ASSESSMENT OF NEURONAL DYSFUNCTION IN PATIENT GROUPS WITH HIGH RISK OF NEUROPATHY

Sándor Magony MD

PhD Thesis

Szeged

2022

ASSESSMENT OF NEURONAL DYSFUNCTION IN PATIENT GROUPS WITH HIGH RISK OF NEUROPATHY

Sándor Magony MD

PhD Thesis

Tutor:

Professor Tamás Várkonyi MD, PhD

Department of Medicine

Albert Szent-Györgyi Medical School, University of Szeged

Doctoral School of Interdisciplinary Sciences

Szeged

2022

Relevant publications

Full papers

I**. Magony S,** Nyiraty Sz, Tóth B, Pesei F, Orosz A, Ábrahám Gy, Kempler P, Lengyel, Cs, Várkonyi T. Peripheral sensory nerve hyperaesthesia in women with polycystic ovary syndrome MINERVA ENDOCRINOLOGY 2021 **(impact factor: 1.529)**

II. **Magony S,** Nyiraty Sz, Fehértemplomi K, Tóth B, Pesei F, Orosz A, Lengyel Cs, Kempler P, Várkonyi T. Inzulinpumpa-kezelést igénylő, kedvezőtlen anyagcsere-állapotú 1-es típusú diabeteses betegek autonóm idegrendszeri funkciójának jellemzői DIABETOLOGIA HUNGARICA 2020;28(6)289-296.

III. **Magony S,** Nyiraty Sz, Fehértemplomi K, Tóth B, Pesei F, Orosz A, Lengyel Cs, Kempler P, Horváth V, Várkonyi T. Long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump. DIABETES STOFFWECHSEL UND HERZ 2021;30(3)193-197. (**impact factor 0.262)**

Impact factor of publications related to the thesis: 1.791

Other publications

Palásti P, Zombori T, Kaiser L, **Magony S**, Kakuja F, Vörös A, Morvay Zita, Kincses ZsT, Palkó A, Fejes Zs. „Rés a pajzson" – a pajzsmirigy modern képalkotó vizsgálata multidiszciplináris szemszögből ["Gap in the shield" – imaging of the thyroid gland from the multidisciplinary perspective] ORVOSI HETILAP 2021;162(14)530-541. **(impact factor: 0.54)**

Brzózka Á, Kuthi L, Hajdú E, **Magony S**, Király IE, Kincses ZsT, Szatmári A, Szalma E, Palkó A, Fejes Zs. Testicularis adrenalis rest tumor a congenitalis adrenalis hyperplasia hátterében ORVOSI HETILAP 2020;161(16)623-631. **(impact factor: 0.54)**

Ku CR, Brue T, Schilbach K, Ignatenko S, **Magony S**, Chung YS, Kim BJ, Hur KY, Kang HC, Kim JH et al. Long-acting FC-fusion rhGH (GX-H9) shows potential for up to twice-monthly administration in GH-deficient adults EUROPEAN JOURNAL OF ENDOCRINOLOGY 2018;179(3)169-179. **(impact factor 5.107)**

Csajbók É, Páll I, **Magony S**, Sepp K, Valkusz Zs. A felnőttkori transzszexualizmus és kezelése MAGYAR BELORVOSI ARCHIVUM 2016;69(2-3)134-140

Magony S, Borthaiser Z, Valkusz Zs. Terhesség és anyai pajzsmirigy betegség MAGYAR NŐORVOSOK LAPJA 2016;79(1)16-19.

Orosz A, Csajbok E, Czekus Cs, Gavaller H, **Magony S**, Valkusz Zs, Varkonyi T, Nemes A, Baczko I, Forster T. et al. Increased Short-Term Beat-To-Beat Variability of QT Interval in Patients with Acromegaly. PLOS ONE 2015;(10)4e0125639 **(impact factor 3.057)**

Magony S, Valkusz Zs, Csajbok E, Sepp K, Gardi J, Szecsi M, Julesz J, Wittmann T. A kalcium-anyagcsere útvesztői [In the labyrinth of calcium metabolism] ORVOSI HETILAP 2013;154(9)351-355.

Sepp K, Nagy M, Csajbók É, **Magony S**, Valkusz Zs, Wittmann T. Második primer tumor előfordulása differenciált pajzsmirigy-carcinomás betegekben MAGYAR BELORVOSI ARCHIVUM 2013;66(2)87-93.

Bella Zs, Fulop B, Csajbok E, **Magony S**, Valkusz Zs, Herczegh Sz, Jori J, Bodosi M, Czigner J, Barzo P. Endoszkópos posterior transseptalis hypophysisműtét: A sebészi technika fejlődése a teljes endoszkópos tumoreltávolításig 61 eset elemzése alapján [Endoscopic, posterior transseptal pituitary surgery – Learning curve of the surgical technique and equipment in 61 operations] IDEGGYOGYASZATI SZEMLE / CLINICAL NEUROSCIENCE 2012;65(7-8)271-279. **(impact factor 0.348)**

Szécsi M, Ondré D, Tóth I, **Magony S**, Wölfling J, Schneider Gy, Julesz, J. Determination of rat 5alpha-reductase type 1 isozyme activity and its inhibition by novel steroidal oxazolines ACTA BIOLOGICA HUNGARICA (1983-2018) 2010;61(3) 274-281. **(impact factor 0.793)**

Csajbók É, **Magony S**, Valkusz Zs, Pánczél P, Julesz, J. Poliglanduláris autoimmun szindróma különböző megjelenése HLA-identikus egypetéjű ikrekben MAGYAR BELORVOSI ARCHIVUM 2009;62(5)391-398.

Magony S, Valkusz Zs, Csajbók É, Gardi J, Kiss I, Julesz J. A diagnosztika és terápia lehetőségei acromegaliában – esetismertetés. MAGYAR BELORVOSI ARCHIVUM 2009;62(5)385-390.

Valkusz Zs, **Magony S**, Csajbók É, Julesz J. Mellékvesekéreg-carcinoma - mindig reménytelen? Esetismertetések és áttekintés MAGYAR BELORVOSI ARCHIVUM 2009;62(5)399-403.

Magony S, Julesz J. Daganatos betegségek endokrin megnyilvánulásai - áttekintés MAGYAR BELORVOSI ARCHIVUM 2008;61(3)166-170.

Magony S, Julesz J. Terhesség és prolactinoma MAGYAR BELORVOSI ARCHIVUM 2007;60(5)433-436.

Hubina E, Czirják S, Görömbey Z, Julesz J, Kovács GL, Kovács L, Laczi, F, **Magony S**, Mezősi E, Nemes O et al. A felnőttkori növekedésihormon-pótló kezelés 10 éves hazai tapasztalatai MAGYAR BELORVOSI ARCHIVUM 2006;59(2)71-82.

Laczi F, **Magony S**, Julesz J. Korszerű lehetőség az acromegalia gyógyszeres kezelésére: octreotid, a tartós hatású somatostatin-analóg [Current alternative in the pharmacotherapy of acromegaly: the long-acting somatostatin analogue octreotide]
ORVOSI HETILAP 2002;143(19.Suppl. 2.)1062-1066.

Pócsik A, Pikó B, Vörös E, **Magony S**, Borbola, Gy. Bifocalis germinoma. MAGYAR RADIOLÓGIA 1998;72(1)19-21.

Published abstracts related to the thesis

Nyiraty Sz, **Magony S,** Fehértemplomi K, Tóth B, Pesei, F, Orosz A, Lengyel Cs, Kempler P, Ábrahám Gy, Várkonyi T. Subcutan inzulinpumpa-kezelést igénylő, rossz anyagcsere-állapotú 1-es típusú diabeteses betegek autonóm idegrendszeri funkciójának jellemzői DIABETOLOGIA HUNGARICA 2019; 27(S1): 43-44.

Magony S, Nyiraty Sz, Pesei F, Tóth B, Orosz A, Lengyel Cs, Ábrahám Gy, Kempler P, Várkonyi T. Korai szenzoros hyperaesthesia polycystás ovarium szindrómában szenvedő nőbetegekben DIABETOLOGIA HUNGARICA 2018; 26(S)167-68.

