Spinal Inhibitory Dysfunction in Patients With Painful or Painless Diabetic Neuropathy

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OBJECTIVE

Impaired rate-dependent depression of the Hoffman reflex (HRDD) is a marker of spinal inhibitory dysfunction and has previously been associated with painful neuropathy in a proof-of-concept study in patients with type 1 diabetes. We have now undertaken an assessment of HRDD in patients with type 1 or type 2 diabetes.

RESEARCH DESIGN AND METHODS

A total of 148 participants, including 34 healthy control subjects, 42 patients with painful diabetic neuropathy, and 62 patients with diabetic neuropathy without pain, underwent an assessment of HRDD and a detailed assessment of peripheral neuropathy, including nerve conduction studies, corneal confocal microscopy, and thermal threshold testing.

RESULTS

Compared with healthy control subjects ($P < 0.001$) and patients without pain ($P < 0.001$), we found that HRDD is impaired in patients with type 1 or type 2 diabetes with neuropathic pain. These impairments are unrelated to diabetes type and the presence or severity of neuropathy. In contrast, patients without neuropathic pain ($P < 0.05$) exhibited enhanced HRDD compared with control subjects.

CONCLUSIONS

We suggest that loss or impairment of HRDD may help to identify a subpopulation of patients with painful diabetic neuropathy mediated by impaired spinal inhibitory systems who may respond optimally to therapies that target spinal or supraspinal mechanisms. Enhanced RDD in patients without pain may reflect engagement of spinal pain–suppressing mechanisms.

Painful diabetic peripheral neuropathy (DPN) can affect up to one-third of people with diabetes and results in somnipathy, depression, and a poor health-related quality of life (1–3). The majority of patients achieve limited pain relief with considerable side effects when using first-line antineuropathic pain medication at maximum tolerated doses or in combination (4). There has been a resurgence of interest in identifying new drug targets or predictive biomarkers for specific pain mechanisms that may be more effectively targeted using existing therapies (5–7). One potential mechanism is spinal disinhibition, where decreased tonic spinal inhibitory processes result in lack of suppression or even amplification of painful and nonpainful peripheral signaling (8).

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PATHOPHYSIOLOGY/COMPLICATIONS

In rodent models of type 1 diabetes, behavioral indices of neuropathic pain are driven by spinal disinhibition (9,10). Despite impaired inhibition/increased excitability in the dorsal spinal cord, diabetic rats exhibiting tactile allodynia and exaggerated hyperalgesia show an increase in both basal and evoked spinal levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (11). It has been suggested that this paradoxical finding reflects a switch of GABAA receptor function so that it is no longer inhibitory and becomes pronociceptive (9). In support of this, the potassium/chloride cotransporter KCC2, which is critical in determining intracellular chloride levels and the direction of ion flow through the ionotropic GABAA receptor, is reduced in the dorsal spinal cord of diabetic rats (9,10,12).

A biomarker of altered spinal inhibition is rate-dependent depression of the Hoffmann reflex (HRDD) (13). The stimulation protocol for the Hoffmann reflex (H-reflex) evokes two waveforms: a direct nerve to muscle M-wave and the longer latency, trans-spinally mediated H-wave. Originally believed to represent a purely monosynaptic trans-spinal reflex, it is now accepted that the H-reflex arc is modulated by oligosynaptic connections (13–15). The H-reflex has several properties that can be used to evaluate spinal function. HRDD is the measure of the change in amplitude of the H-reflex component over consecutive stimulations and can be measured noninvasively in humans using a modification of standard nerve conduction studies. Loss of inhibitory function in the spinal cord results in reduced depression of the H-reflex amplitude during successive stimulations. For example, the impairment of HRDD has been used as a marker of disinhibition of spinal sensory processing caused by spinal cord injury in both animals and humans (16–18) and has been linked to loss of GABAergic inhibition (16,19).

