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Association between visceral, cardiac and sensorimotor polyneuropathies in diabetes mellitus ☆

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ABSTRACT

Aims: Gastrointestinal complaints are common in diabetes mellitus. However, its association to peripheral sensorimotor and autonomic neuropathies is not well investigated. The aim was to assess skin, muscle, bone and visceral sensitivity in diabetes patients with sensorimotor neuropathy, and correlate these with gastrointestinal symptoms and degree of cardiac autonomic neuropathy.
Methods: Twenty patients with sensorimotor neuropathy (65% type 2 diabetes, aged 58.3 ± 12.0 years, diabetes duration 15.8 ± 10.0 years) and 16 healthy controls were recruited. Cutaneous sensitivity to von Frey filaments, mechanical allodynia, muscle/bone/rectosigmoid sensitivities, and heart rate variability were examined. Gastrointestinal symptom scores (PAGI-SYM) and health-related quality of life (SF-36) were also recorded.
Results: Patients displayed hypesthesia to von Frey filaments ($p = 0.028$), but no difference to muscle and bone pain sensitivities. Also, patients were hyposensitive to multimodal rectal stimulations (all $p < 0.05$), although they suffered more gastrointestinal complaints. Heart rate variability was reduced in the patient cohort. Rectal mechanical and cutaneous sensitivities correlated ($p < 0.001$), and both were associated with heart rate variability as well as PAGI-SYM and SF-36 scores ($p < 0.01$).
Conclusions: In diabetic sensorimotor neuropathy there is substantial evidence of concomitant cutaneous, cardiac and visceral autonomic neuropathies. The neuropathy may reduce quality of life and explain the higher prevalence of gastrointestinal complaints.

1. Introduction

Diabetes mellitus (DM) is one of the most common causes of chronic morbidity world-wide, leading to late complications in a great number of patients. Neuropathy, a condition in which the nerve signal quality and velocity is reduced, is one of the most prevalent late complications, affecting around 50 % of all diabetes patients (Tesfaye et al., 2010). In its typical form, diabetic sensorimotor polyneuropathy (DSPN) affects both large myelinated as well as small unmyelinated nerve fibers. The condition is most commonly investigated through examination of large fiber conduction velocity, leaving the small nerve fibers unassessed (Dyck et al., 2011). However, measuring small fiber patency may be even more relevant, but is more complicated with various tests being advocated, such as corneal confocal microscopy, quantitative sensory testing, laser Doppler imager flare, sudomotor tests and intraepidermal nerve fiber density (Papanas & Ziegler, 2011; Rage et al., 2011; Tesfaye et al., 2010; Vas, Green, & Rayman, 2012).

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The entire gastrointestinal (GI) tract may also be affected in DM, producing a wide variety of symptoms. Many patients are undiagnosed and under-treated, because the GI tract has not traditionally been associated with DM and its complications. Complaints such as bloating, vomiting, pain, constipation and diarrhea are more prevalent in DM compared to the general population, affecting 30–70% (Bytzer et al., 2001; Ko, Chan, Chan, Tsang, & Cockram, 1999; Ricci et al., 2000). Although the visceral complications in diabetes were already known and described in a review by Rundles in 1945, the pathophysiological mechanisms are still not clear (Rundles, 1945). Visceral autonomic and enteric neuropathies, autoantibodies, changed intestinal microbiota, direct glucose effects, increased stiffness of the GI wall, exocrine pancreatic insufficiency, disordered gut–brain axis and humoral alterations are among the involved mechanisms, and likely etiology is multifactorial (Brock et al., 2012; Bures et al., 2010; Darwiche, Almer, Bjorgell, Cederholm, & Nilsson, 2001; Frokjaer et al., 2007; Granberg, Ejskjaer, Peakman, & Sundkvist, 2005; Rayner, Samsom, Jones, & Horowitz, 2001). Recent studies have demonstrated esophageal hyposensitivity and altered central sensory processing in DM patients with symptoms of gastroparesis (Brock et al., 2012). In line with this, microstructural changes in the brain areas involved in visceral sensory processing have been discovered, using magnetic resonance diffusion tensor imaging (Frokjaer et al., 2013).

To date, no study has examined the relationship between sensitivities in different tissues (skin, mucles, bone and viscera) in patients with DSPN. Using the rectosigmoideum as a visceral proxy, the aims of the present study were 1) to investigate somatic and visceral sensitivity in patients with DSPN using a multimodal, multitissue approach, 2) to characterize the association between sensorimotor (somatic), visceral and cardiac autonomic sensitivities and 3) to correlate these findings to health-related life quality and gastrointestinal symptom scores.

2. Methods

2.1. Subjects

Twenty patients with DM and sensorimotor neuropathy (average age 58.3 years, 10 women,) were recruited from Haukeland University Hospital, Bergen, Norway (Department of Internal Medicine and Section for Clinical Neurophysiology) and Saint George Hospital, Székesfehérvár, Hungary. The presence of sensorimotor neuropathy was defined according to the respective national guidelines, in line with the 2005 statement by the American Diabetes Association (Boulton et al., 2005). Sixteen healthy controls, matched for age and gender (9 women, age 62.6 years) were recruited through newspaper advertisement in Bergen. Subject characteristics are summarized in Table 1. Presence of retinopathy was based on anamnestic information and patient journals.

