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Impaired rate-dependent depression of the H-reflex in type-2 diabetes, prediabetes, overweight and obesity

A cross-sectional study

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Abstract

Type-2 diabetes is a chronic metabolic disorder characterized by hyperglycemia, resulting from deficits in insulin secretion or insulin resistance. According to the International Diabetes Federation, approximately 463 million people suffered from this condition in 2019, with a rapidly increasing impact in low-and middle-income countries. Obesity is a well-known risk factor for diabetes, and current data project a continuous increase in diabetes prevalence worldwide in obese individuals. Among the common complications, diabetic peripheral neuropathy (DPN) causes sensory symptoms, including pain that contributes to foot ulceration, and if not controlled, limb amputation may occur. The diagnosis of DPN is a clinical problem. Rate-dependent depression (RDD) of the Hoffmann reflex in the lower limbs has been proposed as a test to determine the presence of neuropathic pain in subjects with type-1 and type-2 diabetes. Recently, impaired RDD has been described in obese and diabetic rodent models. In this study, we characterized the RDD by evaluating the H-reflex at 0.2, 1, 2, 5, and 10 Hz in 39 patients with type-2 Diabetes mellitus (T2DM) and 42 controls without diabetes, subsequently classified as overweight/obese and prediabetic. A significant decrease in the RDD of the H-reflex was found in T2DM subjects at 1, 2, 5, and 10 Hz ($P < .001$) stimulation frequencies compared to controls, but not at 0.2 Hz ($P = .48$). A major finding of this study is that impaired RDD was also found in 11/25 overweight and obese subjects in at least 2 stimulation frequencies, being 10 of those classified in prediabetic levels according to their HbA1C values. The RDD of the H-reflex could be used as a quantitative and sensitive tool to study T2DM subpopulations with peripheral neuropathy. RDD could be used as a screening tool in combination with clinical tests to diagnose DPN and evaluate the progression of this condition.

Abbreviations: BMI = body mass index, DPN = diabetic peripheral neuropathy, MNSI = michigan neuropathy screening instrument, RDD = rate-dependent depression of the H reflex, T2DM = type-2 diabetes mellitus, WHtR = waist to height ratio.

Keywords: diabetic peripheral neuropathy, H-reflex, overweight and obesity, prediabetes, rate-dependent depression, type 2 diabetes

1. Introduction

Type-2 Diabetes mellitus (T2DM) is a metabolic disorder resulting in chronic hyperglycemia caused by deficits in insulin secretion or insulin resistance, with disturbances in carbohydrate, fat, and protein metabolism.[1] The International Diabetes Federation estimated 463 million adults living with this condition worldwide in 2019, with a prevalence of 10.8% in urban areas. The prevalence of diabetes is rising in low- and middle-income countries.[2] Prospective studies have shown that central obesity is a strong risk factor for T2DM.[3] Modeling using data from the WHO Global Health Observatory associated obesity and diabetes prevalence, confirming the continuity of the "diabetic crisis" in the following years..[4] In fact, "Diabesity" is a term referring to the close relationship between T2DM and obesity.[5] Among the complications of T2DM, diabetic peripheral neuropathy (DPN) contributes to the development of foot ulceration, resulting in a higher risk of lower limb amputations; however, up to 50% of DPN patients may be asymptomatic.[6,7] By the time of T2DM diagnosis, DPN was already present in 8% of the Rochester cohort,[8] while 10%

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to 15% was reported in Western countries,[9] with a prevalence up to 6% around the globe.[6]

Diagnostic tools for DPN include nerve conduction studies, devices that quantify temperature and vibration perception using quantitative sensory testing,[10] electrophysiological scores, microneurography,[11] questionnaires accompanied by clinical examination, and skin biopsy and/or corneal confocal microscopy.[12–14] Nevertheless, the repertoire of diagnostic tools is not fully available in most hospitals, particularly in middle-and low-income countries, making it difficult for an early and accurate diagnosis following the progression of DPN.[15]

Recently, Rate-Dependent Depression (RDD) of the H-reflex has been suggested as a biomarker for determining the presence of neuropathic pain in diabetic patients.[16] This application was established after the observation that RDD was impaired in experimental rat models of type-1 and -2 diabetes presenting with tactile allodynia.[17] Interestingly, intrathecal administration of bicuculline[17] or L-655708, an inverse agonist of the α_5GABA_A receptors,[18] removed tactile allodynia and restored RDD. It has been suggested that the loss of the RDD is a consequence of GABAergic dysfunction produced by alterations in Cl^- homeostasis mediated through downregulation of the KCC2 co-transporter in dorsal horn neurons involved in circuitry processing nociceptive information.[16,17]

In both animals and humans, RDD was observed under non-pathological conditions when the H-reflex was evoked with paired-pulse stimulation frequencies >1 Hz.[19,20] In contrast, altered RDD was observed in mice fed with a high-fat diet that consequently developed obesity.[21] In line with the evidence in animals, in humans, RDD impairment was reported in individuals with type-1 and type 2 diabetes with painful and non-painful neuropathy.[22,23] Recently, further exploration of optimal RDD parameters has been conducted to study spinal disinhibition in diabetic subpopulations by using stimulation frequencies <3 Hz.[24]

The first aim of this study was to characterize the RDD of the H-reflex in subjects with T2DM compared with subjects without diabetes as controls. The second aim was to subdivide the control group to determine whether RDD impairment was also present in individuals with prediabetes and overweight/obesity.