Accumulating preclinical evidence in animal models of type 1 diabetes has demonstrated that behavioral indices of painful neuropathy arising from loss of spinal GABAergic inhibitory function are associated with a loss of HRDD (9,10). The potential of these findings to translate to the clinical population was indicated in our exploratory study in patients with type 1 diabetes in which we identified impaired HRDD in approximately one-half of patients with painful diabetic neuropathy (12). While preclinical studies in a rat model of type 2 diabetes have identified loss of HRDD (12), it is not known whether this extends to patients with type 2 diabetes, who represent the majority of people with painful diabetic neuropathy (20). We have therefore assessed HRDD in conjunction with detailed peripheral structural and functional phenotyping of neuropathy in a large cohort of patients with type 1 or type 2 diabetes with and without neuropathic pain.

RESEARCH DESIGN AND METHODS

Research ethics committee approval was granted (East Midlands–Leicester South Research Ethics Committee reference 17/EM/0076), and written informed consent was obtained from each participant. Study conduct adhered to the tenets of the Declaration of Helsinki.

Study Participants

Patients with type 1 diabetes (n = 47), patients with type 2 diabetes (n = 81), and control subjects (n = 36) underwent nerve conduction studies and assessment of HRDD. The H-reflex was absent in 24 patients, 10 with type 1 and 14 with type 2 diabetes, as well as in 2 control subjects, and these subjects were excluded from the study (Fig. 1). Participants underwent further assessment of neuropathic symptoms and signs, thermal threshold testing, and corneal confocal microscopy (CCM). Detailed demographic data, medical history, current medications, age, sex, ethnicity, type and duration of diabetes, comorbidities, height, weight, blood pressure, HbA_{1c}, lipids, and renal function were documented. Other common causes of neuropathy were excluded on the basis of family history as well as testing for serum B_{12}, folate, immunoglobulins, electrophoresis, and antinuclear antibody. The modified Toronto Diabetic Neuropathy Expert Group recommendations (21) were used to allow for the inclusion of small-fiber abnormalities (22) to diagnose DPN on the basis of the presence of symptoms and signs and an abnormality in either nerve conduction studies or CCM. The Neuropathy Symptom Profile (NSP) questionnaire and Visual Analog Scale (VAS) pain scores recording current, average, and maximum pain ratings over the previous 24 h were documented. Patients were stratified into painful (DPN+) and painless (DPN−) cohorts on the basis of the Toronto consensus that "the symptoms are distal, symmetrical, often associated with nocturnal exacerbations, and commonly described as prickling, deep aching, sharp, like an electric shock, and burning with hyperalgesia" (21) for >3 months, which is consistent with the requirement of pain chronicity defined by the International Association for the Study of Pain (23). All patients with a current, average, or maximum VAS pain score >0 were placed in the pain cohort. The pain group was further subdivided into mild (VAS <4) and moderate/severe (VAS 4–10) (Fig. 1*A*) (24).

Experimental Procedures

Vibration and Thermal Detection

Vibration detection threshold (VDT) was evaluated using a Rydel Seiffer 64-Hz tuning fork with fixed weights on the first metatarsophalangeal joint of the right foot. The vibration amplitude of the tuning fork at the point of sensation loss is used to assess VDT. The scale reading moves from 0 to 8, exponentially with decreasing vibration amplitude, and a low value indicates a loss of vibration sensation at high-vibration amplitude. Cold detection threshold (CDT) and warm detection threshold (WDT) were recorded on the dorsum of the right foot using a TSA 2 NeuroSensory Analyzer (Medoc, Ltd., Ramat-Yishai, Israel).

CCM

CCM using a laser scanning Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering, Heidelberg, Germany) was undertaken in both eyes using an established protocol (25). Corneal nerve fiber density (CNFD) (total number of main nerves/mm^2), corneal nerve fiber length (CNFL) (total length in mm of main nerves and nerve branches/mm^2), and corneal nerve branch density (CNBD) (total number of branches/mm^2) were quantified.