Table 1
Baseline characteristics.

Variables	Patients (n = 20)	Controls (n = 16)	*p*-value
Age (years)	58.3 (± 12.0)	62.6 (± 10.5)	NS
Gender (male/female)	10/10	7/9	NS
Body mass index BMI (kg/m^2)	28.0 (± 3.62)	25.8 (± 3.38)	NS
Diabetes duration (years)	15.8 (± 10.0)	–	–
Diabetes type (1/2)	35/65 %	–	–
HbA1c (%)	8.0 (± 1.0)	–	–
Retinopathy (%)	28	–	–
Creatinine level (μmol/l)[a]	68.5 (± 15.8)	71.3 (± 13.2)	NS
Smoking status (present/past/never)	2/8/9	2/10/4	NS
Hypertension (%)	81	19	0.001
Beta-blocker (%)	27	0	0.04
ACEI/angiotensin receptor blocker (%)	71	13	0.004
Statin use (%)	67	19	0.02
Metformin (%)	57	–	–

Data are means (± SD) unless otherwise indicated. NS = not significant.
[a] All estimated glomerular filtration rates > 60 ml/min/1.73 m^2.

The exclusion criteria were clinical conditions that might influence the visceral sensitivity (i.e. inflammatory bowel disorder, previous abdominal surgery, peptic ulcers), as well as neurological or psychiatric disorders that might interfere with study results. Medications known to affect GI motility or pain perception were ceased at least 24 h prior to experimental testing. Oral and written informed consents were obtained from all subjects, and the study was approved by the local ethic committees at Haukeland and St. George Hospitals.

2.2. Questionnaires

Subjects were asked to rate their gastrointestinal symptoms during the preceding two weeks using the validated Patient Assessment of Upper Gastrointestinal Disorder Severity Symptom Index (PAGI-SYM) (Revicki et al., 2004). Symptoms were graded from 0 (no symptoms) to 5 (very severe symptoms), and the scores were combined into six subscales; postprandial fullness/early satiety, nausea/vomiting, bloating, upper and lower abdominal pain and heartburn/regurgitation. The Short Form Health Survey (SF-36) was applied to assess quality of life. Eight subscales and two summary scales were calculated according to previously validated methods (Garratt, Schmidt, Mackintosh, & Fitzpatrick, 2002).

2.3. Experimental protocol

Sensory testing was performed following an overnight fast and after administration of a suppository to empty the rectum (dioctyl sodium sulfosuccinate and sorbitol, Klyx©, Ferring AS, Copenhagen, Denmark). All subjects underwent a hyperinsulinemic clamp procedure, aiming at a blood glucose level of 6.0 mmol/L in order to limit any effect of hyperglycemia and hyperinsulinemia on the visceral sensitivity (Frokjaer, Softeland, Graversen, Dimcevski, & Drewes, 2010; Softeland et al., 2011). The subjects were instructed in the use of a 0–10 electronic Visual Analogue Scale (VAS), which was later employed during the sensory testing. The use of VAS was facilitated through anchor words, where 0 = no perception; 3 = vague perception of moderate sensation; 5 = pain detection threshold; 7 = moderate pain and 10 = worst perceivable pain. VAS was recorded continuously during testing. The scale has been widely used in somatic and visceral sensory experiments, and has been described in detail previously (Brock et al., 2008; Dimcevski et al., 2007). Somatic and visceral sensory testing was completed in two hours on average, with another two hours needed for clamp preparation, blood glucose adjustment and the rectal suppositories. The order of testing was as indicated by the subsections below.

2.3.1. Somatic sensory testing

2.3.1.1. First sensation of von Frey filaments. Quantitative sensation of light touch was measured by use of Optihair von Frey-like filaments of increasing diameter at the base of the dorsum of the first toe, dominant foot (Marstock Nervtest, Schriesheim, Germany). The weight corresponding to first sensation was determined (Rolke et al., 2006).

2.3.1.2. Brush-induced allodynia. Dynamic allodynia was tested on a 2 × 6 cm area on the dorsum of the dominant foot. The SENSELab Brush-06 (Somedic AB, Hörby, Sweden) was used, aiming at a swipe speed of 4 cm/s (Samuelsson, Leffler, Johansson, & Hansson, 2007). VAS score following the first and the sixth consecutive swipes were assessed.

2.3.1.3. Temporal summation. Temporal summation was examined by giving 10 pin-pricks at a rate of 1/s using the 26 g von Frey monofilament within a 1 × 1 cm area just proximal of the first toe of the dominant foot. VAS was assessed at first and last prick. In case the patient was unable to feel the 26 g monofilament, the filament size corresponding to VAS 1 was used (Rolke et al., 2006).

2.3.1.4. Muscle pressure algometry. Pressure sensitivity on the dominant lateral side of the anterior tibial muscle was examined by use of a handheld electronic pressure algometer (Somedic AB, Hörby, Sweden). The probe had a surface area of 1 cm^2. Pressure increased by 30 kPa/s until VAS 7 was reached. This examination was performed three times, and the mean pressure was calculated (Staahl et al., 2007).

