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Quantitative sensory testing of post-traumatic hyperalgesia at the diabetic foot

Dissertation

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Für meine Familie

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Wienemann, T.; Chantelau, E.A.; Koller, A. (2014) Effect of painless diabetic neuropathy on pressure pain hypersensitivity (hyperalgesia) after acute foot trauma. In: *Diabetic Foot Ankle* 5: 24926. doi: 10.3402/dfa.v5.24926.

Introduction and objective: Mechanical pain perception thresholds are elevated in the feet of patients with painless diabetic neuropathy (PLDN). It is not known whether posttraumatic hyperalgesia is similarly affected. To explore the issue, cutaneous and deep pressure pain perception thresholds were measured in patients with PLDN and diabetic foot syndrome (defined as painless foot ulcer or Charcot arthropathy), and in control subjects, with and without acute foot trauma.

Methods and patients: Two case-control studies were conducted. 18 PLDN patients with a chronic diabetic foot syndrome, 10 non-neuropathic subjects with an acute painful non-ulcerative foot trauma (e.g. a sprain), and 20 uninjured healthy control subjects were examined cross-sectionally (study 1). In addition, 12 PLDN patients with diabetic foot syndrome and elective bone surgery in the foot, and 13 non-neuropathic subjects with an acute skeletal foot trauma were studied prospectively over one week (study 2). Cutaneous pressure pain perception thresholds (CPPPT) and deep pressure pain perception thresholds (DPPPT) were measured at hands and feet, by the psychophysical method of quantitative sensory testing (QST), using calibrated pinprick stimulators (CPPPT), and a handheld algometer (DPPPT).

Results: Non-neuropathic subjects had lower CPPPTs and DPPPTs at the injured and the contralateral foot, as compared to the uninjured feet of the control group (study 1), suggesting hyperalgesia due to peripheral and central sensitization. By contrast, patients with diabetic foot syndrome displayed elevated CPPPTs and DPPPTs at both feet, exceeding the safety limit of measurement in 100% (CPPPT), 72% (DPPPT over joint) and 28% (DPPPT over muscle) of patients (study 1); baseline findings were similar in study 2. In study 2, the non-neuropathic subjects displayed slightly lowered DPPPTs (but not CPPPTs) at both the injured and the uninjured contralateral foot at an early stage after the foot trauma. Likewise, elevated baseline DPPPT -but not CPPPT- at both feet of patients with diabetic foot syndrome declined temporarily early after surgery (study 2).

Conclusion: Hyperalgesia after a trauma was impaired in feet with PLDN and diabetic foot syndrome. Elevated baseline DPPPT declined after trauma, but not as low as in the control subjects' feet. This feature, which may be related to the applied stimulation technique and the effects of spatial summation, deserves further study.

Einleitung und Ziele der Arbeit: Bei schmerzloser diabetischer Neuropathie (PLDN) ist die Schwelle für die Wahrnehmung mechanischer Schmerzreize an den Füßen erhöht. Bislang ist unklar, ob die posttraumatische Hyperalgesie ebenfalls eingeschränkt ist. Daher wurden im Folgenden die Druckschmerzschwellen bei Patienten mit PLDN und diabetischem Fußsyndrom (definiert als schmerzlose Fußulzeration oder Charcot-Osteoarthropathie) untersucht, sowie bei Kontrollpersonen mit und ohne Fußtrauma.

Methoden und Patienten: Zwei Fall-Kontroll-Studien wurden durchgeführt. 18 PLDN-Patienten mit chronisch diabetischem Fußsyndrom, 10 Personen ohne Neuropathie, die akut an einer Verletzung des Fuß-Skeletts (z.B. Distorsion) erkrankt waren, und 20 unverletzte gesunde Personen wurden einmalig miteinander verglichen (Studie 1). Des Weiteren wurden zwölf Patienten mit PLDN und diabetischem Fußsyndrom, die elektiv am Fuß-Skelett operiert worden waren, mit 13 Versuchspersonen ohne Neuropathie, die ein akutes Trauma des Fuß-Skeletts erlitten hatten, prospektiv mehrfach innerhalb einer Woche verglichen (Studie 2). Die Wahrnehmungsschwellen für oberflächlichen (CPPPT) und tiefen (DPPPT) Druckschmerz wurde mit der psychophysikalischen Methode der quantitativen sensorischen Testung an den Händen und Füßen ermittelt. Hierzu wurden kalibrierte Pin-Prick-Stimulatoren (für CPPPT) und ein tragbares Algometer (für DPPPT) eingesetzt.

Ergebnisse: Die Personen ohne Neuropathie hatten niedrigere CPPPT und DPPPT, d.h. Hyperalgesie, am verletzten und kontralateralen Fuß im Vergleich zur Kontrollgruppe mit unverletzten Füßen (Studie 1). Dies ist vermutlich auf periphere und zentrale Sensibilisierung zurückzuführen. Im Gegensatz dazu hatten Patienten mit einem diabetischen Fußsyndrom stark erhöhte CPPPT und DPPPT an beiden Füßen. In 100% (CPPPT) 72% (DPPPT am Gelenk) und 28% (DPPPT an Muskeln) der Fälle lagen diese sogar oberhalb der Messgrenze (Studie 1). Die Ausgangsbefunde in der zweiten Studie waren ähnlich. Personen ohne Neuropathie hatten kurz nach dem Fußtrauma leicht erniedrigte DPPPT (aber nicht CPPPT) sowohl am gesunden als auch am verletzten Fuß (Studie 2). Die prätraumatisch stark erhöhten DPPPT - aber nicht die CPPPT - an beiden Füßen der Patienten mit diabetischem Fußsyndrom nahmen posttraumatisch vorübergehend ab (Studie 2).

Schlussfolgerung: An den Füßen von Patienten mit einer PLDN und diabetischem Fußsyndrom ist die mechanische Hyperalgesie nach einem Trauma vermindert. Insbesondere die erhöhte Schmerzschwelle für tiefe Druckstimulation sinkt nach einem Trauma vorübergehend, jedoch nicht so deutlich wie bei den Kontrollpersonen. Dieses Phänomen könnte methodisch und/oder durch räumliche Summation bedingt sein; es bedarf weiterer Abklärung.

CPPPT	cutaneous pressure pain perception threshold
DPPPT	deep pressure pain perception threshold
N	Newton (unit of force), 1 N=1000 mN = equivalent to approx. 0.1 kg
NRS	numeric rating scale , ranging from 0 (no pain) to 10 (worst imaginable pain)
Pa	Pascal= (unit of pressure), 1000 Pa=1 kPa= 0,1 N/cm²= equivalent to approx. 10 g/cm²
PLDN	painless diabetic neuropathy
QST	quantitative sensory testing
VPT	vibration perception threshold

1. Introduction

1.1 Nociception: pathogenic and clinical features

Nociception is the sensory modality that detects external or internal noxious stimuli to anatomical structures of living beings, with pain as the resulting perception. Nociceptive pain alerts the central nervous system of a threatening process going on with its tissues, but does not tell anything about the stimulus itself. For example, perception of pain of a stinging character does not inform of the external stinging object, be it a needle or a honey-bee. By contrast the other five sensory modalities (acoustic, olfactoric, gustatoric, visual, mechanic) inform of any external phenomenon of perception, but in general do not cause any bodily symptoms.