2. Materials and Methods

Patients from a community health center (ISEM, State of Mexico Health System) and a local university campus (State of Mexico Autonomous University) were invited to participate in this study. Recruitment and protocol testing were conducted between July 2021 and January 2022. Clinical screening and electrophysiological tests were performed at the Laboratory of Physiology of the Faculty of Medicine of the State of Mexico Autonomous University. Convenience sampling consisting of 39 subjects with T2DM (50.0 ± 12.3 y/o) and 42 without a previous diagnosis of diabetes who served as controls (32.3 ± 9.9 y/o) were enrolled in this cross-sectional study. The study followed the tenets of the Declaration of Helsinki and Mexican Law for Conducting Research on Humans (NOM-012-SSA3-2012). The study protocol was approved by the Ethics Committee of the Faculty of Medicine of the Autonomous University of the State of Mexico (CONBIOETICA-15-CEI-002-20210531, registry 004.2021). The participants provided written informed consent to participate in this study.

2.1. Clinical screening

For both the T2DM subjects and the Control group, individuals of both sexes (25-60 y/o) were included. Participants with T2DM were monitored by their community health system. For the T2DM group, subjects with at least 5 years of diagnosis were included. Exclusion criteria included demyelinating diseases, peripheral nerve pathologies other than DPN, alcoholism, venous insufficiency (class C2-C6, CEAP classification), and history of cancer. Clinical history was obtained for all subjects. HbA1C data were obtained no longer than 1 month at the time of the study. Capillary glucose was measured at the beginning of the session (Accu-Check Active®). Weight, height, and waist circumference were measured to determine body mass index (BMI) and WHtR (waist to height ratio). The Global Physical Activity Questionnaire was administered to all the participants. The Michigan Neuropathy Screening Instrument (MNSI) was applied to all participants, and scores >2 were considered positive for DPN. The Achilles and patellar tendon reflexes, vibration test (128 Hz fork), and 10-g Semmes Weinstein monofilament test were performed. The STROBE guidelines were followed.

2.2. RDD of the h-reflex protocol

The subjects were placed in prone position during the protocol. The temperature of the limbs was maintained constant (32°C-35°C) during the test. Standard procedures for H-reflex recording were as follows.[25] Initially, a handheld electrode (STMHUM, Biopac Systems, Inc.) was used to explore the anatomical site over the popliteal fossa to stimulate the tibial nerve and evoke consistent H-reflex responses. For this purpose, 1 ms width, monophasic square pulses were given at 0.2 Hz starting at 1 V in 1 V steps. Once the H-reflex was identified (latency of approximately 30 ms), a bar electrode (MFI Medical; distance between electrodes, 3 cm) was secured. The H-reflex was then evoked by a voltage electrical stimulator (BSLSTM, Biopac Systems, Inc.), and electromyographic responses were recorded bipolarly over the soleus muscles with surface electrodes (Red Dot, 3M). Signals were amplified, digitized at 10 kHz, band-pass filtered (5-500 Hz) (MP36, Biopac Systems, Inc.), and stored on a computer for offline analysis. Curves of the stimulation intensity versus amplitude of the H-reflex were constructed to determine the optimal range of stimulation to evaluate RDD in the control and T2DM groups. Voltages around 50% of the maximal amplitude of the H-reflex were used. To assess the RDD of the H-reflex, a train of 10 pulses was administered at 1, 2, 5, and 10 Hz in the left leg. Marshall et al[22] described that the RDD is of a lesser magnitude at the second pulse, with the third pulse being depressed compared to the second pulse. In this study, we decided to evaluate RDD in controls and T2DM subjects delivering 10 pulses to gain a better understanding of the phenomenon. Some subjects manifested discomfort, especially at 5 and 10 Hz stimulation, but they did not express intolerable pain; however, whenever the subject decided, 5 or 10 Hz tests were not completed. Stimulation frequencies were applied sequentially with at least 1-minute intervals between stimulations.

2.3. Data analysis

Latencies and peak-to-peak amplitudes of the H-reflex (10 responses) for each frequency were manually determined using Clampfit 10.7 (Molecular Devices). Data are reported as the mean (± SD). The amplitude of the 2^{nd} to 10^{th} pulses for each stimulation frequency was expressed as a percentage relative to the amplitude of the first pulse to determine RDD (Hn/H1). Data are presented in plots expressing RDD rate (%) versus pulse number. Then, for each subject, the mean RDD was determined as the average of the amplitude of the H-reflex from the 2^{nd} to the 10^{th} pulse divided by the amplitude of the first H-reflex. The Shapiro–Wilk test was performed to test the normality of the data, and when data were normally distributed ($P > .05$), Student t test was used to compare 2 groups; otherwise, the Mann-Whitney rank-sum test was used. Statistical significance was set at $P < .05$. Data were analyzed using Sigma Plot 14 (Systat Software).

3. Results

Eighty-one subjects were enrolled in this study: 39 with T2DM (23 F) and 42 controls (17 F). The T2DM group had a mean BMI of 28.0 ± 4.12, while that of the control group was 26.3 ± 4.46. Seventeen subjects in the control group and 5 subjects in the T2DM group were considered to have normal weight (BMI 18.5-24.9). Demographics and variables related to clinical screening (BMI, WHI, waist circumference, physical activity, capillary glucose, HbA1C, and MNSI score) are shown in Table 1 for the T2DM and control groups. T2DM subjects enrolled in this study had 10.0 ± 5.2 years after diagnosis (Table 2). A total of 20/39 T2DM individuals had signs and symptoms of painful neuropathy, while 19/39 had non-painful neuropathy. A total of 32/39 subjects reported metformin consumption, whereas an insulin prescription was reported in 21/39 subjects. Four subjects reported pregabalin consumption, and gabapentin was prescribed to the other 4 subjects. A total of 17/39 subjects reported taking B-complex vitamins (Table 2).

