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**CUFF ALGOMETRY FOR ESTIMATION OF HYPERALGESIA AND
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CUFF ALGOMETRY FOR ESTIMATION OF HYPERALGESIA AND PAIN SUMMATION

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ABSTRACT

OBJECTIVE: Cuff algometry is useful to assess pain sensitivity mechanisms, but effects of cuff position and stimulation pattern are not clear.

METHODS: In 20 healthy volunteers, cuff pain detection threshold (PDT) and pain tolerance (PTT) were recorded with cuffs accommodating two individual chambers at four locations (eight positions) along the leg, using ramp inflation (1 kPa/s) until subjects indicated PDT and PTT. Repeated stimulations (1-s stimulation, 4-s break) with a staircase increase in stimulus intensity (5 kPa/step) were used to assess PDT and PTT on a single location. Spatial pain summation was calculated as the ratio between PTTs recorded with one chamber or simultaneously with two neighbour chambers. Temporal pain summation was assessed by repeated cuff stimulations (1-s stimulation, 1-s break) and the pain intensity was recorded on a visual analogue scale (VAS); the PTT from ramp and staircase assessments were used as stimulus intensity.

RESULTS: For the most distal cuff position, the PTT was higher compared with other leg positions except when in proximity to the knee ($P<0.01$). The PDT was higher for the distal part compared with the mid-portions of the lower and upper leg ($P<0.01$). Compared with other leg locations, the spatial summation ratio was highest at the proximal lower leg ($P<0.02$). The staircase versus ramp pattern revealed higher PDT and PTT ($P<0.01$), as well as pronounced temporal pain summation ($P<0.01$).

CONCLUSION: The mid-portion of the lower leg is recommended for cuff placement, and the staircase paradigm provides relevant stimulus intensity for assessment of temporal pain summation.

Key words: Cuff algometry, temporal summation of pain, hyperalgesia, cuff positioning, inflation pattern

INTRODUCTION

Hyperalgesia and sensitization of central pain mechanisms are often assessed by quantitative sensory testing in pain conditions (1). A reliable approach for assessing hyperalgesia is handheld pressure algometry which has been used extensively (2-4). Handheld pressure algometry is however criticized for being operator dependent and assessing limited tissue volume (2,5) due to the manual control of pressure increment rate and direction of pressure from a small stimulation probe. An alternative to the hand-held pressure algometry, cuff pressure algometry is user independent and standardized, reducing some of the factors related to outcome variation in hand-held algometry (5,6). A recent study demonstrated good reliability for cuff pressure algometry (7) and it has been used in clinical studies including e.g. fibromyalgia, epicondylalgia, whiplash and osteoarthritis patients (8-12). Common for previous studies are cuff algometry with a cuff mounted on the lower leg, with few exceptions using the thigh and arm. The specific position of the cuff seem to influence cuff pain detection threshold (PDT) and cuff pain tolerance (PTT) (5,11,13) although the difference between cuff positions on the leg has not been systematically studied.

Facilitated spatial and temporal summation of pain have been used in patient studies to assess potential sensitization of central pain mechanisms (11,14,15). Spatial summation of pain is often defined as reduced pain thresholds when assessed in larger, compared with smaller, areas (16,17). Small versus larger heat stimulation probes (18) or one cuff versus two cuffs (11) demonstrates spatial summation of pain. An example of facilitated spatial summation is found in knee osteoarthritis patients, demonstrating more spatial summation compared with controls (14). Temporal summation of pain is the increased pain perception occurring when a painful stimulus at the same intensity is repeated with a frequency above 0.3 Hz (19,20). Previous studies have demonstrated temporal pain summation using heat, pressure, and cuff stimulations in healthy subjects (21,22). Facilitated temporal summation of

pain is e.g. found in whiplash associated disorder patients (23). The stimulation intensity as well as the first stimulation being painful are important for evoking temporal pain summation (24). Common for cuff algometry to assess temporal summation of pain is the use of a tonic ramp pattern (5,9,13,14) to detect PDT and PPT and using these to adjust the stimulation intensity for assessing temporal summation of pain. However, to ensure a painful first stimulation when assessing temporal summation of pain, a better approach may be to determine the pain and tolerance thresholds based on phasic stimuli with the same duration as used for temporal pain summation.

It was hypothesized that 1) the pain sensitivity and spatial pain summation differ between cuff locations, and 2) a novel paradigm for temporal pain summation where the stimulation intensity is based on pain sensitivity assessments with a phasic inflation pattern, would obtain more pronounced temporal pain summation than the conventional temporal pain summation protocol.

METHODS

The aim of this study was to asses cuff pressure algometry using different cuff locations and inflation patterns as well as assessing potential similarities in the pain sensitivity when using the classical hand-held and cuff pressure algometry.

Materials

Twenty healthy subjects (10 females) with a median age of 26 years (range 19 – 48 years) were recruited from a university setting. The exclusion criterion were physical exhausting activities 24 hours prior to the study, consumption of alcohol, caffeine, nicotine or analgesics on the morning of the study, and history of pain affecting the lower limb and/or trunk. Prior to the study all subjects signed a consent form after receiving written and oral information. The

study was approved by the local ethics committee (N-20140002) and followed the Helsinki declaration.

Protocol

In one session, pressure and cuff algometry parameters were assessed on eight different positions of the leg. Moreover, in two **neighbor** chambers, cuff algometry was performed simultaneously with two different inflation paradigms (ramp and staircase) and subsequently assessment of temporal summation of pain. The sequence of procedures and locations were randomized except for temporal summation of pain which always was assessed last. The subjects were placed in a comfortable and relaxed supine position during the experimental procedure. **The entire session had a duration of approximate 90 minutes. (Fig. 1)** All data was collected by the same assessor.

Cuff algometry

The computer-controlled cuff pressure algometer (Nocitech, Denmark and Aalborg University, Denmark) (10) consisted of a double chambered 13-cm wide silicone high-pressure tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany), a computer-controlled compressor, a 10 cm electronic visual analogue scale (VAS), and a stop-button for immediate release of air in the tourniquet cuff. The proximal and distal chamber of the cuff were controlled and inflated independently. The tourniquet was mounted around the dominant leg without cloths between the cuff and skin, ensuring a tight fit around the leg. **In order to determine any differences in pain sensitivity between locations, four** locations (equivalent to eight chamber positions when using a double chamber cuff) were chosen with intend of covering as much of the leg as possible (Fig. **2a**). All four locations were measured to the lowest part of the tourniquet cuff: 1) 10 cm from the lateral malleolus, 2) 1 cm above the top

part of the tourniquet cuff in location 1, 3) 5 cm above the patella, and 4) 1 cm above the top part of the tourniquet cuff in location 3.

A ramp inflation pattern (Fig. 2c) with a constant inflation of 1 kPa/s (13) was used for measurements at all positions. Subjects were asked to continuously rate perceived pain using the electronic VAS where the extremities “0” and “10 cm” were defined as “no pain” and “maximal pain”. Furthermore, the participants pressed the stop-button when the pain became intolerable. Cuff pain detection threshold (PDT) was defined as the pressure value the first time the VAS score exceeded 1 cm, and cuff pain tolerance (PTT) was defined as the pressure value when the subject terminated the pressure inflation. A maximum pressure of 100 kPa (750 mmHg) was set as upper limit throughout the study (23) and if the subjects reached this before the PDT or PTT were reached these parameters were set to 100 kPa in the further analysis.

Simultaneously in position 5 and 6, a staircase inflation pattern was additionally used to detect PDT and PTT: Repeated stimulations (1-s duration) each increasing by 5 kPa were used to inflate the chambers, separated by a four seconds interval. Thus, the increment rate over time was equivalent with the assessment performed with the constant inflation rate of 1 kPa/s. For each stimulus, the inflation rate was as fast as possible to reach the target pressure. The phasic staircase inflation pattern was used as a contrast to the more tonic ramp inflation pattern in order to assess the ability to obtain more pronounced temporal pain, with a painful first stimulation. With the staircase inflation, subjects were instructed to rate the VAS immediately after the stimulation and not adjusting to zero in-between. For analysis the mean VAS score during the 4 second delay between each stimulus was used for extraction of the PDT (i.e. VAS exceeded 1 cm first time) and PTT was the stimulus intensity where the subjects pushed the stop-button when the pain became intolerable.

All assessments were conducted by inflating the cuff chamber for each of the eight positions, i.e. the proximal and distal chamber at each of the four cuff locations. Additionally a simultaneous inflation of both chambers were performed at each of the four cuff locations. All measurement were repeated three times (sequence randomized), with a 60 seconds interval in order to reduce influence of cuff PPT and PDT values due to repeated assessment (25). A mean of the three measurements was used for further analysis.

Spatial and temporal summation of pain

The degree of spatial pain summation was assessed by the ratio between cuff PPTs assessed with both chambers (at one location) divided with the mean PTT of the two single chambers at the same location (14). Temporal summation of pain was measured by simultaneous chamber stimulation at position 5 and 6. A repetitive inflation pattern delivering 10 painful stimuli of one second duration with a one second interval between stimulations was used to evoke temporal summation. Two different stimulation intensities levels were used for the measurements; one using the PTT from the ramp pattern, and one from the staircase pattern.