The nociceptive pain system is a protective system as it induces defensive and evasive (nocifensive) reactions and behaviour in response to stimuli that potentially or actually damage the anatomical structures they are impacting (noxious stimuli). Nociceptive pain, thus, serves to maintain body homeostasis. Nociception requires high-threshold receptors (nociceptors) to be excited only by strong stimuli that are capable of causing transient or permanent tissue damage (Handwerker and Kobal 1993; Perl 2007). Hence, nociceptors only become activated by, for example, mechanical energy exceeding a pressure force of 20 mN (Adriaensen et al. 1983; Schmidt et al. 1997; Ziegler et al. 1999; Cain et al. 2001) or thermal energy with temperature exceeding 40–42 C° (Cain et al. 2001) or 46°C (Price et al. 1989). Nociceptor signalling intensity increases with increasing stimulus intensity in a certain range (Andrew and Greenspan 1999a, 1999b; Ziegler et al. 1999; Slugg et al. 2000), and so does perceived pain intensity (Andrew and Greenspan 1999b; Magerl et al. 2001).

Nociceptors not only respond to physical impacts, but also to chemical agents (Roosterman et al. 2006). Furthermore, they become activated by molecules and compounds released from the impacted (compressed, stretched, shrunken, deformed or swollen) cells (Eilers and Schuhmacher 2005). Such compounds are mostly inflammatory factors and mediators like cytokines, bradikinin, histamine, serotonin which themselves excite a nociceptor, or increase its excitability to noxious or innocuous impacts. The same holds true for protons, and neurotrophic factors like nerve growth factors released from the damaged tissues (Schaible 2007; Üçeyler et al. 2009). Hence, not only the intensity of noxious impacts, but also the state of the tissue (e.g. cell destruction, inflammation) is relevant for nociceptor function (Perl 2007; Ringkamp and Meyer 2008). Tissues may release nociceptor-sensitizing substances due to damage from one singular noxious impact of maximum energy, but also in the setting of

multiple subsequent submaximal impacts in short succession without appropriate regenerative pauses (repetitive mechanical stress, see Messing and Kilbom 2001; Brand 2003). Tissue-derived inflammatory factors are responsible for spontaneous ongoing pain after an injury, which persists although the noxious impact causing the injury is no longer active. Tissue-derived substances increasing the excitability of nociceptors are (partly) responsible for the hyperalgesia at the injured site following a trauma (posttraumatic hyperalgesia). In terms of anatomy, nociceptors are naked endings of small fibre afferents, notably of A-delta- and C-fibre afferent neurons. These neurons at their endings arborize into several unmyelinated (termed naked or "free") branches (Handwerker and Kobal 1993). Many of the naked nerve endings (not all) serve as nociceptors. There are two types of nociceptors, A-delta-fibre and C-fibre nociceptors, with different response characteristics and perception qualities (Bigelow et al. 1945). A-delta nociceptors in general have a higher mechanical activation threshold than C-fibre nociceptors (Greenspan and McGillis 1991) and respond more vigorously by higher discharge frequencies (Andrew and Greenspan 1999b). In the plantar skin of the rat, the ratio of A-delta to C-nociceptors is about 1:3 (Leem et al. 1993). Naked nerve endings are most densely distributed in the skin, particularly in the epidermis (termed intraepidermal free nerve endings), and are considerably less in muscle and other deep tissues.

1.2 Hyperalgesia: pathogenic and clinical features

Hyperalgesia is enhanced nociception. Clinically, hyperalgesia corresponds for instance to tenderness (i.e. hypersensitivity to touch and palpation) at an anatomical region that is injured or inflamed. Two types of hyperalgesia may be discerned: primary hyperalgesia, which means enhanced nociception at the point of injury itself, and secondary hyperalgesia, which means enhanced nociception adjacent to the injured site or even at remote tissues. Secondary hyperalgesia is less intensive than primary hyperalgesia (Hardy et al. 1950). Typical examples for hyperalgesia are the tenderness on deep palpation at the lower right abdominal quadrant in case of appendicitis, or the pain elicited by touching or palpating the region of a sprained ankle.

1.2.1 Mechanisms of enhanced nociception: peripheral and central sensitization

Hyperalgesia is consistent with a state of sensitization of the nociceptive system. "Nociception, the detection of noxious stimuli, is a protective process that helps prevent injury by generating both a reflex withdrawal from the stimulus and a sensation so unpleasant that it results in complex behavioural strategies to avoid further contact with such stimuli. An additional important phenomenon that further enhances this protective function is the sensitization of the nociceptive system that occurs after repeated or particularly intense noxious stimuli, so that the threshold for its activation falls and responses to subsequent inputs are amplified." (Latremoliere and Woolf 2009).

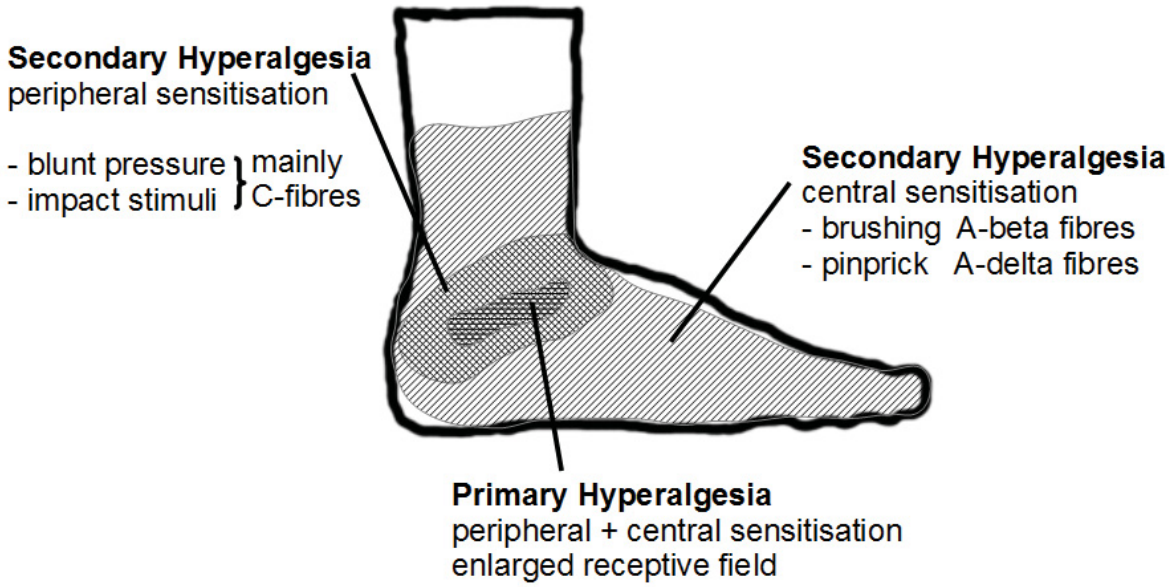

Figure 1. **Primary and secondary hyperalgesia at a sprained ankle.** Primary hyperalgesia (dark grey) is restricted to the area of the direct trauma; hyperalgesia is due to peripheral sensitization and conveyed by afferent C- and A-delta fibres. Secondary hyperalgesia appears in the surrounding tissue; it is due to peripheral plus central sensitization (conveyed by A-delta and C-fibre afferents), or due to central sensitization conveyed by A-delta and A-beta afferents (Kilo et al. 1994; Koltzenburg 2000; Treede et al. 2007).