3.1. Rate dependent depression of the h-reflex in controls and t2dm subjects

Our protocol included 10 pulses to evaluate the RDD of the H-reflex, which, at high stimulation frequencies (i.e., 5 and 10 Hz), subjects may experience unpleasant sensations. However, none of the patients experienced intolerable pain. Once the protocol ended, none of the subjects manifested persistent pain or unpleasant sensations in the explored leg. Representative examples of the H-reflexes evoked at each stimulation frequency are shown in Figure 1 for a normal-weight subject (*left panels*)

Table 1

Main characteristics of control and type-2 diabetes mellitus groups.

	Control (n = 42)	T2DM (n = 39)
Age (yrs ± SD)	32.3 ± 9.9	48.0 ± 15.3
Median	28.5	50
Sex (M/F)	25/17	16/23
BMI (± SD)		
BMI classification	26.3 ± 4.4	28.07 ± 4.12
Underweight	0	0
Normal	17	6
Overweight	15	27
Obesity I	9	4
Obesity II	1	2
WHI (± SD)	0.56 ± 0.07	0.61 ± 0.05
Waist (m)	0.99 ± 0.08	0.96 ± 0.09
Physical activity	3	3
Glucose (mg/dL ± SD)	95.4 ± 13.38	219.5 ± 87.8
HbA1C (% ± SD)	5.87 ± 0.38	11.6 ± 3.03
MNSI score (±SD)	3.96 ± 2.2	8.26 ± 3.5

BMI = body mass index; MNSI = Michigan neuropathy screening instrument, T2DM = type-2 diabetes mellitus, WHI = waist-height index.

Table 2

Clinical history of type-2 diabetes mellitus subjects (n = 39).

Yrs after diagnosis (±SD)	10.08 ± 5.29
Prescriptions	
Metmorfin	32/39
Insulin	9/39
Gabapentin	4/39
Pregabalin	4/39
B-complex vitamins	17/39

T2DM = type-2 diabetes mellitus.

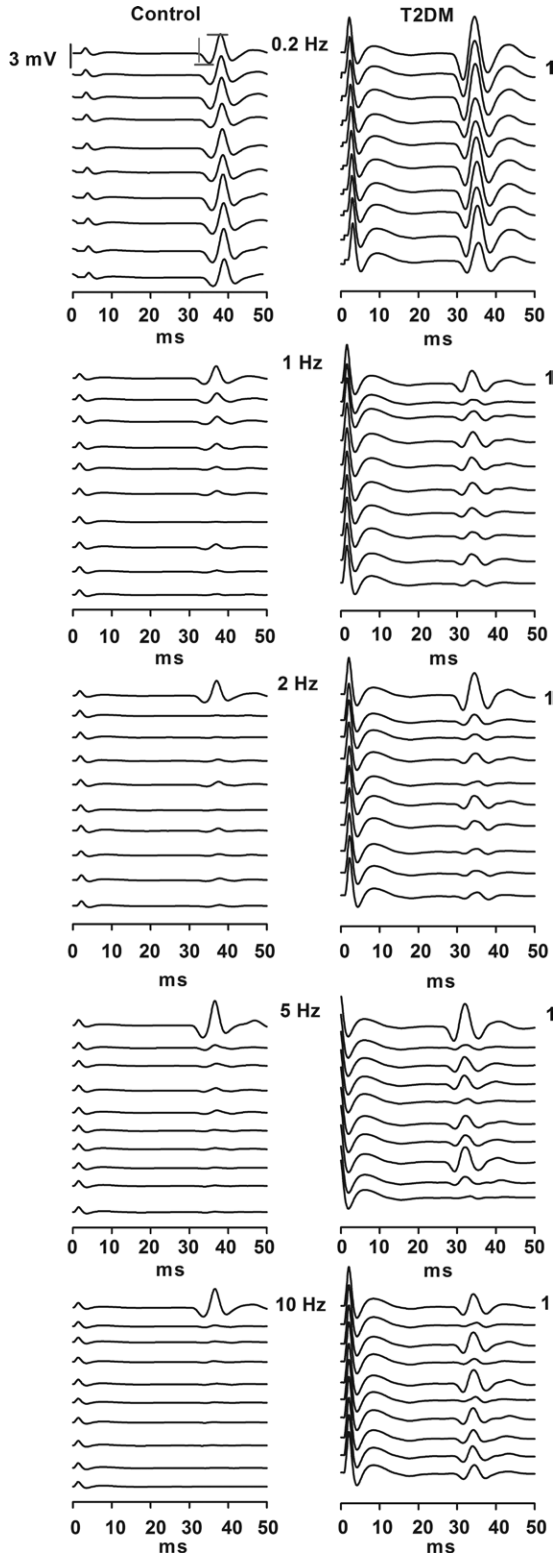

Figure 1. RDD in control and T2DM subjects. Representative traces of the H-reflex were evoked at 0.2, 1, 2, 5, and 10 Hz in control (*left panel*) and T2DM (*right panel*) subjects. There is no loss of RDD at 0.2 Hz; however, in the T2DM subject, a loss of the RDD of the H-reflex was observed from 1 to 10 Hz. The uppermost trace in each panel corresponds to the first H-reflex evoked. The pink vertical line and blue horizontal lines indicate the time at which the latency and the peak-to-peak amplitude of the H-reflex were measured, respectively (see Methods). RDD = rate-dependent depression of the Hreflex, T2DM = type-2 diabetes mellitus.