2.3.1.5. Bone pressure algometry. Pressure was applied on the flat side of the dominant tibial bone, approx. 15 cm distal of the patella. The same pressure algometer was used, however the probe had a smaller surface area of 0.031 cm^2. Pressure increased with 970 kPa/s, and the mean of three test runs was calculated (Andresen, Pfeiffer-Jensen, Brock, Drewes, & Arendt-Nielsen, 2013).

2.4. Visceral sensory testing

The rectal sensitivities to thermal, mechanical and electrical stimulations were examined by use of a multimodal probe (Ditens A/S, Aalborg, Denmark). The probe, which has an outer diameter of 6.2 mm, was positioned in the rectosigmoidum through a small anoscope, as described in greater detail previously (Brock et al., 2008).

2.4.1. Visceral thermal stimulation

Heat sensitivity was examined by circulating heated water through a rectal bag pre-filled with 60 ml water, using a volume-controlled pump (Ole Dich Instrument Makers, Hvidovre, Denmark). The balloon temperature increased gradually from 37 °C to a maximum of 60 °C. The stimulation was halted when reaching a sensation of VAS 7, and to minimize the unpleasantness the warm water was immediately evacuated. The balloon temperature corresponding to VAS 7 was recorded (Brock et al., 2008).

2.4.2. Visceral mechanical stimulation

Mechanical volume sensitivity was examined by distending the rectal bag, infusing 37 °C water through the pump. In order to ensure proper accommodation towards the pressure sensation, three preconditioning balloon distensions until a sensation of VAS 5 were performed. Then, sensitivity to mechanical stimulation was examined by recording the bag volume necessary to induce the sensation of VAS 7 (Brock et al., 2008).

2.4.3. Visceral electrical stimulation

The probe contained two stainless steel electrodes at the tip, and mucosal contact was ensured by measuring the impedance. Subjects in which it was not possible to achieve impedance below 5 kΩ were discarded from the subsequent statistical analysis. The electrical stimulation was given as a single 2 ms square pulse, starting at 1 mA and increasing gradually in 1–3 mA steps using a voltage-controlled current source stimulator (IES 230, JNI Biomedical ApS, Klarup, Denmark). Due to safety considerations, maximum current intensity was 50 mA. Intermittent sham stimuli were given in order to ensure blinding to the ascending stimulation intensity as well as to avoid the effect of accommodation and expectation. The current intensities needed to induce a sensation of VAS 1, 3, 5 and 7 were assessed.

2.5. Cardiac autonomic nervous system tests

All subjects were investigated on a separate occasion and in a fasting state. The autonomic nervous system patency was assessed through three test of heart rate variability (HRV) using the Heart Rhythm Scanner PE (Biocom Technologies, Poulsbo, WA, USA). The system investigates both time- and frequency domain measures of the HRV and its use has been described and validated elsewhere (1996; Zhang, 2007). Recordings were reviewed offline by the first author. Minor problems, such as mis-sensed beats were corrected. Subjects in which there were multiple ectopic beats or frank arrhythmias were excluded from further HRV analysis. The following tests were performed:

2.5.1. HRV during at 5-min rest

Subjects were instructed to rest in a semi-reclined position, and the surroundings were designed to avoid emotional arousal. See Table 2 for a complete list of parameters that were included in further analysis.

2.5.2. HRV upon deep respiration

Deep respiration triggers the baroreflex, which leads to heart rate fluctuations, primarily through a parasympathetic route. Subjects were instructed to breathe deeply with a rate of six breaths per minute, for a total recording period of one minute. The examination was repeated if necessary, to ensure deep and regular respiratory movements.

2.5.3. HRV as well as 30:15 ratio when standing up

This ratio is thought to predominately represent the capacity of the parasympathetic nervous system. Subjects rested in a semi-reclined position for five minutes and were then instructed to stand up in one steady movement. HRV in both positions was calculated, as

Table 2
Results of the cardiac autonomic tests.

Heart rate variability parameter		Patients	Healthy controls	*p*-value
HRV at rest	Mean HR	76.1 (±9.4)	61.3 (±9.0)	<0.001
	SDNN	20.4 (17–34)	43.2 (29–66)	0.009
	RMS-SD	21.9 (14–25)	31.2 (19–65)	0.056
	TP	130.5 (80–358)	394.0 (249–949)	0.025
	LF Norm	44.3 (21–76)	71.2 (57–87)	0.036
	HF Norm	55.7 (24–79)	28.8 (13–43)	0.036
	LF/HF	0.80 (0.27–3.3)	2.47 (1.3–6.8)	0.036
HRV during deep respiration	SD of HR	3.25 (±2.0)	5.63 (±2.8)	0.012
	Mean variance of HR	7.11 (±5.6)	13.3 (±8.1)	0.020
	E/I Ratio	1.08 (1.03–1.17)	1.19 (1.12–1.39)	0.017
HRV when standing up	30:15 Ratio	1.07 (1.03–1.16)	1.39 (1.25–1.44)	<0.001
	Time HR max	14.8 (9.3–24.5)	5.75 (3.5–11.5)	0.004
	Time HR min	22.3 (16.3–33.3)	17.8 (11.5–20.0)	0.07

