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Early sensory neurophysiological changes in prediabetes

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ABSTRACT

Aims/Introduction: To elucidate whether axonal changes arise in the prediabetic state and to find a biomarker for early detection of neurophysiological changes.

Materials and Methods: We enrolled asymptomatic diabetes patients, as well as prediabetic and normoglycemic individuals to test sensory nerve excitability, and we analyzed those findings and their correlation with clinical profiles.

Results: In nerve excitability tests, superexcitability in the recovery cycle showed increasing changes in the normoglycemic, prediabetes and diabetes cohorts (–19.09 ± 4.56% in normoglycemia, –22.39 ± 3.16% in prediabetes and –23.71 ± 5.15% in diabetes, $P = 0.002$). Relatively prolonged distal sensory latency was observed in the median nerve (3.12 ± 0.29 ms in normoglycemia, 3.23 ± 0.38 ms in prediabetes and 3.45 ± 0.43 ms in diabetes, $P = 0.019$). Superexcitability was positively correlated with fasting plasma glucose ($r = 0.291$, $P = 0.009$) and glycated hemoglobin ($r = 0.331$, $P = 0.003$) in all participants.

Conclusions: Sensory superexcitability and latencies are the most sensitive parameters for detecting preclinical physiological dysfunction in prediabetes. In addition, changes in favor of superexcitability were positively correlated with glycated hemoglobin for all participants. These results suggest that early axonal changes start in the prediabetic stage, and that the monitoring strategy for polyneuropathy should start as early as prediabetes.

INTRODUCTION

Diabetic neuropathies include distal symmetric polyneuropathy, chronic idiopathic sensory axonal neuropathy and small fiber neuropathy. The neurological complications of diabetes might arise as early as the time of diagnosis. Of all individuals with prediabetes, 11–25% have peripheral neuropathies[1]. The possible mechanisms of axonal dysfunction, including disruption of Schwann cell metabolism, microvascular abnormalities and endothelial dysfunction through the polyol, hexosamine/protein kinase C, and advanced glycation end-product pathways, are related to hyperglycemia, dyslipidemia and insulin resistance[2–4]. Hyperglycemia also causes excessive glycolysis, which overloads the mitochondria and causes excessive reactive oxygen species generation. Hexosamine pathway activation and extracellular advanced glycation end-product binding to receptors as a result of hyperglycemia might increase oxidative stress and trigger an inflammatory response. These phenomena of bioenergetic failure, osmotic and oxidative stress, and inflammation result in axonal dysfunction.

The nerve injury and metabolic derangement that occur in prediabetes patients might be reversible and transiently improved in the first year with diet control and exercise[5]. Consequently, early diagnosis of neurological dysfunction is important for preventing neuropathic deterioration. Clinical practitioners urgently require a sensitive tool to detect early changes in nerves in diabetes and prediabetes patients. Many studies focusing on neuropathy in diabetes patients through traditional nerve conduction studies (NCSs) have been published, and the results show that NCSs are not a sensitive tool for diabetic polyneuropathies[6–8]. In patients with prediabetes or impaired glucose tolerance, neuropathy predominantly involving small fibers was established to contribute to neuropathic pain, and autonomic dysfunction was established[9–12]. Therefore, traditional NCSs, which are mainly for large nerve fibers, are

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not sensitive enough to detect early nerve injury[13]. This lack of sensitivity limits the clinical neurological assessment of polyneuropathy in prediabetes or early diabetes patients[9].

In 1999, a nerve excitability test was developed to provide complementary information to traditional neurophysiological studies[14,15]. This non-invasive test can provide clinical neurologists with nodal and paranodal ion channel activity levels, membrane potentials, and myelin properties *in vivo*[14–16]. Kiernan *et al.*[16] established a protocol measuring the "sensory" axonal nerve excitability, and confirmed its efficacy in studying the electrophysiology and channel function of sensory axons. Clinical application has been studied for different neurological diseases, such as cervical radiculopathy[18], cisplatin-induced neuropathy[19–21], uremic polyneuropathy[22,23] and diabetic neuropathies[24–26]. In previous nerve excitability tests among diabetes patients, the excitability parameters of sensory nerves changed earlier than those of motor nerves[26] and were correlated with glycated hemoglobin (HbA1c) in individuals with asymptomatic diabetes[25,26]. Therefore, a nerve excitability test could be an early tool for detecting neurophysiological changes in patients with hyperglycemia. The purpose of the present study was to use this tool to detect whether sensory axonal fiber changes begin in prediabetes and are associated with plasma glucose.