Nerve Conduction and H-Reflex Studies

Nerve conduction and H-reflex studies were performed using a Dantec Keypoint system (Dantec Dynamics Ltd., Bristol, U.K.). Participants sat semirecumbent at 45° with limb temperature maintained between 32 and 35°C. Sural sensory

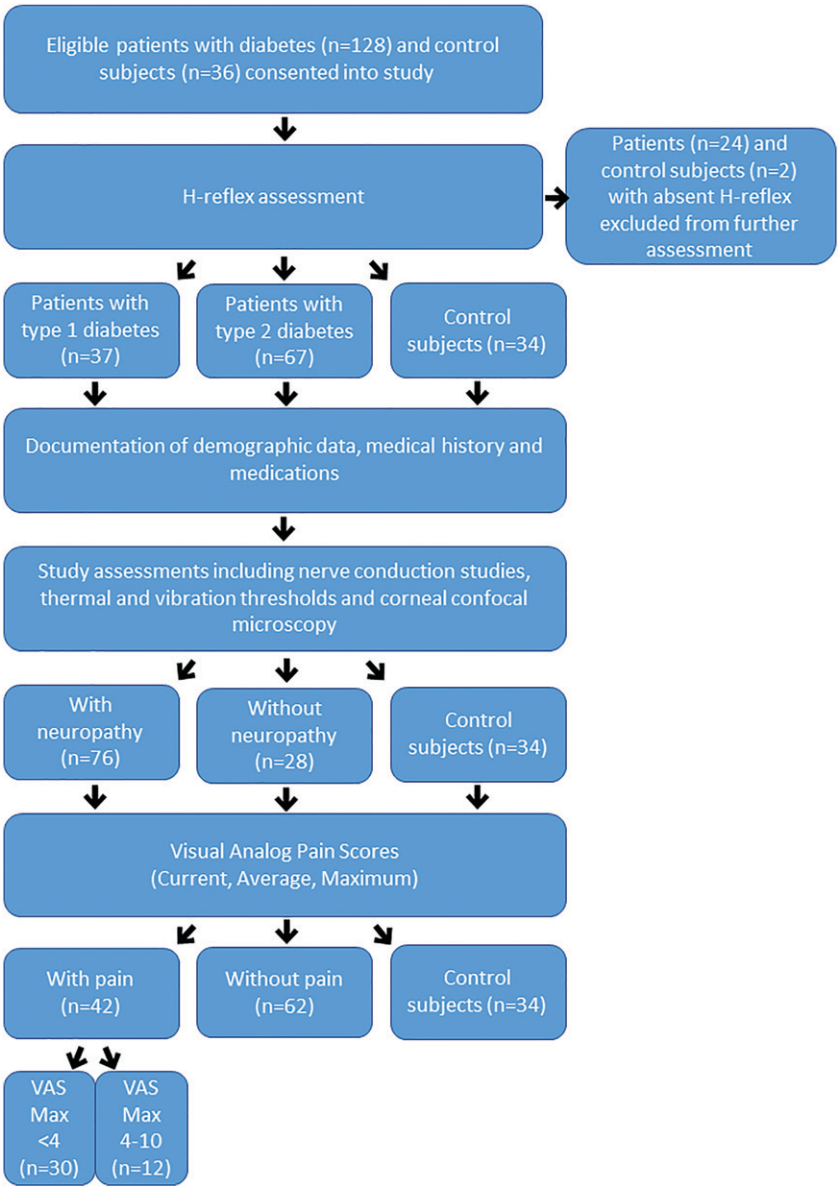

Figure 1—Study flow diagram. Max, maximum.

amplitude (SNAP) and conduction velocity (SNCV) along with peroneal motor nerve amplitude (PMNAP) and conduction velocity (PMNCV) were recorded. For H-reflex studies, tibial nerve stimulation was performed using 1-ms monophasic square-wave pulses delivered using surface silver-silver chloride electrodes to the popliteal fossa. Surface silver-silver chloride recording electrodes with a diameter of 9 mm were placed on the long axis of the soleus. H-reflex recruitment curves were obtained to determine peak-peak H-reflex maximal amplitude by incrementing the stimulation current by 1 mA. A minimal interstimulation interval of 10 s was observed. For HRDD, a submaximal stimulus strength (to achieve a response of 75% of the maximum) was used. The HRDD measurement consists of H-wave responses to trains of 10 stimuli delivered at 1 Hz. HRDD was calculated as the mean of responses 2–10 (meanH2–10) of a 1-Hz stimulus train, expressed as a percentage of response number H1. Therefore, a higher value of HRDD indicates a smaller degree of depression than a lower value and vice versa. The meanH2–10 was used to mitigate against random and time course fluctuations.