Data are presented as means (± SD) or median (IQ-range).
Parameters HRV at rest: *Time domain*; Mean HR = mean heart rate during rest, SDNN = standard deviation from the mean heartbeat interval value (net effect of the autonomic regulation), RMS-SD = root mean square of the standard deviation (activity level of the parasympathetic regulation). *Frequency domain*; TP = total power (power spectrum of RR intervals throughout the frequency ranges — net autonomic function), LF Norm = low frequency activity (represents sympathetic tone), HF Norm = high frequency activity (represents parasympathetic tone), LF/HF = low/high frequency ratio (balance between sympathetic and parasympathetic activity).
Parameters HRV during deep respiration: SD of HR = standard deviation of the HR value, mean variance of HR = mean variation of heart rate among all breathing cycles, E/I-ratio = mean ratio between the longest and shortest RR-interval during deep respiration (all are measures of baroreflex sensitivity and capacity — predominantly parasympathetic tests).
Parameters HRV when standing up: 30:15 Ratio = ratio between maximum HR within the first 15 s after standing up and minimum HR within the first 30 s after standing up (predominantly parasympathetic test), time HR max (combined sympathetic and parasympathetic effect), time HR min (predominantly parasympathetic test).

well as the ratio between maximum heart rate within the first 15 s after standing up and minimum heart rate within the first 30 s after standing up.

2.6. Statistics

Normally distributed results are presented as mean (±SD) whereas non-normally distributed results as median (interquartile (IQ) range). One-way analysis of variance (ANOVA) was used to compare the two groups in terms of skin sensitivity, cardiac autonomic parameters and the questionnaires. Two-way ANOVA was used when comparing overall and VAS-level specific visceral, muscle and bone sensitivities. Pearson correlation analysis was performed in normally distributed data, and Spearman Rank correlation otherwise. Statistical significance was defined as $p \leq 0.05$. SigmaPlot 11 (Systat Software Inc., San Jose, CA, USA) was used for statistical analysis.

3. Results

All subjects underwent the hyperinsulinemic clamp without any adverse events. One patient did not complete any visceral stimulation due to strong discomfort upon insertion of the anoscope.

3.1. First sensation of von Frey filaments

Patients showed hypesthesia compared to controls, needing significantly thicker von Frey filaments in order to reach first sensation. Median thickness in patients 3.0 g (0.8–12.5), controls 1.0 g (0.4–1.4), $p = 0.028$.

3.2. Brush-induced allodynia

There was a trend towards decreased sensitivity but no change to allodynia in patients. Least square mean of VAS score at the 1st swipe in patients was 1.55 (±0.9) vs. 2.03 (±0.6) in controls, $p = 0.07$. At the 6th swipe: patients 1.7 (±1.3) vs. controls 1.97 (±0.50), $p = 0.2$.

3.3. Temporal summation

Three patients did not feel the standard 26 g monofilament and were tested using the monofilament size representing the sensation of VAS 1. Another patient did not feel any of the available filament sizes. Overall, in both patients and controls there was a dynamic pattern through the repetitive stimuli showing significant temporal summation, as the first prick mean VAS score was 2.2 (±1.1) whereas the tenth prick mean VAS score was 3.2 (±1.4), $F = 9.1$, $p = 0.004$. However, sub-group analysis did not reveal different summation in patients compared to controls (all $p > 0.05$).

3.4. Muscle and bone algometry

No differences between the patients and controls could be detected in terms of muscle and bone pain sensitivity. Mean pressure inducing a muscle sensation of VAS 7 was 415 (±232) kPa in patients, and 481 (±220) kPa in controls, $p = 0.13$. Corresponding bone pressures were 138 (±107) kPa in patients, and 150 (±50) kPa in controls, $p = 0.50$.

3.4.1. Visceral thermal stimulation

Fig. 1 illustrates the sensitivities to rectal thermal stimulations in the two groups. Patients were hyposensitive compared to controls, with an overall baseline corrected temperature increase of 18.0 (±9.1) °C vs. 13.3 (±7.0) °C in controls ($F = 14$, $p < 0.001$).

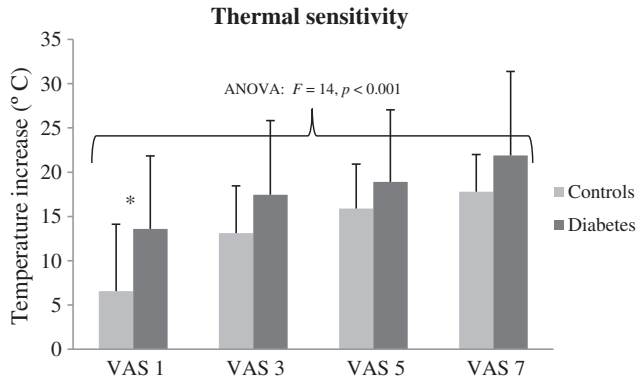

Fig. 1. The rectal sensitivity to thermal stimulation. ANOVA analysis included all VAS levels, and the post-hoc results of the individual VAS levels are provided. Patients showed overall hyposensitivity to heat. Y-axis describes the baseline-corrected temperature increase needed to induce the sensation of corresponding VAS scores. *$p \leq 0.05$. Error bars represent SD.