Várkonyi T, **Magony S**, Nyiraty Sz, Pesei F, Tóth B, Orosz A, Lengyel Cs, Ábrahám Gy, Kempler P. Evaluation of autonomic and sensory nerve function in women with polycystic ovary syndrome Paper: P31 (2018) 28th Annual Meeting of the Diabetic Neuropathy Study Group of the European Association for the Study of Diabetes (EASD), Rome, Italy, 4-7 September 2018.

Table of contents

Abbreviations

AN – autonomic neuropathy

BMI – body mass index

CPT – current perception threshold

CRT – cardiovascular reflex test

DCCT – Diabetes Care and Complications Trial

EDIC – Epidemiology of Diabetes Interventions and Complications

HOMA – Homeostasis Model Assessment

PCOS – polycystic ovary syndrome

SE – standard error.

1 Introduction and aims of the study

The aims of the preventive medicine are protecting, promoting, and maintaining the state of well-being and health, avoiding disease, disability, and death on individuals and on the populations. The preventive medicine through interdisciplinary ways approaches many factors influencing the patients' health. Neuropathy is one of the most detrimental and diversified neurological conditions that damages the balance of several physiologic processes. It considerably impairs patients' quality of life and also associated with an increased morbidity and mortality [1]. The literature data clearly prove that it is an important task to organize studies to explore the possible presence of neuropathy in those patients who have several risk factors of it or in those who have a long-standing hyperglycemia that directly impairs the neuronal functions. The underlying cause of polyneuropathy is typically found outside the nervous system [2]. Preventing the manifestation or the progression of neuropathy is essential in high-risk patients. Therefore, it is very important to explore all possible conditions which might lead to this complication. In addition to high blood glucose, there are a number of pathological conditions which may potentially cause neuropathy. Common risk factors are proven of distal symmetric sensorimotor polyneuropathy in diabetes and cardiovascular diseases including age, hypertension, dyslipidaemia, oxidative stress and obesity [3]. Cardiovascular autonomic neuropathy (AN) is also commonly associated with well-known macrovascular risk factors in type 1 and type 2 diabetic patients including high blood pressure, hypertriglyceridemia and smoking [4, 5]. The complexity of the pathogenesis of neuropathy has been shown by the Steno 2 trial: in addition to close glucose control, intensified multifactorial intervention, the use of renin–angiotensin system blockers, aspirin, and lipid-lowering agents has been shown to slow the progression of neuronal complications in patients with type 2 diabetes [6]. In accordance with the findings indicating that severe hyperglycaemia is not the exclusive cause of the neuronal damage, both autonomic and sensory neuropathies were documented in patients with prediabetes [7]. The prevalence of polyneuropathy in prediabetes is associated with obesity, visceral fat and peripheral arterial disease [8]. The decisive role of visceral fat is revealed by the fact that the incidence of neuropathy in obese patients – even with normal blood glucose levels – is higher compared to lean controls [9]. Based on these findings, it is clear that insulin resistance is the underlying common condition. Insulin resistance may develop in the neurons and results in injury to the peripheral and central nervous systems playing a role in the pathogenesis of neuropathy and Alzheimer's disease [10]. Higher insulin levels exert a detrimental effect on the neuronal systems, as hyperinsulinemic/euglycemic clamps revealed

increased muscle and cardiac sympathetic activity in healthy subjects [11], and an altered response to orthostatic stress in older adults with type 2 diabetes [12]. In a cross-sectional study on 2,035 patients with metabolic syndrome, peripheral neuropathy was associated with insulin resistance independently of the additional components of metabolic syndrome [13]. Insulin resistance has a decisive role in the pathogenesis of polycystic ovary syndrome (PCOS), and it is associated with a high number of risk factors for the development of neuropathy, including impaired glucose metabolism, hypertension, obesity and hyperlipidaemia [14]. In addition, the excess of androgens in women with PCOS might be a contributing factor in the development of cardiovascular diseases and probably neuropathy as well. Although PCOS is the most common endocrine disorder in females, there is a very limited amount of data available on the manifestation of neuronal dysfunctions in the patients. Higher sympathetic cardiac autonomic modulation during the evaluation of heart rate variability has been described in women with PCOS [15]. Based on the lack of the previous characterization of the neuronal systems in PCOS, we designed a study as a first part of this thesis with the aim to the assess the cardiovascular autonomic and peripheral sensory functions in women with this disease.

It is widely accepted that in type 1 diabetes AN is an independent risk factor for mortality, that particularly explained by the relatively increased activity of the sympathetic nervous system due to an attenuated parasympathetic function [16]. Further etiologic factors of the poor life expectancy in patients with AN are the impaired cardiovascular adaptation, the development of diastolic dysfunction and the increased rate of arrhythmias that may be associated with sudden cardiac death or respiratory arrest [17]. As AN is a critical determinant of the cardiovascular state, it is mandatory to apply all the possible therapeutic options to prevent the development or to reduce the progression of this neuronal complication. In type 1 diabetes, one of the most important tool to achieve these aims is to keep the glycemic control strictly in the target range. It is not known how long it takes for metabolic optimization to have a beneficial effect on the functioning of the autonomic nervous system, but nobody queries the harmful effect of hyperglycemia on the neurons. It is a well-known fact, that an association exists between long-term glycemic control and the incidence of neuropathy, as it was proven in several previous studies on high number of patients. Pirart's early results in the 25-year long follow-up of 4400 patients demonstrated that the incidence of neuropathy depends on the quality of metabolic control and the duration of diabetes. His results prove that the incidence of retinopathy, nephropathy, and neuropathy depends not only on the duration of diabetes but also on the quality of the metabolic setting [18]. At the closeout of Diabetes Care and Complications Trial (DCCT) after 6.5 years of follow-up of 1441 patients, the investigators reported that intensive

insulin treatment significantly reduced the incidence of diabetic neuropathy, similarly to findings for diabetic retinopathy and nephropathy [19]. By the end of DCCT the prevalence of AN remained the same in the intensively treated group, while it almost doubled in participants applying conservative treatment. The incidence of AN reduced by 45% with intensive treatment during the course of the DCCT [19]. The observational Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up was established to monitor the long-term effects of the prior intensive treatment in the DCCT cohort on the development and progression of neuropathy. At 13-14 years of EDIC the groups receiving previous intensive or conservative insulin treatment differed primarily in their R-R variation to deep breathing. This cardiovascular test remained significantly higher in the group with intensive treatment in the original DCCT, compared with the group applying conservative treatment in that period of the trial. The R-R variation to deep breathing is a sensitive marker of the parasympathetic function and becomes abnormal early during the progression of AN. This observation underlines the importance of the role of the early glycemic control in the long-term progression of AN and proves the dominance of the parasympathetic impairment in the initial phase of AN [20]. The EURODIAB-IDDM study identified a number of risk factors for AN in type 1 diabetes, including long-term glycemic control and duration of diabetes [5]. A metaanalysis of 17 randomized trials revealed, that the effective glucose control significantly prevented the development of clinical neuropathy in type 1 diabetes mellitus, whereas this association in type 2 diabetes is less evident [21]. No doubt that the long-term glycemic control plays an important role in the development of neuropathy in type 1 diabetes, but some remarkable observations in small number of patients suggest that even short-term changes of glucose levels may also affect the nervous system function in both types of diabetes or even in healthy subjects. Eight months after starting subcutaneous pump therapy, peripheral nerve conduction measurements and vibratory sensory threshold improved compared to those receiving conservative insulin therapy in type 1 diabetic patients [22]. Four weeks following initiation of intensive insulin therapy, improvements in vibratory sensation were observed in Japanese type 2 diabetic patients [23]. In addition 20 days of consistently maintained normoglycemia, vibration sensation in type 2 diabetes also showed improvement in the lower and upper extremities when tested by two methods [24]. In experimental conditions only 2 hours of hyperglycemia increased corrected QT interval at ECG in type 2 diabetic patients [25] and in healthy men as well [26]. Furthermore, a 150 minute-long hyperglycemia increased the supine heart rate and altered a parasympathetic reflex test in healthy subjects [27]. Moreover, acute hyperglycaemia inhibited basal and stimulated pancreatic polypeptide secretion in non-diabetic participants suggesting

