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Title Page

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Reducing glucose variability with continuous subcutaneous insulin infusion is associated with reversal of axonal dysfunction in type 1 diabetes mellitus

Running head:

Reducing glucose variability

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Dr J Kamel has full access to all the data in the study and takes responsibility for its accuracy.

Abstract:

Introduction: We assess whether improvement in control of type 1 diabetes mellitus (T1DM) with continuous subcutaneous insulin infusion (CSII) can protect peripheral nerve function.

Methods: 12 patients with T1DM treated with multiple daily insulin injections (MDII) were assessed with nerve excitability testing prior to, and three months following initiation of CSII.

Results: Although commencing treatment with CSII for three months improved mean HbA1c, it did not significantly alter nerve excitability or glycemic variability (GV). In four patients, some deterioration in GV was observed, whilst eight patients had improvement in standard deviation (SD) and mean amplitude of glycemic excursions (MAGE). For these eight patients, there was normalization of depolarising and hyperpolarising threshold electrotonus and recovery cycle superexcitablity.

Discussion: When CSII initiation is able to reduce glycemic variability in T1DM, reversal of axonal dysfunction is seen, likely due to normalization of Na+/K+ pump function and restoration of transaxonal membrane potential.

Key words: Glycemic variability; type 1 diabetes mellitus; nerve excitability, continuous subcutaneous insulin infusion; neuroprotection

Introduction:

The mechanisms underlying nerve dysfunction in type I diabetes (T1DM) are not entirely understood. However, it is hypothesized that dysglycemia mediates reversible functional impairment prior to the onset of more permanent structural axonal degeneration.

The above is supported by abnormal Na-K-ATPase activity being implicated in nerve function impairment seen in diabetes,(1) which is thought to cause axonal depolarization changes in nerve excitability testing. In addition, a reduction in nodal Na+ conductance has been thought to be contributory,(2) as this would account for the reductions in refractoriness and strength-duration time constant (SDTC) that are typically seen in people with diabetes. Such changes have been described in patients after ingesting tetrodotoxin, a potent Na+ channel blocker.(3)

Kitano et al.(2) were able to demonstrate that improvement in diabetes control in a relatively short period of time (4 weeks), by initiating multiple daily insulin injections, resulted in significant functional changes in motor axonal excitability, with increased strength-duration time constant and increased refractory periods suggesting restoration of the trans-axonal Na+ gradient.

The Diabetes Control and Complications Trial has clearly demonstrated that tighter glucose control (as measured by improving hemoglobin A1c (HbA1c)) slows the development and progression of microvascular complications in T1DM (retinopathy, nephropathy and neuropathy).(4) Persisting benefit of prior intensive therapy on neuropathy is seen in the Epidemiology of Diabetes Interventions and Complications study at 14 years follow-up.(5)

HbA1c is a commonly used marker of longer-term diabetes control. Although it gives an indication of mean glucose levels, it fails to identify the prevalence of hypoglycemic and hyperglycemic periods that may impact development of diabetic complications. Measures of glucose variability (GV) have been developed to account for this, but only recently has this been shown to correlate with diabetic complications including retinopathy,(6) and neuropathy.(7) These studies show that it may be the larger excursions, rather than average blood glucose levels, that mediate neuronal injury.

Continuous subcutaneous insulin infusion (CSII) can offer superior blood glucose control over multiple daily insulin injections (MDII) by reducing the number of hypoglycemic episodes, as well as offering an improvement in HbA1c in patients

with previously suboptimal control.(8) Nerve excitability studies, which provide information regarding the activity of axonal ion channels, energy-dependent pumps, and ion exchangers that are involved in nerve impulse conduction,(9) have suggested that patients with T1DM on CSII have superior and normal nerve excitability profiles when compared with those treated with MDII.(10)

Nerve excitability abnormalities have been demonstrated to occur in early and even subclinical diabetic neuropathy, *before* the development of axonal loss.(11-13) These functional changes in excitability may be reversible.(2) Although there have not been any long-term longitudinal studies to directly confirm that abnormalities in nerve excitability are required, or predict the development of diabetic neuropathy, these factors have a common relationship with degree of glycemic control. There is at least indirect evidence that changes in nerve excitability are relevant and part of the pathophysiology of diabetic neuropathy.

The primary aim of this study is to evaluate nerve function of patients with T1DM treated with MDII as they transition to CSII, and to determine whether this change in treatment is associated with a neuroprotective effect through restoration of axonal dysfunction. The secondary aim is to determine whether any improvement

seen is related to improvement in acute glucose levels at the time of testing, HbA1c or degree of GV.