The use of PTT was chosen to ensure painful stimulations during temporal summation measurements. Subjects were instructed to continuously rate the pain intensity on the VAS during the ten stimulations and not adjust to zero in-between stimuli. A mean VAS score in the pause between each of the 10 stimulations were extracted for analyses. The VAS scores were normalized by subtraction of the VAS score from the first stimulation. The sum of normalized VAS scores was extracted (VAS sum). Furthermore, the mean values of VAS scores from the first four normalized VAS scores (VAS-I) and the last three normalized VAS scores (VAS-III) were extracted; the ratio between VAS-III and VAS-I was extracted.

Hand-held pressure algometry

In order to compare cuff algometry with hand-held pressure algometry extensively used in previous studies, a series of pressure pain thresholds (PPT) were obtained. A hand-held algometer (Somedic, Hörby, Sweden) equipped with a 1 cm² probe covered by a disposable latex sheath and a wired stop-button were used to record PPT. The PPTs were assessed at four locations (Fig. 2b) sited approximately at the middle of the corresponding tourniquet cuff location and on specific muscles: 1) The tibialis anterior muscle approximately 20 cm from the lateral malleolus, 2) the medial gastrocnemius muscle approximately 8 cm from the lower part of the patella, 3) the lateral vastus muscle approximately 9 cm from the upper part of the patella, and 4) the rectus femoris muscle approximately 22 cm from upper part of the patella. The pressure was manually increased with 30 kPa/s until the subjects pressed the button when the pressure went from being a pressure to a pain sensation (13). PPTs were collected in triplets for each location with a 60 s interval between each measurement at the same location. The mean of the three PPT measurements was used for further analysis.

Statistics

Results are presented as mean and standard error of the mean (SEM), unless otherwise specified. All statistical analyses were performed using IBM SPSS Statistics 22. All data passed the Kolmogorov-Smirnov test for normality. Repeated measures analysis of variance (RM-ANOVA) was applied to determine differences between chamber positions (eight positions) and Bonferroni post-hoc tests were applied when relevant. A RM-ANOVA was used to investigate the influence of inflation patterns on normalized VAS scores after repeated stimulation with *stimulation intensity* (PTT-ramp, PTT-staircase) and *stimulation number* (2-10) as factors. Two-way ANOVAs with *location* (four) and *single/double* (average-of-single-cuff, double-cuff) as factors were applied to determine influence of location on spatial

summation. T-tests with Bonferroni corrections were applied to determine differences between the two inflation patterns. Thus, all data was controlled for influence of gender as a between-subjects effect, as well as chamber sequence within one location, and location sequence within- and between-subjects by including these variables as factors in separate additional ANOVA's. P-values less than 0.05 were considered significant.

RESULTS

Cuff pressure pain sensitivity at different locations

During PTT measurement 45% subjects reached maximum pressure, mainly at position 1 and 4, while one subject did not reach 1 cm on the VAS during PDT measurements at position 1. None of the subjects reached maximum pressure using both chambers at position 3, which was used for temporal summation. Males obtained higher cuff algometry values than females using the proximal and distal chamber individually for PDT (ANOVA: $F = 4.19$, $P < 0.048$), using all chamber combinations for PTT (ANOVA: $F = 14.90$, $P < 0.002$), all temporal summation VAS scores for both inflation patterns (RM-ANOVA: $F = 10.04$, $P < 0.005$), and hand-held PPT at location 3 and 4 (ANOVA: $F = 7.11$, $P < 0.016$).

The RM-ANOVA of PDT across positions demonstrated that the PDT recorded at position 1 was significantly higher than at position 3 and 6 (Fig. 3A; RM-ANOVA: $F = 4.65$, $P < 0.01$; Bonferroni: $P < 0.01$).

Additionally, cuff PTT (Fig. 3A) recorded at position 1 was higher compared with positions 2, 3, 6, 7, and 8 (RM-ANOVA: $F = 12.24$, $P < 0.01$; Bonferroni $P < 0.01$). For position 2, PTT was higher than at position 8 (Bonferroni: $P < 0.01$). Finally, PTT at position 4 was higher compared with positions 3, 6, 7, and 8 (Bonferroni: $P < 0.01$).

Spatial pain summation at different locations

Inflating the chambers at two positions simultaneously produced significantly lower pressure values compared with the mean of the same two positions when recorded individually for both PDT (Fig. 3B; Two-way ANOVA: $F = 13.56$, $P < 0.01$) and PTT (Fig. 3B; Two-way ANOVA: $F = 13.63$, $P < 0.01$). Furthermore, when inflating both chambers at position 1 and 2, the PTT was higher compared with the simultaneous inflation of cuff position 7 and 8 (Two-way ANOVA: $F = 3.94$, $P < 0.02$; Bonferroni: $P < 0.02$). The spatial summation ratios of PDT and PTT were higher when assessing positions 3 and 4 compared with assessments of positions 5 & 6, and 7 & 8 (Fig. 3C; Two-way ANOVA: $F = 4.54$, $P < 0.01$; Bonferroni: $P < 0.02$).

Effects of staircase and ramp inflation pattern

Using the staircase compared with the ramp inflation pattern, the PDT and PTT were higher when stimulating with the chamber at positions 5 and 6 individually, and when both chambers at position 5 and 6 was inflated simultaneously (Table 1; T-test: $F = 14.9$, $P < 0.01$). Compared with the ramp pattern, the lowest degree of spatial summation was observed when using the staircase pattern (Table 1; T-test, $F > 7.52$, $P < 0.09$).

Temporal summation of pain

Normalized VAS scores from repeated stimulation demonstrated a significant interaction between the stimulation intensity (ramp or staircase PTT) and stimulation number (RM-ANOVA: $F = 5.94$, $P < 0.02$). Using PTT based on the ramp pattern resulted in a VAS score after stimulation 3 which was lower than stimulation 4 to 10 (Bonferroni: $P < 0.03$). Likewise, VAS scores after stimulation 4 and 6, respectively, was lower than stimulation 8 to 10 (Bonferroni $P < 0.03$). All staircase stimulations resulted in normalized VAS scores

significantly lower than all the following VAS scores, the only exception being stimulation 6 and stimulation 7 (Bonferroni $P < 0.03$).

The temporal summation induced with the PTT level obtained from the staircase pattern demonstrated higher VAS sum (25.42 ± 2.6 cm) compared with temporal summation induced with the PTT estimated from the ramp pattern (20.53 ± 2.8 cm; T-test $P < 0.002$). A significant increase in VAS scores between each pairwise stimulus was found for staircase pattern induced temporal summation for all except between stimulation 6 and 7 (Fig. 4; Bonferroni: $P < 0.03$). Using the ramp pattern, the only significant pairwise difference in VAS scores was between stimulation 2 and 3 (Fig. 4 Bonferroni: $P < 0.02$). The ratio between the last and first normalized VAS scores was 1.40 ± 0.19 for ramp pattern with a tendency to be higher (1.93 ± 0.19 , T-test: $P < 0.01$) for the staircase pattern.

Pressure algometry at different locations

PPTs assessed by hand held pressure algometry were 568 ± 50 kPa, 418 ± 36 kPa, 437 ± 29 kPa, and 478 ± 27 kPa for location 1 to 4, respectively. The PPT at location 1 was higher than location 2 (Two-way ANOVA: $F = 3.32$, $P < 0.024$; Bonferroni $P < 0.03$). The hand held algometer PPTs were not significantly correlated with cuff measurements (PDT, PTT).

DISCUSSION

This study demonstrated significant differences in the pain sensitivity between different assessment positions at the leg when evaluated by cuff algometry. The novel assessment paradigm of the pain tolerance level based on a staircase pattern resulted in higher stimulation levels to be used for assessment of temporal summation of pain, and resulted in pronounced temporal summation of pain compared with the traditional approach.

Effects of cuff location

The significantly higher cuff PDT and PTT using the chamber at position 1 could be explained by the underlying structures here having less soft tissue compared with other locations on the leg, and therefore are able to obtain higher values. The same pattern can be seen for cuff PTT at position 4, which may be due to the chambers proximity to the knee with less soft tissue. This explanations falls in line with a previous study indicating that the cuff pressure algometry assesses muscle and deep tissue sensitivity (10).

The positions of the cuff were determined by measuring from prominent structures and the cuff itself, however due to different leg length between subjects, one or more positions overlapped for eight of the subjects, and it can be speculated whether these overlapping positions have influenced the cuff PPT and PDT values due to repeated assessment of the same tissue without adequate pauses in-between. Furthermore several cuff assessment performed on each subject present a limitation, as they may have evoked pain sensitization. Likewise the different positions assessed may have evoked pain inhibition. Nonetheless, the randomized position and assessment sequence along with the 60 second interval in-between assessments was included in the experimental design to reduce these limitations (19,24).