an actual vagal inhibition of high glucose levels [28]. These latter observations point to a reversable decreased parasympathetic activity and a relatively increased sympathetic activity in case of currently high glucose. The data from the literature clearly suggest that assuring a well-treated glucose metabolism is the only way to prevent the development or to reduce the progression of neuropathy. The most intensive form of the treatment and optimization of the glycemic control in type 1 diabetes is the introduction of subcutaneous pump therapy which ensures the continuous insulin supply by the basal rate of the administration supplemented with bolus doses before main meals. Analyzing the previous articles that was not clear what time should be elapsed in better glycemic condition to achieve an improvement of the neuronal function. The available data from small studies extend from hours to years defining the glycemic condition affecting the parameters of neuropathy. In the second part of this thesis we explored the characteristics of autonomic cardiovascular function in those type 1 diabetic patients whose glycemic control necessitated insulin pump therapy.

Based on previous data and our preliminary assumptions discussed above our main goals were:
- to assess the peripheral sensory and the cardiovascular autonomic function in women with polycystic ovary syndrome,
- to explore the association of the measured neuronal functions and the possible risk factors of neuropathy (age, BMI, serum insulin, blood glucose, HOMA index, testosterone, androstendione),
- to characterize the nature of the cardiovascular autonomic dysfunctions in type 1 patients at the introduction of their insulin pump therapy,
- to follow the cardiovascular autonomic functions in type 1 diabetic patients with short-term insulin pump treatment (2 months),
- to describe the long-term trends (6 years) of alterations in the cardiovascular autonomic reflexes in type 1 diabetic patients with insulin pump treatment.

2 Patients

2.1 Assessment of neuronal function in women with polycystic ovary syndrome

27 women with PCOS were involved in the study (age: 28.7±1.8 years, mean±SE). Fasting blood glucose, fasting serum insulin, testosterone, androstenedione, body weight and

height were measured in PCOS patients, and in controls with the exception of serum insulin levels. Homeostasis Model Assessment (HOMA) [29] was expressed from fasting insulin and glucose values in PCOS patients. The patients had no symptoms of neuropathy. The mean BMI of the patient group was 29.7 kg/m2, indicated that most of them were overweight or obese. None of the patients had been diagnosed with diabetes; their mean fasting blood glucose was 4.5±0.09 mmol/L. 24 healthy women with normal weight acted as controls. These individuals had no significant differences in comparison to the patient group in the descriptive parameters except for BMI (age: 28.1±1 years, fasting glucose: 4.1±0.07 mmol/L, BMI: 22.6±0.8 kg/m^2). The HOMA index of the PCOS group indicated insulin resistance (2.64±0.59 vs 1.92±0.33, $p<0.05$; PCOs vs control). PCOS was diagnosed following the Rotterdam consensus [30], based on the presence of at least two of the following criteria: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, ultrasound evidence of polycystic ovaries. Other aetiologies of hyperandrogenic state (congenital adrenal hyperplasias, androgen-secreting tumours, Cushing's syndrome) were excluded. Patients with acute infection or chronic alcohol consumption were not involved in the study.

2.2 Short-term follow-up of the cardiovascular autonomic function among patients with type 1 diabetes treated with insulin pump

38 (23 women and 15 men) patients with type 1 diabetes and 10 healthy controls (6 women and 4 men) participated in this study. Patients were young adults (29.5±1.3 years±SE) at the time of the study, the diabetes was started in childhood or puberty (duration of diabetes: 13.8±1.5 years), and all patients received insulin treatment immediately after diagnosis. Their mean BMI was 23.2±0.6. The first autonomic test was performed in all patients within 1 week before the start of their insulin pump therapy. Before the pump treatment the blood glucose fluctuations in all patients were hectic, often exceeding 10 mmol / l / day (24/38 patients). The HbA1c was higher than 8% in all cases (38/38 patients), their mean HbA1c was 8.7%±0.2. The incidence of complications associated with diabetes was detected as 16 patients with retinopathy, 10 patients with nephropathy, and 10 patients with confirmed diabetic neuropathy. Before the insulin pump treatment, we observed a rapid progression of complications in 6 patients, while 2 female patient received pump treatment due to planned pregnancy. The second autonomic function test was performed 2 months after the initiation of pump treatment. Patients were tested for HbA1c at the beginning of pump treatment and 2 months later. The members of the control group did not differ significantly from the patient group in terms of descriptive

parameters (mean age: 27.8 ± 2 years, mean BMI: 24.1±0.4).

2.3 Long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

This study involved 13 patients (7 women and 6 men) with type 1 diabetes. The patients were young adults at the time of the study initiation (30.4±2.7 years, mean±SE). Their disease started in childhood or puberty (duration of diabetes at baseline: 16.5 ± 2.7 years). All patients received intensive insulin treatment immediately after their diagnosis until the starting of insulin pump therapy. The BMI of the patient group at the initiation of the pump treatment was 24.2±1.0 kg/m2, while the HbA1c was 8.85±0.2% was at the same time. The first cardiovascular reflex tests were performed in all patients within 1 week before starting their subcutaneous insulin pump therapy. The assessments of the cardiovascular autonomic function were performed after 2 months and 6 years of the pump treatment. The HbA1c levels were determined at all of the three tests.

3 Methods

3.1 Assessment of AN

The presence and severity of AN was characterized using the four standard cardiovascular reflex tests (CRT) in all patient [31]. These measurements provide a non-invasive, clinically relevant, reproducible and standardized gold-standard assessment of the autonomic function [32]. Three of these tests record the changes in heart rate during specific manoeuvres, while the fourth test is designed to monitor blood pressure changes [16]. Most of the tests aiming to detect heart rate changes are used primarily but not exclusively for the assessment of parasympathetic innervation, while the blood pressure response predominantly indicates the impairment of sympathetic functions [33]. The heart rate changes were analyzed during deep breathing, in positions of lying and standing up (30/15 ratio) and during and after of Valsalva manoeuvre. Systolic blood pressure was determined in response from lying to standing up.

3.1.1 Heart rate tests

Heart rate variation to deep breathing

Normally the heart rate is increased during inspiration and decreased by expiration. The patient was asked to breath deeply at a rate of six breaths per minute (inhale for five seconds in and exhale for five seconds). The result is expressed as the difference between maximum and minimum heart rates (beat/min) during the six breathing cycles.

Heart rate response to Valsalva manoeuvre

During the strain period of Valsalva manoeuvre the blood pressure drops and the heart rate rises under physiologic conditions. Following the procedure, the blood pressure rises and the heart rate slows. The patient was instructed to blow into a mouth-piece connected to a modified manometer and holding it at a pressure of 40 mmHg for 15 seconds while an electrocardiogram was recorded continously. The Valsava ratio is calculated at the evaluation as the ratio of the longest R-R interval after the manouevre to the shortest R-R interval during the procedure.

Heart rate response to standing (30:15 ratio)

Following the position change from lying to standing the heart beat frequency is immediately increased and peaks at the 15^{th} beat after standing up. Then a relative bradycardia occurs in healthy subjects peaking at about the 30^{th} beat. At the start of the test the patient was lying at rest while the heart rate was recorded continuously at the electrocardiogram. Then the patient was asked to stand up without interrupting the detection of the heart rate monitoring. The 30/15 ratio was expressed as the ratio of the longest R-R interval at around the 30^{th} beat to the shortest R-R interval at around the 15^{th} beat after standing up.