Materials and Methods:

Study population

Consecutive and eligible adult patients were recruited and evaluated from a type 1 diabetes clinic at our tertiary centre. Inclusion criteria were patients treated with MDII that were preparing to switch delivery of insulin treatment to CSII. Patients that had any disorder other than diabetes that could potentially impair nerve function were excluded. This included vitamin B12 deficiency, thyroid or other autoimmune diseases, malignancy, use of neurotoxic drugs or drugs with sodium channel blocking activity (e.g. phenytoin, carbamazepine), as well as family history of inherited neuropathy. Patients that had diabetic (or other) renal disease or other biochemical abnormalities such as hypo- or hyperkalemia that could confound nerve excitability results were also excluded. Normative values for nerve excitability were obtained from 30 age-matched control volunteers. Participants were all under the age of 60. Height, weight and body mass index (BMI) were recorded in all

participants. This study was approved by our local Human Research Ethics Committee, and written informed consent was obtained from all participants.

Initial evaluation

Participants were assessed for the presence of diabetes-related neuropathy with the Michigan Diabetic Neuropathy Score (MDNS).(14) Patients with large fibre neuropathy were still included, as nerve excitability testing is an assessment of surviving nerve function, and we were interested in evaluating reversibility of any axonal changes in those with established diabetic neuropathy or not.

The clinical examination portion of MDNS was performed using a 128 Hz tuning fork to assess vibration perception and a 10 g monofilament placed on the dorsum of the great toe to assess light touch perception. Nerve conduction studies (NCS) were performed with an electromyographic (EMG) device (Dantec Keypoint G4 Workstation). We performed median and fibular motor, and sural, median and ulnar sensory studies on the right side, with all amplitudes measured as baseline-to-peak. Results were compared to our laboratory's normal values. Testing was performed at

32 degrees Celsius in the upper extremity and 30 degrees Celsius in the lower extremity.

Nerve excitability testing

Multiple measurements of excitability were performed using a computerized program (QTRAC, Digitimer, London, UK) and applying the nerve excitability protocol TRONDNF (copyright, Prof. Hugh Bostock, Institute of Neurology, London, UK). Nerve stimulation was performed using a DS5 isolated bipolar constant current stimulator (Digitimer, London, UK) with the active electrode over the median nerve at the wrist, and the remote electrode 10cm proximally over forearm muscle. Skin temperature was continually measured at the site of median nerve stimulation and maintained above 32.0 degrees Celsius, and warming with a heat pack was used prior to testing if required. The median compound muscle action potential (CMAP) was recorded from abductor pollicis brevis.

The TRONDNF protocol measures stimulus response (SR) curves using a 1 ms duration current of decreasing intensity (starting with an intensity that is just maximal). For the remaining tests, a target CMAP of 40% maximum was tracked with automated threshold tracking. This included measurement of i. Strength-

duration time constant (SDTC) by assessing the relationship between various stimulus durations and the change in stimulus intensity required to produce the same size response; ii. Threshold electrotonus (TE) by measuring the change in threshold during and after 100 ms subthreshold 20% and 40% depolarising and hyperpolarising currents; iii. Recovery cycle (RC) by measuring the change in threshold from 2 to 200 ms following supramaximal stimulation; and iv. Current threshold relationship (I/V) by assessing the change in threshold following 200 ms duration conditioning currents administered in 10% steps from 50% depolarising to 100% hyperpolarising.(15)

Nerve excitability testing was performed in study participants with TIDM just prior to, and three months following initiation of CSII treatment. This interval was chosen as initiation of CSII is followed by six weeks of intensive glycemic stabilization, and then a buffer of a further six weeks was added to this. Another reason this three-month period was to allow adequate turnover of red blood cells such that hemoglobin glycated during MDII had been cleared.

Glycemic control

Measurements of glycemic control in participants with TIDM included a random capillary blood glucose at the time of each nerve excitability test, and an HbA1c reading. Prior to initiation of CSII, GV was assessed using interstitial glucose measurements obtained from a subcutaneously implanted glucose sensor for a six-day period (Medtronic Enlite sensor with iPro2 CGM system). Three months following CSII initiation, at the time of repeat nerve excitability testing, GV was reassessed using interstitial glucose measurements obtained for another six-day period using real-time CGM linked to the Medtronic MiniMed 640G insulin pump. During these periods, interstitial glucose was calculated and recorded every five minutes. Two measurements of GV were calculated including standard deviation (SD) and MAGE.(16) SD is a simple measure of the range of glucose readings over a period of time, and it is also conveniently supplied in each CGM report. Of all the other numerous potential measurements of GV, MAGE was calculated in our patients, as it has been more consistently used in the literature for decades than other measures. Rather than simply reflecting the dispersion of glucose values, it is a measurement of glucose swings in response to meals, exercise and insulin administration, and it is these rapid excursions that have been postulated as a mechanism of neurotoxicity.(17)

Statistical Analysis

For assessing changes in nerve excitability over time in participants with T1DM, unless otherwise stated due to non-normality, paired t-test was performed. For comparing nerve excitability of patients to non-diabetic controls, unless otherwise stated due to non-normality, unpaired t-test was performed. For assessing correlations of nerve excitability parameters with CBG, HbA1c and measures of GV, unless otherwise stated due to non-normality, Pearson R was determined. Findings were considered statistically significant when $p < 0.05$. Unless indicated, data is expressed as mean +/- standard error of the mean (SEM).