METHODS

Criteria for patient enrollment

A total of 40 patients (aged 42–80 years) at Wanfang Hospital (Taipei, Taiwan) who had been diagnosed with prediabetes were enrolled to undergo a nerve excitability test and an NCS. Prediabetes is defined by the American Diabetes Association as meeting one of the three following criteria: HbA1c of 5.7–6.4%, fasting glucose of 100–125 mg/dL or a result of 140–199 mg/dL on the 2-h oral glucose tolerance test[27,28]. A total of 20 age-matched normoglycemic (NG) volunteers (aged 47–83 years) and 20 patients with diabetes (aged 42–70 years) were also enrolled. Diabetes was diagnosed according to the American Diabetes Association criteria[27,28], and the patients had received medical treatment. We excluded individuals with carpal tunnel syndrome, abnormal renal function (serum creatinine >1.2 mg/dL) and polyneuropathies caused by other etiologies.

The protocol for this research project was approved by a suitably constituted institutional ethics committee (TMU-Joint Institutional Review Board, Approval No. N201510049), and it conforms to the provisions of the Declaration of Helsinki.

Clinical evaluation

The enrolled patients underwent laboratory tests to determine their fasting plasma glucose, HbA1c and lipid profiles (total cholesterol, triglyceride and low-density lipoprotein cholesterol); additionally, their body mass index was calculated.

For the study of asymptomatic diabetes and prediabetes, we excluded patients with dysesthesia, hypoesthesia, numbness or weakness in their limbs. A neurological examination was also carried our. Furthermore, an NCS was performed on all participants in a neurophysiological laboratory at Wanfang Hospital, and the participants were required to have results within the normal ranges to be included in the study.

Nerve excitability test

Nerve excitability studies were carried out on all participants by stimulating the median nerve at the wrist according to TRONDNF protocols, with the skin temperature on the wrist maintained at ≥32.0°C[15]. An isolated linear bipolar constant-current stimulator (DS5; Digitimer, Welwyn Garden City, UK) provided the stimulus current. The changes in current required to produce a target potential corresponding to 50% of the maximal compound muscle action potential or sensory nerve action potential were tracked. Commercialized software (QTRAC version 10/11/2012; Institute of Neurology, London, UK) controlled the stimulation current and recorded the threshold changes[15,17].

The TRONDNF protocol was established by Kiernan *et al.*[15] for the nerve excitability test. Four different electrostimulation tests were automatically carried out in the TRONDNF: (i) a test to establish the stimulus–response curve; (ii) a test to determine the strength–duration relationship, the rheobase and the strength–duration time constant (SDTC); (iii) a test to determine the threshold electrotonus (TE); that is, the potential change produced by 1-ms test pulses under 100-ms subthreshold conditioning, polarizing currents in both depolarizing (TEd) and hyperpolarizing (TEh) directions; and (iv) the recovery cycle, the threshold changes in response to a test stimulus pulse after a supramaximal conditioning stimulus with interstimulus intervals from 2 to 200 ms. The important parameters in the nerve excitability test include the SDTC, TEd, TEh, superexcitability and late subexcitability. SDTC is determined by nodal sodium permeability. TEd and TEh are determined mainly by internodal membrane properties and potential. Superexcitability is inhibited by paranodal fast potassium channel (K_f) function, and late subexcitability is determined by internodal slow potassium channel (K_s) function. Using these parameters, we can estimate the nodal and internodal function of the diseased axons.

Statistical analysis

We used Statistical Package for the Social Sciences (SPSS) for Windows version 21 (SPSS Inc., Chicago, IL, USA). Levene's test for equality of variances was carried out on all variables. We compared the demographic profiles, nerve conduction results and nerve excitability parameters in the three groups by analysis of variance (ANOVA). We use Bonferroni's method as a post-hoc test to analyze the pairwise differences between groups. Linear correlation was used to determine whether NCS and/or nerve excitability parameters were correlated with clinical profiles. We defined P-values ≤0.05 as significant.

RESULTS

Patient clinical profiles

The demographic and clinical features of the normoglycemic, prediabetic and diabetic cohorts are shown in Table 1. The

mean HbA1c levels were 5.30% in normoglycemia, 5.9% in prediabetes and 6.7% in diabetes ($P < 0.001$; Table 1). The mean fasting plasma glucose levels were 87.1 mg/dL in normoglycemia, 101.5 mg/dL in prediabetes and 128.75 mg/dL in diabetes ($P < 0.001$).