3.1.2 Blood pressure response to standing (orthostatic hypotension)

In healthy subjects, pooling of blood in the lower extremities upon standing up causes a minor fall in the blood pressure which is rapidly counterbalanced by peripheral vasoconstriction. Severe postural hypotension is a characteristic sign of AN. This test is based on blood pressure determinations in a lying position and after standing up. The postural fall is defined as the difference between systolic pressure after 10 minutes of lying and systolic pressures measured at 1^{st}, 5^{th} and 10^{th} minutes after standing up. The largest difference from the systolic pressure in lying is defined as the blood pressure response to standing up.

3.1.3 Autonomic score

Finally each CRT was scored as 0 (normal), 1 (borderline) or 2 (abnormal) and by this method an autonomic score (0-8) was calculated to express the overall severity of AN. Age-corrected normal reference values were applied based on the definition of Ewing and the recommendations of the Toronto Neuropathy Expert Group [16, 33].

3.2 Assessment of peripheral sensory neuropathy by application of Neurometer

The peripheral sensory function was studied with a Neurometer (Neurotron Incorporated, Baltimore, MD, USA). This device is intended to quantify the function of different nerve fibres and provides a simple, non-invasive, and quantitative measure of peripheral sensory function [34]. Low voltage electric sine wave stimulation was applied transcutaneously on the upper and lower extremities, and the current perception threshold (CPT) values were determined. The sensory function of the median and peroneal nerves was tested in our study. The surface electrodes, 1 cm in diameter, were placed on the terminal phalanx of the index and the great toe. The electrodes were fixed only on intact skin surface, because wounds or scars would have disturbed the peripheral sensations. The amplitude of the delivered stimuli was between 0.01 and 9.99 mA. The stimulus was initially increased until a sensation was reported, then short stimuli (2 to 5 s) were applied at progressively lower amplitudes until a minimal threshold for consistent detection was determined. The CPT values of the upper and lower limbs were detected at three different stimulating frequencies (2 kHz, 250 Hz, and 5 Hz).

3.3 Statistical methods

Comparisons of PCOS and diabetic patients to the control patients were performed using the unpaired Student’s t-test for normally distributed parameters. The possible associations between the measured values were analysed with the Spearmen correlation test. Multiple linear regression analysis was used for predicting the value of one dependent variable based on two or more independent variables. CRT-s, CPT-s and the descriptive parameters were expressed as mean values ± standard error (SE). A p value of <0.05 was regarded as statistically significant. The statistical analyses were performed using the SigmaStat 4.0 Systat Software and Statistica 12 packages.

These studies were conducted in accordance with the ethical standards of the Helsinki Declaration and approved by the Human Research Ethics Committee of University of Szeged. All patients provided a written informed consent form before they were enrolled.

4 Results

4.1 Results of the cardiovascular autonomic and peripheral sensory functional tests in women with polycystic ovary syndrome

The heart rate responses to deep breathing, the Valsalva manoeuvre and standing up, as well as the systolic blood pressure response to standing up from a lying position were obtained from both the PCOS and control patients and compared across the groups. Statistical evaluation revealed no significant differences in these test results reflecting the parasympathetic and sympathetic cardiovascular function (heart rate responses to deep breathing: 24.9±1.9 vs 24.5±1.6 beats/min; Valsalva ratio: 1.68±0.07 vs 1.86±0.06; 30/15 ratio: 1.4±0.06 vs 1.49± 0.06; orthostatic systolic blood pressure drop: 2.5±0.8 vs 2±0.8 mmHg, mean±SE, PCOS vs control; $p>0.05$ respectively, Figures 1-4).

The CPT levels in the median nerve at all three testing frequencies were significantly lower in the PCOS patients than in the controls (Table 1). Comparison of the CPT values in the peroneal nerve yielded similar findings, as these were significantly lower than in the control group (Table 2). These observations reflect peripheral sensory hyperaesthesia in PCOS patients compared to the healthy control subjects. To explore the possible role of insulin resistance in the abnormal sensory function, correlation tests were performed between HOMA indexes and the CPT values. No associations were observed between the CPT values measured in the median or the peroneal nerves, and the degree of insulin resistance expressed by the HOMA index (Table 3). Most of the analyses revealed no significant correlations between the BMI of PCOS patients and the CPT values measured in any of the extremies (Table 4). The only exception was a significant negative correlation between BMI and the CPT in the peroneal nerve at 5 Hz stimulation ($r = -0.39$; $p<0.05$). There was no statistical association between testosterone or androstenedione levels and the measured parameters of neuropathy. Multiple linear regression analysis yielded no predictors for sensory hyperaesthesia from the measured parameters (age, BMI, serum insulin, blood glucose, HOMA index, testosterone,

androstendione).

4.2 Results of the study of short-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

The duration of type 1 diabetes at the time of the pump treatment initiation correlated strongly with the score for the overall severity of AN ($r=0.51$, $p<0.05$, Figure 5). The positive correlation suggests that the overall rate of AN is more severe with longer duration of diabetes with poor metabolic conditions.

The duration of diabetes and the result of the most sensitive parasympathetic test, the heart rate response to respiration, also significantly correlated at the implementation of pump therapy (Figure 6). The correlation is negative, as the lower the rate in heart rate response, associated with the longer the duration of diabetes ($r=-0.63$, $p<0.001$).

During the 2-month follow-up, AN score slightly significantly decreased (Figure 7). The baseline value of the AN score in the diabetic patients' group was significantly higher than in the controls (AN score: 2.2±0.2 vs 0.9±0.1, $p<0,05$). The AN scores measured 2 months later in insulin pump-treated diabetic patients did not differ significantly from the healthy subjects (1.5±0.2 vs 0.9±0.1, $p>0.05$).

The heart rate response to deep breathing increased significantly during the two months of the pump treatment (18.6±2,1 vs 22.4±2 beats/min, $p<0.05$, Figure 8), suggesting an improvement in parasympathetic function during this period. As in the case of the AN scores, the heart rate responses to deep breathing in diabetic patients were significantly lower than in the controls, while there was no difference from controls after 2 months of insulin pump treatment (baseline: 18.6±1.5 vs 32.6±3.8 beats/min., $p<0.001$, follow-up: 22.4±2 vs 32.6±3.8 beats/min $p>0.05$).

Regarding the further reflex tests, we observed that the change in heart rate for Valsalva manouevre and standing up, as well as the systolic blood pressure decrease to orthostasis, did not change significantly over 2 months. The results of these cardiovascular tests were not different from the controls at baseline or at the 2-month follow-up (Figures 9, 10, and 11.).

Patients' HbA1c levels decreased from 8.7%±0.2 to 8.1±0.2% at 2-month follow-up, this change was not significant.

4.3 Result of the study of long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

At baseline a moderate severity of autonomic neuropathy was revealed, as we observed it in the study of short-term follow-up of the autonomic function among patients with type 1 diabetes newly treated with insulin pump (Figure 12). During the follow-up an improvement of the total autonomic score was detected two months after the implementation of pump (2.85±0.3 vs 1.23±0.3, $p<0.01$). The AN score measured six years later was identical to the initial value (2.85±0.3 vs 2.85±0.4 $p>0.05$**)** (Figure 12). The heart rate responses to deep breathing, to Valsalva manouevre and to standing up as well as the blood pressure response to standing up did not differ significantly during the follow-up (Figure 13, 14, 15 and 16). In 3 of the 4 tests there was a non-significant tendency of an improvement by the second month (Figures 13, 17 and 16), while a progression was not revealed by the 6th year in comparison to the initial values at any of the tests (Figures 13, 14, 15 and 16). Regarding the metabolic state the HbA1c decreased by 0.7% after 2 months as a mean (8.85±0.2% vs 8.12±0.3 %, $p=0.07$) and it was significantly lower by the end of the follow-up (8.85±0.2% vs 7.85±0.3 %, $p<0.05$) (Figure 17).

5 Discussion

The best way to prevent the development or the progression of neuropathy is to seek for it in patients with high risk. This is the most effective way to intervene the pathogenetic process in time in case of positive results. We have chosen two groups with pathogenetic conditions that responsible for the development of neuronal dysfunction. We decided to analyze patients with the potentially early and patients with presumably advanced forms of neuropathy, this is the explanation why we included patients with PCOS and long-standing type 1 diabetes with poor glycemic control. Moreover, the theoretically benefical effect of insulin pump treatment was also studied in the latter group.