Statistical Methods

Statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA) and SPSS version 27.0 for Windows (IBM Corporation, Armonk, NY) statistical software. Parametric data were analyzed using unpaired *t* test or one-way ANOVA followed by Tukey multiple comparison for means between groups. Nonparametric data were analyzed using the Kruskal-Wallis test followed by Dunn post hoc test for multiple comparisons. ANCOVA (post hoc least squares difference) was used to compare variables between groups while statistically controlling for the effects of age. Correlations were calculated using Spearman rank test and expressed as a coefficient (*r*) with significance level. $P < 0.05$ was considered biologically relevant. Data sets are available from the corresponding author upon request.

RESULTS

Patients With Type 1 and Type 2 Diabetes Have Comparable Degrees of HRDD

Patients with type 1 diabetes were significantly younger ($P < 0.001$), had a longer duration of diabetes ($P = 0.033$), and a lower BMI ($P = 0.0029$) compared with patients with type 2 diabetes. However, HbA_{1c}, and measures of both large- and small-fiber neuropathy did not differ significantly between patients with type 1 or type 2 diabetes (Table 1). There was no significant difference in group mean HRDD between patients with type 1 or type 2 diabetes or healthy control subjects (Fig. 2*A*) and between patients with and without diabetic neuropathy (Fig. 2*B*).

Patients With Painful and Painless Diabetic Neuropathy Have Abnormal HRDD

Demographics and clinical characteristics are summarized in Table 2. Patients with diabetes were significantly older than control subjects and had significantly higher BMI and HbA_{1c}. Nerve conduction study, CDT, WDT, VDT, and CCM parameters were significantly impaired in patients with diabetes compared with control subjects. There was no significant difference in demographic or neuropathy parameters between patients with or without painful diabetic neuropathy. However, HRDD was significantly impaired in patients with painful diabetic neuropathy compared with patients without painful diabetic neuropathy ($P \leq 0.001$) and control subjects ($P < 0.001$). In contrast, HRDD

Table 1—Demographic and neuropathy parameters for patients with type 1 and type 2 diabetes and control subjects

	Patients with type 1 diabetes (n = 37)	Patients with type 2 diabetes (n = 67)	Control subjects (n = 34)
Sex			
Female	12	24	20
Male	25	43	14
Ethnicity			
White	34	44	25
Asian	2	19	7
Black	1	4	2
Age (years)	51.0 (42.5–65)++++	66.0 (60–73.75)****	46.5 (31–55)
Duration of diabetes (years)	17.0 (7.5–34.5)+	15.0 (7.25–19)	
HbA_{1c} (%)	7.2 (6.7–8.4)	7.3 (6.6–8.1)	5.4 (4.9–5.7)
HbA_{1c} (mmol/mol)	55.5 (49.25–68)****	56.0 (49–64.5)****	35.0 (30.5–38.75)
BMI (kg/m^2)	25 (23–31.66)++	29.2 (26.5–34.08)****	23.8 (22.5–25.4)
VAS current	20 (8–46.5)		
VAS average 24 h	35.5 (15.8–64.5)		
VAS worst 24 h	56 (30–80)		
SNAP (μV)	9.1 (4.9–16)**	6.5 (3.6–12)****	17.0 (15–22)
SNCV (m/s)	41.2 (40–46.7)****	43.8 (40–46.7)***	48.3 (45.2–51.9)
PMNAP (mV)	4.2 (2.25–6.3)	3.3 (2.25–4.9)**	4.9 (3.4–7.5)
PMNCV (m/s)	41.3 (38–44.5)****	41.4 (38.4–43.7)****	47.5 (43.4–50)
CDT (°C)	27.8 (23.3–29.6)***	28.0 (24.1–29.9)**	29.8 (28.5–30.5)
WDT (°C)	40.3 (36.4–46.3)**	40.0 (37.7–43.6)***	36.3 (34.8–39.3)
VDT (0–8)	7 (4–8)**	7 (5.3–8)*	8 (6.6–8)
CNFD (n/mm^2)^	25.73 ± 1.44**	24.23 ± 1.05***	32.36 ± 1.59
CNFL (mm/mm^2)^	18.62 ± 1.24**	18.72 ± 0.91**	25.11 ± 1.35
CNBD (n/mm^2)^	51.02 ± 5.45	47.58 ± 4.14	59.74 ± 6.85
RDD meanH2–10 at 1 Hz^	40.51 ± 3.46	48.79 ± 2.78	41.66 ± 3.92