3.4.2. Visceral mechanical stimulation

Fig. 2 depicts the sensitivities to rectal mechanical stimulation. Patients were hyposensitive; overall balloon volume 204 (±125) ml in patients vs. 147 (±87.7) ml in controls, $F = 14$, $p < 0.001$.

3.4.3. Visceral electrical stimulation

Sensitivities to rectal electrical stimulation are shown in Fig. 3. Two patients were unable to complete the examination due to discomfort, whereas four healthy controls were excluded due to lack of mucosal contact. Patients were hyposensitive; overall current intensity was 40.3 (±11.3) mA vs. 34.9 (±11.4) mA in healthy controls, $F = 7$, $p = 0.009$.

3.5. Autonomic nervous system tests

Heart rate variability tests were completed in all subjects except five patients; one was lost to follow-up, two were excluded due to atrial fibrillation and two due to frequent ectopic heart beats. Patients demonstrated significant changes in 12 out of 13 parameters, affecting both sympathetic and parasympathetic fibers of the autonomic nervous system (Table 2).

3.6. Questionnaires

3.6.1. SF-36

The SF-36 was completed in 12 patients and 16 controls. Patients' health-related quality of life was reduced for most subscales

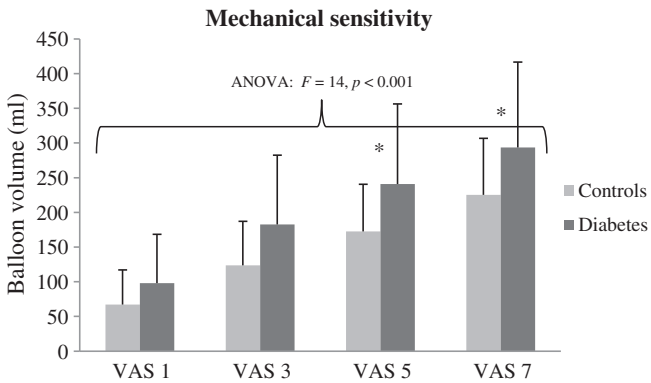

Fig. 2. The rectal sensitivity to mechanical stimulation. ANOVA analysis included all VAS levels, and the post-hoc results of the individual VAS levels are provided. Patients showed overall hyposensitivity. Y-axis describes the balloon volume needed to induce the corresponding VAS scores. *$p \leq 0.05$. Error bars represent SD.

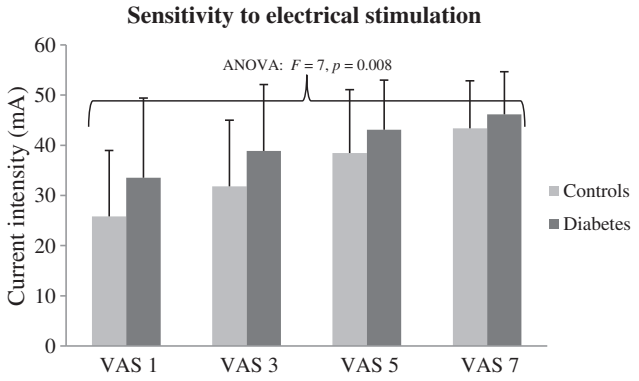

Fig. 3. The rectal sensitivity to electrical stimulation. ANOVA analysis included all VAS levels, and the post-hoc results of the individual VAS levels are provided. Patients showed overall hyposensitivity. Y-axis describes the current intensity needed to induce the corresponding VAS scores. The current increment blunts as the VAS increases, as a growing number of subjects reached the pre-defined maximal current intensity (50 mA). Error bars represent SD.

compared to controls, see Fig. 4. In particular, patients reported their physical health as poorer than in the aged matched control group (physical functioning, role limitation due to physical health, bodily pain, general health and vitality, all $p < 0.001$). Mental health and social functioning were also reduced, although the changes were less pronounced, $p < 0.05$. Looking at the summary scores, there was a highly significant reduction in the physical summary score; patients median score 37 (IQ-range 24–46) vs. healthy controls 55 (50–57), $p < 0.001$. However, there was no difference between the two groups in the mental summary score, $p > 0.05$.

3.6.2. PAGI-SYM

The PAGI-SYM questionnaire was completed in 10 patients and 16 controls. The two groups differed in terms of total score ($p = 0.008$) as well as four out of the six subscales; postprandial fullness ($p = 0.017$), upper abdominal pain ($p = 0.035$), lower abdominal pain ($p = 0.004$) and heartburn/regurgitation ($p = 0.004$). No difference could be found with respect to nausea/vomiting ($p = 0.146$) or bloating ($p = 0.076$).

3.7. Correlations

3.7.1. Correlations between visceral and monofilament skin sensitivity

The size of von Frey filament upon first sensation was positively associated with the overall rectal mechanical sensitivity ($r = 0.45$, $p < 0.001$), i.e. the more cutaneous hypesthesia, the more rectal mechanical hyposensitivity. There were no such associations to rectal electrical or thermal sensitivities.