Sensitization of the nociceptive system can take place at the level of the emitter (peripheral sensitization of the nociceptor) and at the level of the receiver (central sensitization of the nervous system). Peripheral sensitization represents a physiologic hyperexcitability of nociceptors (reduction in threshold and amplification in responsiveness due to inflammatory factors and mediators released from injured tissue), whereas central sensitization represents nociceptive hyperexcitability of the central nervous system. While peripheral sensitization implies that subliminal inputs are recruited in the presence of peripheral stimuli, central

sensitization implies amplification of effects that nociceptive inputs have in the central nervous system "due to an enhanced functional status of neurons and circuits in nociceptive pathways" irrespective of the presence, intensity, or duration of particular peripheral stimuli (Latremoliere and Woolf 2009).

Peripheral and central sensitization combine in posttraumatic hyperalgesia: an increased nociceptor signal response to a given stimulus (more intensive signalling generated in relation to a given stimulus force) and a lowered nociceptor threshold of traumatised structures (onset of signalling at lowered stimulation force) consistent with peripheral sensitization, and enhanced excitability of the central nervous system (consistent with central sensitization).

Burn pain, fracture pain or postoperative pain, are useful animal models of posttraumatic hyperalgesia (Nozaki-Taguchi and Yaksh 1998; Xu and Brennan 2010, 2011). In experiments, postoperative pain is produced for instance by a deep incision into an animal's hindpaw (Xu and Brennan 2010). Nociception is subsequently measured by observation and quantification of evasive behaviour (flinching, guarding), or by quantification of the animals' vocalization in response to painful stimuli. These assessments represent forms of quantitative sensory testing. Suitable techniques for human studies are available in the framework of recently developed psychophysical sensory testing protocols.

1.3 Measuring nociceptive capacity by psychophysical tests

Quantitative sensory testing (QST) is an established, non-invasive, psychophysical method to quantify sensory functions, particularly of the peripheral somatosensory system. This paradigm is comparable to optometry (measuring visual acuity) and audiometry (measuring auditory acuity), which are well established methods in ophthalmology and otology, respectively. QST protocols, amongst others, address perception thresholds and tolerance thresholds of cold and warm stimuli, of thermal pain stimuli, of mechanical stimuli (touch, pressure, vibration), and of mechanical pain stimuli. A perception threshold is defined by the least energy that induces a sensation. A pain perception threshold, for example, "expresses the minimum stimulus inducing pain" (Fischer 1987). In human experiments, pain perception thresholds are likely to vary depending on the instructions the person under study gets for indicating his/her perception of "pain": whether perceiving "a sharp or burning sensation" or "an uncomfortable sensation" should be judged as "painful sensation" (Greenspan 2007). In the present studies, participants were asked to report verbally "when pressure starts to hurt". Perceived pain can have various qualities, for instance can it be "dull" or "aching" or

"burning", or "stinging", "sharp" or "pricking". A sharp pricking or stinging sensation is typical for so-called first pain mediated by A-delta nociceptors, whereas a dull burning pain is typical for C-fibre mediated nociception (Bigelow et al. 1945; Handwerker and Kobal 1993; Greenspan and McGillis 1999; Andrew and Greenspan 1999b; Magerl et al. 2001).

Concerning mechanical pain perception threshold measurements, not only the type of the stimulus (punctate skin stimulation versus blunt deep tissue stimulation) is of relevance, but also the anatomical region and the type and composition of tissues being stimulated (Perl 2007). Moreover, the cooperation of the subject under study, the way the measurements are carried out by the operator, and the expectations of the latter will affect the results (Greenspan 2007). Like all human psychophysical measurements, pain perception measurements rely on the awareness and expectations of the person under study and, hence, are non-objective (Ylinen 2007).

The German Research Network on Neuropathic Pain (DFNS) has established a standardized protocol and reference values for 7 sensory tests measuring 13 parameters. This protocol was originally devised "to characterize the somatosensory phenotype of the patients as precisely as possible" (Rolke et al. 2006). Two of these tests measure pain perception stimulated by two different mechanical stimuli: cutaneous pressure pain perception threshold is measured by calibrated pinprick stimulators, and pressure pain perception threshold of deep tissues is measured by handheld pressure algometer. Both stimuli work by indentation of skin and subdermal tissues (Greenspan and McGillis 1991; Treede et al. 2002; Xiong et al. 2010; Finocchietti et al. 2011). Pressure pain perception threshold is defined as the minimum force required for causing a tissue indentation that elicits a painful sensation (see below).

1.3.1 Published data on experimental pressure nociception thresholds at the feet

How sensitive are feet to mechanical noxious impacts? Previous QST studies focusing on mechanical nociception at the feet of otherwise healthy subjects had measured deep pressure pain perception thresholds at feet during rest (Rolke et al. 2005; Xiong et al. 2010; Xiong et al. 2011), and in traumatised state (posttraumatic hyperalgesia), either after repetitive submaximal traumatisation (Messing and Kilbom 2001) or single casualty trauma (Ramiro-González et al. 2012). Animal experiments have measured offloading induced by experimental injuries, e.g. of a rat's hindpaw (Xu and Brennan 2010), and withdrawal to pinprick stimulation (Nozaki-Taguchi and Yaksh 1998) as equivalents of deep or cutaneous pressure pain perception threshold.

1.3.1.1 Thresholds at resting feet

Rolke et al. have measured DPPPT at hands and feet with a handheld algometer (indentation area 1 cm²), reporting higher thresholds at the feet than at the hands (Rolke et al. 2005). Xiong et al. (2011) measured perception thresholds of deep pressure pain (or deep pressure discomfort) at various locations all over the foot sole in 20 healthy subjects. Using an indentation apparatus with 1cm² contact area they found that thresholds were highest over the metatarsal heads (380–515 kPa) and heel (584 kPa), and lowest over the instep (236 kPa). The data confirm anatomical and tissue differences in relation to percutaneous pressure pain stimulation.

1.3.1.2 Thresholds at feet stressed by repetitive submaximal trauma

Messing and Kilbom (2001) have measured by algometer stimulation (1 cm² indentation area) deep pressure pain perception thresholds at the foot sole of healthy subjects in relation to foot use. 10 kitchen workers (approximately 1500 steps/hour) and 11 white collar employees mostly working in sitting position (approximately 8 steps/hour) were assessed (Messing and Kilbom 2001). Except before starting work, the kitchen workers had always about 50 kPa lower deep pressure pain perception thresholds than the sedentary employees. Moreover, the kitchen workers' deep pressure pain perception threshold had decreased by about 32% (80 kPa) at the end versus the beginning of their work shift, while it decreased by only 2% (15 kPa) in the sitting persons. The data indicate plantar (primary) hyperalgesia in response to heavy walking activity (nearly uninterrupted repetitive submaximal stress). In line with these data, Alfuth and Rosenbaum (2011) have reported reduced cutaneous pressure detection thresholds (measured by Semmes-Weinstein monofilaments) at the foot sole in relation to walking activity.