and a T2DM subject (*right panels*). Note that in both examples, the amplitude of the H-reflex at 0.2 Hz was not depressed, as expected. In the control subject (BMI 24.8; WHtR 0.55; capillary glucose 76.4 mg/dL; HbA1C 5.4), RDD was present at 1-, 2-, 5, and 10 Hz stimulation frequencies. In contrast, in the T2DM subject (BMI, 23.1; WHtR, 0.45; capillary glucose, 270 mg/dL; HbA1C, 12.2), RDD was impaired at all tested frequencies ≥ 1 Hz (Fig. 1). Impairment is observed as an absence or decrease in the depression of the H-reflex.

Graphs of RDD expressed as a percentage of Hn/H1 (see Methods) are shown in Figure 2. No RDD was observed at 0.2 Hz in both groups (control, *black line* vs T2DM, *gray line*), as expected. In contrast, RDD was present at stimulation frequencies ≥1 Hz in controls, but not in T2DM subjects (Fig. 2).

Mean latencies of the H-reflex were grouped (for all stimulation frequencies) and compared between the control (25.31 ± 1.57 ms) and T2DM (26.47 ± 1.09 ms) groups, with a statistically significant difference ($P < .001$). Then, comparisons were made between the control and T2DM groups at each stimulation frequency (Fig. 3). Statistical differences were found for 0.2 Hz (Control, 25.30 ± 1.59 ms vs T2DM, 26.48 ± 1.12 ms; $P < .01$); 1 Hz (Control, 25.33 ± 1.58 ms vs T2DM, 26.46 ± 1.12 ms; $P < .01$); 2 Hz (Control, 25.31 ± 1.56 ms vs T2DM, 26.47 ± 1.09 ms; $P < .01$); 5 Hz (Control, 25.32 ms ± 1.59 ms vs T2DM, 26.49 ± 1.10 ms; $P < .01$) and 10 Hz (Control, 25.28 ± 1.60 ms vs T2DM, 26.46 ± 1.13 ms; $P < .01$).

The results of the mean RDD for all the stimulation frequencies are presented in Figure 4. As shown in Figure 2, the amplitude of the H-reflex along the 10 pulses administered at 0.2 Hz was similar, that is, no RDD was present. Accordingly, the mean depression of the RDD was not statistically different at 0.2 Hz between the control (96.27% ± 6.36%) and T2DM groups (93.15% ± 13.42%) ($P = .48$). In the same Figure 4, the RDD of the H-reflex can be observed in the Control group: 1 Hz, 45.01% ± 11.66%; 2 Hz, 42.63% ± 16.45%; 5 Hz, 35.41% ± 10.30%, and 10 Hz 23.41% ± 16.91%. On the other hand, in the T2DM group, a significant loss of RDD was observed: 1 Hz, 69.35% ± 29.40%; 2 Hz, 94.47% ± 26.05%; 5 Hz, 92.41% ± 30.66% and 10 Hz, 88.20% ± 40.26%. Significant differences between the control and T2DM groups were found at1 Hz ($P < .001$), 2 Hz ($P < .001$), 5 Hz ($P < .001$), and 10 Hz ($P < .001$) (Fig. 4).

In the T2DM group, a subclassification was made based on painful (10 subjects, 47.9 ± 15.0 y/o, 7 females) and non-painful peripheral neuropathy (7 subjects, 49.7 ± 8.8 y/o, 3 females). When RDD was compared between the painful and non-painful DPN, no statistical differences were found at 0.2 Hz (90.06% ± 16.41% vs 97.58% ± 6.13%; $P = .30$) and 1 Hz (109.9% ± 8.68% vs 100.14% ± 11.35%; $P = .46$). For the rest of the stimulation frequencies, statistical differences were found (painful vs non-painful DPN): 2 Hz, 87.5% ± 17.52% versus 73.14% ± 5.64% ($P < .01$); 5 Hz, 84.6% ± 14.13% versus 60.42% ± 12.33% ($P < .001$); and 10 Hz 106.97% ± 25.78% versus 47.9 ± 16.7% ($P < .001$).

Latencies were also analyzed in subgroups with painful and non-painful diabetic neuropathy. We found that latencies in the non-painful T2DM group were significantly higher compared to painful neuropathy at 0.2 Hz (27.06 ± 0.91 ms vs 25.66 ± 0.89 ms; $P < .01$); 1 Hz (26.99 ± 0.95 ms vs 25.76 ± 0.92 ms; $P < .01$); 2 Hz (27.02 ± 0.86 ms vs 25.69 ± 0.92 ms; $P < .01$); 5 Hz (27.64 ± 0.89 ms vs 25.70 ± 0.91 ms; $P < .01$) and 10 Hz (27.05 ± 0.96 ms vs 25.61 ± 0.92 ms; $P < .01$) respectively.