The individual anatomy of the subjects legs may have been influenced the present data as pressure pain is affected by muscle hardness as well as the thickness of adipose tissue (26). Body dimensions may therefore be of interest for future studies assessing variations in different locations.

The cuff PDT was defined as when the VAS score was equal to or greater than 1 cm, this definition however made the PDT sensitive to any subject manipulating the VAS slider but not reaching 1 cm, as was the case with one of the subjects. For 9 of the 20 subjects, one or more cuff PTT measurements reached the maximum pressure of 100 kPa during their session; as such these values were therefore at least 100 kPa, but may in reality be higher. The

measurements reaching the maximum pressure was however set as 100 kPa for the data analysis. Although all positions were able to obtain valid cuff PDT and PTT, several subjects reached maximum pressure, the majority at position 1 and 4, a correct value would have resulted in a more pronounced difference between the positions. It is worth to note that this study was conducted on healthy subjects, while previous studies have demonstrated a reduced cuff PTT for patients with e.g. rheumatoid arthritis, fibromyalgia, chronic whiplash associated disorder, and ongoing pain following a total knee replacement (5,13,15,23). Patient groups may not reach maximum pressure as the subjects in this study, but choosing a location where it is less likely to end at maximum pressure would be desirable. The ability to obtain cuff PDT and PTT at all sites is however interesting, as these values will still be obtainable in cases where using a specific part of the leg is unavailable. The present results demonstrate variations in cuff PDT and PTT, depending on the position of the tourniquet cuff. Variable pain perception in different regions of the body is well established for a number of modalities, and with this study also cuff algometry of the leg (16,27,28).

Hand held algometry and cuff pressure algometry are two different methods for assessing pressure pain, as they express pain sensitivity for a small compact and large volume, respectively. Cuff pressure algometry is able to efficiently stimulate deep tissue in contrast to the hand held algometry's single point which affects more superficial structures. Previous studies have demonstrated that larger rounded probes more efficiently induce muscle pain than small flat probes, but the single point pressure algometry used in this study still stands in contrast to cuff pressure algometry (29,30). One study have however found a correlation between cuff pressure algometry and computer controlled pressure algometer using a 1 cm padded probe (25). This study did not find any correlation between the two methods for assessing pressure pain probably because the handheld pressure algometry only assess a very limited tissue volume, or due to inconsistency in pressure; future studies with several hand-

held algometer measurement sites in the area of cuff assessments may however clarify if correlation between hand-held algometer and cuff pressure algometry measurements exists.

Spatial summation of pain

All positions demonstrated spatial summation, but a lesser degree was observed at location 2 when compared to summation at location 3 and 4. This difference from location 2 may be due to the significant difference between sensitivity parameters when assessing position 3 and 4. The same was found when comparing position 1 and 2, although no significant difference was found between spatial summation assessed at location 1 and location 3 & 4. This lesser degree of spatial summation for location 2 could correspond to the previous mentioned issue of the underlying tissue, as position 4 is in proximity of the knee. It is however important no notice that none of the used locations for cuff placement correspond to previously used positions e.g. the widest part of the lower leg, as this location would correspond to position 2 and 3 which was not measured in this study (11,15,25). Location 3 and 4 could also be valid for assessing spatial summation, but especially for position 4 the ease of placement. Although no patient related outcomes were systematically collected, several subjects expressed discomfort with the placement on location 3 and 4, which also should be taken into account. These results demonstrate the importance of consistency in cuff placement, as well as taking the anatomy of the leg into account when designing a study. Despite not being measured in the present study, the previously used position located on the widest part of the lower leg is therefore recommended for future studies. This position provides a high amount of soft tissue, is not dependent on measuring from prominent structures, is easy to place and do not cause patient discomfort as the positions on the thigh.

Temporal summation of pain

Both ramp and staircase pattern determined PTT stimulation intensity demonstrated temporal summation of pain. The staircase stimulation pattern determined PTT delivered a more apparent temporal summation, with significant differences between stimulations in contrast to the ramp pattern. The difference between the ramp and staircase inflation pattern determined PTTs, may be found in different nature of the two stimulation patterns. The lower ramp pattern PDT and PTT values in this study could therefore be explained by temporal summation of pain due to the prolonged stimulation in contrast to the staircase pattern (31,32). Although previous studies have induced temporal summation using the tonic ramp stimulation pattern, the phasic nature of the staircase pattern may provide PDT and PTT values uninfluenced by temporal summation and match the stimulus duration used for repeated stimulations. The 5 kPa increment of the staircase pattern should also be considered as it is less nuanced in contrast to the ramp patterns 1 kPa/s increment. Compared with previous studies, this study obtained a higher degree of temporal summation in contrast to temporal pain summation induced by cuff pressure algometry and computer-controlled pressure algometry with a 1 cm² padded probe (13,33). The present study differed however from previous studies by using both chambers of the cuff (double chamber stimulation) at location 3, in contrast to the widest part of the lower leg used in previous studies (corresponding to position 2 and 3 in the present study) (11,15,25). Furthermore, a mean of PTT from both chambers were used in contrast to previously used mean of cuff PDT and cuff PTT from a single chamber (13,23). For this study the mean of both cuff PDT and cuff PTT was higher than the mean of PTT from both chambers, but still managed to induce temporal summation. With the significantly higher PDT and PTT thresholds and the more apparent intense temporal summation of pain, the staircase pattern paradigm is therefore recommended when used to assess temporal summation.

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Conclusion

This study illustrates the importance of cuff positioning for assessing cuff PDT, PTT, and spatial summation of pain, as well as the assessment paradigm for assessing temporal pain summation. For future studies it is recommended to use the widest part of the lower leg for cuff placement, and the novel staircase pattern assessment paradigm of the pain tolerance level subsequently to be used for assessment of temporal pain summation.

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Conflict of interest statement

Nocitech is partly owned by Aalborg University.

References

1. Pavlaković G, Petzke F. The Role of Quantitative Sensory Testing in the Evaluation of Musculoskeletal Pain Conditions. *Current Rheumatology Reports*. 2010;12(6):455-61.
2. Kinser AM, Sands WA, Stone MH. Reliability and validity of a pressure algometer. *J Strength Cond Res*. 2009;23(1):312-4.
3. Rolke R, Andrews Campbell K, Magerl W, Treede RD. Deep pain thresholds in the distal limbs of healthy human subjects. *Eur J Pain*. 2005;9(1):39-48.
4. Frank L, McLaughlin P, Vaughan B. The repeatability of pressure algometry in asymptomatic individuals over consecutive days. *Int J Osteopath Med*. 2013;16(3):143-52.
5. Jespersen A, Dreyer L, Kendall S, Graven-Nielsen T, Arendt-Nielsen L, Bliddal H, et al. Computerized cuff pressure algometry: A new method to assess deep-tissue hypersensitivity in fibromyalgia. *Pain*. 2007;131(1-2):57-62.
6. Bartholdy C, Zangger G, Hansen L, Ginnerup-Nielsen E, Bliddal H, Henriksen M. Local and Systemic Changes in Pain Sensitivity After 4 Weeks of Calf Muscle Stretching in a Nonpainful Population: A Randomized Trial. *Pain Pract*. 2015.
7. 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.
8. Izumi M, Petersen KK, Arendt-Nielsen L, Graven-Nielsen T. Pain referral and regional deep tissue hyperalgesia in experimental human hip pain models. (1872-6623 (Electronic)).
9. Amris K, Jespersen A, Bliddal H. Self-reported somatosensory symptoms of neuropathic pain in fibromyalgia and chronic widespread pain correlate with tender point count and pressure-pain thresholds. *Pain*. 2010;151(3):664-9.

10. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry. *The Journal of Pain*. 2002;3(1):28-37.

11. Jespersen A, Amris K, Graven-Nielsen T, Arendt-Nielsen L, Bartels EM, Torp-Pedersen S, et al. Assessment of Pressure-Pain Thresholds and Central Sensitization of Pain in Lateral Epicondylalgia. *Pain Med*. 2013;14(2):297-304.

12. Soriano-Maldonado A, Klokke L, Bartholdy C, Bandak E, Ellegaard K, Bliddal H, et al. Intra-Articular Corticosteroids in Addition to Exercise for Reducing Pain Sensitivity in Knee Osteoarthritis: Exploratory Outcome from a Randomized Controlled Trial. *PLoS One*. 2016;11(2):e0149168.

13. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. *Pain*. 2013;154(9):1588-94.

14. Graven-Nielsen T, Wodehouse T, Langford RM, Arendt-Nielsen L, Kidd BL. Normalization of widespread hyperesthesia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. *Arthritis Rheum*. 2012;64(9):2907-16.

15. Vladimirova N, Jespersen A, Bartels EM, Christensen AW, Bliddal H, Danneskiold-Sams, et al. Pain Sensitisation in Women with Active Rheumatoid Arthritis: A Comparative Cross-Sectional Study. *Arthritis*. 2015;2015:5.

16. Defrin R, Ronat A, Ravid A, Peretz C. Spatial summation of pressure pain: effect of body region. *Pain*. 2003;106(3):471-80.