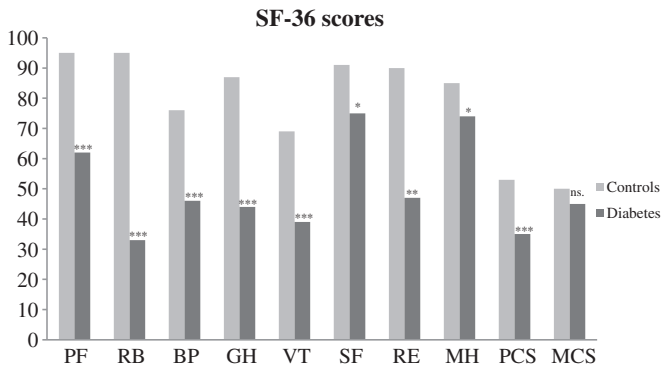

Fig. 4. Short Form-36 scores. The following subscales were calculated: physical functioning (PF), role limitations due to physical health (RP), bodily pain (BP), general health (GH), energy fatigue/vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH). Two summary scores were established, the physical (PCS) and mental (MCS) component summaries. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, ns = not significant.

3.7.2. Correlations between types of visceral stimulation

Sensitivity to rectal electrical stimulation correlated positively with mechanical sensitivity ($r = 0.52$, $p = 0.006$). No other significant associations between types of visceral stimulation could be found (all $p > 0.08$).

3.7.3. Correlations between visceral sensitivity and heart rate variability

As shown in Fig. 5A, the rectal mechanical sensitivity correlated with SDNN at rest ($r = -0.52$, $p < 0.01$). Furthermore there were several less strong associations; rectal mechanical sensitivity and total spectral power ($r = -0.40$, $p = 0.04$), E/I-ratio ($r = -0.36$, $p = 0.05$), 30:15 ratio when standing up ($r = -0.38$, $p = 0.04$). Similarly, we noted associations between rectal electrical sensitivity and SDNN at rest ($r = -0.42$ and $p = 0.05$), and rectal thermal sensitivity and the 30:15 ratio when standing up ($r = -0.37$, $p = 0.05$). No other significant correlations were found. In summary, the more impaired HRV, the more visceral hyposensitivity.

3.7.4. Correlations between SF-36, visceral and cardiac autonomic nervous function

Physical summary score of the SF-36 (PSC) correlated with the mechanical sensitivity of the rectum ($r = -0.49$, $p = 0.009$), and with several key components of the HRV, such as SDNN at rest ($r = 0.59$, $p = 0.003$), E/I-ratio ($r = 0.60$, $p = 0.001$) and 30:15 ratio ($r = 0.557$, $p = 0.005$), see Fig. 5B–C. Overall, subjects with poor physical health had impaired HRV and rectal hyposensitivity.

3.7.5. Correlations between PAGI-SYM, visceral and cardiac autonomic nervous function

The 30:15-ratio upon standing up correlated with both upper abdominal pain ($r = -0.63$, $p = 0.002$) and lower abdominal pain ($r = -0.53$, $p = 0.009$) scores. Additionally, there were several weaker associations; between E/I-ratio and upper abdominal pain scores ($r = -0.48$, $p = 0.02$), between SDNN at rest and both upper and lower abdominal pain scores (both $r = -0.47$, $p = 0.02$), and between rectal electrical sensitivity and upper abdominal pain ($r = 0.46$, $p = 0.03$).

4. Discussion

The present study showed that diabetes patients with sensorimotor neuropathy demonstrated cutaneous hypesthesia, clear signs of diffuse autonomic impairment affecting both the visceral sensory system (rectal hyposensitivity to multimodal stimulations) as well as cardiac autonomic nervous function expressed as reduced heart rate variability. Also, the patients had increased prevalence of gastrointestinal complaints and reduced quality of life. Furthermore, there were meaningful associations between skin hypesthesia, visceral hyposensitivity, degree of cardiac autonomic neuropathy, reduced physical wellbeing and abdominal pain scores.

4.1. Methodological considerations

Patients were recruited on the basis of clinical suspicion of DSPN (i.e. symptoms and pathological 10 g monofilament test), 10 out of 20 were confirmed by nerve conduction studies. As such, not all cases fulfilled the Toronto criteria, which were formulated after the study protocol was written (Tesfaye et al., 2010). However, the patient group showed a clearly reduced sensitivity to von Frey filament test, confirming peripheral hyposensitivity. Furthermore, one can speculate that any visceral hyposensitivity found in this study most likely would be more pronounced if all patients were selected based on stricter definitions of peripheral neuropathy. The study cohort