Results:

The patients (7 female, 5 male) had a mean age of 32.9 +/- 3.2 years (range 21 to 59 years) and mean body mass index (BMI) of 26 +/- 1.3 kg/m^2. There was no significant difference in these parameters or temperature between the patient and control group (see Table 1). Duration of diabetes was 16.5 +/- 4.0 years (range 0.5

to 45 years). Ten patients had no evidence of peripheral neuropathy detectable on MDNS either from the clinical exam or NCS component (i.e. class 0 – no neuropathy), whilst one patient had class 1 (mild) neuropathy and one patient had class 2 (moderate) neuropathy. As mentioned, these latter two patients were still included to assess for any functional improvement in surviving axons. A summary of the patient's NCS results is shown in Table 2.

Patient baseline vs. control group

When compared to the age, gender and BMI-matched control group, the 12 patients on MDII had abnormal nerve excitability profiles. Threshold electrotonus curves demonstrated a 'fanning in' with a reduction in percentage threshold change of TEd at 10-20ms and TEh at 90-100ms. Patients also demonstrated abnormalities in the recovery cycle with reduced superexcitability (%), which together with the threshold electrotonus changes is consistent with membrane depolarization.

However, there was no difference in subexcitability (see Table 3). There was also no difference in SDTC or resting I/V slope between the patient and control group.

Longitudinal assessment

Three months after initiation of CSII in the 12 study patients, there was no statistically significant overall change in any nerve excitability parameter. This was despite the fact that there was a significant reduction in mean HbA1c from 8.0% (64 mmol/mol) to 7.2% (55 mmol/mol) ($p<0.05$). However, overall there was no significant reduction in measurements of GV, with neither standard deviation ($p=0.14$) nor MAGE ($p=0.23$) improving. There was only a weak correlation between change in HbA1c and change in SD ($R=0.39$) and no significant correlation between change in HbA1c and change in MAGE ($R=0.16$).

Correlations were undertaken between CBG at the time of testing, HbA1c and CGM measures (including average glucose, SD and MAGE). No significant correlation was seen between CBG, HbA1c, or average glucose and any nerve excitability parameter. However, MAGE correlated with TEd(10-20ms) ($p<0.05$), and SD demonstrated the

strongest correlations with multiple TE and RC parameters (see Figure 1). The two measures of glycaemic variability, SD and MAGE correlated strongly with each other.

Subgroup analysis of the CSII group showed that in eight patients there was an improvement in GV three months after initiation of CSII, in terms of both SD and MAGE, whilst four patients actually showed some deterioration.

The eight patients that achieved an improvement in GV (defined by any reduction in MAGE and SD) demonstrated the same baseline TE and RC abnormalities seen in the TIDM group overall. Following initiation of CSII in this subgroup there was normalization of TE parameters with significant improvement at several points including TEd(10-20ms), TEd(peak), TEd(90-100ms), TEh(10-20ms) and TEh(90-100ms) (all $p<0.05$) whilst TEd(90-100ms)failed to reach statistical significance ($p=0.07$). Following initiation of CSII there were no significant TE differences between the patient and control group in any parameter. Normalization of RC was also seen with improved superexcitabitliy ($p<0.05$) to meet that of the control group (see Figure 2). Subexcitability, SDTC and resting I/V slope remained unchanged, and comparable to the control group.

Discussion:

In this study, we found that our patients with T1DM with longstanding disease duration and moderate overall glycemic control on MDII therapy had abnormal nerve excitability profiles compared with age-matched controls. These patients were largely asymptomatic (10/12 patients) with respect to peripheral neuropathy symptoms, and thus nerve excitability changes were predominantly a subclinical finding. When switched to CSII therapy, the patients that were able to achieve improvement in GV, rather than improvement in HbA1c or mean glucose, demonstrated a normalization of these abnormalities.

Our patients with T1DM treated with MDII (at baseline) had similar nerve excitability abnormalities when compared to previous studies in the literature, especially with regards to the TE changes. Although our patients demonstrate a similar decrease in superexcitability, subexcitability was normal at baseline, which is in contrast to some other studies that show a decrease in subexcitability as well.(10),(18),(11) These RC changes are thought to be due to reduced nodal driving currents due to Na+ channel inactivation with less charging of the internode (leading to reduced superexcitability) and less activation of internodal slow K+

channels (leading to reduced subexcitability).(19) However another study from Arnold et al, focusing on patients with T1DM without clinical evidence of neuropathy showed similar abnormal nerve excitability abnormalities to that seen in our patients, including normal subexcitability.(12) Ten of the 12 patients in our group had MDNS scores of 0 (i.e. no clinical evidence of neuropathy), so this patient group may generally be a closer representation to patients in this study. Similarly, although resting I/V slope has been shown to be sensitive to changes in membrane potential,(20), more recent studies demonstrate that this can still be normal in patients with TIDM (10,12), and there was no difference seen in our patients either. The overall baseline nerve excitability abnormalities of 'fanning in' of the threshold electrotonus curves and decrease in superexcitability seen in our patients are in line with these studies and accounted for by membrane depolarization.