17. Greenspan JD, Thomadaki M, McGillis SL. Spatial summation of perceived pressure, sharpness and mechanically evoked cutaneous pain. *Somatosens Mot Res*. 1997;14(2):107-12.

18. Staud R, Vierck CJ, Robinson ME, Price DD. Spatial summation of heat pain within and across dermatomes in fibromyalgia patients and pain-free subjects. *Pain*. 2004;111(3):342-50.
19. Anderson RJ, Craggs JG, Bialosky JE, Bishop MD, George SZ, Staud R, et al. Temporal summation of second pain: Variability in responses to a fixed protocol. *European Journal of Pain*. 2013;17(1):67-74.
20. Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain*. 2009;10(9):895-926.
21. Suzan E, Aviram J, Treister R, Eisenberg E, Pud D. Individually based measurement of temporal summation evoked by a noxious tonic heat paradigm. *J Pain Res*. 2015;8:409-15.
22. Christensen AW, Rifbjerg-Madsen S, Christensen R, Amris K, Taylor PC, Loch H, et al. Temporal summation of pain and ultrasound Doppler activity as predictors of treatment response in patients with rheumatoid arthritis: protocol for the Frederiksberg hospitals Rheumatoid Arthritis, pain assessment and Medical Evaluation (FRAME-cohort) study. *BMJ Open*. 2014;4(1):e004313.
23. Lemming D, Graven-Nielsen T, Sorensen J, Arendt-Nielsen L, Gerdle B. Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women. *J Rehabil Med*. 2012;44(8):648-57.
24. Finocchietti S, Arendt-Nielsen L, Graven-Nielsen T. Tissue characteristics during temporal summation of pressure-evoked pain. *Exp Brain Res*. 2012;219(2):255-65.
25. Henriksen M, Klokke L, Bartholdy C, Graven-Nielsen T, Bliddal H. The Associations between Pain Sensitivity and Knee Muscle Strength in Healthy Volunteers: A Cross-Sectional Study. *Pain Research and Treatment*. 2013;2013:7.

26. Finocchietti S, Morch CD, Arendt-Nielsen L, Graven-Nielsen T. Effects of adipose thickness and muscle hardness on pressure pain sensitivity. *Clin J Pain*. 2011;27(5):414-24.

27. Kosek E, Ekholm J, Nordemar R. A comparison of pressure pain thresholds in different tissues and body regions. Long-term reliability of pressure algometry in healthy volunteers. *Scand J Rehabil Med*. 1993;25(3):117-24.

28. Poller C, Hopster K, Rohn K, Kastner SB. Evaluation of contact heat thermal threshold testing for standardized assessment of cutaneous nociception in horses - comparison of different locations and environmental conditions. *BMC Vet Res*. 2013;9:4.

29. Finocchietti S, Nielsen M, Morch CD, Arendt-Nielsen L, Graven-Nielsen T. Pressure-induced muscle pain and tissue biomechanics: a computational and experimental study. *Eur J Pain*. 2011;15(1):36-44.

30. Manafi-Khanian B, Arendt-Nielsen L, Frokjaer JB, Graven-Nielsen T. Deformation and pressure propagation in deep somatic tissue during painful cuff algometry. *Eur J Pain*. 2015.

31. Adnadjevic D, Graven-Nielsen T. Temporal summation of muscle pain evoked by very fast pressure sequences and rotation. *Somatosens Mot Res*. 2015;32(2):99-105.

32. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. *Pain*. 2002;100(1):19-26.

33. Arendt-Nielsen L, Nie H, Laursen MB, Laursen BS, Madeleine P, Simonsen OH, et al. Sensitization in patients with painful knee osteoarthritis. *Pain*. 2010;149(3):573-81.

FIGURE LEGENDS

Figure 1: Experimental protocol and timing. The sequence of assessments was randomized among subjects.

Figure 2: Cuff positions, hand-held algometer sites, and stimulation patterns. **A:** Cuff positions on leg. **B:** Hand-held algometer assessment sites. **C:** Ramp and staircase stimulation pattern. The continuous rising ramp pattern is illustrated by the straight black line, whereas the grey bars illustrate the staircase stimulation pattern.

Figure 3: Mean (+SEM, n = 20) cuff PDT and PTT values at all positions. **A:** Cuff PDT and PTT values for all chamber positions. Different from position 3 and position 6 (*, $P < 0.03$); position 2, 3, 6, 7, and 8 (#, $P < 0.07$); position 8 (□, $P < 0.04$); position 3, 6, 7, and 8. (+, $P < 0.01$) **B:** Cuff PDT and PTT for two neighbor chambers and mean values of proximal and distal chamber for all 4 locations. Lower than mean PTT (of PTTs from proximal and distal chamber) values at same location (*, $P < 0.01$), and higher than location 4 PTT (#, $P < 0.02$). **C:** Mean (+SEM, n=20) PDT and PTT cuff ratio (both chambers divided with the mean of the two single chambers) at all four locations. Higher than location 3 and 4. (*, $P < 0.02$).

Figure 4: Temporal summation of pain assessed at location 3 (position 5 & 6). Mean (+SEM, n=20) normalized VAS scores after 10 cuff pressure pain stimulations using the ramp and staircase stimulation pattern. VAS scores were normalized by subtraction of the VAS scores from the first stimulation. Significant different compared with previous stimulation for the staircase inflation pattern (*, $P < 0.03$) and the ramp inflation pattern (#, $P < 0.02$).

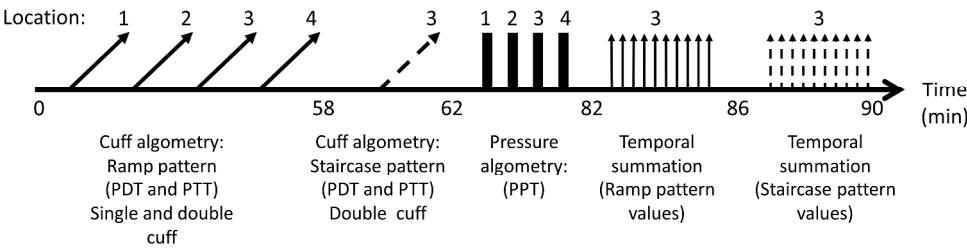


Figure 1. Cuff positions, hand-held algometer sites, and stimulation patterns.
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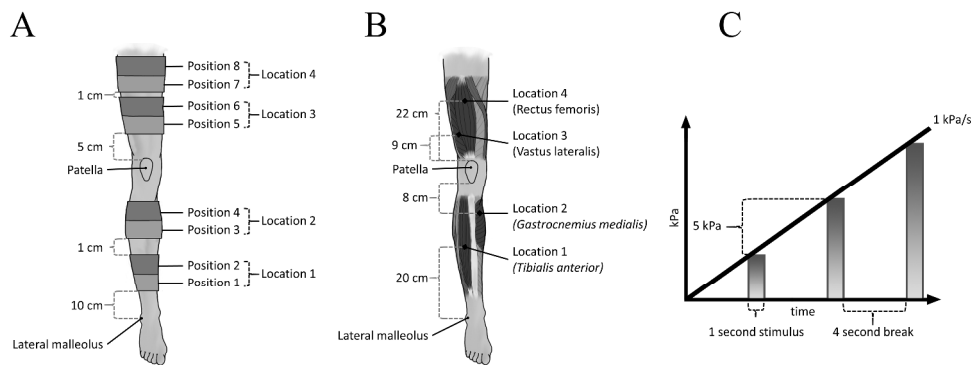


Figure 2: Cuff positions, hand-held algometer sites, and stimulation patterns. A: Cuff positions on leg. B: Hand-held algometer assessment sites. C: Ramp and staircase stimulation pattern. The continuous rising ramp pattern is illustrated by the straight black line, whereas the grey bars illustrate the staircase stimulation pattern.
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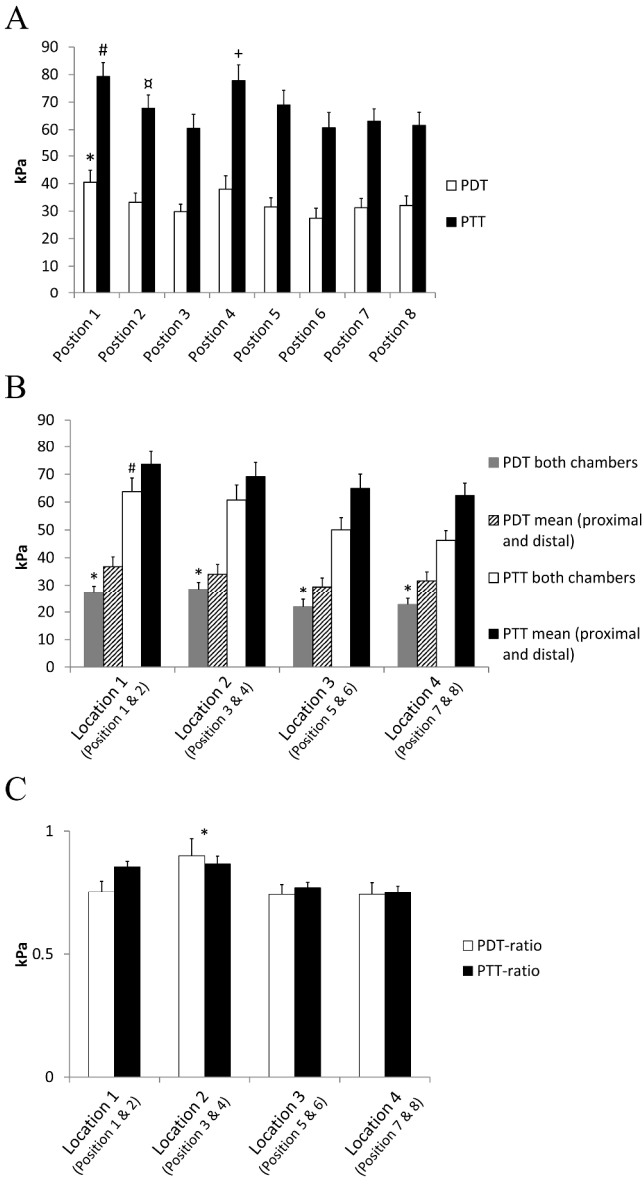