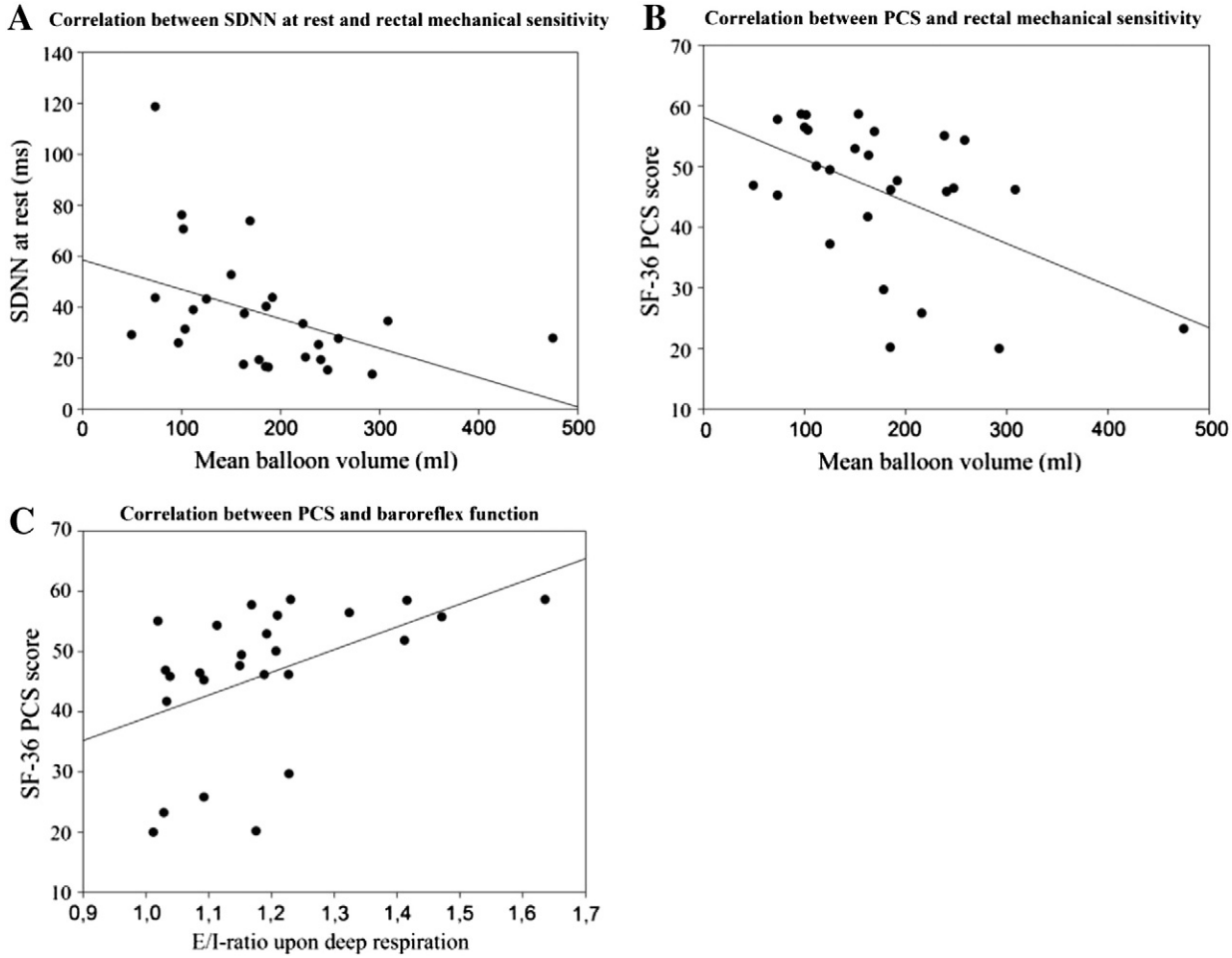

Fig. 5. A: A negative correlation could be found between SDNN at rest and rectal mechanical sensitivity, indicating that subjects with reduced SDNN (i.e. decreased heart rate variability) were hyposensitive to mechanical stimulation (volume) in the rectum. B: A negative correlation could be found between the SF-36 physical component summary score and rectum mechanical sensitivity, indicating that subjects with reduced PCS (i.e. poorer perceived physical health) were hyposensitive to mechanical (volume) stimulation in the rectum. C: A positive correlation could be found between the SF-36 physical component summary score and heart rate variability upon deep respiration (baroreflex sensitivity), indicating that subjects with reduced PCS (i.e. poorer perceived physical health) had reduced baroreflex patency.

consisted of both type 1 and type 2 diabetes (35:65 ratio). Although the two conditions differ in central pathophysiological aspects, they share the presence of microvascular complications, which are regarded as diabetes specific. In our study, the type 2 DM patients were somewhat older than type 1, and had shorter disease duration. Still, sub-group analyses detected no significant differences in terms of cutaneous, muscle, bone and visceral sensitivities between the two groups. Also, the cardiac autonomic tests and questionnaires yielded scores that did not differ significantly. Thus, we consider our results to be applicable in DPSN patients.

The study population did not include diabetes patients without neuropathy, or an unselected diabetes cohort. Several correlations between visceral, cutaneous and cardiac autonomic neuropathies were detected, indicating a co-development of these complications. Still, the chronological order of the neurological deficits remains to be unraveled. Finally, not all participants were able to complete the entire experimental protocol, yielding a somewhat weaker statistical power than originally anticipated. On this basis, borderline significant results should be interpreted with caution.