With axonal depolarization mediated by Na+/K+ pump dysfunction one would expect an increase in SDTC, but previous studies typically demonstrate a decrease due to a reduction in Na+ conductance,(18) so in our cohort it is possible that these competing effects cancel each other out. Also, SDTC has been shown to be associated with factors of microangiopathy including estimated glomerular filtration rate (eGFR) and triglyceride (TG) level, rather than diabetes control itself,(11) and other metabolic or acid-base disturbance can also have an impact.(21) However, patients

with renal impairment or electrolyte disturbances were excluded from this study. SDTC can also be very sensitive to changes in temperature,(22) although this was unlikely a significant confounding factor in this study as temperature was quite reliably maintained above 32 degrees Celsius in all participants.

Interpretation of nerve excitability changes can be difficult even when one variation in conductance is present, as compensation of other channels or pumps may occur and reflect in the results. When more than one change occurs, as is the case with diabetes where it is generally accepted that there is a combination of Na+/K+ pump dysfunction, together with decrease in Na+ conductance, interpretation becomes more complex. It is therefore possible that the Na+/K+ pump dysfunction is the first change to occur and masks the more minor contributions of Na+ channel inactivation such that this is not always apparent on nerve excitability testing.

HbA1c did not significantly correlate with any excitability parameter, and this has been noted previously.(12) However, SD and MAGE, two measures of GV, correlated with both TE and RC parameters. It could be seen from these measures that glycemic control either did not improve or worsened in four patients. In one patient, (Patient 12, see Table 4) their glycemic control was excellent prior to insulin pump therapy and although there was no significant change following the change in

treatment their measurements were still considered to be optimal. There were three patients who were unable to achieve a stable treatment regime within the first three months. Reasons for worsening GV were multi-faceted. Self-management factors including inaccuracy with carbohydrate counting, adherence to insulin boluses pre-meals and adjustment to insulin delivery during exercise were identified as significant contributory factors in these patients. Otherwise there were no identifiable characteristics pertaining to patient demographic, duration of diabetes, pre-pump level of disease control, or presence of any established neuropathy that could predict which patient would achieve improvement in GV and thus nerve excitability. However, we note that the small sample size of this study could potentially obscure certain factors.

It is possible that similar improvements in GV, and thus nerve excitability, could be achieved through other simpler methods such as adjusting multiple daily insulin injections, or by more drastic measures such as islet cell transplantation. However, such interventions were not studied here, but rather we demonstrate successful CSII initiation as one means to achieve this within three months.

We did not study sensory nerve excitability, but rather focussed on motor nerve excitability in this study. Sensory studies are technically more challenging,

particularly when attempted in the lower limbs, and it is not infrequent for the quality of recordings to be affected by artefact, even after optimising conditions (e.g. temperature, skin impedances). Furthermore, it has previously been demonstrated that patients treated with MDII do not demonstrate significance differences in sensory excitability with patients treated with CSII. (23)Therefore, for our longitudinal assessment we hypothesised that motor nerve excitability alone would be a more efficient method sufficient to demonstrate any treatment effect.

A main limitation of our paper is small sample size. However, the eight patients that achieved improved GV still demonstrated statistically significant improvement in nerve excitability profiles. Although the patients that had worsening GV did not demonstrate worsening nerve excitability, this subpopulation was likely too small a sample size and larger studies are warranted to confirm this. Given the overall correlation of GV with TE and RC parameters seen amongst our entire patient group, this would suggest that nerve excitability profiles would both improve and worsen in line with GV.

This is one of few studies in current literature that demonstrate a longitudinal improvement in nerve function in the same group of patients with diabetes. This is likely because of the relatively infrequent employment of nerve excitability testing.

Prior to this, longitudinal assessments in even pivotal studies such as Diabetes Control and Complications Trial only used physical examination and routine nerve conduction studies. Although this assessment may be powered to detect slowing of progression or stability of neuropathy, it is unlikely to detect slight restoration of axonal function that can be seen with nerve excitability testing in a smaller sample size. One study has demonstrated longitudinal improvement in nerve excitability in patients with predominantly TIIDM following initiation of intensive insulin with MDII.(2) However, glycemic variability was not measured in these patients.

Mechanisms of diabetic neuropathy include glucose-driven oxidative stress with overproduction of superoxide by mitochondria.(24) In addition, increased activity of the sorbitol pathway compromises the glutathione cycle and rather than hydrogen peroxide metabolising to water, it is diverted to the production of superhydroxide. Accumulation of sorbitol can also cause direct tissue toxicity as well as tissue oedema from osmotic forces. Other mechanisms include the advanced glycation end-product pathway due to increased non-enzymatic glycation of proteins, increased flux through the hexosamine pathway, and activation of protein kinase C.(25) It was previously generally thought that it was persistent hyperglycemia that led to the above pathophysiological changes. However, studies have shown that GV as expressed by MAGE correlated strongly with urinary 8-iso

prostaglandin F2α, a marker of oxidative stress, while sustained hyperglycemia as expressed by mean glucose concentrations, did not.(26,27) Conversely there are other studies that show no significant association with GV, and thus the way by which GV contributes to peripheral neuropathy, and other microvascular complications in diabetes, remains unclear. Although we did not measure levels of oxidative stress, our study supports the fact that neurons cannot accommodate larger episodic glucose swings, but further studies are required to elucidate the exact mechanism behind this.