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	Ramp	Staircase	Percentage increase
PDT position 5 (kPa)	31.5 ± 15.1	43.8 ± 18.6*	28.0
PDT position 6 (kPa)	27.5 ± 15.9	35.3 ± 12.1*	22.0
PDT position 5 and 6 (kPa)	21.9 ± 12.6	32.3 ± 10.2*	32.2
PTT position 5 (kPa)	69.1 ± 23.6	85.3 ± 19.8*	19.0
PTT position 6 (kPa)	60.8 ± 24.4	75.5 ± 22.3*	19.5
PTT position 5 and 6 (kPa)	50.1 ± 19.3	69.3 ± 19.2*	27.7
PDT Spatial summation ratio	0.7 ± 0.2#	0.8 ± 0.2	12.5
PTT Spatial summation ratio	0.8 ± 0.1#	0.9 ± 0.1	12.5

Table 1: Mean (±SD, n=20) cuff PDT, cuff PTT and spatial summation ratio, and percentage increase for assessments at position 5 and 6 using the ramp and staircase inflation pattern. Significantly higher PDT and PTT than ramp pattern (*, $P < 0.03$). Significantly higher degree of spatial summation than staircase pattern (#, $P < 0.02$)

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**CUFF ALGOMETRY FOR ESTIMATION OF HYPERALGESIA AND
PAIN SUMMATION**

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Running title: Cuff algometry for estimation of hyperalgesia

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ABSTRACT

OBJECTIVE: Cuff algometry is useful to assess pain sensitivity mechanisms, but effects of cuff position and stimulation pattern are not clear.

METHODS: In 20 healthy volunteers, cuff pain detection threshold (PDT) and pain tolerance (PTT) were recorded with cuffs accommodating two individual chambers at four locations (eight positions) along the leg, using ramp inflation (1 kPa/s) until subjects indicated PDT and PTT. Repeated stimulations (1-s stimulation, 4-s break) with a staircase increase in stimulus intensity (5 kPa/step) were used to assess PDT and PTT on a single location. Spatial pain summation was calculated as the ratio between PTTs recorded with one chamber or simultaneously with two neighbour chambers. Temporal pain summation was assessed by repeated cuff stimulations (1-s stimulation, 1-s break) and the pain intensity was recorded on a visual analogue scale (VAS); the PTT from ramp and staircase assessments were used as stimulus intensity.

RESULTS: For the most distal cuff position, the PTT was higher compared with other leg positions except when in proximity to the knee ($P < 0.01$). The PDT was higher for the distal part compared with the mid-portions of the lower and upper leg ($P < 0.01$). Compared with other leg locations, the spatial summation ratio was highest at the proximal lower leg ($P < 0.02$). The staircase versus ramp pattern revealed higher PDT and PTT ($P < 0.01$), as well as pronounced temporal pain summation ($P < 0.01$).

CONCLUSION: The mid-portion of the lower leg is recommended for cuff placement, and the staircase paradigm provides relevant stimulus intensity for assessment of temporal pain summation.

Key words: Cuff algometry, temporal summation of pain, hyperalgesia, cuff positioning, inflation pattern

INTRODUCTION

Hyperalgesia and sensitization of central pain mechanisms are often assessed by quantitative sensory testing in pain conditions (1). A reliable approach for assessing hyperalgesia is handheld pressure algometry which has been used extensively (2-4). Handheld pressure algometry is however criticized for being operator dependent and assessing limited tissue volume (2,5) due to the manual control of pressure increment rate and direction of pressure from a small stimulation probe. An alternative to the hand-held pressure algometry, cuff pressure algometry is user independent and standardized, reducing some of the factors related to outcome variation in hand-held algometry (5,6). A recent study demonstrated good reliability for cuff pressure algometry (7) and it has been used in clinical studies including e.g. fibromyalgia, epicondylalgia, whiplash and osteoarthritis patients (8-12). Common for previous studies are cuff algometry with a cuff mounted on the lower leg, with few exceptions using the thigh and arm. The specific position of the cuff seem to influence cuff pain detection threshold (PDT) and cuff pain tolerance (PTT) (5,11,13) although the difference between cuff positions on the leg has not been systematically studied.

Facilitated spatial and temporal summation of pain have been used in patient studies to assess potential sensitization of central pain mechanisms (11,14,15). Spatial summation of pain is often defined as reduced pain thresholds when assessed in larger, compared with smaller, areas (16,17). Small versus larger heat stimulation probes (18) or one cuff versus two cuffs (11) demonstrates spatial summation of pain. An example of facilitated spatial summation is found in knee osteoarthritis patients, demonstrating more spatial summation compared with controls (14). Temporal summation of pain is the increased pain perception occurring when a painful stimulus at the same intensity is repeated with a frequency above 0.3 Hz (19,20). Previous studies have demonstrated temporal pain summation using heat, pressure, and cuff stimulations in healthy subjects (21,22). Facilitated temporal summation of

pain is e.g. found in whiplash associated disorder patients (23). The stimulation intensity as well as the first stimulation being painful are important for evoking temporal pain summation (24). Common for cuff algometry to assess temporal summation of pain is the use of a tonic ramp pattern (5,9,13,14) to detect PDT and PPT and using these to adjust the stimulation intensity for assessing temporal summation of pain. However, to ensure a painful first stimulation when assessing temporal summation of pain, a better approach may be to determine the pain and tolerance thresholds based on phasic stimuli with the same duration as used for temporal pain summation.

It was hypothesized that 1) the pain sensitivity and spatial pain summation differ between cuff locations, and 2) a novel paradigm for temporal pain summation where the stimulation intensity is based on pain sensitivity assessments with a phasic inflation pattern, would obtain more pronounced temporal pain summation than the conventional temporal pain summation protocol.

METHODS

The aim of this study was to assess cuff pressure algometry using different cuff locations and inflation patterns as well as assessing potential similarities in the pain sensitivity when using the classical hand-held and cuff pressure algometry.

Materials

Twenty healthy subjects (10 females) with a median age of 26 years (range 19 – 48 years) were recruited from a university setting. The exclusion criterion were physical exhausting activities 24 hours prior to the study, consumption of alcohol, caffeine, nicotine or analgesics on the morning of the study, and history of pain affecting the lower limb and/or trunk. Prior to the study all subjects signed a consent form after receiving written and oral information. The

study was approved by the local ethics committee (N-20140002) and followed the Helsinki declaration.

Protocol

In one session, pressure and cuff algometry parameters were assessed on eight different positions of the leg. Moreover, in two neighbor chambers, cuff algometry was performed simultaneously with two different inflation paradigms (ramp and staircase) and subsequently assessment of temporal summation of pain. The sequence of procedures and locations were randomized except for temporal summation of pain which always was assessed last. The subjects were placed in a comfortable and relaxed supine position during the experimental procedure. The entire session had a duration of approximate 90 minutes. (Fig. 1) All data was collected by the same assessor.

Cuff algometry

The computer-controlled cuff pressure algometer (Nocitech, Denmark and Aalborg University, Denmark) (10) consisted of a double chambered 13-cm wide silicone high-pressure tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany), a computer-controlled compressor, a 10 cm electronic visual analogue scale (VAS), and a stop-button for immediate release of air in the tourniquet cuff. The proximal and distal chamber of the cuff were controlled and inflated independently. The tourniquet was mounted around the dominant leg without cloths between the cuff and skin, ensuring a tight fit around the leg. In order to determine any differences in pain sensitivity between locations, four locations (equivalent to eight chamber positions when using a double chamber cuff) were chosen with intend of covering as much of the leg as possible (Fig. 2a). All four locations were measured to the lowest part of the tourniquet cuff: 1) 10 cm from the lateral malleolus, 2) 1 cm above the top

part of the tourniquet cuff in location 1, 3) 5 cm above the patella, and 4) 1 cm above the top part of the tourniquet cuff in location 3.