1.3.1.3 Thresholds at feet stressed by singular maximal trauma

Ramiro-Gonzalez et al. have measured deep tissue pressure pain hypersensitivity at the foot within two days following an acute ankle sprain (posttraumatic hyperalgesia)(Ramiro-González et al. 2012). The cross-sectional study was comprised of 20 patients and 19 control subjects, whose deep pressure pain perception thresholds were examined by pressure algometer (indentation area 1 cm²) over the calcaneofibular ligament, the deltoid ligament, the talofibular ligament, and the medial and lateral malleolus. For comparison, deep pressure pain perception threshold was also measured at the hands. The mean intensity of spontaneous pain

at the sprained ankle was 4.8 on visual analogue scale from 0 to 10. Relative to their hands' thresholds, patients with ankle sprain had 10–15% lower DPPPT at the region of the sprain (consistent with peripheral sensitization), and also at the contralateral foot (consistent with central sensitization). Foot DPPPT was inversely correlated to the intensity of the spontaneous ongoing ankle pain. These data confirm posttraumatic (primary and secondary) hyperalgesia of deep tissues due to peripheral and central sensitization in case of acute ankle sprain.

1.3.1.4 Posttraumatic thresholds: animal studies

In animal experiments, acute foot trauma was produced by cutting with a scalpel deep into the plantar side of a rat hindpaw, thereby damaging skin, fascia and deep muscle tissue. The animals displayed primary mechanical hyperalgesia for about 5–6 days post incision, as well as secondary mechanical hyperalgesia (Xu and Brennan 2010). Thereafter, the reduced mechanical nociception thresholds returned to baseline. Incised deep tissues (fascia and muscle) rather than skin contribute to ongoing spontaneous pain (ongoing nociceptor activity) and evasive behaviour (Xu and Brennan 2010). In other experiments, unilateral mild burn trauma to a rat hindpaw was induced (Nozaki-Taguchi and Yaksh 1998). Subsequently, the pinprick pain perception thresholds decreased at the region of burn (primary hyperalgesia), at the surrounding area (secondary hyperalgesia), and also at the contralateral paw (indicating central sensitization).

In aggregate, these human and animal data clearly provide evidence of physiologic posttraumatic (secondary) hyperalgesia at the feet, documented mostly by a temporary reduction of mechanical (pressure) nociception threshold. Most studies have assessed subdermal deep tissue. The skin, however, was rarely studied. Skin seems to be involved in posttraumatic hyperalgesia only if the subdermal tissue trauma is large enough to extend tissue damage or inflammation to the epidermis. Of note, posttraumatic reduction of pressure nociception thresholds was found either after a single heavy trauma, or after repetitive submaximal traumata of a foot.

1.4 Nociception at the feet of patients with painless diabetic neuropathy

1.4.1 Painless diabetic neuropathy (PLDN): pathogenic and clinical features

PLDN is a condition characterised by a degeneration of small afferent fibres (Javed et al. 2014), namely A-delta- and C-fibres, which transmit mechanoreceptor, thermoreceptor and nociceptor signals to the central nervous system. At the skin level, each of the afferent A-delta and C-neurons divides into multiple branches, the naked ending of each branch serving as receptor for mechanical, thermal or noxious stimuli (see 1.1). There is ample evidence from human and animal studies that the neurons and their branched naked intraepidermal endings diminish in patients with poorly-controlled diabetes mellitus (Kennedy et al. 2005). Diabetes duration of more than 10 years, old age and tall height are further risk factors in human beings. Painless diabetic neuropathy is length-dependent and starts at the anatomical region of the toes; its pathology is typical of a dying-back neuropathy. A comparable condition is the so-called trench-foot, a small-fibre neuropathy due to cold injury (Irwin et al. 1997). The clinical consequences of PLDN are gradually increasing numbness and insensitivity to thermal and mechanical innocuous and noxious stimuli, ascending from the toes to the midfoot, rearfoot and shank/ lower leg. The most important complication of PLDN is the diabetic foot syndrome defined as painlessness of injuries to soft foot tissues and skeleton. Unperceived mechanical injuries are predominant, but also thermal injuries (mostly scalding) may be found, from trivial traumatisation occurring in every-day life. Similar features may be found in patients suffering from leprosy (Sharad et al. 2000; Malaviya 2003; Ooi and Srinivasan 2004; Kennedy et al. 2005; Lund et al. 2007; Wilder-Smith and Van Brakel 2008) or hereditary sensory neuropathy (Kennedy et al. 2005; Axelrod and Simson 2007; Auer-Grumbach 2008). Peripheral neuropathy can also be mimicked by myelopathy, syringomyelia or tabes dorsalis (Baumgärtner et al. 2002; Rogers et al. 2011).

1.4.2 Diabetic foot syndrome- clinical picture

The diabetic foot syndrome is defined as a painless foot injury of any kind in patients with diabetes mellitus. Most common are foot wounds, so-called diabetic foot ulcers; skeletal injuries called neuropathic osteoarthropathy or Charcot-foot are less prevalent. The inciting event is an acute foot trauma without nociception. The trauma may be a skin abrasion, puncture wound, burn from hot water or fire, soft tissue contusion, skeletal contusion, ligament tear or a fracture. The nociceptive withdrawal reflex is missing. Furthermore, there is no appropriate nociception at the feet in response to inflammation of infectious or non-infectious (traumatic) origin. "Patients with diabetes and severe sensorimotor neuropathy can present with a bruised and swollen foot as a result of a bony injury but can remain free from pain and still fully able to bear weight without complaint. Such injuries may have occurred without any clear antecedent history or after apparently trivial trauma. In this scenario, the absence of pain is no reassurance against a bone injury. Walking with ease on a red, hot swollen foot is highly abnormal and a thorough clinical and radiological assessment is essential" (Coll 2009).

1.4.3 Quantitative sensory studies of nociception at the feet of patients with PLDN

Despite the clinically apparent nociceptive deficits outlined above, studies on the nociceptive capacities at the feet in patients with diabetes mellitus are rare. Studies of thermal stimuli unequivocally showed elevated cutaneous perception/detection thresholds for heat pain and cold pain in patients with PLDN, reviewed by Chantelau (2015). Small pilot studies of mechanical stimuli at both feet of patients with chronic (unilateral) diabetic foot syndrome showed elevated thresholds of vibration perception, cutaneous pressure pain perception, and deep pressure pain perception at the feet, as compared to control subjects' feet, and also as compared to the hands of patients and controls alike. While CPPPT was always > 512 mN (the safety limit of measurement), DPPPT was in the normal range in many patients' feet (Chantelau et al. 2012). Neither mechanical traumatic nociception, nor posttraumatic hyperalgesia has ever before been studied in patients with PLDN, by thermal or mechanical experimental stimuli.

2. Aim of the research: quantification of posttraumatic hyperalgesia in painless diabetic neuropathy by measuring pressure pain perception thresholds.