Data are n, median (interquartile range) (nonparametric, Mann-Whitney or Kruskal-Wallis test with Dunn post hoc test), or mean ± SE (parametric). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ compared with control subjects. +$P < 0.05$, ++$P < 0.01$, ++++$P < 0.0001$ compared with patients with type 2 diabetes. ^ANCOVA adjusted for age (post hoc least squares difference).

was significantly exaggerated in patients with diabetes without pain compared with control subjects ($P < 0.05$) (Fig. 2*C* and 2*D*). There was no significant difference in HRDD between patients with mild and moderate to severe neuropathic pain. There was no significant difference in HRDD between female and male patients with or without pain or between female and male control subjects.

Correlations

There was no significant correlation across the whole patient cohort, or across the pain or no pain groups individually, between HRDD and either age, duration of diabetes, BMI, HbA_{1c}, or any of the measures of peripheral neuropathy. There was no significant correlation across the control cohort between HRDD and either age or BMI. Within the group of patients with painful diabetic neuropathy, there was no correlation between HRDD and VAS pain scores.

CONCLUSIONS

We previously demonstrated impaired HRDD in a group of patients with type 1 diabetes and painful neuropathy (12). The current study now extends this finding to a larger group of patients with type 1 diabetes and type 2 diabetes. HRDD was significantly impaired in patients with painful diabetic neuropathy compared with patients without pain and control subjects. The impairment was not related to the presence and severity of diabetic neuropathy. A further novel finding was that HRDD was enhanced in patients without painful diabetic neuropathy compared with control subjects.

Previous studies have shown differences in thermal thresholds (26), and we have shown greater corneal nerve loss (27) in patients with painful diabetic neuropathy. In the current study, there were no significant differences in any of the markers of altered small-fiber function or structure between patients with and without painful neuropathy. This may relate, in part, to the relatively small number of patients studied or reflect their mild to moderate severity of neuropathy. It is well documented that H-reflex amplitudes attenuate with increasing severity of diabetic neuropathy (28,29) and indeed are typically absent in patients with severe neuropathy (29). Patients without an H-reflex were not included in the study, resulting in a cohort of patients with predominantly mild or moderate neuropathy.

In our exploratory study of a small group of patients with type 1 diabetes, the deficits in HRDD showed a correlation with pain ratings such that greater impairment of spinal inhibitory function