A ramp inflation pattern (Fig. 2c) with a constant inflation of 1 kPa/s (13) was used for measurements at all positions. Subjects were asked to continuously rate perceived pain using the electronic VAS where the extremities “0” and “10 cm” were defined as “no pain” and “maximal pain”. Furthermore, the participants pressed the stop-button when the pain became intolerable. Cuff pain detection threshold (PDT) was defined as the pressure value the first time the VAS score exceeded 1 cm, and cuff pain tolerance (PTT) was defined as the pressure value when the subject terminated the pressure inflation. A maximum pressure of 100 kPa (750 mmHg) was set as upper limit throughout the study (23) and if the subjects reached this before the PDT or PTT were reached these parameters were set to 100 kPa in the further analysis.

Simultaneously in position 5 and 6, a staircase inflation pattern was additionally used to detect PDT and PTT: Repeated stimulations (1-s duration) each increasing by 5 kPa were used to inflate the chambers, separated by a four seconds interval. Thus, the increment rate over time was equivalent with the assessment performed with the constant inflation rate of 1 kPa/s. For each stimulus, the inflation rate was as fast as possible to reach the target pressure. The phasic staircase inflation pattern was used as a contrast to the more tonic ramp inflation pattern in order to assess the ability to obtain more pronounced temporal pain, with a painful first stimulation. With the staircase inflation, subjects were instructed to rate the VAS immediately after the stimulation and not adjusting to zero in-between. For analysis the mean VAS score during the 4 second delay between each stimulus was used for extraction of the PDT (i.e. VAS exceeded 1 cm first time) and PTT was the stimulus intensity where the subjects pushed the stop-button when the pain became intolerable.

All assessments were conducted by inflating the cuff chamber for each of the eight positions, i.e. the proximal and distal chamber at each of the four cuff locations. Additionally a simultaneous inflation of both chambers were performed at each of the four cuff locations. All measurement were repeated three times (sequence randomized), with a 60 seconds interval in order to reduce influence of cuff PPT and PDT values due to repeated assessment (25). A mean of the three measurements was used for further analysis.

Spatial and temporal summation of pain

The degree of spatial pain summation was assessed by the ratio between cuff PPTs assessed with both chambers (at one location) divided with the mean PTT of the two single chambers at the same location (14). Temporal summation of pain was measured by simultaneous chamber stimulation at position 5 and 6. A repetitive inflation pattern delivering 10 painful stimuli of one second duration with a one second interval between stimulations was used to evoke temporal summation. Two different stimulation intensities levels were used for the measurements; one using the PTT from the ramp pattern, and one from the staircase pattern. The use of PTT was chosen to ensure painful stimulations during temporal summation measurements. Subjects were instructed to continuously rate the pain intensity on the VAS during the ten stimulations and not adjust to zero in-between stimuli. A mean VAS score in the pause between each of the 10 stimulations were extracted for analyses. The VAS scores were normalized by subtraction of the VAS score from the first stimulation. The sum of normalized VAS scores was extracted (VAS sum). Furthermore, the mean values of VAS scores from the first four normalized VAS scores (VAS-I) and the last three normalized VAS scores (VAS-III) were extracted; the ratio between VAS-III and VAS-I was extracted.

Hand-held pressure algometry

In order to compare cuff algometry with hand-held pressure algometry extensively used in previous studies, a series of pressure pain thresholds (PPT) were obtained. A hand-held algometer (Somedic, Hörby, Sweden) equipped with a 1 cm² probe covered by a disposable latex sheath and a wired stop-button were used to record PPT. The PPTs were assessed at four locations (Fig. 2b) sited approximately at the middle of the corresponding tourniquet cuff location and on specific muscles: 1) The tibialis anterior muscle approximately 20 cm from the lateral malleolus, 2) the medial gastrocnemius muscle approximately 8 cm from the lower part of the patella, 3) the lateral vastus muscle approximately 9 cm from the upper part of the patella, and 4) the rectus femoris muscle approximately 22 cm from upper part of the patella. The pressure was manually increased with 30 kPa/s until the subjects pressed the button when the pressure went from being a pressure to a pain sensation (13). PPTs were collected in triplets for each location with a 60 s interval between each measurement at the same location. The mean of the three PPT measurements was used for further analysis.

Statistics

Results are presented as mean and standard error of the mean (SEM), unless otherwise specified. All statistical analyses were performed using IBM SPSS Statistics 22. All data passed the Kolmogorov-Smirnov test for normality. Repeated measures analysis of variance (RM-ANOVA) was applied to determine differences between chamber positions (eight positions) and Bonferroni post-hoc tests were applied when relevant. A RM-ANOVA was used to investigate the influence of inflation patterns on normalized VAS scores after repeated stimulation with *stimulation intensity* (PTT-ramp, PTT-staircase) and *stimulation number* (2-10) as factors. Two-way ANOVAs with *location* (four) and *single/double* (average-of-single-cuff, double-cuff) as factors were applied to determine influence of location on spatial

summation. T-tests with Bonferroni corrections were applied to determine differences between the two inflation patterns. Thus, all data was controlled for influence of gender as a between-subjects effect, as well as chamber sequence within one location, and location sequence within- and between-subjects by including these variables as factors in separate additional ANOVA's. P-values less than 0.05 were considered significant.

RESULTS

Cuff pressure pain sensitivity at different locations

During PTT measurement 45% subjects reached maximum pressure, mainly at position 1 and 4, while one subject did not reach 1 cm on the VAS during PDT measurements at position 1. None of the subjects reached maximum pressure using both chambers at position 3, which was used for temporal summation. Males obtained higher cuff algometry values than females using the proximal and distal chamber individually for PDT (ANOVA: $F = 4.19$, $P < 0.048$), using all chamber combinations for PTT (ANOVA: $F = 14.90$, $P < 0.002$), all temporal summation VAS scores for both inflation patterns (RM-ANOVA: $F = 10.04$, $P < 0.005$), and hand-held PPT at location 3 and 4 (ANOVA: $F = 7.11$, $P < 0.016$).

The RM-ANOVA of PDT across positions demonstrated that the PDT recorded at position 1 was significantly higher than at position 3 and 6 (Fig. 3A; RM-ANOVA: $F = 4.65$, $P < 0.01$; Bonferroni: $P < 0.01$).

Additionally, cuff PTT (Fig. 3A) recorded at position 1 was higher compared with positions 2, 3, 6, 7, and 8 (RM-ANOVA: $F = 12.24$, $P < 0.01$; Bonferroni $P < 0.01$). For position 2, PTT was higher than at position 8 (Bonferroni: $P < 0.01$). Finally, PTT at position 4 was higher compared with positions 3, 6, 7, and 8 (Bonferroni: $P < 0.01$).

Spatial pain summation at different locations

Inflating the chambers at two positions simultaneously produced significantly lower pressure values compared with the mean of the same two positions when recorded individually for both PDT (Fig. 3B; Two-way ANOVA: $F = 13.56$, $P < 0.01$) and PTT (Fig. 3B; Two-way ANOVA: $F = 13.63$, $P < 0.01$). Furthermore, when inflating both chambers at position 1 and 2, the PTT was higher compared with the simultaneous inflation of cuff position 7 and 8 (Two-way ANOVA: $F = 3.94$, $P < 0.02$; Bonferroni: $P < 0.02$). The spatial summation ratios of PDT and PTT were higher when assessing positions 3 and 4 compared with assessments of positions 5 & 6, and 7 & 8 (Fig. 3C; Two-way ANOVA: $F = 4.54$, $P < 0.01$; Bonferroni: $P < 0.02$).

Effects of staircase and ramp inflation pattern

Using the staircase compared with the ramp inflation pattern, the PDT and PTT were higher when stimulating with the chamber at positions 5 and 6 individually, and when both chambers at position 5 and 6 was inflated simultaneously (Table 1; T-test: $F = 14.9$, $P < 0.01$).

Compared with the ramp pattern, the lowest degree of spatial summation was observed when using the staircase pattern (Table 1; T-test, $F > 7.52$, $P < 0.09$).

Temporal summation of pain

Normalized VAS scores from repeated stimulation demonstrated a significant interaction between the stimulation intensity (ramp or staircase PTT) and stimulation number (RM-ANOVA: $F = 5.94$, $P < 0.02$). Using PTT based on the ramp pattern resulted in a VAS score after stimulation 3 which was lower than stimulation 4 to 10 (Bonferroni: $P < 0.03$). Likewise, VAS scores after stimulation 4 and 6, respectively, was lower than stimulation 8 to 10 (Bonferroni $P < 0.03$). All staircase stimulations resulted in normalized VAS scores

significantly lower than all the following VAS scores, the only exception being stimulation 6 and stimulation 7 (Bonferroni $P < 0.03$).