Two important potential manifestations of neuropathy were analysed in our patients with PCOS. The cardiovascular autonomic function carries a very important predictive value regarding life expectancy even in this population with insulin resistance. During the initial phase of AN, an alteration of the parasympathetic function is a characteristic finding [35]. In prediabetes or diabetes, this has been shown by the early abnormalities in tests assessing heart rate responses to different procedures [13]. Parasympathetic neuropathy at this phase is

associated with a relative overdrive of sympathetic function. It is also well-known that increased sympathetic tone is characteristic of insulin-resistant conditions [36]. On the other hand, current hyperinsulinemia enhanced muscle sympathetic nerve activity in healthy subjects proving the direct relationship between high insulin levels and the autonomic dysfunction [37]. Moreover, in a trial including women with PCOS, a spectral analysis of heart rate variability revealed a normal parasympathetic function with an increased sympathetic cardiac autonomic modulation suggesting an imbalance of the autonomic tone [34].

Our data revealed that heart rate and blood pressure responses in PCOS patients and healthy control subjects are similar. The putative harmful effect of insulin resistance, hyperandrogenism or obesity did not lead to a deficit in the parasympathetic or the sympathetic regulation in patients with PCOS. As the mean age of the patients was 28 years, they were probably too young to be seriously exposed to the effects of insulin resistance exerted on autonomic function. It is very difficult to determine the duration of insulin resistance in PCOS. Moreover, in these patients the degree of insulin resistance may not have reached the threshold of sensitivity of the autonomic system, as the mean value of the HOMA index was only slightly higher than the previously described normal range [38]. This is the first study to evaluate data of the cardiovascular reflex tests of PCOS patients, and the findings indicate intact autonomic functions. This method is not suitable to estimate the hyperactivity of the parasympathetic or sympathetic systems, but its sensitivity, reproducibility and specificity ensures an accurate characterization of the potentially altered conditions. Our data support a hypothesis that in young PCOS patients, insulin resistance and the additional components of the syndrome without diabetes do not exert a negative effect on autonomic function beside the potential risk.

The evaluation of the sensory function revealed characteristic new findings in these patients. In both extremities and at all stimulating frequencies, the women with PCOS consequently perceived the electric stimulation at lower intensities than the healthy controls. As their threshold of perception was lower, they presumably became more sensitive to various stimuli than the healthy women. The Neurometer, our method of choice allows to selectively stimulate three types of the sensory nerve fibres. Comparisons with conventional nerve function tests show that high frequency detection thresholds correlate best with tests of large fibre function, and low frequency detection thresholds indicate small fibre integrity [39]. We tested the large and small myelinated fibres at 2000 Hz and 250 Hz, respectively. The sensory conduction of the small unmyelinated fibres was determined at a testing frequency of 5 Hz. The large fibres are responsible for the detection of tactile stimuli and vibration, while the small fibres convey the sensation of heat and pain [40]. In accordance with the detected lower perception thresholds

we found that in the large and small sensory fibres of the median as well as the peroneal nerves, hyperaesthesia was present in women with PCOs. Several aspects of these findings provide new approach to the pathophysiology of peripheral nerve damage. The described hyperaesthesia was also found in a minority of patients with prediabetes or diabetes at a very early stage of neuropathy, or it is associated with painful symptoms of neuropathy [13, 41]. The nature of the progression of sensory neuropathy is still a subject of debate in the literature, but it is widely accepted that hyperaesthesia is a very early and mostly silent manifestation of neuropathy, which quickly turns into hypaesthesia as neuropathy worsens [42]. Most of the patients are tested in the late phase of hypaesthesia, as this is the period when the symptoms appear, and patients seek medical aid. Hyperaesthesia is not a unique form of very early diabetic neuropathy. This neuronal complication has been proven to be present in diseases with other pathogenetic backgrounds. Using the Neurometer, Keresztes K and co-workers found hyperaesthesia mainly in the peroneal nerve at all three frequencies in primary biliary cirrhosis with autoimmune pathogenesis [43]. In our PCOS patients, lower perception thresholds were not associated with painful neuropathy, as our subjects did not present with any symptoms. The pathogenesis of the observed sensory abnormality might be partially explained by obesity, as higher BMI correlated significantly with lower perception thresholds in the peroneal nerve at the 5 Hz stimulating frequency. The same phenomenon was observed in the population-based Rotterdam Study on 908 participants without any symptoms or signs of polyneuropathy. High weight and body mass index were independently associated with reduced sural sensory nerve amplitudes and peroneal motor amplitudes [44]. This association was further supported on diabetic patients by the fact that abdominal obesity was found to be associated with peripheral neuropathy, and previous intervention studies have shown that body weight loss decreases the incidence of neuropathy [45]. This presumed detrimental effect of obesity on the nerves of the lower limbs requires further scientific exploration. Hyperaesthesia in our patients with insulin resistance might share a common pathogenesis with the well-known phenomenon of incipient hyperfunctions, as in the case of characteristic very early manifestations of retino- and nephropathy in diabetic patients. Increased microvascular circulation in the retina precedes the clinical manifestations of retinopathy [46], while hyperfiltration is an early characteristic sign of nephropathy [47]. Hyperaesthesia in the early stage of neuropathy might be also considered to be an enhanced physiologic function in the course of the developing complication. All three types of fibres in both the upper and lower extremities are targets in an extended pathogenetic process, because the manifestations of developing sensorimotor polyneuropathy generally appear first in the lower limb and mainly in large fibres [40]. Our observations point to a more

generalized early alteration without a selectivity to site or function in patients with PCOS and underlines the magnitude of seeking for neuronal dysfunctions in patients without symptoms but with high risk. The potential beneficial effects of some therapeutic interventions for the prevention of progression (benfotiamine, alpha-lipoic acid, metformin) is a matter of further clinical studies.

The concept of the short-term and long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump based on the well-known fact that an unfavorable metabolic situation increases the risk of developing autonomic neuropathy. In the EURODIAB IDDM study, which processed data from 3004 type 1 patients, the incidence of autonomic neuropathy was 36%, with 2 abnormal tests in 6% and 1 abnormal test in 30% [5]. The prevalence of parasympathetic autonomic neuropathy was significantly correlated not only with HbA1c but also with a number of other parameters, including the duration of diabetes. In the case of all type 1 diabetic patients in our study, despite previous therapeutic efforts, a stable metabolic state could not be achieved, so it became necessary to introduce insulin pump therapy.

We had the opportunity to follow type 1 diabetic patients whose glycemic control necessitated the application of a subcutaneous insulin pump treatment. In our study, a significant association was found at baseline between the duration of the disease expressed in years and the severity of autonomic neuropathy, suggesting that both prevalence and the degree of neuropathy are related to the length of chronic metabolic disorder in accordance with the literature. The analysis of a patient group with type-1 diabetes at the initiation of their insulin pump treatment ensures a unique option to draw conclusions on the consequences of poor glycemic control. The overall grade of AN was not high, as the mean AN score was 2.85, but the relationship was strong between the duration of diabetes and the severity of the autonomic dysfunction. In the presence of longer disease duration, a more severe AN was proven by the reflex tests. The patients had at least a 10-year long type 1 diabetes and before the pump treatment several unsuccessful therapeutic efforts were performed due to their unstable glucose metabolism. These data are in accordance with our previous findings that the variability of the glucose levels is in close relationship with the severity of AN [48]. The detailed analysis of the possible correlation between each of the four reflex tests and the duration of diabetes revealed that the results of the most sensitive parasympathetic tests [49], the heart rate response to deep breathing are less physiologic in the presence of a longer metabolic disease. According to some opinions, this test alone may be suitable for detecting the presence of autonomic neuropathy, while performing other procedures at the same time may support determining the severity [50, 51].