These exact mechanisms are complex and likely multi-factorial. What can be seen from current literature is that is that there is growing evidence that functional axonal changes occur *before* the development of axonal cell death which represents the final step in the cascade of events .(11-13) These changes can be seen in asymptomatic patients without clinical evidence of neuropathy. Given that these changes appear to be reversible with improvement in GV, this potentially provides a therapeutic window to prevent the development of neuropathy.

Conclusion:

Simply initiating continuous insulin will not alter nerve excitability. However, when successful in reducing glycemic variability, CSII therapy is associated with reversal

and even a normalization of axonal dysfunction in TIDM. The improvement in TE and superexcitability seen are consistent with restoration of transaxonal membrane potential, likely due to reversal of Na+/K+ pump dysfunction. CSII therefore has the potential to provide a long-term neuroprotective effect against the development of diabetic polyneuropathy.

Abbreviations:

T1DM, type 1 diabetes mellitus; GV, glycemic variability; HbA1c, glycosylated hemoglobin (%); CBG, capillary blood glucose; MAGE, mean amplitude of glycemic excursions; SD, standard deviation; TE, threshold electrotonus; RC, recovery cycle; SDTC, strength-duration time constant; I/V, current threshold relationship; CGM, continuous glucose monitoring; MDII, multiple daily insulin injections; CSII, continuous subcutaneous insulin infusion

Conflicts of Interest:

None of the authors has any conflict of interest to disclose.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

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Figure 1. Correlations between standard deviation (SD) of continuous glucose monitoring (CGM) readings and motor nerve excitability parameters. MAGE: mean amplitude of glycemic excursions.

Figure 2. Mean motor nerve excitability plots in patients who had an improvement in GV (glycemic variability) after initiation of CSII (continuous subcutaneous insulin infusion): **A.** threshold electrotonus (TE) and **B.** recovery cycle (RC). Red line indicates baseline mean data for patients on MDII (multiple daily insulin injections). Green line indicates the mean data for the same patients three months following successful initiation of CSII. There is reversal of both TE and RC abnormalities to meet that of normal controls (black dashed line). Bar graphs representations of TEd(10-20ms), TEh(90-100ms), superexcitability and subexcitability in the same patients switched from MDII to CSII three months later, compared to controls. * $p<0.05$

Table 1. Demographic details of patient and control group

	Patients (n=12)	Controls (n=30)	P-value
Age (years)	32.9 ± 3.2	35.0 ± 2.5	0.67
Gender (F:M)	7:5	13:17	0.49
BMI	26.0 ± 1.3	25.4 ± 0.9	0.68
Temperature (°C)	32.5 ± 0.1	32.6± 0.1	0.62

Data expressed as mean ± SEM
BMI: body mass index

Table 2. Summary of nerve conduction study data of patients

NCS parameter	Median	Range		Patients Abnormal	Normal Reference
		Low	High		
Sural Amp (μV)	16.4	0	21.6	2	>6.0
Sural CV (m/s)	46.5	33.3	54.8	1	>38.0
Fibular distal CMAP (mV)	4.2	0.2	6.7	1	>2.5
Fibular distal latency (ms)	4.0	3.1	5.6	1	<5.5
Fibular CV (m/s)	43.0	33.1	45.6	1	>38.0

Table 3. Median motor excitability findings for patients with type 1 diabetes mellitus treated with multiple daily insulin injections **vs. controls**

	Patients (12)	Controls (30)	P-value
TEd(10-20ms)	65.8 ± 1.7	70.1 ± 0.7	0.02*
TEd(peak)	64.8 ± 1.7	69.6 ± 0.7	0.005**
TEd(90-100ms)	43.0 ± 0.9	45.5 ± 0.7	0.07
TEh(90-100ms)	-106.9 ± 5.7	-124.2 ± 3.2	0.02*
Superexcitability	-20.3 ± 1.9	-24.2 ± 0.9	0.04*
Subexcitability	14.4 ± 1.2	14.8 ± 0.8	0.70
Resting I/V slope	0.64 ± 0.02	0.58 ± 0.01	0.29

SDTC (ms)	0.48 ± 0.04	0.48 ± 0.01	0.92

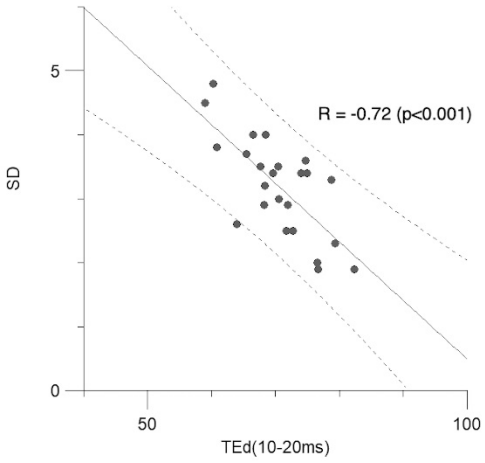
Unless otherwise stated, values are given as mean percentage change in threshold ± SEM.
* p<0.05; ** p<0.01