Graven-Nielsen et al. (2012) have rightly emphasised that algometer stimulation versus "the clinical nociceptive origin" may excite "different populations of nociceptors" (Graven-Nielsen et al. 2012). However, in humans -as opposed to animals- there is no appropriate model to evaluate how the nociceptive system of the skin or the skeleton actually works in the event of an acute clinical injury. Therefore, the condition of clinical hyperalgesia was addressed, immediately after an acute foot trauma. Nociception thresholds were measured by experimental mechanical stimuli (pressure) in patients with PLDN and acute foot injury. Pressure stimuli were directed to cutaneous nociceptors, as well as to nociceptors residing in subdermal deep tissues. Measurements focused on secondary posttraumatic hyperalgesia and refrained from measuring primary hyperalgesia –directly at the point of the foot injury. This design was chosen in order to avoid aggravating the injury by strong mechnical stimulation with up to 1400 kPa pressure which may be required when assessing the deep pressure pain perception threshold in patients with painless diabetic neuropathy (Chantelau et al. 2012).

Study 1: This cross-sectional study aimed at measuring perception thresholds of mechanical stimuli (vibration, cutaneous and deep pressure pain) at the hands and feet in otherwise healthy subjects with unilateral acute foot trauma (e.g. sprain), and in patients with PLDN and either uninjured feet or feet with chronic diabetic foot syndrome.

Study 2: This prospective follow-up study aimed at exploring the evolution of perception thresholds of mechanical stimuli (vibration, cutaneous and deep pressure pain) at the hands and feet over one week in otherwise healthy subjects with an acute foot trauma (accidental), and in patients with chronic diabetic foot syndrome and an acute foot trauma (elective bone surgery).

It was hypothesised that pressure pain perception thresholds at the feet in patients with PLDN, and in particular those with diabetic foot syndrome, would fail to display secondary posttraumatic hyperalgesia, whereas control subjects would display reduced pressure pain perception thresholds at the area surrounding an acute foot trauma.

3. Published original articles

Study 1

Wienemann, T., Chantelau, E.A., Richter, A. (2012) Pressure pain perception at the injured foot: the impact of diabetic neuropathy. In: *Journal of Musculoskeletal and Neuronal Interactions* 2012;12:254-261 (Erratum: *Journal of Musculoskeletal and Neuronal Interactions* 2013;13:264)

The study was approved by the Ethics Commission of the Medical Faculty of the Heinrich Heine-University Düsseldorf (project number 3718).

Study 2

Wienemann, T.; Chantelau, E.A.; Koller, A. (2014) Effect of painless diabetic neuropathy on pressure pain hypersensitivity (hyperalgesia) after acute foot trauma. In: *Diabetic Foot Ankle* 5: 24926. doi: 10.3402/dfa.v5.24926.

The study was approved by the Ethics Commissions of the Hannover Medical School (project number 1466-2012), the Medical Faculty of the University Erlangen-Nürnberg (project number 170_12Bc), and the Wilhelms-University Münster (project number 2013-048-b-S).

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3.1 Authors' contributions

EAC conceived the idea and supervised the execution of the studies. TW and EAC designed the studies. TW recruited the participants, did the majority of the measurements and data analyses, and drafted and wrote the papers. EAC critically reviewed the data and the manuscripts. AR and AK participated in the coordination of the studies and contributed helpful comments. EAC is overall guarantor of the entire material.

4. Discussion

4.1. Results of the studies

The studies show for the first time that physiologic posttraumatic hyperalgesia is impaired in feet of patients with painless diabetic neuropathy. Cross-sectional comparison between otherwise healthy subjects with and without an acute foot trauma, and patients with chronic diabetic foot syndrome, showed that acute foot trauma was associated with physiological secondary hyperalgesia in otherwise healthy subjects. In this study group, pressure pain perception thresholds declined temporarily after the trauma compared to subjects without a foot trauma.

Concomitantly, there was spontaneous ongoing foot pain rated 3.5/10 NRS on the day of the trauma, which subsequently declined on day 3 and day 7 after the trauma. This feature is also consistent with posttraumatic hyperalgesia.

A chronic foot injury (ulcer) in patients with diabetic foot syndrome, however, was not associated with low pressure pain perception thresholds, suggestive of impaired posttraumatic hyperalgesia (study 1). Longitudinal observation of the evolution of posttraumatic hyperalgesia in otherwise healthy subjects with an acute trauma to the foot skeleton (e.g. ankle sprain), and in patients with diabetic foot syndrome subjected to elective foot surgery, revealed reduced pressure pain thresholds early after the trauma in both groups. The patients with diabetic foot syndrome, however, had elevated pre-trauma baseline perception thresholds, which after the trauma did not decline to the threshold levels of the control subjects (study 2). Spontaneous ongoing foot pain was absent (rated 0/10 on NRS) before and after the surgical foot trauma, consistent with absence of posttraumatic hyperalgesia in patients with diabetic foot syndrome.

Using a healthy comparable part of a subject as a normal reference has been recommended for evaluation of sensory conditions which affect only parts of the body (Ylinen 2007, Kuni et al. 2015). Or, as Rolke et al. put it: ”intra-individual site-to-site comparisons will be more sensitive than comparison of patient data with absolute normative values.“ (Rolke et al. 2005). Hence, when the present foot thresholds (grossly affected by PLDN in cases of diabetic foot syndrome) were expressed in percent of the hand thresholds (merely unaffected by PLDN), a clear pattern emerged (see Fig. 2a,b).

From the uninjured control subjects in study 1 it may be inferred that in study 2 the baseline pre-trauma DPPPT in the traumatised control subjects at the MTP joint might have been

around 150% of the hands' joint DPPPT, and at musculus abductor hallucis it might have been around 120% of the hands' muscle DPPPT. Based on these figures, an even greater posttraumatic reduction in DPPPTs could be assumed than shown by comparing DPPPTs on the day of the trauma, and the subsequent days 3 and 7. However, it needs to be emphasised that these differences, like most other analyses of the present data, failed to reach statistical significance, which might be due to small sample sizes and patient heterogeneity (see below). Nevertheless the present DPPPTs obtained in the healthy control subjects with acute foot trauma, do fit well with those published earlier by Ramiro-Gonzalez et al. (2012), in fact, they are nearly identical in absolute terms or expressed as hand:foot-ratio. The consistency of both, the earlier and the present, data sets suggests that the present DPPPT measurements are valid, not only in the healthy control subjects but also in the patients with PLDN (Figures 2a, 2b).