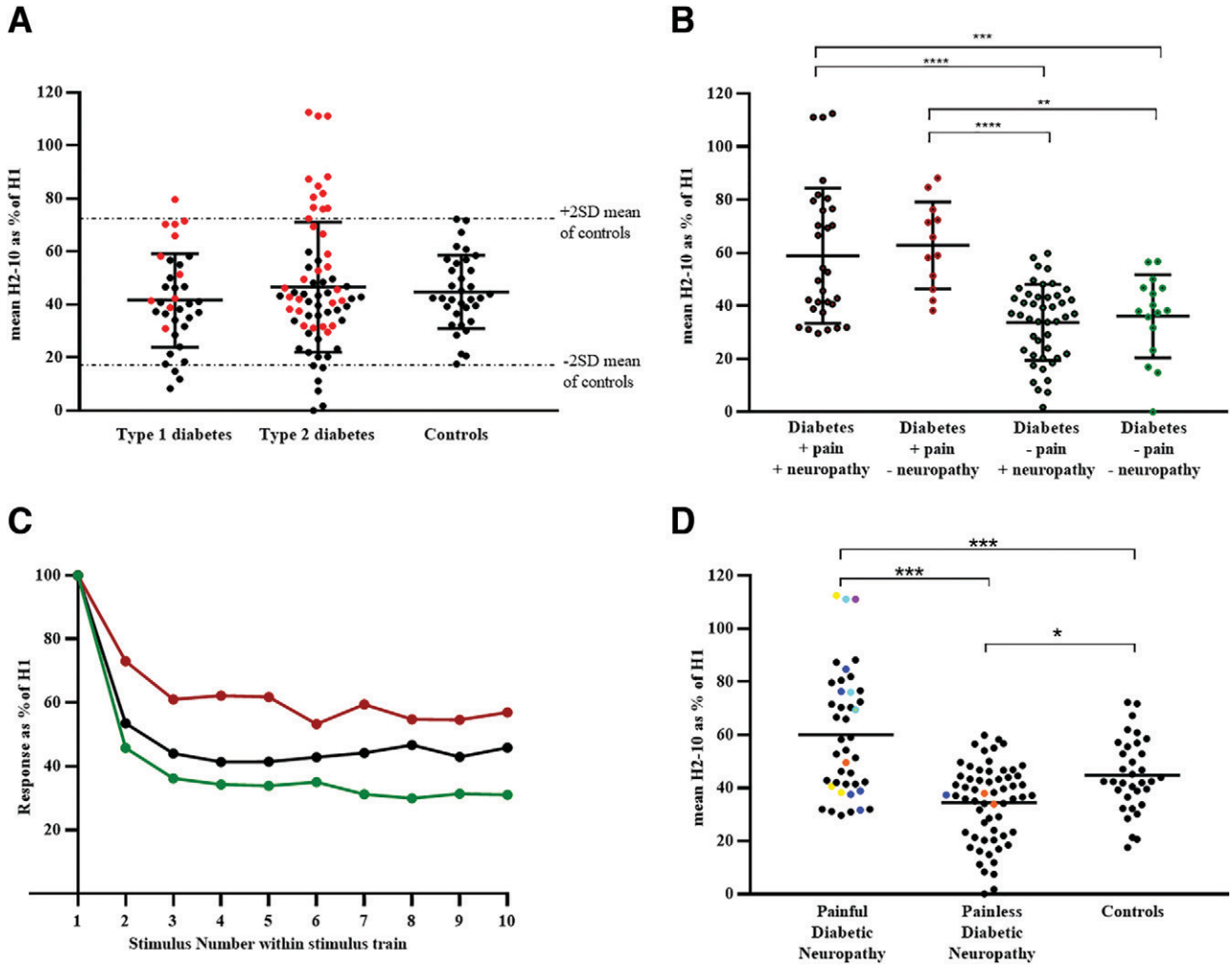

Figure 2—*A*: Individual HRDD (meanH2–10 as % of H1) at 1 Hz in patients with type 1 or type 2 diabetes and control subjects. Patients reporting painful symptoms are shown in red. The dotted line represents ±2 SDs of the mean value of control subjects. *B*: Individual HRDD (meanH2–10 as % of H1) at 1 Hz in control subjects, patients with diabetic neuropathy with pain, patients without diabetic neuropathy with pain, patients with diabetic neuropathy without pain, and patients without diabetic neuropathy without pain. *C*: Group mean H-wave amplitude responses to stimulus train at 1 Hz in control subjects (black), patients with painful diabetic neuropathy (red), and patients with painless diabetic neuropathy (green). *D*: Individual HRDD (meanH2–10 as % of H1) at 1 Hz in control subjects, patients with painful diabetic neuropathy, and patients with painless diabetic neuropathy. Neuropathic pain medications: duloxetine (cyan), gabapentin/pregabalin (yellow), selective serotonin reuptake inhibitor (blue), amitriptyline (orange), and tricyclic antidepressant (purple). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ by one-way ANOVA follow by Tukey multiple comparison test.

