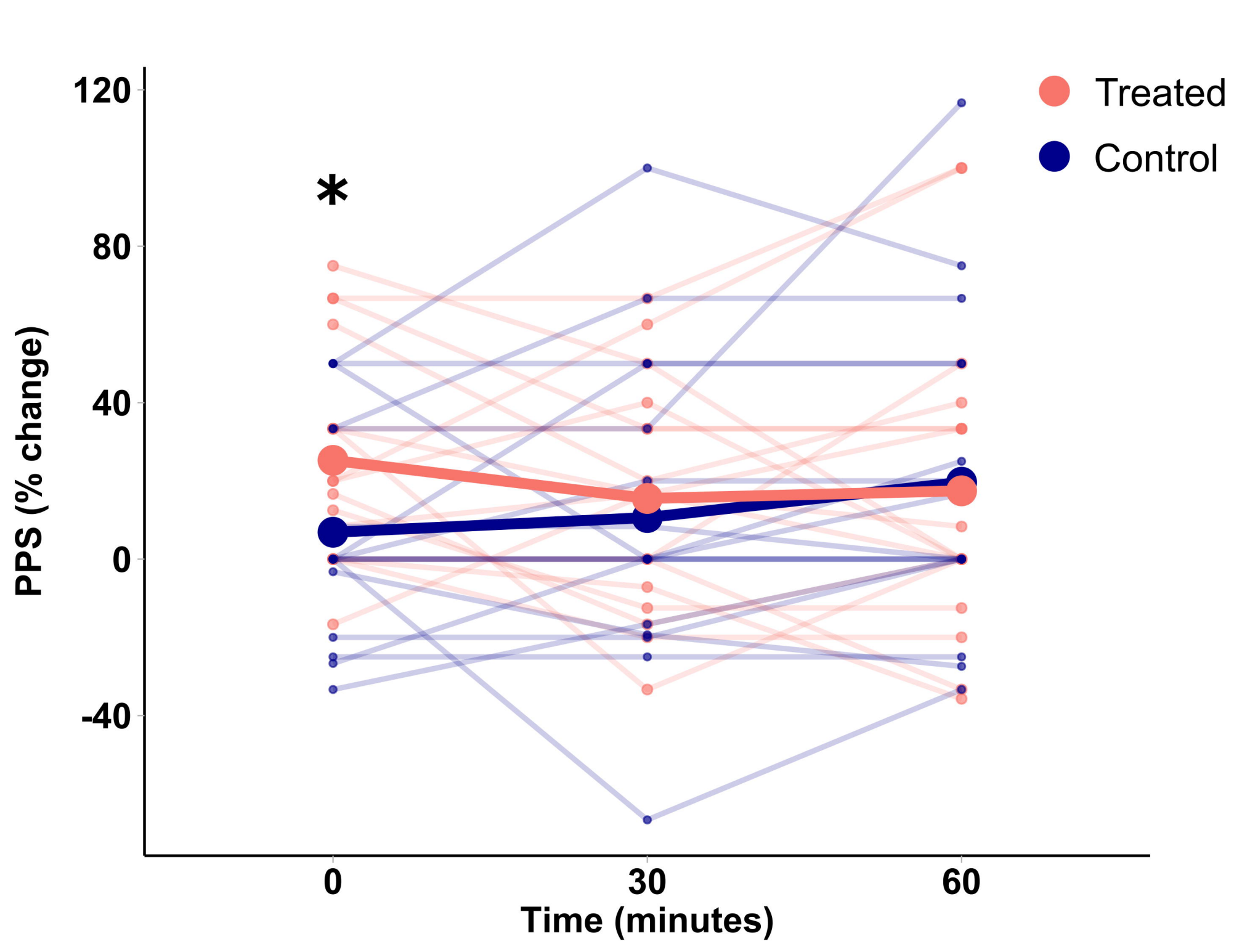
495


A
SWD
VAS
B
SWD
VAS
30 min
30 min
30 min
PPT
PPS
Baseline
0 minutes
30 minutes
60 minutes


A
B
Right arm
Tolerance threshold
Pain threshold
VAS
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
Time (minutes)


Subject (ID number)
15
10
5
0
Baseline
0 min
30 min
60 min
24 h
Time


Treated
Control
**
50
25
0
-25
-50
PPT (% change)
0
30
60
Time (minutes)


120
80
40
0
-40
PPS (% change)
*
Treated
Control
0
30
60
Time (minutes)