In addition to fasting plasma glucose, body mass index was higher in prediabetes than in normoglycemia (25.45 ± 4.05 vs 22.01 ± 2.33, $P = 0.003$). The NG cohort was noted to have higher total cholesterol than the prediabetes cohort (209.06 ± 35.16 mg/dL vs 176.49 ± 38.43 mg/dL, $P = 0.011$) or the diabetes cohort (175.21 ± 33.93 mg/dL, $P = 0.021$). The NG cohort had increased low-density lipoprotein cholesterol compared with the prediabetes cohort (132 ± 32.05 mg/dL vs 106.37 ± 34.70 mg/dL, $P = 0.024$). The difference in triglycerides among groups was not statistically significant (Table 1).

Nerve conduction studies

All participants underwent NCS, the results of which are shown in Table 2. The results are within the normal range defined by the NCS laboratory at Wanfang Hospital.

Nerve excitability test

Regarding the sensory axonal nerve excitability properties of participants with prediabetes, the superexcitability increased

Table 1 | Demographic data and clinical profiles of the participants

Clinical profile	Normoglycemia ($n = 20$)Mean (SD)	Prediabetes ($n = 40$)Mean (SD)	Diabetes ($n = 20$)Mean (SD)
Male/female (*n*)	10/ 10	15/ 25	13/ 7
Age (years)	62.35 (11.08)	60.20 (9.14)	57.55 (9.30)
HbA1c (%)†	5.3 (0.29)	5.9 (0.23)	6.7 (0.81)
Fasting plasma glucose (mg/dL)‡	87.1 (5.96)	101.5 (13.71)	128.75 (31.76)
BMI (kg/m^2)§	22.01 (2.33)	25.45 (4.05)	23.44 (2.60)
Cholesterol (mg/dL)¶	209.06 (35.16)	176.49 (38.23)	175.21 (33.93)
LDL (mg/dL)**	132 (32.05)	106.37 (34.70)	106.74 (25.96)
Triglycerides (mg/dL)	99.05 (40.85)	108.86 (49.17)	137.79 (64.05)

†One-way ANOVA showed $P < 0.001$, with Bonferroni's post-hoc test showing $P < 0.001$ between each two of these three cohorts. ‡‡One-way ANOVA showed $P < 0.001$, with Bonferroni's post-hoc test showing $P < 0.018$ for normoglycemia versus prediabetes and $P < 0.001$ for diabetes versus prediabetes and normoglycemia. §One-way ANOVA showed $P = 0.003$, with Bonferroni's post-hoc test showing $P = 0.004$ for normoglycemia versus prediabetes. ¶One-way ANOVA showed $P = 0.007$, with Bonferroni's post-hoc test showing $P = 0.011$ for normoglycemia versus prediabetes and $P = 0.021$ for normoglycemia versus diabetes. **One-way ANOVA showed $P = 0.024$, with Bonferroni's post-hoc test showing $P = 0.028$ for normoglycemia versus prediabetes and $P = 0.070$ for normoglycemia versus diabetes. BMI, body mass index; LDL, low-density lipoprotein; SD, standard deviation.

Table 2 | Comparison of sensory nerve neurophysiology studies in participants with normoglycemia, prediabetes and diabetes

	NormoglycemiaMean (SD)	PrediabetesMean (SD)	Diabetes Mean (SD)
Nerve excitability tests			
Latency (ms)†	3.12 (0.29)	3.23 (0.38)	3.45 (0.43)
SDTC	0.58 (0.13)	0.56 (0.13)	0.52 (0.09)
Superexcitability (%)‡	−19.09 (4.56)	−22.39 (3.16)	−23.71 (5.15)
Subexcitability (%)	11.12 (2.97)	11.26 (2.58)	10.30 (2.65)
RRP (ms)	3.34 (0.62)	3.18 (0.40)	3.15 (0.48)
Refractoriness (%)	20.26 (19.53)	16.32 (15.58)	10.49 (14.91)
TEh (90–100 ms)	−149.23 (19.69)	−149.27 (20.40)	−152.66 (26.77)
Nerve conduction study§			
Median distal latency (ms)	2.32 (0.30)	2.61 (0.35)	2.62 (0.27)
Median SNAP amplitude (μV)	40.83 (14.11)	33.23 (12.17)	35.57 (7.11)
Median NCV (m/s)	61.33 (8.31)	54.79 (7.45)	54.00 (5.42)
Sural SNAP amplitude (μV)	11.39 (4.94)	14.13 (7.18)	14.50 (5.91)
Sural NCV (m/s)	51.00 (5.46)	52.00 (8.00)	39.20 (17.28)