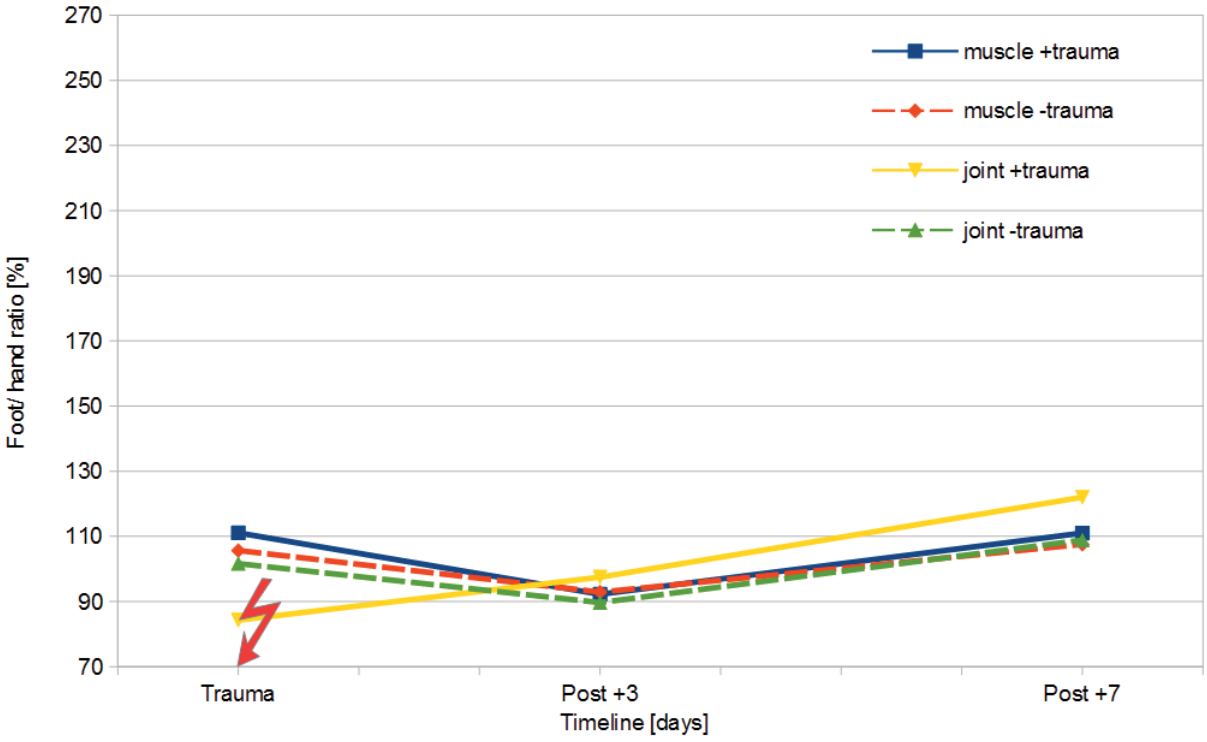

Figure. 2a: **Ratio of hand DPPPTs (average of both hands) to foot DPPPTs in otherwise healthy control subjects (data from study 2)**. Posttraumatic reduction in DPPPT is similar to that observed by Ramiro-Gonzalez et al. (Ramiro-González et al. 2012)

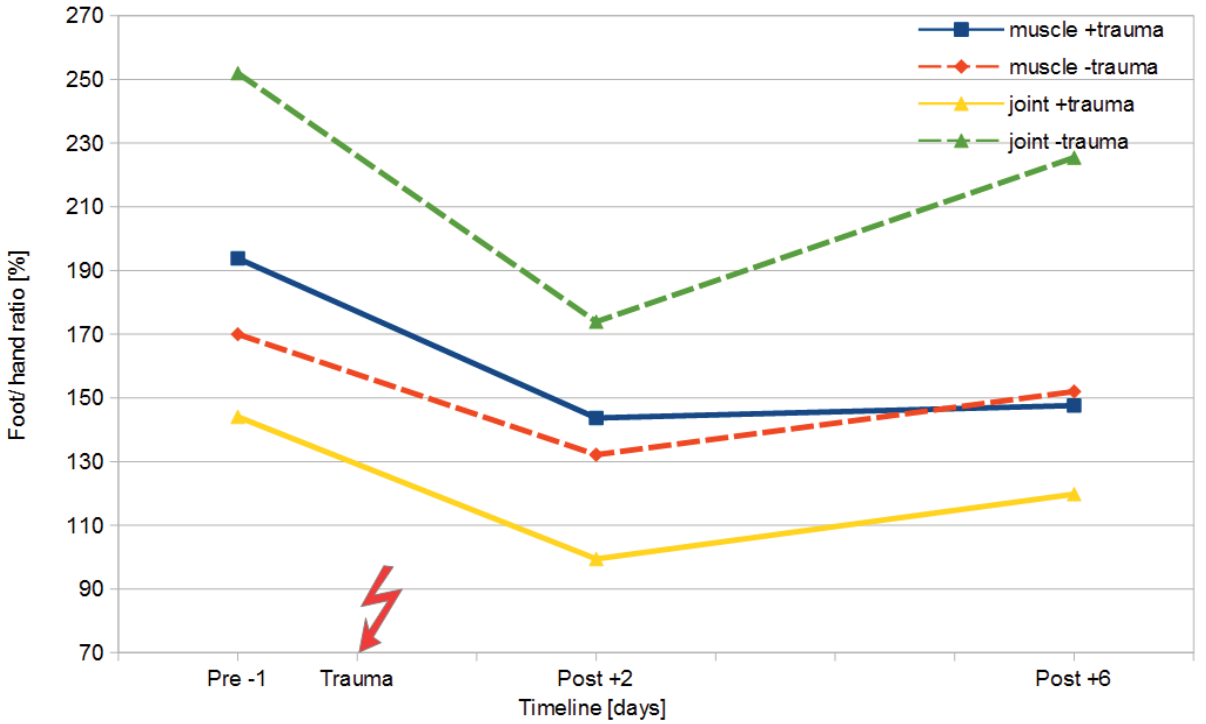

Figure 2b: **Ratio of hand DPPPTs (average of both hands) to foot DPPPTs in patients with diabetic foot syndrome (data from study 2)**. Posttraumatic reduction of DPPPTs is evident.

The findings in the healthy control subjects with acute foot trauma reproduce the normal physiology and, thus, do not need further interpretation. However, the DPPPT data obtained in the patients with diabetic foot syndrome are difficult to interpret. Despite PLDN there was a posttraumatic reduction of DPPPT, however, not to the same nadir as in the control subjects. A conclusion that these PLDN patients experienced nearly-physiologic posttraumatic hyperalgesia would not be valid, since none of them had perceived the foot trauma as painful nor had indicated spontaneous ongoing pain it the affected foot. However, when stimulated by blunt pressure algometer (see below) on an indentation area of 1 cm² at their feet, they indicated considerable pressure pain intensity. Moreover, several patients displayed DPPPT at the feet that were in the range of the control subjects. Nevertheless, patients with diabetic foot syndrome are accustomed to step and even walk on their injured foot without any complaints (Coll 2009).

There are no prior observations in the literature to compare with the present findings, except a single pilot study (Chantelau et al. 2012) showing DPPPT in the normal range in some (not all) patients with diabetic foot syndrome. The present data could suggest that the reduction in DPPPT following a foot trauma in the patients with diabetic foot syndrome was not enough to be clinically meaningful –the reduction did not reach the nadir of the healthy control subjects.

This would imply that posttraumatic hyperalgesia would become clinically relevant only at a certain (absolute ? relative ?) nadir of perception thresholds. An alternative explanation could question the concept of deep tissue pressure “pain” as stimulated by the pressure algometer. The present findings would support a contention that deep pressure “pain” is merely “pressure discomfort” rather than pain, because the pressure algometer likely stimulates low-threshold mechanoreceptors and not high-threshold nociceptors in the framework of spatial summation. These issues are discussed below.