This observation leads to a conclusion that mainly parasympathetic impairment is expected in type 1 patients at the initiation of pump treatment. Moreover, this condition is frequently associated with a relative dominance of the sympathetic function resulting in increased cardiovascular risk for these patients [52]. The data suggest that in this patient population, exposure to diabetes prior to pump application caused a moderate degree of AN, the first manifestation of which was impaired parasympathetic function. Reducing parasympathetic damage as soon as possible also results in a reduction in cardiovascular risk, as there is also relative sympathycotonia, which is a markedly poor prognosis for the cardiovascular system [17]. A significant short-term improvement was found in the overall cardiovascular autonomic function during the follow-up of the pump treatment. 2 months after the pump application the autonomic score became significantly lower. This parameter is accumulated from the scores of cardiovascular reflex tests thus it ensures a general characterization of the autonomic function. The analysis of the separate tests did not reveal a significant change in the results by the second month but 3 of the 4 tests reflected a tendency of an improving cardiovascular function. The significant reduction of the autonomic score might be a cumulative additive result of the partial improvement in the parasympathetic and sympathetic functions. The beneficial effect during such a short period of the intensified glycemic control on the cardiovascular autonomic function was not published earlier in type 1 diabetic patients. These data might support the hypothesis that the moderate impairment of autonomic regulation might be sensitive for the short-term changes of the metabolic conditions and the pathogenetic process is particularly reversable. The results of the follow-up after 6 years reflect the same severity of neuropathy as it was recorded at the baseline tests. This means that the autonomic function was preserved during a 6 year-long period with the most intensive insulin treatment. The degree of the glycemic control characterized by HbA1c didn't change by the second month but became significantly lower by the 6^{th} year, although the mean value didn't reach the glycemic target. As HbA1c didn't decrease markedly during the follow-up despite of the preserved autonomic function proves a hypothesis that the global stability of the glycemic control has a more important role in the prevention than the average glycemia characterized by HbA1c. The initial moderate tendency of improvement in the autonomic function seemed to be temporary by the 6^{th} year. The literature doesn't supply real evidence from prospective trials on high number of patients about the time interval that must be elapsed to utilize an impact of the better glycemia on the autonomic function. In DCCT the prevalence of AN almost doubled in the conventional group during 6.5 years, while remained the same in the intensive group [53]. Simultaneous pancreas-kidney transplantation improved Valsalva ratio in type 1 diabetic patients by the 3^{rd} year of follow-up

[54]. In a smaller study, the heart rate variation was significantly less impaired within 24 months in a group of type 1 diabetic patients with an HbA1c less than 8.3% as compared to those having higher HbA1c [55]. Our trial suggests that the beneficial effect might begin as early as months after the intensive treatment and lasts up to 6 years. Our observations on type 1 diabetic patients draw the attention to the importance of the earliest stabilization of glycemic control as far as possible in order to prevent the progression of autonomic neuropathy.

6 Conclusions and new findings

1. The cardiovascular autonomic function is not altered in young women with PCOS besides the presence of putative risk factors.
2. A hypersensitive peripheral sensory condition is proven in the upper and lower extremities in PCOS patients. This sensory hyperfunction may be regarded as a very early manifestation of neuropathy.
3. Patients with poorly controlled type 1 diabetes has a moderately severe cardiovascular autonomic neuropathy at the start of their pump therapy when their metabolism required a change in therapy.
4. Duration of type 1 diabetes has been consistently associated with the severity of parasympathetic neuropathy as a manifestation of early autonomic nervous system damage.
5. During two-month pump treatment, cardiovascular autonomic function moderately improved after a relatively rapid correction of the metabolic state.
6. The severity of the cardiovascular autonomic neuropathy was preserved during a 6 year-long period with the most intensive insulin treatment in type 1 diabetes.

7 Tables and figures

Frequency	Controls	PCOs	p value
2 kHz	2.88±2	1.64±1.7	<0.01
250 Hz	1.27± 0.12	0.73±0.08	<0.01
5 Hz	1± 0.18	0.49±0.06	<0.05

Table 1. Peripheral sensory function of the median nerve in women with PCOs and controls (CPT in mA, mean±SE)

Frequency	Controls	PCOs	p value
2 kHz	4.4±0.28	3.29±0.19	<0.05
250 Hz	2.02±0.56	1.34±0.49	<0.01
5 Hz	1.56±0.1	0.83±0.09	<0.01

Table 2. Peripheral sensory function of the peroneal nerve in women with PCOs and controls (CPT in mA, mean±SE)

Correlated parameters	**r**	**p value**
Median nerve		
HOMA-CPT at 2 kHz	0.30	0.11
HOMA-CPT at 250 Hz	0.15	0.44
HOMA-CPT at 5 Hz	0.01	0.93
Peroneal nerve		
HOMA-CPT at 2 kHz	0.08	0.65
HOMA-CPT at 250 Hz	0.06	0.73
HOMA-CPT at 5 Hz	0.33	0.08

Table 3. Correlation between the HOMA index and the peripheral sensory function of the patients

Correlated parameters	**r**	**p value**
Median nerve		
BMI-CPT at 2 kHz	-0.04	0.83
BMI-CPT at 250 Hz	-0.15	0.42
BMI -CPT at 5 Hz	-0.24	0.22
Peroneal nerve		
BMI-CPT at 2 kHz	-0.14	0.48
BMI-CPT at 250 Hz	-0.27	0.15
BMI-CPT at 5 Hz	-0.39	<0.05

Table 4. Correlation between BMI and the peripheral sensory function of the patients

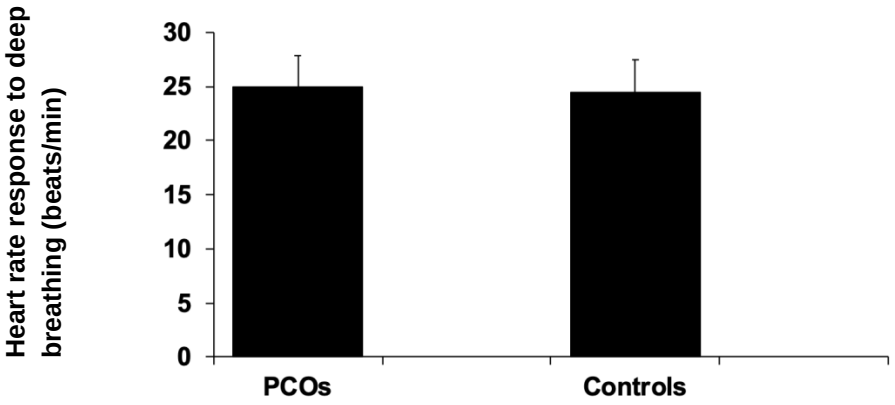


Figure 1. Heart rate response to deep breathing in women with PCOS and controls. p>0.05, PCOS vs controls.

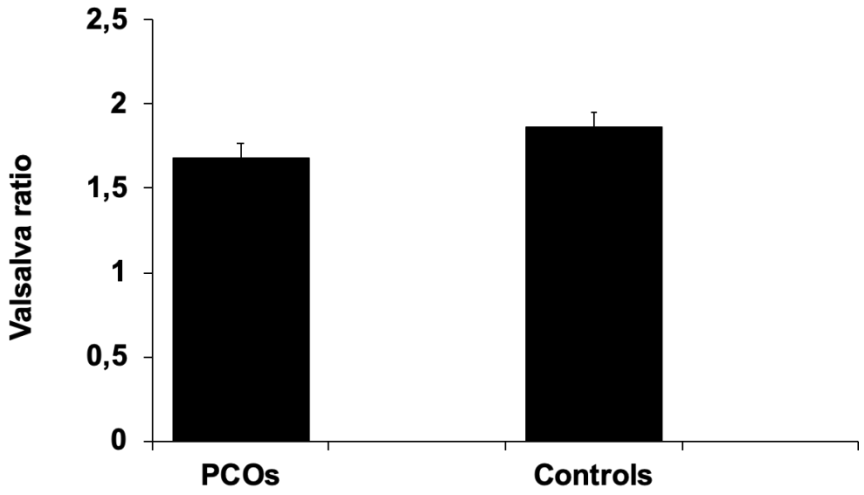


Figure 2. Heart rate response to Valsalva manouevre in women with PCOS and controls. p>0.05, PCOS vs controls