The skin temperature at the wrist was maintained at ≥32.0°C for all studies. †One-way ANOVA showed $P < 0.019$, with Bonferroni's post-hoc test showing $P = 0.018$ for normoglycemia versus diabetes. ‡One-way ANOVA showed $P < 0.002$, with Bonferroni's post-hoc test showing $P = 0.013$ for normoglycemia versus prediabetes and $P = 0.002$ for normoglycemia versus diabetes. §The normal ranges defined by the nerve conduction study laboratory at Taipei Municipal Wanfang Hospital: median distal sensory latency <2.8 ms, median sensory nerve action potential (SNAP) amplitude >10 μV, median sensory nerve conduction velocity (NCV) 48.7–65.5 m/s, sural SNAP amplitude >5 μV, sural NCV: 41.5–58.3 m/s. CMAP, compound muscle action potential; RRP, relative refractory period; SDTC, strength-duration time constant; TEh, threshold electrotonus in hyperpolarization.

significantly (–22.39 ± 3.16% in prediabetes and –19.09 ± 4.56% in NG, $P = 0.013$; Figures 1d,2b; Table 2). The participants with diabetes had greater superexcitability than the NG participants (–23.71 ± 5.15%, $P = 0.002$, Figures 1d,2b; Table 2). The latencies in prediabetes and diabetes were mildly prolonged, but the only significant difference was between diabetes and normoglycemia (Figure 2a). There was no difference among the three cohorts in other parameters: SDTC (Figure 1b; Table 2), subexcitability, refractoriness (%), relative refractory period (Figure 1d; Table 2) and TE (Figure 1c; Table 2).

Correlations between axonal excitability parameters and clinical profiles

The sensory superexcitability of all enrolled participants was positively correlated with both fasting plasma glucose (correlation coefficient 0.291, $P = 0.009$) and HbA1c levels (correlation coefficient 0.331, $P = 0.003$; Figure 3). All measured parameters, including other NCS and nerve excitability parameters, were uncorrelated with plasma fasting glucose, HbA1c and lipid profiles. The linear regression implied that plasma fasting glucose and HbA1c were more important than other metabolic factors including body mass index, bodyweight, total cholesterol, low-density lipoprotein, and triglyceride level in determining sensory axonal function (superexcitability).

DISCUSSION

The present results showed that sensory superexcitability increased in prediabetes and diabetes patients. Superexcitability is determined by the membrane potential or the function of the paranodal K_f channel. Two main factors increase superexcitability: the hyperpolarization of membrane potential and a decrease in K_f function[16,17,29]. Membrane hyperpolarization might not be the cause in prediabetes, because no changes of parameters, such as increased threshold current, reduced the SDTC, increase in TE and reduced subexcitability, were found[30–32]. Therefore, we assumed the change of superexcitability was a result of K_f channel dysfunction. Calvo *et al.*[33] documented that expression of fast potassium channels (including Kv1.1 and Kv1.2) at juxtaparanodal region is markedly reduced in the injured sensory axon animal model. In addition, axonal hyperexcitability and increasing spontaneous discharge occurred. Zenker *et al.*[34] found reduced presence of the