The muscle and bone pressure protocols have been validated and applied in several previous studies (Andresen et al., 2013; Staahl et al., 2007). Muscle algometry is predominantly a measure of deep muscle strain sensitivity, and to a lesser degree superficial structures, whereas bone algometry predominantly stimulates the periosteal mechanosensitive nociceptors.

Autonomic nervous system patency has traditionally been investigated based on the methods proposed by Ewing & Clarke (1986). In this study we investigated two of the original Ewing parameters (i.e. heart rate response to standing up and to deep respiration), and in addition the HRV at 5-min rest. Although a reference database with normal values exists, our patients had an age distribution with several elderly participants, emphasizing the need for a matched control group as HRV decreases in the elderly (Phillips & Powley, 2007).

The investigation of visceral sensations is complicated due to the relative inaccessibility of gut organs, the diffuse nature of visceral pain and the complex organization of visceral afferents running in parallel with the autonomic nerves. Previous studies have investigated esophageal sensitivity in diabetic autonomic neuropathy, however an esophageal probe can be hard to tolerate, in particular in these patients who frequently suffer from nausea and reflux (Frokjaer et al., 2007). Our research group has substantial experience when it comes to both esophageal and rectosigmoid sensory stimulations as a proxy of visceral sensitivity, and the multimodal probe has been employed in several previous studies on neuropathic conditions as well as in healthy individuals (Brock, Arendt-Nielsen, Wilder-Smith, & Drewes, 2009). The probe design allows the investigation of three modalities in one session, thus approaching a more complete and physiologically meaningful examination at the same time addressing the limitations of the individual test modalities. Thermal sensations are thought to be predominantly mucosal whereas mechanical stimulation mainly

activates a stretch sensation of the muscle layer. Electrical stimulation bypasses all the sensory receptors, directly activating the nerves (Burgell & Scott, 2012).

Finally, a strong central nervous system component with functional and neuroplastic changes has been shown in both somatic and visceral diabetic neuropathies, the influence of which this study did not aim to investigate (Brock et al., 2012; Frokjaer et al., 2009; Selvarajah, Wilkinson, Davies, Gandhi, & Tesfaye, 2011).

5. Clinical considerations

This study revealed an association between GI complaints and reduced HRV as well as a positive correlation between GI complaints and rectal hyposensitivity. By contrast, functional disorders such as irritable bowel syndrome are characterized by visceral *hyper*sensitivity (Kanazawa, Hongo, & Fukudo, 2011). In addition to disease-related causes, other mechanisms have been proposed to explain the high prevalence of GI symptoms in DM. These include psychological stress of a chronic disorder such as DM (de et al., 2012; Talley et al., 2001), and side effects of common diabetes medications i.e. metformin and glucagon-like peptide 1-analogues (Aroda & Ratner, 2011; Icks, Haastert, Rathmann, & Wareham, 2002). The present findings indicate that visceral and/or autonomic neuropathies could be central components in the pathogenesis of visceral symptoms and pain in this patient population. Studies in streptozotocin-induced diabetic rats also indicate that the function of rectal visceral afferents deteriorates at an early stage of DM (Beyak, Bulmer, Sellers, & Grundy, 2009), and enteric neurodegeneration appears early in such models (Chandrasekharan & Srinivasan, 2007).

Interestingly, diabetes patients had normal pain sensitivity to muscle and bone pressure stimulation, in spite of a clear visceral and skin hyposensitivity. Thus, these deep pain perception pathways seem to be less affected in DSPN, although little is known about the epidemiology of myalgia and ostealgia in DM. Similar apparent discrepancies are found in patients with chronic painful pancreatitis, a condition with a marked neuropathic component. These patients show visceral and skin hyposensitivity, but normal muscle pain pressure sensitivity (Dimcevski et al., 2006; Dimcevski et al., 2007).

6. Therapeutical considerations

The results indicate that once the patient has developed sensorimotor neuropathy, there are already coexisting diffuse neuropathic complications in the cardiac and visceral autonomic nervous systems, associated with GI complaints and reduced HRV. This finding is novel, but not surprising, as small fiber neuropathy has been found to precede that of larger myelinated nerve fiber damage in several studies (Loseth, Stalberg, Jorde, & Mellgren, 2008; Sumner, Sheth, Griffin, Cornblath, & Polydefkis, 2003). Early detection using HRV or other sensitive small fiber tests followed by therapeutic interventions should be attempted in larger, clinical studies. Thus far, neuroprotective interventions apart from glycemic control have been unsuccessful. However, newly developed c-peptide analogues, and the recently implemented incretin-based therapies show theoretical potential in this respect (Holst, Burcelin, & Nathanson, 2011; Wahren, Kallas, & Sima, 2012). No human randomized controlled trial with small fiber neuropathy as primary end-point has yet been published.

7. Conclusion

In diabetes patients with sensorimotor neuropathy there are concurrent rectal hyposensitivity and reduced heart rate variability, indicating an affection of both visceral and cardiac autonomic nerves. The degree of rectal hyposensitivity is associated to reduced HRV, gastrointestinal symptoms and reduced quality of life, thus indicating a clinical significance of the results. Clinicians should consider autonomic complications in patients presenting with peripheral sensorimotor neuropathy. Early detection of small fiber neuropathy and continued research into neuroprotective interventions are needed.

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