was associated with higher severity of clinical pain (12). However, in this larger cohort of patients with painful diabetic neuropathy, some of whom were taking medication for neuropathic pain, the impairment of HRDD was not related to the severity of pain. Intuitively, patients with painful diabetic neuropathy and evidence of spinal disinhibition, a mechanism that is proposed to result in reduced suppression of nociceptor afferent inputs from the periphery, might be expected to experience greater pain severity. However, many factors influence the presence and severity of pain. Indeed, according to the International Association for the Study of Pain, "pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors" (30).

Unlike in rodents where impairments in HRDD are more uniform, considerable variance of HRDD was seen in our previous study and current cohort of patients with painful diabetic neuropathy, and indeed, a proportion of patients with painful diabetic neuropathy did not demonstrate impaired HRDD. This likely reflects the complex etiology of painful neuropathy mediated at multiple peripheral, spinal, and supraspinal levels.

We propose that HRDD is a biomarker of a pain mechanism in painful diabetic neuropathy rather than of pain per se. In this respect, within our cohort there will likely also be patients with painful diabetic neuropathy in whom the pain is due to an alternative dominant mechanism that could cause pain of equivalent severity (31). Therefore, across a group of patients with pain due to multiple and different dominant mechanisms, the lack of correlation is not that surprising. Indeed, in previous mechanistic studies addressing the role of deficient descending pain modulation in painful diabetic neuropathy, although abnormalities in conditioned pain modulation predicted the response to the selective serotonin-noradrenaline reuptake inhibitor duloxetine, they did not correlate with pain severity (6).

Table 2—Demographic and neuropathy parameters, with and without pain, for patients with diabetes and control subjects

	Diabetes with pain (n = 42)	Diabetes without pain (n = 62)	Control subjects (n = 34)
Type 1 diabetes	11	26	
Type 2 diabetes	31	36	
Sex, *n*			
Female	18	18	20
Male	24	44	14
Ethnicity			
White	32	46	25
Asian	8	13	7
Black	2	3	2
Age (years)	61.5 (49.8–69.5)***	65.0 (51.5–71)****	46.5 (31–55)
Duration of diabetes (years)	12.5 (4.8–20.3)	16.0 (10.0–23.3)	
HbA_{1c} (%)	7.0 (6.2–7.5)	7.5 (6.8–8.4)	5.4 (4.9–5.7)
HbA_{1c} (mmol/mol)	53.0 (44.5–58)***	58.5 (51–68.25)****	35.0 (30.5–38.75)
BMI (kg/m^2)	29.2 (25.5–34.9)****	27.3 (24.3–31.9)**	23.8 (22.5–25.4)
SNAP (μV)	7.7 (3.7–15)***	6.9 (4.3–11.5)****	17.0 (15–22)
SNCV (m/s)	43.1 (40–46.7)***	42.4 (40–46.7)****	48.3 (45.2–51.9)
PMNAP (mV)	3.8 (2.4–5.7)	3.5 (2.4–5.9)*	4.9 (3.4–7.5)
PMNCV (m/s)	41.4 (38.1–43.7)****	41.2 (38.6–44)****	47.5 (43.4–50)
CDT (°C)	27.8 (23.3–29.6)***	28.0 (24.1–29.9)**	29.8 (28.5–30.5)
WDT (°C)	40.3 (36.4–46.3)**	40.0 (37.7–43.6)***	36.3 (34.8–39.3)
VDT	7 (4–8)**	7 (5.3–8)*	8 (6.6–8)
VAS current	20 (8–46.5)		
VAS average 24 h	35.5 (15.8–64.5)		
VAS worst 24 h	56 (30–80)		
CNFD (n/mm^2)^	23.84 ± 1.84***	25.30 ± 1.31***	32.36 ± 1.59
CNFL (mm/mm^2)^	18.16 ± 1.563***	18.43 ± 1.11***	25.11 ± 1.35
CNBD (n/mm^2)^	46.64 ± 5.18	47.61 ± 4.20	59.74 ± 6.85
HRDD meanH2–10 at 1 Hz^	60.40 ± 2.74***	34.92 ± 2.33*+++	43.46 ± 3.31

Data are *n*, median (interquartile range) (nonparametric, Mann-Whitney or Kruskal-Wallis test with Dunn post hoc test), or mean ± SE (parametric). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ compared with control subjects. +++$P < 0.001$ compared with painful diabetic neuropathy. ^ANCOVA adjusted for age (post hoc least squares difference).