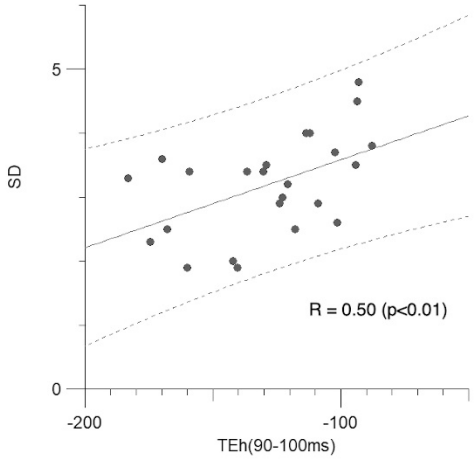
Table 4. Description of each enrolled patient with glucose, HbA1c and

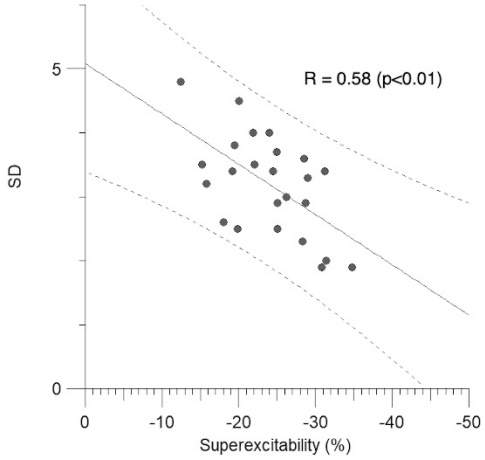
Patient	Age (yrs)	Sex	BMI	Years of DM	MDNS Grade		Sural Amp (μV)	BSL		HbA1c		MAGE		SD	
					Exam	NCS		Pre	Post	Pre	Post	Pre	Post	Pre	Post
1*	42	M	35.1	31	0	0	20.8	9.6	9	8	7	7.1	7.4	3.3	3.6
2	21	F	20.7	1	0	0	16.8	8.4	8	7.3	7.1	9.7	7.7	3.4	3.2
3	38	F	23.1	2	0	0	19.0	11	8.5	7.2	7.4	7.9	6.6	3.0	2.5
4	26	F	32.0	13	0	0	12.6	13.2	18.4	8.5	8.8	9.7	8.4	4.5	4.0
5	38	M	23.5	28	1	1	4.3	22	6.3	12	7.8	11.1	8.8	4.8	3.5
6*	59	F	23.0	41	2	2	0	5.8	13.9	8.5	6.4	5.6	6.2	2.3	2.5
7	22	F	28.1	13	0	0	21.6	8.6	11.5	8.4	7.7	7.9	7.5	3.4	3.2
8*	39	M	27.8	18	0	0	11.4	9.2	3.8	6.5	6.9	5.5	6.9	2.6	3.8
9	26	M	26.6	21	0	0	12.3	10	9.7	7.9	7.6	9.7	7.2	4.0	2.9
10	28	F	23.9	6	0	0	18.5	6.6	7.2	7.9	6.9	7.2	7.1	3.7	2.9
11	26	F	24.8	23	0	0	16.0	5.3	7.2	6.7	5.9	7.9	3.1	3.5	1.9
12*	30	M	22.9	1	0	0	20.0	3.9	5.3	5.4	5.2	3.1	3.3	1.9	2.0
Mean	32.9		26.0	16.5			14.4	9.5	9.1	8.0	7.2	7.8	6.8	3.3	2.9
SEM	3.1		1.2	4			1.9	1.4	1.2	0.4	0.3	0.6	0.4	0.3	0.2

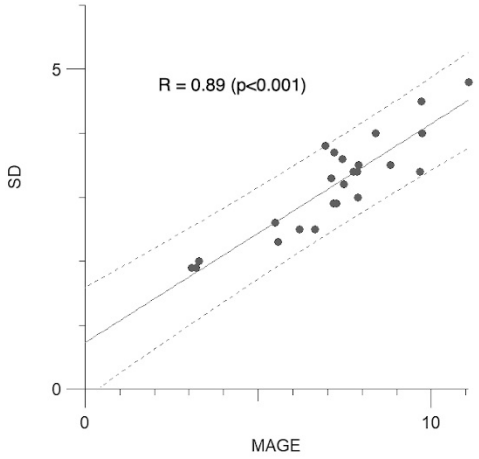
MDNS Grade includes physical examination component and nerve conduction study results
BMI: body mass index
MDNS: Michigan Diabetic Neuropathy Score
BSL: blood sugar level, performed at time of nerve excitability testing
MAGE: mean amplitude of glycemic excursions
SD: standard deviation of BSLs