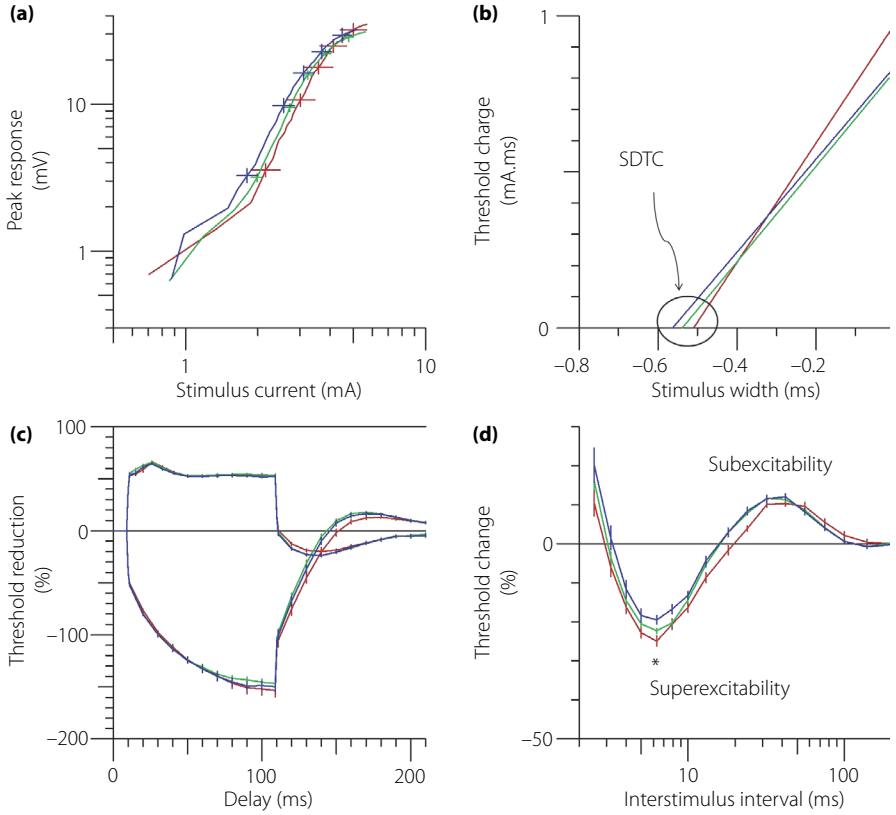

Figure 1 | (a) The peak response showed a similar threshold in all three groups. (b) There was no difference in the strength–duration time constant (SDTC) between the three groups. (c) The threshold electrotonus did not show a fanning-out pattern in depolarizing or hyperpolarizing conditions. (d) The recovery cycle showed increased superexcitability in the prediabetes and asymptomatic diabetes groups compared with the normoglycemic group. However, there was no difference in subexcitability, refractoriness or relative refractory period among the three groups. Blue line: normoglycemia; green line: prediabetes; red line: asymptomatic diabetes.

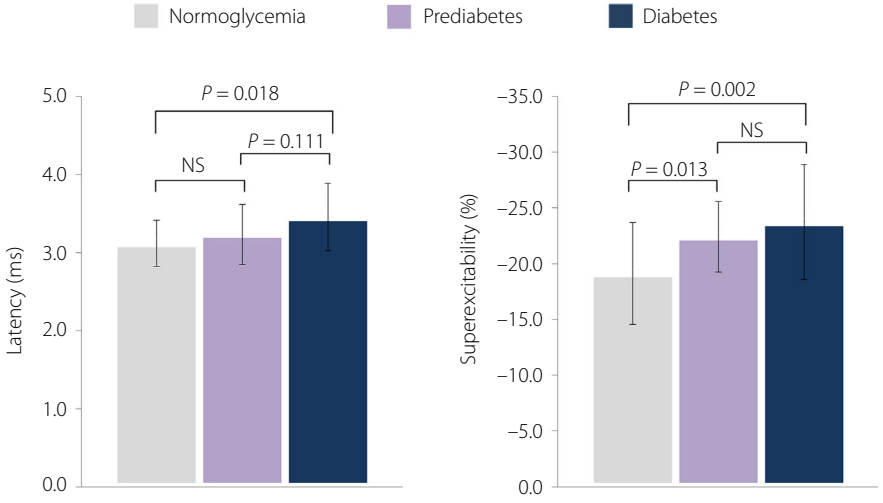

Figure 2 | Analysis showed significantly prolonged distal sensory latency (P = 0.019) and increased superexcitability (P = 0.002) in the participants with higher glycemic levels; the P-values were determined by ANOVA with Bonferroni's post-hoc test. Error bars, stamdard deviation. NS, the mean difference is non-significant.