CPPPT in the control subjects with acute foot trauma was lower than in the uninjured control subjects (study 1). This difference was confirmed by the foot: hand ratio of CPPPT. In study 2, only the foot: hand ratio showed a slightly reduced posttraumatic CPPPT in healthy control subjects on the day of their acute foot trauma, again at the injured and the contralateral foot.
The cross-sectional and longitudinal observations of skin nociception are consistent with physiologic secondary pinprick hyperalgesia due to central sensitization in the healthy controls (Treede et al. 1992). The data is consistent with previous studies of experimental skin hyperalgesia in humans using cutaneous capsaicin (LaMotte et al. 1991; Kilo et al. 1994), freeze lesion (Kilo et al. 1994) or electrical stimulation (Klede et al. 2003) models, showing secondary hyperalgesia to pinprick but not to blunt cutaneous stimulation (LaMotte et al. 1991; Kilo et al. 1994; Treede et al. 2002). However, in the present patients with diabetic foot syndrome, pre-and post-trauma CPPPTs were elevated and remained unchanged, indicating the absence of secondary prinprick hyperalgesia (and of central sensitization), which is consistent with PLDN.

The study also suggested that in patients with diabetic foot syndrome baseline DPPPTs might be higher at the metatarsophalangeal joint (forefoot) than at the instep, implying a distal-to-proximal gradient of pressure pain sensitivity. Such a gradient, which was less obvious in control subjects, would be consistent with the distal-to-proximal distribution of painless diabetic neuropathy. On the other hand, the gradient could be tissue-specific rather than site-specific as the instep deep tissues comprise mostly muscle, whereas the forefoot tissues comprise fascia, etc. (see below). It would be interesting to see whether such a distal-to-proximal difference in PLDN severity would be apparent also for CPPPT (assuming identical skin composition at the plantar forefoot and midfoot). A distal-to-proximal gradient of

DPPPT between foot and hand thresholds in healthy subjects has previously been reported by Rolke et al. (2005); however a distal-to-proximal gradient in DPPPT has not as yet been reported for a small anatomical region like the foot.

Vibration perception threshold (VPT), representing non-nociceptive A-beta-afferent function, was not altered by the foot trauma in every study group (with and without PLDN) indicating that neither peripheral nor central sensitization of A-beta-afferent mediated perceptions was induced by the acute foot trauma.

4.2 Methodological issues

The present data needs to be considered with great caution because of certain methodological shortcomings related to: sample size and study participants, methods of stimulation, spatial summation of sensory input.

4.2.1 Patients and sample size

In total, 73 subjects were enrolled in both studies. They were 30 consecutive patients with chronic diabetic foot syndrome (12 of whom with acute surgical trauma), 23 otherwise healthy subjects with acute trauma of the foot skeleton (e.g. sprain) and 20 healthy control subjects. The altogether five study groups were small, and heterogeneous in age and gender, due to limited facilities and constraints of study design. Age, height, gender, type of diabetes, duration of diabetes, co-morbidities, may all affect QST measurements at the feet, but could not be accounted for in the analyses. Hence, the precision of the group data was probably low. According to previous statistical power calculations (Brennum et al. 1989; Staahl et al. 2006), group sizes of about 50 subjects are necessary for showing a DPPPT difference of 20% with an alpha of 0.05 in a parallel study design.

4.2.2 Mechanics of pressure pain perception stimulation

Pressure pain perception was stimulated in two different anatomical structures (skin versus subdermal tissues) with two different QST instruments working, however, by the same physical principle of percutaneous tissue indentation. Punctuated application of pressure on a surface of <1 mm² seems to stimulate only epidermal mechano-receptors and nociceptors, whereas pressure applied to a larger surface predominantly stimulates perceptions from subdermal tissues (Takahashi et al. 2005). The depth of indentation depends on the

compliance of the tissues and the underlying structures. For instance, at the dorsal side of a finger or toe, indentation of 1 mm or less may be possible, whereas at the palmar or plantar side, at a tip of a finger or toe, skin and subdermal tissues may be indented by several millimetres (Greenspan and McGillis 1991; Xiong et al. 2011).

Another important issue when measuring perception of pressure or of pressure pain is spatial summation. Spatial summation is the psychophysical phenomenon of decreasing perception threshold with increasing area of stimulation. The underlying physiologic mechanism includes co-stimulation of multiple identical receptors (e.g. mechano-receptors or nociceptors) at a circumscript anatomical region, multiple sensory endings of the same neuron, or sensory endings of adjacent receptive field of other neurons. Concomitant simultaneous stimulation induces a multitude of identical signals which cumulate in an increased signal reaching the CNS. As a consequence, the perception (pain, pressure) is more intense. Thereby, the perception threshold decreases: the more receptors become activated simultaneously, the less individual nociceptor activation is required to achieve a percept in the CNS. “Single impulses induced in C-mechano-heat-receptors often do not induce any sensation indicating that a central nervous threshold that requires temporal and/or spatial summation has to be exceeded” (Handwerker 2007). Hence, spatial summation causes pinprick-stimulated CPPPTs to decline consistently with increasing probe size, even with probe sizes as small as 0.5 to 0.01 mm² (Greenspan and McGillis 1991). With probe sizes of up to 2 cm², as applied by algometer stimulation of deep tissues, spatial summation is also evident (Defrin et al. 2003; Nie et al. 2009; Andresen et al. 2013).

Most essential for the observation of spatial summation of stimulus input is that the stimulus intensity is reported in terms of force per space (area). Regarding mechanical stimulation, stimulus intensity must be reported as force (e.g. N) per unit area (e.g. cm²), rather than as force per radius or circumference of a stimulator probe (Greenspan and McGillis 1991; Greenspan et al. 1997).

4.2.2.1 Cutaneous pressure pain stimulation

In the present studies, cutaneous pressure pain was stimulated by punctuate stimulators applying calibrated pressure forces on a tiny contact area of <1 mm². Punctuate stimulators are thin filaments or rods of various bending forces or weight. When pressed vertically onto the skin, the cutis and underlying tissues are indented to various depths, according to the force/weight of the stimulator and/or the compliance (softness) of the skin. Punctuate stimulators activate nociceptors and mechanoreceptors located in the epidermis, in particular intraepidermal A-delta- and C-fibre endings, and probably also epidermal Merkel discs attached to the endings of A-beta fibres. However, when the epidermal A-delta and C-fibre nociceptors are blocked by topical lidocaine ointment, touch and dull pressure pain may still be stimulated in the subdermal deep tissues to a depth of around 10 mm (Graven-Nielsen et al. 2004). The latter corresponds to the finding that pinching a foot skinfold with blunt forceps on a stimulation area of 16 mm², revealed a similar pressure pain perception threshold in healthy control subjects, and in patients with diabetic foot syndrome, whose epidermis presumably was completely devoid of nociceptors (Le Quesne and Fowler 1986). Although the pain quality was not measured, it may have been dull or burning (consistent with C-fibre nociception) rather than stinging or sharp (consistent with A-delta fibre nociception), since Greenspan and McGillis (Greenspan and McGillis 1991) have reported that punctuate pressure probe sizes > 0.5 mm² evoke a pain percept that is “not sharp pain”. Likewise, Defrin et al. (2003), have reported that pressure pain induced by a small algometer stimulus area perceived as a prick”, while a larger blunt stimulus area induced a perception of “pressure”.