3.2. Loss of RDD in prediabetic, overweight, and obese subjects

Subjects without a previous T2DM diagnosis enrolled as controls (n = 42) were later subdivided according to their BMI in overweight/obesity and normal weight subgroups. Interestingly, we also found impaired RDD in the overweight/obesity subgroup. Representative recordings of this phenomenon are shown in Figure 5. Note that in this subject, classified as obese grade I (BMI 35.5; WHtR 0.71; capillary glucose 89 mg/dL; 5.8 HbA1C), RDD was impaired at 2 and 5 Hz, while at 1 and 10 Hz, RDD seemed to be normal (Fig. 5). Then, RDD was evaluated in a subgroup formed by 25 overweight/obese individuals (10 F, 34.9 ± 9.8 y/o; BMI, 29.1 ± 3.0; WHtR, 0.57 ± 0.04; capillary glucose, 95.4 ± 13.3 mg/dL; HbA1C, 5.8 ± 0.3). At 0.2 Hz no statistical difference was found when RDD was compared in normal-weight subjects (97.4% ± 4.53%) versus overweight/obese (94.87% ± 13.87%) ($P = .91$). For the rest of the stimulation frequencies, statistical differences (normal weight vs overweight/obese) were found at 1 Hz, 41.52% ± 12.22% versus 70.57% ± 29.34%, $P < .01$; 2 Hz, 40.23% ± 19.55% versus 54.58% ± 26.00%, $P < .01$; 5 Hz, 32.43% ± 10.51% versus 46.64% ± 22.41%, $P < .01$; and 10 Hz, 16.86% ± 16.09% versus 36.07 ± 20.76%, $P < .001$ as shown in Figure 6.

Considering that in the normal weight subgroup, stimulation frequencies ≥1 Hz produced RDD values below 50% (Fig. 6), data above this cut point were arbitrarily considered

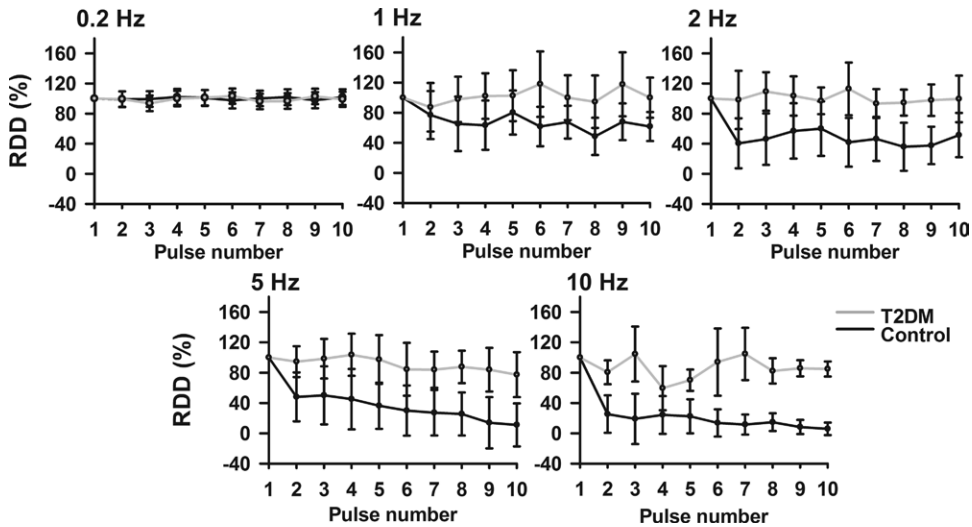

Figure 2. Loss of RDD (%) in T2DM during a 10 pulses protocol compared to controls. Mean and SD (±) of the RDD (%) at 0.2, 1, 2, 5, and 10 Hz stimulation frequencies in control (black line, n = 42) and T2DM (gray line, n = 39) groups. RDD = rate-dependent depression of the Hreflex, T2DM = type-2 diabetes mellitus.

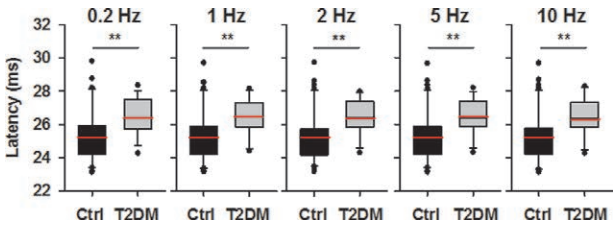

Figure 3. Latency of the H-reflex is affected in T2DM subjects compared to controls. Statistical differences in latencies were found at all stimulation frequencies tested (0.2, 1, 2, 5, and 10 Hz) when comparing Control (black plots) versus T2DM (gray plots) groups. The red line in the plots represents the median. $^{**}P < .01$, *t* test. T2DM = type-2 diabetes mellitus.

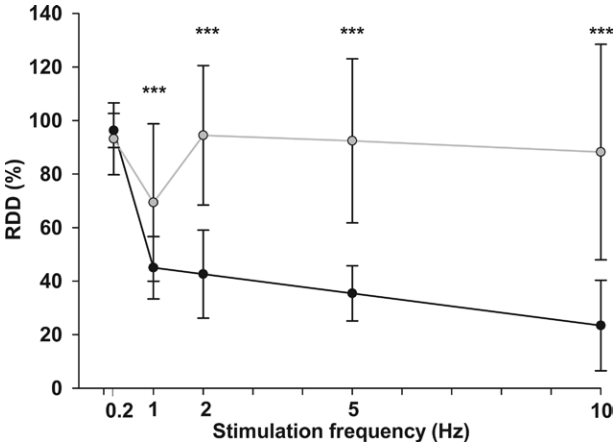

Figure 4. Mean RDD in controls and T2DM subjects. Impaired RDD is shown in T2DM (*gray line*) but not in control subjects (black line) at 1 Hz ($P < .001$), 2 Hz ($P < .001$), 5 Hz ($P < .001$), and 10 Hz ($P < .001$). No statistical differences were found at 0.2 Hz ($P = .48$), *t* test. RDD = rate-dependent depression of the Hreflex, T2DM = type-2 diabetes mellitus.