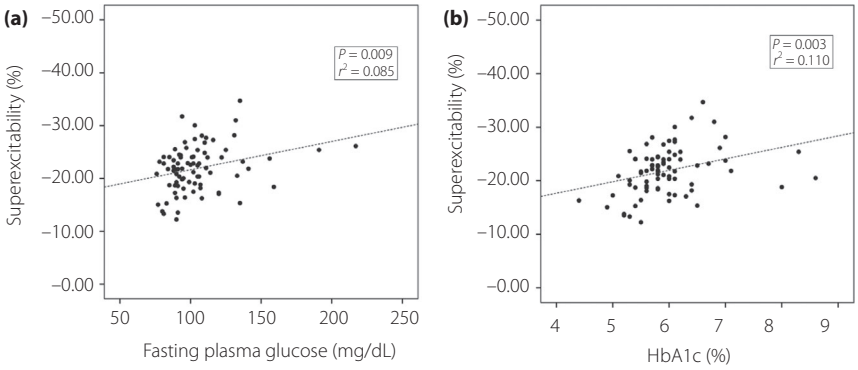

Figure 3 | The results show a correlation between increased sensory axonal superexcitability and increased (a) fasting plasma glucose or (b) glycated heomglobin (HbA1c).

Kv1.2 in juxtaparanodal regions of axons in both a type 2 diabetes animal model and in human peroneal nerve biopsy samples. These studies imply fast potassium channel dysfunction plays an important role in sensory axonal hyperexcitability[33–35]. Therefore, we surmised that the increased superexcitability in prediabetes is related to the reduction in K_f function. In Figure 4, we showed the possible neurophysiological changes in prediabetic axons. Hyperglycemia causes intracellular sorbitol accumulation and affects mitochondrial function. These alterations lead to increasing metabolic stress and energy failure[2–4]. Consequently, the Na^+/K^+ pump will be hypoactive, reducing both the sodium and potassium gradients across the axonal membrane[30]. The reduced ion gradients will also decrease paranodal K_f function[36,37]. The metabolic change is mild in prediabetes patients; therefore, the membrane potential might not be affected[11]. Other nerve excitability parameters are not different from those in individuals with NG.

Misawa *et al.*[38] reported that reduced activation of paranodal K_f conductance is related to increased superexcitability in hyperglycemia. Kitano *et al.* also reported that reduced SDTC in diabetes reduced nodal Na^+ conductance[39,40]. Those discoveries suggest that the pathogenesis of diabetic neuropathy starts from nodal and paranodal impairment. Consequently, we hypothesized that changes in prediabetic nerve function might also start in the paranodal area. Superexcitability is the most sensitive parameter for paranodal ion conductance changes; this finding is compatible with the present results for increasing superexcitability, which is the earliest change in prediabetes.

In the present study, sensory axonal superexcitability tended to increase with normoglycemia, prediabetes and diabetes. These changes were not affected by acute plasma glucose concentration, but were related to glycemic variability[41]. Our previous study also reported downward shifting of the sensory

recovery cycle and "fanning out" of TE progress from asymptomatic to symptomatic diabetes[26]. These findings suggest functional changes precede structural changes in diabetes polyneuropathy; they can also explain why the NCS is not a sensitive tool for clinical detection or screening. The results of the present study suggest that preventing the progression of neuropathy should start at the beginning of glucose instability.

In the present study, none of the patients or healthy controls had any symptoms or signs of neuropathy. Slight changes in sensory nerve excitability were detected in patients with asymptomatic prediabetes, indicating the start of axonal changes. As observed in epidemiological and some skin biopsy studies, injury to the peripheral nerves might start in the prediabetic stage[10,12,42]. We suspect that the possible pathogenesis is the same in prediabetes patients and early diabetes patients without neuropathy. Animal model studies also support the view that the pathophysiology of peripheral nerve dysfunction in patients with prediabetes or metabolic syndrome is similar to that in early diabetes patients without structural or pathological changes[43–45].

We found that sensory superexcitability was positively correlated with fasting plasma glucose and HbA1c in all participants. Similar correlations between nerve excitability parameters (superexcitability and late subexcitability) and clinical profiles have been discovered in diabetes patients[26,41]. However, aggressive glycemic control is an effective approach to reduce the risk of polyneuropathy only in type 1 diabetes patients[11]. A possible explanation is that complicated metabolic and inflammatory factors contribute to neuropathy in long-term type 2 diabetes. In the present study, the correlation of HbA1c with sensory hyperexcitability suggests that glucose control in prediabetes or the early stage of diabetes might slow the deterioration of axonal function. However, this hypothesis requires further empirical support.