Therefore, this novel translational observation raises the intriguing possibility that HRDD could be used, either in the clinic or in the setting of a clinical trial, to identify or stratify patients with painful neuropathy driven predominantly by impaired spinal inhibition who may respond preferentially to medications, such as duloxetine, that target spinal inhibition. Indeed, the absence of mechanism-specific stratification may well explain the relatively modest outcomes in most clinical trials of drugs for painful diabetic neuropathy (32).

Testing of HRDD is unlikely to be applicable to all patients, particularly those with severe diabetic neuropathy because of the increased likelihood of a severely attenuated or absent H-reflex. However, HRDD assessment is applicable for 75–80% of patients with diabetes. Painful symptoms are reported in up to one-third of patients with mild and moderate diabetic neuropathy as well as in approximately one-quarter of patients with diabetes without confirmed neuropathy (1).

Enhanced HRDD was observed in a subgroup of the patients with diabetes without pain such that mean HRDD for the entire group was significantly higher than in the control group. This subgroup shared similar indices of peripheral neuropathy with the subgroup of patients with diabetes with pain and the most dramatic loss of HRDD and was indistinguishable from patients with diabetes, no pain, and normal HRDD. It is well documented that spinal nociceptive transmission can be reduced by engagement of inhibitory pathways in the descending pain modulatory system (DPMS) (33). Conversely, loss of endogenous pain suppression as a result of a reduced capacity for inhibition and/or enhanced facilitation in DPMS pathways has been implicated in diverse pain states, including painful diabetic neuropathy (6,34–36). Whether the dynamic alterations in the DPMS that either enhance or suppress inhibition in the spinal cord are linked to the mechanisms underlying painful diabetic neuropathy–related spinal inhibitory dysfunction and HRDD requires further study.

Our novel observation of enhanced HRDD in some patients with painless diabetic neuropathy raises the possibility that spinal inhibitory systems, as reflected by HRDD, can also be augmented to suppress peripheral nociceptive inputs that may otherwise cause pain. It will be of interest to assess whether the patients with no

pain but exaggerated HRDD show peripheral hyperexcitability or spontaneous activity, electrophysiological indices that have been linked to neuropathic pain in some patients (37).

A potential limitation of the study is that the control group was significantly younger than the patient groups with diabetes. While this has the potential to affect the significance of neuropathy parameters between patients with diabetes and control subjects, the patient cohorts with and without pain were well matched for age. Furthermore, there was no significant correlation between age and HRDD, and the findings for HRDD between patients with and without pain and control subjects were highly significant following adjustment for age using ANCOVA. We also acknowledge that this is a cross-sectional study comprising relatively small cohorts of patients. A small proportion of patients were taking antineuropathic pain medication, and we did not evaluate the effect of drugs on either HRDD or VAS pain scores.

In conclusion, we show reduced and enhanced HRDD in patients with and without painful diabetic neuropathy, respectively, which was not associated with the presence or severity of diabetic neuropathy. Prospective and pharmacological intervention studies are required to systematically address the utility of HRDD to target therapies in the clinic and for trial enrichment in clinical trials of new therapies for painful diabetic neuropathy.

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Author Contributions. A.W. researched data, performed statistical analyses, and wrote the manuscript. A.K., M.F., L.D'O., S.A., C.A., and S.H. researched data. S.H., R.A.M., and N.A.C. reviewed and edited the manuscript. A.G.M. researched data and reviewed and edited the manuscript. A.G.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

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