abnormal. Thus, in this subgroup of 25 overweight/obese subjects, impaired RDD was observed at 1 Hz (16/25), 2 Hz (13/25), 5 Hz (7/25), and 10 Hz (4/25). Overall, 19/25 subjects from the overweight/obese subgroup met the criteria for prediabetes according to the HbA1C levels: 5.7% to 6.4%.[26] Particularly, impaired RDD in at least 2 stimulation frequencies (1 and 2 Hz) was observed in 11/25 subjects, while from those 11 individuals, 10 can be considered in a HbA1C prediabetic levels.

Latencies were also compared between overweight/obesity and normal weight subjects, founding significantly higher latencies for the first subgroup: 0.2 Hz (25.90 ± 1.70 ms vs 24.43 ± 0.89 ms, $P < .001$); 1 Hz (25.93 ± 1.67 ms vs 24.44 ± 0.91 ms, $P < .001$); 2 Hz (25.90 ± 1.66 ms vs 24.43 ± 0.89 ms, $P < .01$); 5 Hz (25.95 ± 1.67 ms vs 24.40 ± 0.90 ms, $P < .001$) and 10 Hz (25.91 ± 1.66 ms vs 24.34 ± 0.92 ms, $P < .001$).

3.3. H-reflex was absent in 22 T2DM subjects

In 22 T2DM subjects (14 females, 51.05 ± 12.47 y/o), it was not possible to evoke the H-reflex. On average, these subjects had a BMI of 27.84 ± 4.74 and WHtR of 0.62 ± 08. Fourteen subjects were classified as overweight (BMI 28.04 ± 2.33), 2 subjects had obesity grade I (BMI 30.58 and 33.35), 2 subjects had obesity grade II (BMI 35.19 and 36.62), 1 subject had malnourishment (BMI 15.28), and 3 had normal weight (BMI, 23.11; 21. 60 and 24.11). MNSI was 9.47 ± 2.99. Ten subjects had signs and symptoms compatible with a painful neuropathy. In this group, capillary glucose was 227.13 ± 95 mg/dl, while HbA1C was 13.06 ± 2.26. None of the participants in this group regularly practiced physical activity. In Table 3, detailed information about prescriptions in this subgroup is provided. Capillary glucose, Hb1AC, BMI, WHtR, and MNSI mean values were compared between T2DM subjects with and without the H-reflex (Fig. 7). No statistical differences were found when comparing capillary glucose levels ($P = .54$); however, Hb1AC levels were significantly different between the subgroups ($P < .001$). The BMI was not significantly different between the subgroups ($P = .68$) or WHtR ($P = .30$). Finally, MNSI was statistically different between the subgroups ($P = .01$) (Fig. 7).

4. Discussion

An estimated 463 million people worldwide have T2DM.[2] In Mexico, where this study was conducted, an estimated 10.3% of the population over 20 years old (around 8.6 million people) had diabetes diagnosed in 2018.[27] DPN is a common complication of T2DM, and its prevalence is rapidly growing worldwide, particularly in low-income countries. The landscape is not significantly different for rich countries, resulting in a worrying perspective for the following decades.[6]

Spinal disinhibition, evaluated through the RDD of the H-reflex, has been proposed as a valuable tool to study DPN; in particular, impaired RDD has been observed in painful neuropathy in Type-1 and Type-2 diabetes.[23,24,28] In this study, we characterized RDD in T2DM subjects compared with controls (individuals without diabetes). The control group was then subdivided into normal-weight and overweight/obese subgroups. In

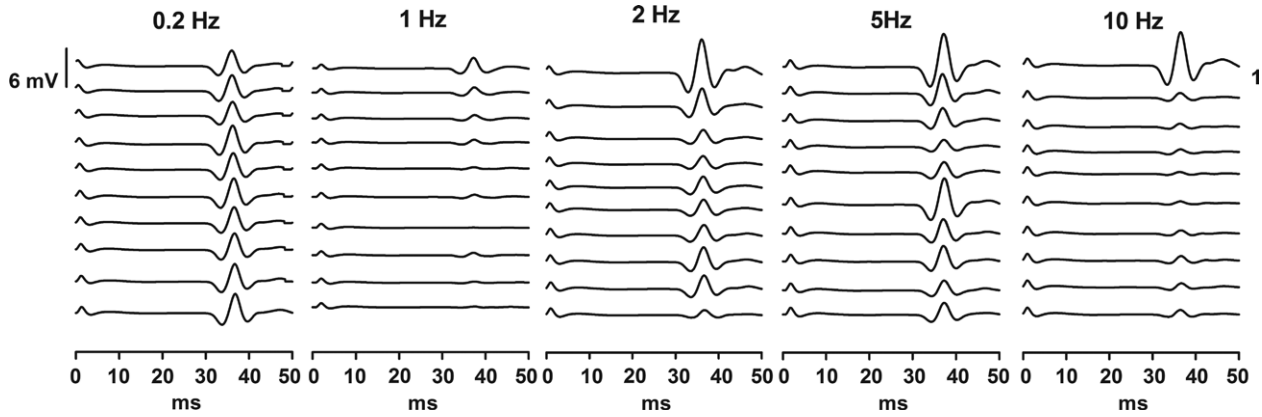

Figure 5. Impaired RDD in an obese subject (Grade I) without diabetes. Representative traces of impaired RDD were observed at 2 Hz and 5 Hz but not at 1 Hz and 10 Hz. The uppermost trace in each panel corresponds to the first H-reflex evoked. RDD = rate-dependent depression of the Hreflex.