The temporal summation induced with the PTT level obtained from the staircase pattern demonstrated higher VAS sum (25.42 ± 2.6 cm) compared with temporal summation induced with the PTT estimated from the ramp pattern (20.53 ± 2.8 cm; T-test $P < 0.002$). A significant increase in VAS scores between each pairwise stimulus was found for staircase pattern induced temporal summation for all except between stimulation 6 and 7 (Fig. 4; Bonferroni: $P < 0.03$). Using the ramp pattern, the only significant pairwise difference in VAS scores was between stimulation 2 and 3 (Fig. 4 Bonferroni: $P < 0.02$). The ratio between the last and first normalized VAS scores was 1.40 ± 0.19 for ramp pattern with a tendency to be higher (1.93 ± 0.19 , T-test: $P < 0.01$) for the staircase pattern.

Pressure algometry at different locations

PPTs assessed by hand held pressure algometry were 568 ± 50 kPa, 418 ± 36 kPa, 437 ± 29 kPa, and 478 ± 27 kPa for location 1 to 4, respectively. The PPT at location 1 was higher than location 2 (Two-way ANOVA: $F = 3.32$, $P < 0.024$; Bonferroni $P < 0.03$). The hand held algometer PPTs were not significantly correlated with cuff measurements (PDT, PTT).

DISCUSSION

This study demonstrated significant differences in the pain sensitivity between different assessment positions at the leg when evaluated by cuff algometry. The novel assessment paradigm of the pain tolerance level based on a staircase pattern resulted in higher stimulation levels to be used for assessment of temporal summation of pain, and resulted in pronounced temporal summation of pain compared with the traditional approach.

Effects of cuff location

The significantly higher cuff PDT and PTT using the chamber at position 1 could be explained by the underlying structures here having less soft tissue compared with other locations on the leg, and therefore are able to obtain higher values. The same pattern can be seen for cuff PTT at position 4, which may be due to the chambers proximity to the knee with less soft tissue. This explanations falls in line with a previous study indicating that the cuff pressure algometry assesses muscle and deep tissue sensitivity (10).

The positions of the cuff were determined by measuring from prominent structures and the cuff itself, however due to different leg length between subjects, one or more positions overlapped for eight of the subjects, and it can be speculated whether these overlapping positions have influenced the cuff PPT and PDT values due to repeated assessment of the same tissue without adequate pauses in-between. Furthermore several cuff assessment performed on each subject present a limitation, as they may have evoked pain sensitization. Likewise the different positions assessed may have evoked pain inhibition. Nonetheless, the randomized position and assessment sequence along with the 60 second interval in-between assessments was included in the experimental design to reduce these limitations (19,24).

The individual anatomy of the subjects legs may have been influenced the present data as pressure pain is affected by muscle hardness as well as the thickness of adipose tissue (26). Body dimensions may therefore be of interest for future studies assessing variations in different locations.

The cuff PDT was defined as when the VAS score was equal to or greater than 1 cm, this definition however made the PDT sensitive to any subject manipulating the VAS slider but not reaching 1 cm, as was the case with one of the subjects. For 9 of the 20 subjects, one or more cuff PTT measurements reached the maximum pressure of 100 kPa during their session; as such these values were therefore at least 100 kPa, but may in reality be higher. The

measurements reaching the maximum pressure was however set as 100 kPa for the data analysis. Although all positions were able to obtain valid cuff PDT and PTT, several subjects reached maximum pressure, the majority at position 1 and 4, a correct value would have resulted in a more pronounced difference between the positions. It is worth to note that this study was conducted on healthy subjects, while previous studies have demonstrated a reduced cuff PTT for patients with e.g. rheumatoid arthritis, fibromyalgia, chronic whiplash associated disorder, and ongoing pain following a total knee replacement (5,13,15,23). Patient groups may not reach maximum pressure as the subjects in this study, but choosing a location where it is less likely to end at maximum pressure would be desirable. The ability to obtain cuff PDT and PTT at all sites is however interesting, as these values will still be obtainable in cases where using a specific part of the leg is unavailable. The present results demonstrate variations in cuff PDT and PTT, depending on the position of the tourniquet cuff. Variable pain perception in different regions of the body is well established for a number of modalities, and with this study also cuff algometry of the leg (16,27,28).

Hand held algometry and cuff pressure algometry are two different methods for assessing pressure pain, as they express pain sensitivity for a small compact and large volume, respectively. Cuff pressure algometry is able to efficiently stimulate deep tissue in contrast to the hand held algometry's single point which affects more superficial structures. Previous studies have demonstrated that larger rounded probes more efficiently induce muscle pain than small flat probes, but the single point pressure algometry used in this study still stands in contrast to cuff pressure algometry (29,30). One study have however found a correlation between cuff pressure algometry and computer controlled pressure algometer using a 1 cm padded probe (25). This study did not find any correlation between the two methods for assessing pressure pain probably because the handheld pressure algometry only assess a very limited tissue volume, or due to inconsistency in pressure; future studies with several hand-

held algometer measurement sites in the area of cuff assessments may however clarify if correlation between hand-held algometer and cuff pressure algometry measurements exists.

Spatial summation of pain

All positions demonstrated spatial summation, but a lesser degree was observed at location 2 when compared to summation at location 3 and 4. This difference from location 2 may be due to the significant difference between sensitivity parameters when assessing position 3 and 4. The same was found when comparing position 1 and 2, although no significant difference was found between spatial summation assessed at location 1 and location 3 & 4. This lesser degree of spatial summation for location 2 could correspond to the previous mentioned issue of the underlying tissue, as position 4 is in proximity of the knee. It is however important to notice that none of the used locations for cuff placement correspond to previously used positions e.g. the widest part of the lower leg, as this location would correspond to position 2 and 3 which was not measured in this study (11,15,25). Location 3 and 4 could also be valid for assessing spatial summation, but especially for position 4 the ease of placement. Although no patient related outcomes were systematically collected, several subjects expressed discomfort with the placement on location 3 and 4, which also should be taken into account. These results demonstrate the importance of consistency in cuff placement, as well as taking the anatomy of the leg into account when designing a study. Despite not being measured in the present study, the previously used position located on the widest part of the lower leg is therefore recommended for future studies. This position provides a high amount of soft tissue, is not dependent on measuring from prominent structures, is easy to place and do not cause patient discomfort as the positions on the thigh.

Temporal summation of pain

Both ramp and staircase pattern determined PTT stimulation intensity demonstrated temporal summation of pain. The staircase stimulation pattern determined PTT delivered a more apparent temporal summation, with significant differences between stimulations in contrast to the ramp pattern. The difference between the ramp and staircase inflation pattern determined PTTs, may be found in different nature of the two stimulation patterns. The lower ramp pattern PDT and PTT values in this study could therefore be explained by temporal summation of pain due to the prolonged stimulation in contrast to the staircase pattern (31,32). Although previous studies have induced temporal summation using the tonic ramp stimulation pattern, the phasic nature of the staircase pattern may provide PDT and PTT values uninfluenced by temporal summation and match the stimulus duration used for repeated stimulations. The 5 kPa increment of the staircase pattern should also be considered as it is less nuanced in contrast to the ramp patterns 1 kPa/s increment. Compared with previous studies, this study obtained a higher degree of temporal summation in contrast to temporal pain summation induced by cuff pressure algometry and computer-controlled pressure algometry with a 1 cm² padded probe (13,33). The present study differed however from previous studies by using both chambers of the cuff (double chamber stimulation) at location 3, in contrast to the widest part of the lower leg used in previous studies (corresponding to position 2 and 3 in the present study) (11,15,25). Furthermore, a mean of PTT from both chambers were used in contrast to previously used mean of cuff PDT and cuff PTT from a single chamber (13,23). For this study the mean of both cuff PDT and cuff PTT was higher than the mean of PTT from both chambers, but still managed to induce temporal summation. With the significantly higher PDT and PTT thresholds and the more apparent intense temporal summation of pain, the staircase pattern paradigm is therefore recommended when used to assess temporal summation.

Conclusion

This study illustrates the importance of cuff positioning for assessing cuff PDT, PTT, and spatial summation of pain, as well as the assessment paradigm for assessing temporal pain summation. For future studies it is recommended to use the widest part of the lower leg for cuff placement, and the novel staircase pattern assessment paradigm of the pain tolerance level subsequently to be used for assessment of temporal pain summation.

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Conflict of interest statement

Nocitech is partly owned by Aalborg University.

References

1. Pavlaković G, Petzke F. The Role of Quantitative Sensory Testing in the Evaluation of Musculoskeletal Pain Conditions. *Current Rheumatology Reports*. 2010;12(6):455-61.

2. Kinser AM, Sands WA, Stone MH. Reliability and validity of a pressure algometer. *J Strength Cond Res*. 2009;23(1):312-4.

3. Rolke R, Andrews Campbell K, Magerl W, Treede RD. Deep pain thresholds in the distal limbs of healthy human subjects. *Eur J Pain*. 2005;9(1):39-48.

4. Frank L, McLaughlin P, Vaughan B. The repeatability of pressure algometry in asymptomatic individuals over consecutive days. *Int J Osteopath Med*. 2013;16(3):143-52.