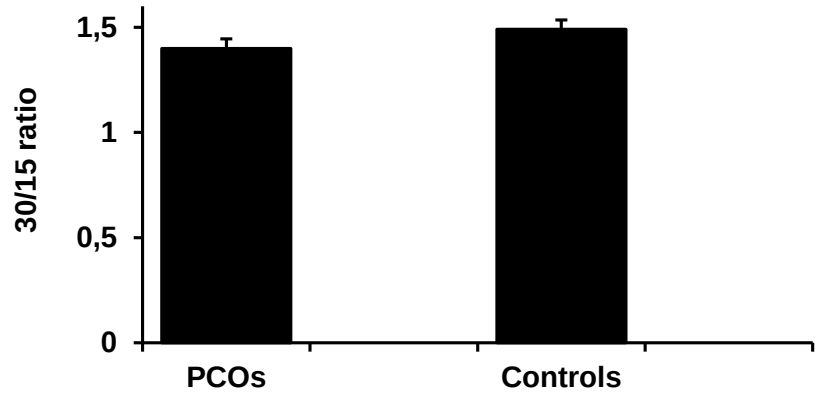


Figure 3. Heart rate response to standing (30/15 ratio) in women with PCOS and controls p>0.05, PCOS vs controls.

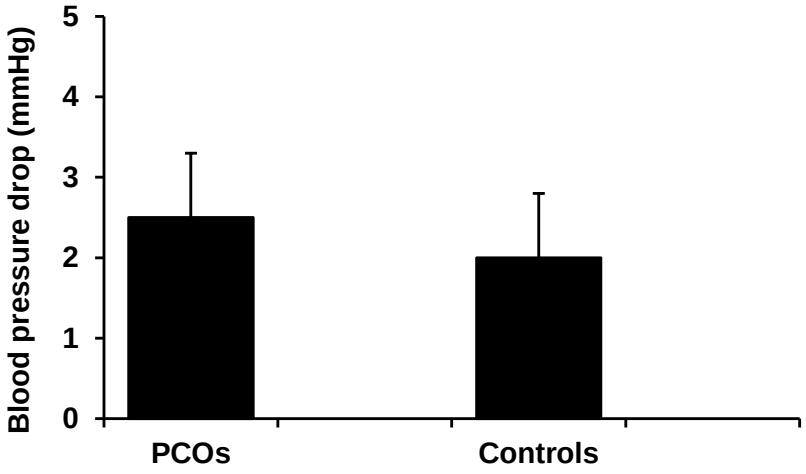
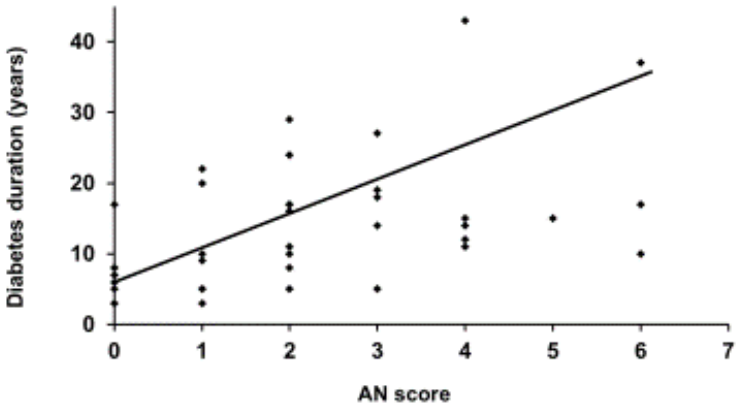
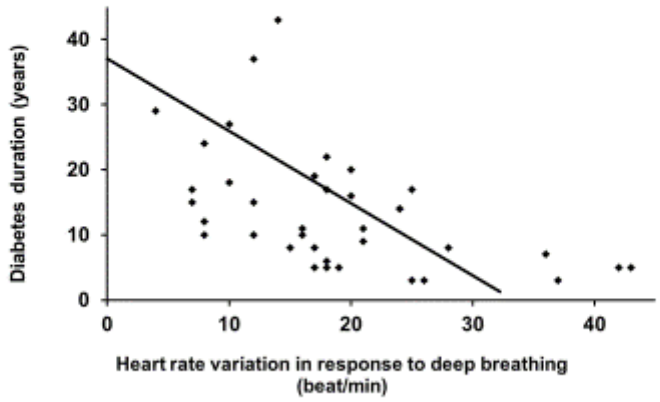


Figure 4. Orthostatic systolic blood pressure response to standing in women with PCOS and controls. p>0.05, PCOS vs controls.



r= 0,51 p<0,05

Figure 5. Relationship between diabetes duration and autonomic neuropathy (AN) score in patients with type 1 diabetes when insulin pump was initiated



r= -0,63 p<0.01

Figure 6. Relationship between diabetes duration and heart rate variation in deep breathing in patients with type 1 diabetes when insulin pump was initiated

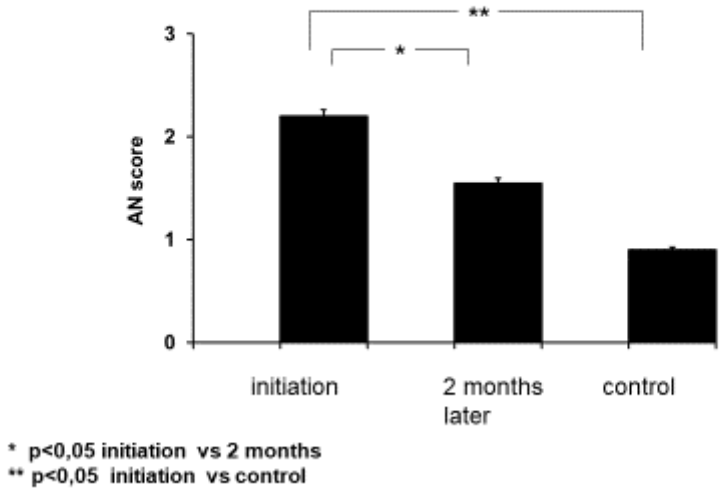


Figure 7. Short-term changes in autonomic neuropathy (AN) score in diabetic patients treated insulin pump versus the control group

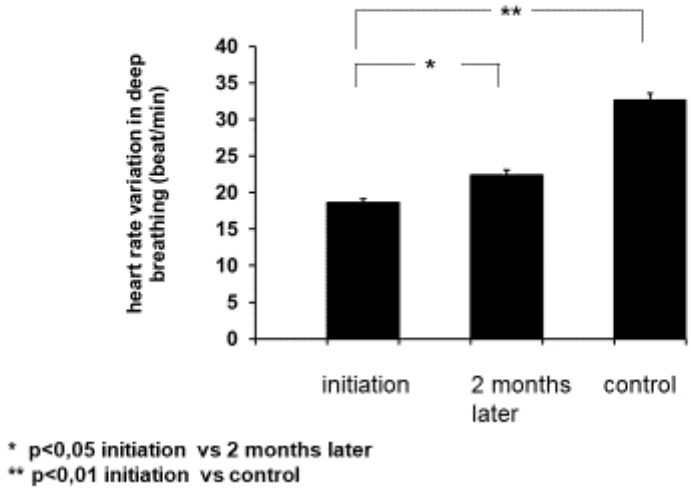


Figure 8. Short-term changes in heart rate variation in deep breathing in diabetic patients treated with insulin pumps versus the control group