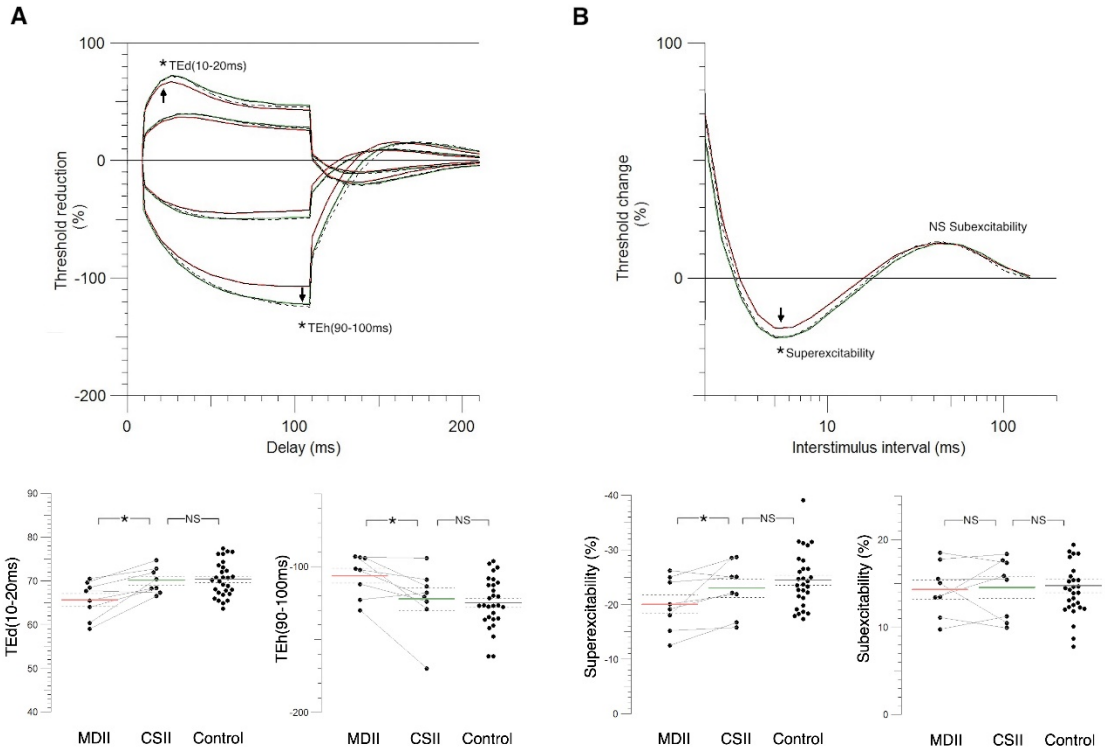
* indicates patient that showed no improvement or worsening of glycaemic variability as measured by MAGE and SD