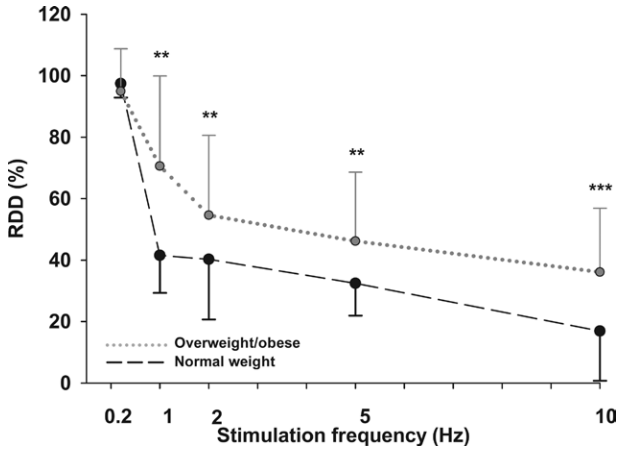

Figure 6. Impaired RDD in overweight and obese subjects. In the overweight/obese subgroup (25 subjects, dotted gray line), RDD was significantly impaired at stimulation frequencies of 1 Hz ($P < .01$), 2 Hz ($P < .01$), 5 Hz ($P < .01$) and 10 Hz ($P < .001$) (*t* test) compared to normal-weight subjects (17 subjects, black line). For simplicity, up and down error bars are shown in the overweight/obese and control subgroups, respectively. RDD = rate-dependent depression of the Hreflex.

Table 3

Prescription in type-2 diabetes mellitus subjects with absence of H-reflex.

Drugs	Total
Gabapentin	4
Pregabalin	4
B complex vitamins	13
Metformin	16
Insulin	12
Linagliptin	1
Atorvastatin	2
Telmisartan	1
Telmisartan/hidroclorotiazide	3
Pentoxifilin	1
Losartan	1
Glibenclamide	3
Ferric sulfate	3
Duloxetin	1
Food supplements	3
SSRIs	1
Teofilin	1
Bezafibrate	1
Levotiroxin	1
Pinaverium bromide	1
Hioscin	1
Diclofenaco	1

SSRI = serotonin selective reuptake inhibitors.

agreement with previous studies,[23,24,28] we found that T2DM subjects had impaired RDD and that reflex H was absent in a portion of individuals. The latter was positively correlated with higher HbA1C values (Fig. 7). Interestingly, 13 overweight and obese subjects also presented a decrease in RDD compared with normal-weight control subjects. Previously, it was reported that diet-induced obesity in mice causes impairment of RDD.[21] Our results represent the first evidence of the loss of RDD in this population and open new questions about the consequences of overweight and obesity on sensory and motor spinal processing.

In this study, we characterized the RDD in T2DM subjects with clinical manifestations of DPN in comparison with controls, delivering 10 consecutive pulses at stimulation frequencies of 0.2, 1, 2, 5, and 10 Hz. Typical protocols for studying RDD involve paired-pulse stimulation at different frequencies.[20,29] Marshall et al[28] applied a 3-pulse protocol and reported that in Type-1 diabetics, the second response is not depressed as the third pulse when evaluating the RDD. Later, the same group applied 10 pulses at 1 Hz.[23] Therefore, we also decided to deliver 10 pulses for better characterization of the RDD and to determine the mean RDD (see Methods), as shown in experimental diabetes.[18] Interestingly, Worthington et al[23] found decreased RDD in painful neuropathy, but not in diabetic patients with non-painful neuropathy. The authors found that RDD was enhanced in this subpopulation when stimulation was delivered at 1 Hz.[23] In our study, we did not find enhanced RDD in any subpopulation of T2DM subjects compared with controls. In contrast, we found that the RDD was significantly different between painful and non-painful neuropathic patients at 2, 5, and 10 Hz. Differences between our patients with non-painful diabetic neuropathy and those presented by Calcutt's group[28] could be partially explained in terms of metabolic control, prescription, and tests employed to classify DPN. A large sample size is warranted to further explore these discrepancies.

Figure 2 shows the variability of the mean amplitude of the H-reflex from the 2nd to 10th pulses, exhibiting RDD in controls but not in T2DM subjects at all stimulation frequencies tested, being more evident at 5 Hz and 10 Hz. In fact, in controls, there was a trend toward a maximal RDD towards the last pulse, while in T2DM, it was not obvious (Fig. 2). The latency of the H-reflex was affected in T2DM patients compared to controls (Fig. 3), as reported in a previous study.[28] In contrast to Marshall et al,[28] we found significantly higher mean latencies in non-painful patients than in painful patients with diabetes. As mentioned before, differences could be explained in terms of differences in the clinical characteristics of the sampled populations. When evaluating the mean RDD, the loss of RDD was statistically significant at all tested frequencies >1 Hz in the T2DM group compared to controls (Fig. 4). Although some subjects manifested unpleasant sensations, particularly at 5 Hz and 10 Hz, no subject manifested intolerable pain in our 10-pulse paradigm. Another interesting finding was that the overweight/obese and among the identified prediabetic subjects also exhibited significantly higher H-reflex latencies compared to controls. In this context, we can assume a subclinical diabetic neuropathy condition, as reported by Marya et al.[30]