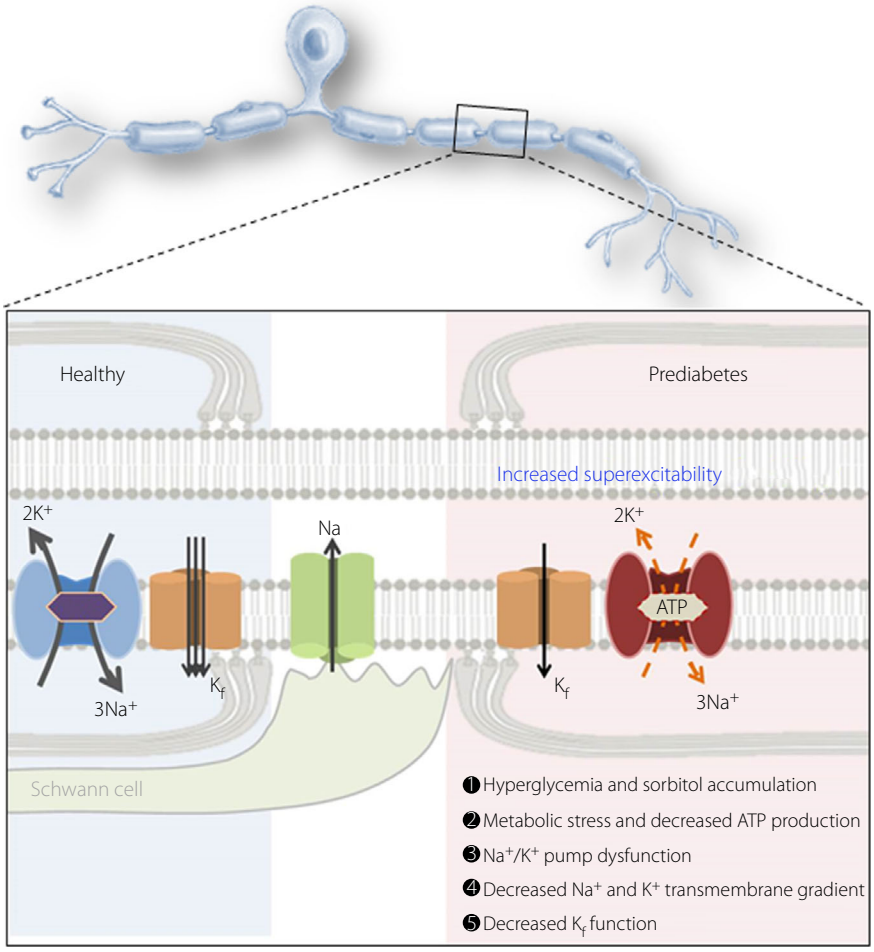

Figure 4 | ① Hyperglycemia, hyperlipidemia and advanced glycation end-products lead to sorbitol accumulation through the polyol pathway; ② increase metabolic stress, and induce anaerobic metabolism and energy failure with decreased adenosine triphosphate (ATP) production; resulting in ③ Na^+/K^+ pump dysfunction; which ④ reduces the transmembrane concentration gradients of both sodium and potassium; consequently, there is ⑤ a decrease in potassium conductance by hypoactive K_f channels, resulting in increased superexcitability.

In prediabetes, peripheral nerve dysfunction might be reversed if environmental factors are corrected. Kitano *et al.* reported that superexcitability shifted toward a normal range after the start of insulin treatment for diabetes[40]. In a prediabetic animal model, the administration of an aldolase reductase inhibitor corrected the peripheral neurological dysfunction induced by a high-fat diet[45]. In addition, lifestyle intervention, including diet control and exercise in patients with impaired glucose tolerance, results in restoration of cutaneous nerve endings and improvement of neuropathic pain[5]. We believe that the physiological changes might be reversed in the prediabetic and early diabetic stages, which is the reason why we focus on prediabetes rather than diabetes patients.

In conclusion, we believe that physiological changes in nerves begin to arise in the prediabetic stage, and that the Na^+/K^+ pumps are hypoactive caused by metabolic changes after hyperglycemia. In prediabetes patients, sensory axons are more vulnerable than motor axons, and the nerve excitability parameter that is most sensitive to hyperglycemia is superexcitability. Sensory axonal superexcitability is the most sensitive parameter in preclinical neurophysiological dysfunction in prediabetes. The present results show that sensory axonal superexcitability has a significantly positive correlation with fasting plasma glucose and HbA1c. Sensory nerve excitability provides a non-invasive tool for early detection to prevent the progression of diabetic neuropathy.

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DISCLOSURE

The authors declare no conflict of interest.

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