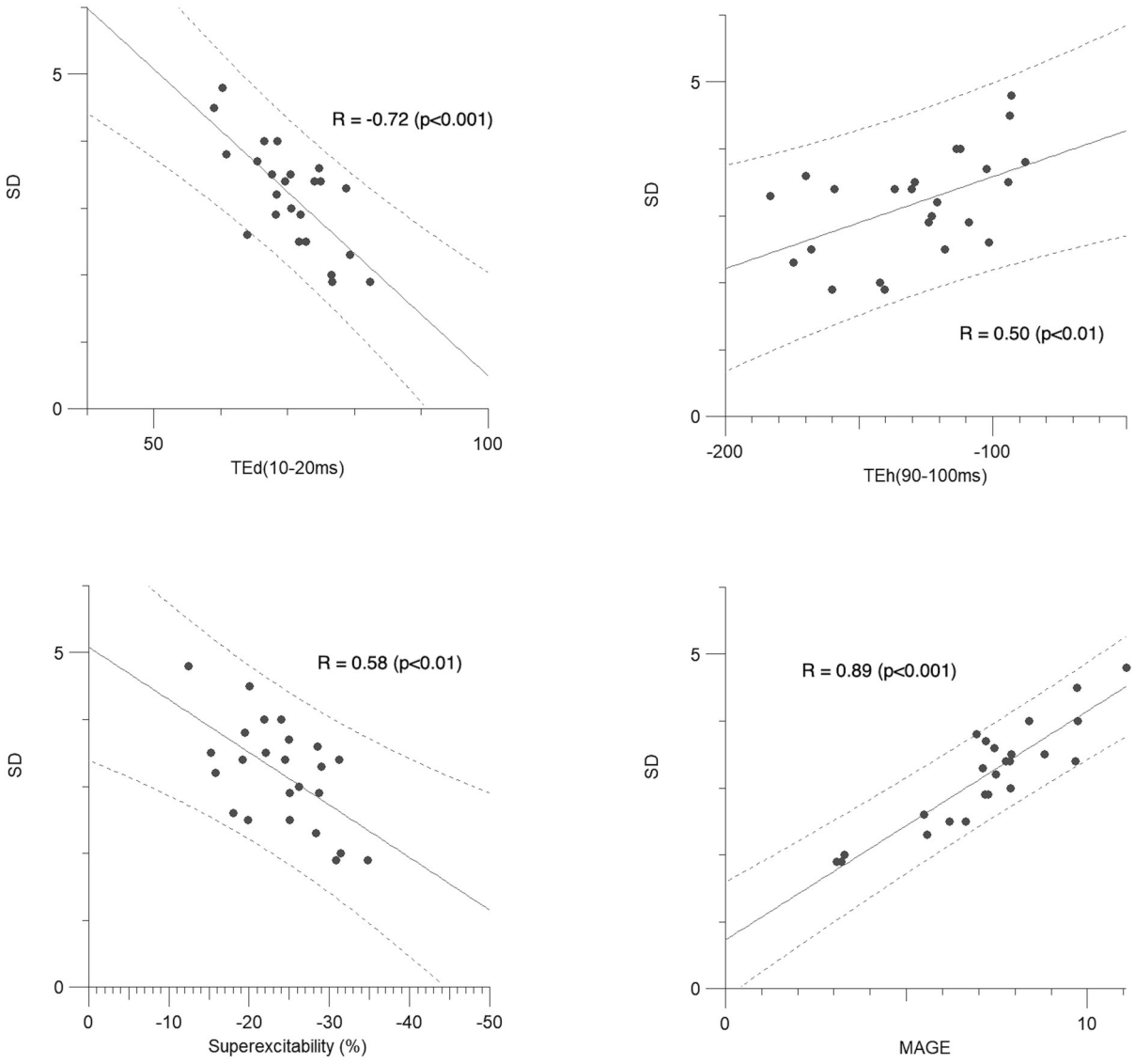












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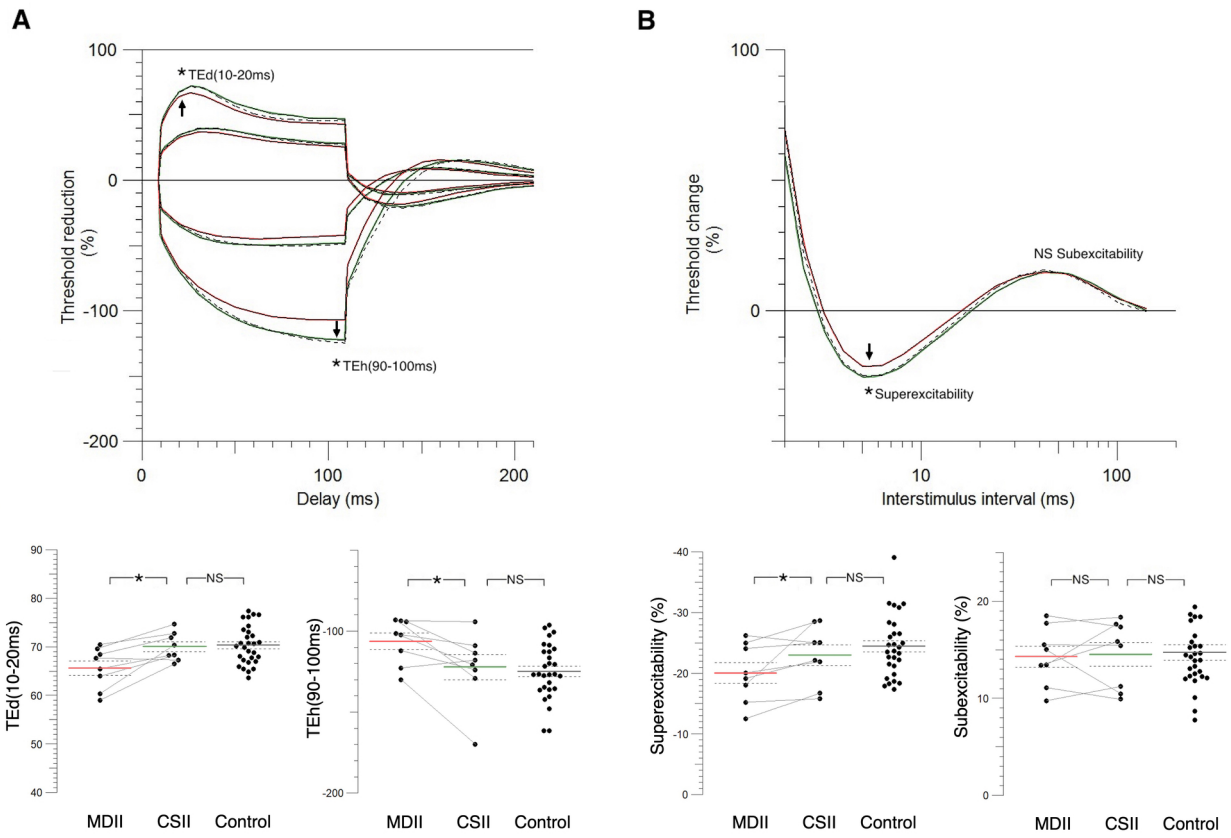

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