In the present studies, punctuate fibre glass pinpricks were applied to a palmar and a plantar skinfold of a finger and toe, respectively, to measure CPPPT. Punctuate stimulators rarely stimulate a singular epidermal mechanoreceptor or nociceptor. Hence, a single intraepidermal nociceptor might have been stimulated at the terminal of an A-delta or C-fibre afferent, or two or three of them. Also, singular A-beta fibre endings may have been co-stimulated (Baumgärtner et al. 2012). The resulting stimulated perception may, thus, have consisted of at least three components: stinging pain (the predominating percept, from stimulation of an A-delta fibre nociceptor), dull burning pain (less intense, from stimulation of a C-fibre nociceptor) and pressure (from stimulation of a C-fibre mechanoreceptor and/or Merkel cells connected to an A-beta fibre), as has been shown from experiments selectively blocking A-delta- fibres, or A-delta plus A-beta fibres. Neither of these perceptions could be elicited in

the patients with diabetic foot syndrome in the present studies. Even the strongest pinprick stimulator (521 mN) causing the deepest indentation was unable to elicit a pain sensation in the present patients with severe PLDN. This finding corroborates data on pinprick stimulation reported by Tjon-A-Tsien et al. (1995) in patients with diabetic foot syndrome.

Taken together, these lines of evidence seem to indicate that punctuate versus blunt pressure stimulation of skin does not excite the same types of receptors, and hence does not evoke perceptions of the same character.

4.2.2.2 Subdermal tissue pressure pain stimulation

Commonly, subdermal tissues are exposed to blunt mechanical pain stimulation by percutaneously applied pressure from handheld algometers. Handheld algometers are equipped with a cylindrical probe to apply pressure of various forces onto contact areas of 0.5–2 cm², thereby indenting the skin and underlying soft tissues to various depths, e.g. at the instep up to 20 mm (Xiong et al. 2010). The standard version is a probe tip with a flat stimulation area of 1 cm². The larger the pressure area, the greater the volume of tissue (and the number of its nociceptors and mechano-receptors) stimulated, and the spatial summation of receptor signals. The deeper the indentation, the greater is the amount of skin at the edges of the probe that is strained during the indentation process. Hence, the shape of the probe (whether its edges are sharp or rounded) and the speed of the indentation process affect the stimulated percept (Finocchietti et al. 2011; Xiong et al. 2011), features that are well known from manual palpation.

Handheld algometer stimulation elicits a pain quality that is dull, deep, aching or cramping, over muscle (Mense 2004). In muscle, A-delta fibre nociceptors obviously do not elicit a separate stinging (pinprick) pain sensation (Mense 2004); hence, the predominating pain percept elicited by external pressure stimulation of muscle is like C-fibre nociception. In muscles, as opposed to skin, the innervation density with nociceptors is low.

In deep tissues, a handheld algometer stimulates a mixed population of receptors for innocuous and noxious pressure and touch, presumably C-fibre endings, together with A-delta-fibre endings and corpuscles attached to A-beta-fibre endings. Due to the relatively large stimulation areas, spatial summation is considerable. As has been noted previously, spatial summation is considerably greater for pressure sensation threshold than for pressure pain threshold, “suggesting that slowly adapting mechanoreceptor input is subject to greater spatial summation centrally than that of nociceptors” (Greenspan et al. 1997).

Low-threshold mechanoreceptors are usually found in vascularized and soft connective tissues, whereas high-threshold mechano-nociceptors are most frequently located in dense connective tissues (ligaments, tendons, collagenous layers of the articular capsule), as noted by Messlinger (2007). In the present studies, various vascularized and soft connective tissues were co-compressed when the thenar, the instep, or a metatarsophalangeal joint was targeted by the algometer stimulus (from outer to inner): glabrous skin, fat tissue, musculus abductor pollicis brevis, musculus opponens pollicis, arteria princeps pollicis (*thenar*); glabrous skin, fat tissue, plantar fascia, musculus abductor hallucis, nervus, arteria and vena plantaris medialis (*instep*); glabrous skin, fat tissue, ligamentum transversum superficiale et profundum of the fascia plantaris, fasciculi longitudinales of the aponeurosis plantaris, tendons and tendon sheaths of musculus flexorum digitorum brevis, arteriae, venae and nervi digitales plantares communes, joint capsule (*metatarsophalangeal joint*). Accordingly, algometer pressure stimulation in the present studies likely excited deep sited mechanoreceptors rather than nociceptors.

In aggregate, these features suggest that algometer stimulated deep tissue pressure pain likely represents a perception of “strong pressure discomfort” evoked by stimulation of low-threshold mechanoreceptors rather than a perception of “pain” as evoked by high-threshold nociceptors located inside the in dense connective tissues (ligaments, tendons, collagenous layers of the articular capsule).

4.3 Limitations of the data

First, the sample size was rather small, due to the well-known large inter-individual variability, a minimum of 50 subjects is usually necessary to demonstrate clinically relevant differences (Brennum et al. 1989; Staahl et al. 2006). Second, the study groups were rather heterogeneous in terms of age and gender –both parameters affect QST measurements. Third, the stimuli were presented only once per site (instead of 3 per site as in Rolke et al. 2005) in order not to overstress tissues in patients with PLDN. Fourth, measurements were taken at predefined locations (CPPPT at a toe skinfold, DPPPT at a metatarsal head and instep) irrespective of the localisation of the acute skeletal injury, which means that the measurements were not always taken in the area of the most pronounced secondary hyperalgesia, i.e. in the vicinity of the injury. These shortcomings limited the precision of the measurements.

5. Conclusions and potential clinical implications

Pending confirmation by larger studies, the present data suggest that secondary hyperalgesia after a skeletal trauma of the foot is impaired in patients with PLDN and diabetic foot syndrome. The clinical implications are evident, for example regarding the management of foot traumata in patients with PLDN. The clinical decision rules for assessing the severity of an ankle sprain (Mayer 2009) or an ankle fracture (Goost et al. 2014) are based on normal nociception at the feet; these rules are unreliable in patients with PLDN (Coll 2009). According to the present data, a clinical assessment of the nociception function of the feet, e.g. by testing a plantar digital skinfold with a 512 mN monofilament, is a precondition for the application of these rules. In case of PLDN, these rules must not be applied; instead, these patients should undergo imaging appropriate studies to judge the severity of their foot injury (Bancroft et al. 2015). Surgical site infection prevention (Centers of Disease Control and Prevention 2015) at feet has to be adapted to PLDN accordingly: more frequent postoperative wound inspections are required in patients with PLDN as compared to subjects who themselves will alert their physicians because they can feel postoperative wound pain.

Moreover, protected weight bearing as advised after a fracture or surgery of a leg/foot etc. cannot be granted in patients with PLDN because of the sensory deficit.

6. Perspectives for future research

A larger study is needed to extend the data and demonstrate any differences in the responses to noxious stimuli that may exist. The impact of methodological intricacies has to be addressed in future research. Progressive testing may affect the results as Jones et al. (2007) have shown that DPPPT declines temporarily over three successive days and increases to baseline value on day four.

Quantitative sensory testing, as a psychological method, may also be influenced by the sequence of the measurements (Gröne et al. 2012). A changed testing order should help to elucidate the effect on the overall measurements. A detailed investigation has to be made to check which structures and nerves are triggered by the algometer. Furthermore the role of spatial summation in the assessment of DPPPT has to be addressed in detail, for example by the use of Algometer stimulation areas of varing sizes. Once the data are verified and an adequate precision is guaranteed the knowledge can be tranferred into clinical practice, assisting patients with PLDN and their physicians to prevent foot ulcerations and establish a better quality of life.

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