The mechanism for the loss of RDD has been attributed to alterations in GABAergic function mediated by KCC2 in the dorsal horn of the spinal cord in animal models, resulting in dysfunctional sensorial and motor processing in experimental diabetes,[17,18,31] suggesting that the loss of spinal inhibition (i.e., disinhibition) evaluated through RDD is a mechanism involved in chronic pain. In particular, α_5GABA_A receptor–mediating GABAergic inhibition plays an important role in allodynia and hyperalgesia during chronic hyperglycemia. Blockade of this receptor with L-655708 reverted tactile allodynia and restored RDD in Type-1 diabetic rats.[18]

A novel finding of this study was that 13/25 overweight and obese subjects also exhibited RDD loss (Figs. 5 and 6). The subjects were enrolled as controls because they did not report any known clinical conditions. However, the link between body fat and diabetes has been well established.[3–5,32] Accordingly, some subjects could be classified as prediabetic since capillary glucose levels at the time of the study were >100 mg/dL in 3/13 subjects, while in 8/13 subjects, HbA1C was ≥6%. Peripheral neuropathy was present in 49% of prediabetic and 50% of new-onset diabetics screened in the PROMISE cohort.[33] Therefore, our study points out that RDD accompanied by standard screening for diabetes in overweight subjects could be used as an auxiliary in diagnosing peripheral neuropathy.

In agreement with a previous reports,[23,28] we also identified a subgroup of subjects in whom the H-reflex could not be

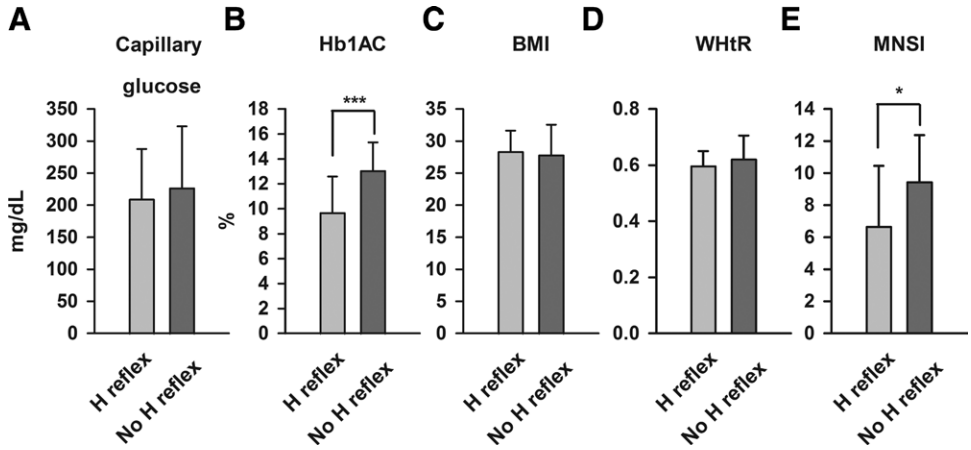

Figure 7. Comparison of anthropometric and clinical variables between T2DM subjects with H-reflex and without H-reflex. Comparisons between T2DM subjects with H-reflex (light gray bars, n = 39) versus absent H-reflex (dark gray bars, n = 22); (A) capillary glucose ($P > .05$); (B) Hb1AC ($P < .001$); (C) BMI ($P > .05$); (D) WHtR ($P > .05$), and (E) MNSI ($P < .05$). *t* test. T2DM = type-2 diabetes mellitus.

evoked. When looking for any parameter that might contribute to this phenomenon, we found that Hb1Ac was significantly different compared to that in subjects with the H-reflex. In line with this, MNSI scores were significantly higher in subjects without the H-reflex (Fig. 7). In contrast, capillary glucose, BMI, and WHtR were not significantly different between subgroups. Longitudinal studies evaluating RDD in T2DM subjects without the H-reflex under strategies aimed at lowering Hb1AC or neuropathy scores could determine if this phenomenon is associated with serious disturbances in glucose control or any other condition.

One limitation of this study is that the diagnosis of neuropathy was not confirmed by nerve conduction studies and sensory tests. However, MNSI scores, clinical examination, and previous results in experimental diabetes[17,18,28,34] suggest that evaluating RDD in diabetic subjects could be relevant for understanding somatosensory dysfunction in the spinal cord and could distinguish between subgroups with painful and non-painful neuropathy.

5. Conclusions

We described a significant decrease in RDD in T2DM and overweight and obese individuals (some classified as prediabetics) at stimulation frequencies ≥1 Hz compared to normal-weight controls, indicating dysfunction in the spinal inhibitory processes. Finally, the H-reflex could not be evoked in a subpopulation of T2DM subjects with significantly higher levels of Hb1AC. Therefore, our study highlighted the relevance of the RDD of the H-reflex not only to characterize the presence of neuropathic pain in diabetic patients, but also to demonstrate the presence of PND in overweight and obese subjects with metabolic syndrome and prediabetic conditions. Additionally, this test could be useful for monitoring changes in spinal sensory processing after pharmacological interventions and other therapeutic approaches in both human and animal models.

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