5. Jespersen A, Dreyer L, Kendall S, Graven-Nielsen T, Arendt-Nielsen L, Bliddal H, et al. Computerized cuff pressure algometry: A new method to assess deep-tissue hypersensitivity in fibromyalgia. *Pain*. 2007;131(1-2):57-62.

6. Bartholdy C, Zangger G, Hansen L, Ginnerup-Nielsen E, Bliddal H, Henriksen M. Local and Systemic Changes in Pain Sensitivity After 4 Weeks of Calf Muscle Stretching in a Nonpainful Population: A Randomized Trial. *Pain Pract*. 2015.

7. 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.

8. Izumi M, Petersen KK, Arendt-Nielsen L, Graven-Nielsen T. Pain referral and regional deep tissue hyperalgesia in experimental human hip pain models. (1872-6623 (Electronic)).

9. Amris K, Jespersen A, Bliddal H. Self-reported somatosensory symptoms of neuropathic pain in fibromyalgia and chronic widespread pain correlate with tender point count and pressure-pain thresholds. *Pain*. 2010;151(3):664-9.

10. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry. *The Journal of Pain*. 2002;3(1):28-37.
11. Jespersen A, Amris K, Graven-Nielsen T, Arendt-Nielsen L, Bartels EM, Torp-Pedersen S, et al. Assessment of Pressure-Pain Thresholds and Central Sensitization of Pain in Lateral Epicondylalgia. *Pain Med*. 2013;14(2):297-304.
12. Soriano-Maldonado A, Klokke L, Bartholdy C, Bandak E, Ellegaard K, Bliddal H, et al. Intra-Articular Corticosteroids in Addition to Exercise for Reducing Pain Sensitivity in Knee Osteoarthritis: Exploratory Outcome from a Randomized Controlled Trial. *PLoS One*. 2016;11(2):e0149168.
13. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. *Pain*. 2013;154(9):1588-94.
14. Graven-Nielsen T, Wodehouse T, Langford RM, Arendt-Nielsen L, Kidd BL. Normalization of widespread hyperesthesia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. *Arthritis Rheum*. 2012;64(9):2907-16.
15. Vladimirova N, Jespersen A, Bartels EM, Christensen AW, Bliddal H, Danneskiold-Sams, et al. Pain Sensitisation in Women with Active Rheumatoid Arthritis: A Comparative Cross-Sectional Study. *Arthritis*. 2015;2015:5.
16. Defrin R, Ronat A, Ravid A, Peretz C. Spatial summation of pressure pain: effect of body region. *Pain*. 2003;106(3):471-80.
17. Greenspan JD, Thomadaki M, McGillis SL. Spatial summation of perceived pressure, sharpness and mechanically evoked cutaneous pain. *Somatosens Mot Res*. 1997;14(2):107-12.

18. Staud R, Vierck CJ, Robinson ME, Price DD. Spatial summation of heat pain within and across dermatomes in fibromyalgia patients and pain-free subjects. *Pain*. 2004;111(3):342-50.

19. Anderson RJ, Craggs JG, Bialosky JE, Bishop MD, George SZ, Staud R, et al. Temporal summation of second pain: Variability in responses to a fixed protocol. *European Journal of Pain*. 2013;17(1):67-74.

20. Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain*. 2009;10(9):895-926.

21. Suzan E, Aviram J, Treister R, Eisenberg E, Pud D. Individually based measurement of temporal summation evoked by a noxious tonic heat paradigm. *J Pain Res*. 2015;8:409-15.

22. Christensen AW, Rifbjerg-Madsen S, Christensen R, Amris K, Taylor PC, Locht H, et al. Temporal summation of pain and ultrasound Doppler activity as predictors of treatment response in patients with rheumatoid arthritis: protocol for the Frederiksberg hospitals Rheumatoid Arthritis, pain assessment and Medical Evaluation (FRAME-cohort) study. *BMJ Open*. 2014;4(1):e004313.

23. Lemming D, Graven-Nielsen T, Sorensen J, Arendt-Nielsen L, Gerdle B. Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women. *J Rehabil Med*. 2012;44(8):648-57.

24. Finocchietti S, Arendt-Nielsen L, Graven-Nielsen T. Tissue characteristics during temporal summation of pressure-evoked pain. *Exp Brain Res*. 2012;219(2):255-65.

25. Henriksen M, Klokke L, Bartholdy C, Graven-Nielsen T, Bliddal H. The Associations between Pain Sensitivity and Knee Muscle Strength in Healthy Volunteers: A Cross-Sectional Study. *Pain Research and Treatment*. 2013;2013:7.

26. Finocchietti S, Morch CD, Arendt-Nielsen L, Graven-Nielsen T. Effects of adipose thickness and muscle hardness on pressure pain sensitivity. *Clin J Pain*. 2011;27(5):414-24.
27. Kosek E, Ekholm J, Nordemar R. A comparison of pressure pain thresholds in different tissues and body regions. Long-term reliability of pressure algometry in healthy volunteers. *Scand J Rehabil Med*. 1993;25(3):117-24.
28. Poller C, Hopster K, Rohn K, Kastner SB. Evaluation of contact heat thermal threshold testing for standardized assessment of cutaneous nociception in horses - comparison of different locations and environmental conditions. *BMC Vet Res*. 2013;9:4.
29. Finocchietti S, Nielsen M, Morch CD, Arendt-Nielsen L, Graven-Nielsen T. Pressure-induced muscle pain and tissue biomechanics: a computational and experimental study. *Eur J Pain*. 2011;15(1):36-44.
30. Manafi-Khanian B, Arendt-Nielsen L, Frokjaer JB, Graven-Nielsen T. Deformation and pressure propagation in deep somatic tissue during painful cuff algometry. *Eur J Pain*. 2015.
31. Adnadjevic D, Graven-Nielsen T. Temporal summation of muscle pain evoked by very fast pressure sequences and rotation. *Somatosens Mot Res*. 2015;32(2):99-105.
32. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. *Pain*. 2002;100(1):19-26.
33. Arendt-Nielsen L, Nie H, Laursen MB, Laursen BS, Madeleine P, Simonsen OH, et al. Sensitization in patients with painful knee osteoarthritis. *Pain*. 2010;149(3):573-81.

FIGURE LEGENDS

Figure 1: Experimental protocol and timing. The sequence of assessments was randomized among subjects.

Figure 2: Cuff positions, hand-held algometer sites, and stimulation patterns. **A:** Cuff positions on leg. **B:** Hand-held algometer assessment sites. **C:** Ramp and staircase stimulation pattern. The continuous rising ramp pattern is illustrated by the straight black line, whereas the grey bars illustrate the staircase stimulation pattern.

Figure 3: Mean (+SEM, n = 20) cuff PDT and PTT values at all positions. **A:** Cuff PDT and PTT values for all chamber positions. Different from position 3 and position 6 (*, P < 0.03); position 2, 3, 6, 7, and 8 (#, P < 0.07); position 8 (□, P < 0.04); position 3, 6, 7, and 8. (+, P < 0.01) **B:** Cuff PDT and PTT for two neighbor chambers and mean values of proximal and distal chamber for all 4 locations. Lower than mean PTT (of PTTs from proximal and distal chamber) values at same location (*, P < 0.01), and higher than location 4 PTT (#, P < 0.02). **C:** Mean (+SEM, n=20) PDT and PTT cuff ratio (both chambers divided with the mean of the two single chambers) at all four locations. Higher than location 3 and 4. (*, P < 0.02).

Figure 4: Temporal summation of pain assessed at location 3 (position 5 & 6). Mean (+SEM, n=20) normalized VAS scores after 10 cuff pressure pain stimulations using the ramp and staircase stimulation pattern. VAS scores were normalized by subtraction of the VAS scores from the first stimulation. Significant different compared with previous stimulation for the staircase inflation pattern (*, P < 0.03) and the ramp inflation pattern (#, P < 0.02).

	Ramp	Staircase	Percentage increase
PDT position 5 (kPa)	31.5 ± 15.1	43.8 ± 18.6*	28.0
PDT position 6 (kPa)	27.5 ± 15.9	35.3 ± 12.1*	22.0
PDT position 5 and 6 (kPa)	21.9 ± 12.6	32.3 ± 10.2*	32.2
PTT position 5 (kPa)	69.1 ± 23.6	85.3 ± 19.8*	19.0
PTT position 6 (kPa)	60.8 ± 24.4	75.5 ± 22.3*	19.5
PTT position 5 and 6 (kPa)	50.1 ± 19.3	69.3 ± 19.2*	27.7
PDT Spatial summation ratio	0.7 ± 0.2#	0.8 ± 0.2	12.5
PTT Spatial summation ratio	0.8 ± 0.1#	0.9 ± 0.1	12.5

Table 1: Mean (±SD, n=20) cuff PDT, cuff PTT and spatial summation ratio, and percentage increase for assessments at position 5 and 6 using the ramp and staircase inflation pattern. Significantly higher PDT and PTT than ramp pattern (*, $P < 0.03$). Significantly higher degree of spatial summation than staircase pattern (#, $P < 0.02$)