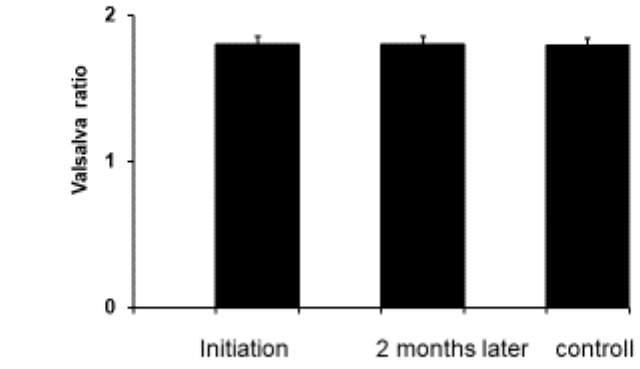


Figure 9. Short-term changes in Valsalva ratio in diabetic patients treated with insulin pump versus the control group

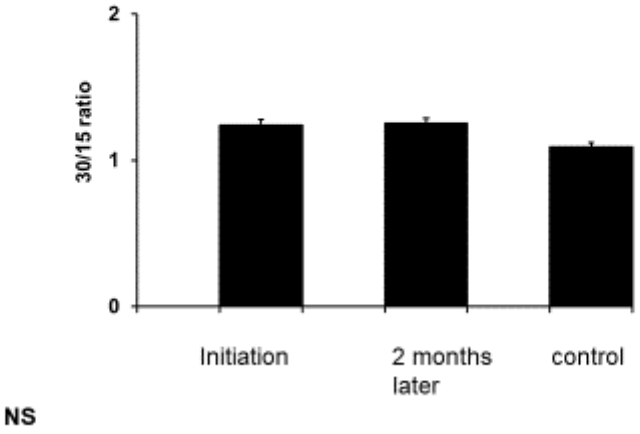


Figure 10. Short-term changes in the 30/15 ratio in diabetic patients treated with insulin pumps versus the control group

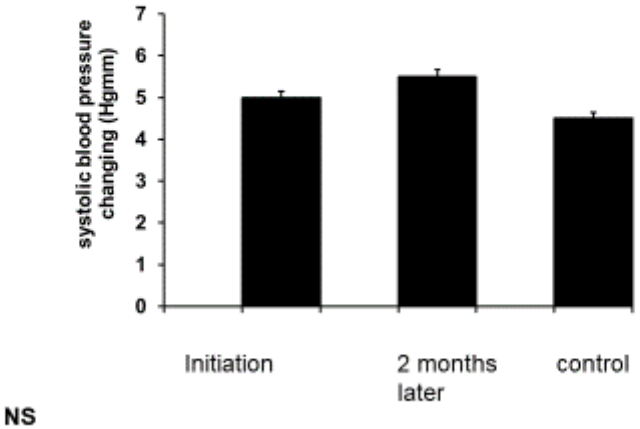


Figure 11. Short-term changes in systolic blood pressure response to standing in diabetic patients treated with insulin pumps versus the control group

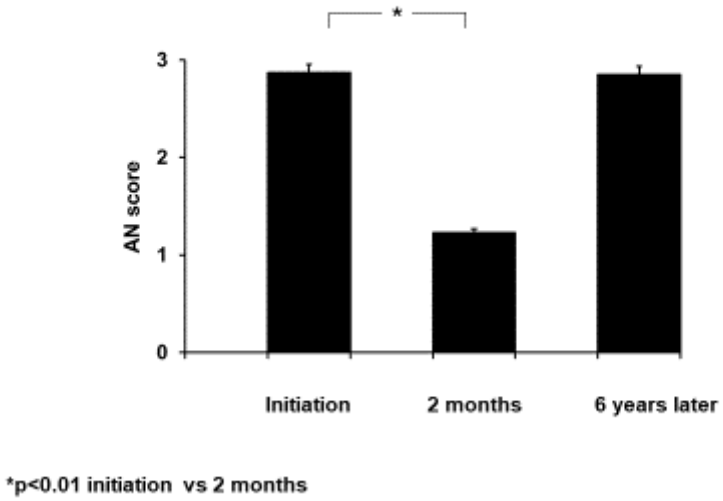


Figure 12. Autonomic neuropathy score at baseline and during the long-term follow up

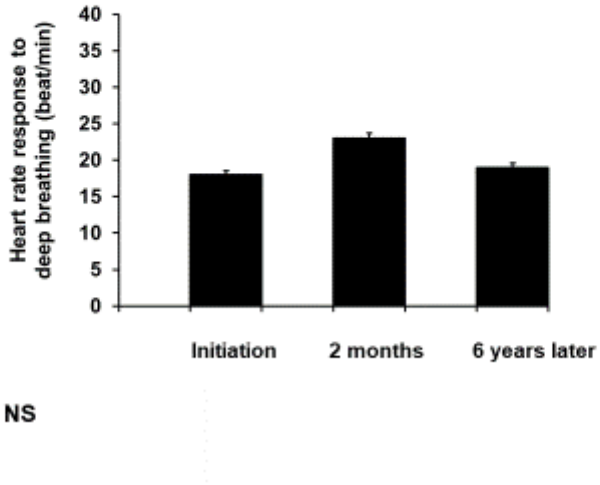


Figure 13. Heart rate response to deep breathing at baseline and during the long-term follow up

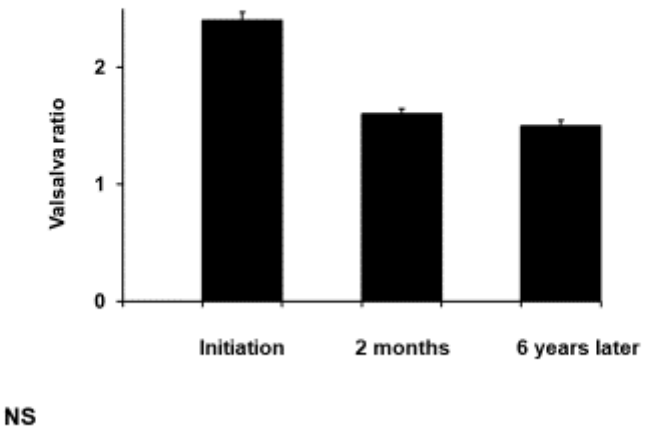


Figure 14. Valsalva ratio at baseline and during the long-term follow up

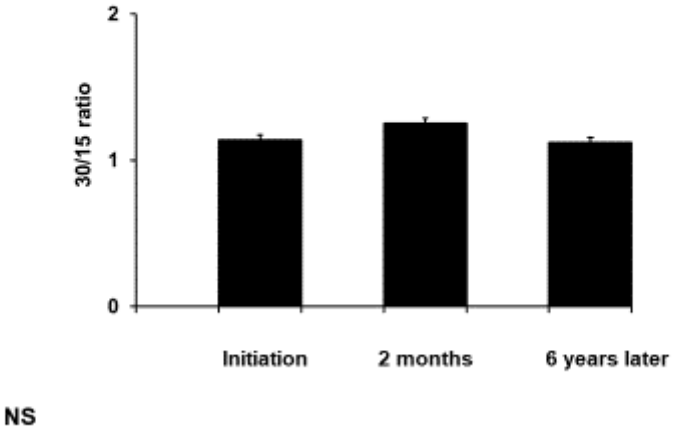


Figure 15. 30/15 ratio at baseline and during the long-term follow up

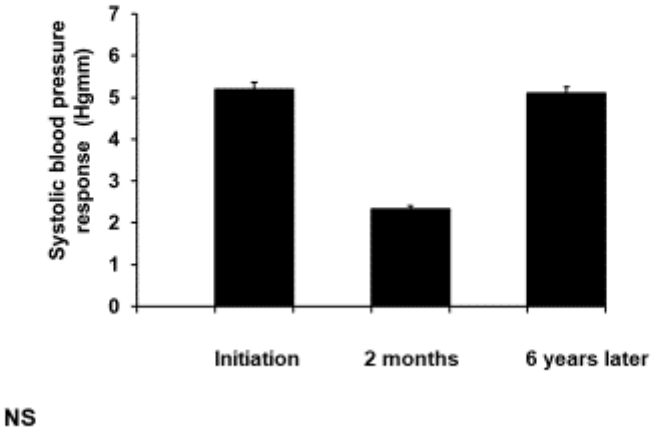
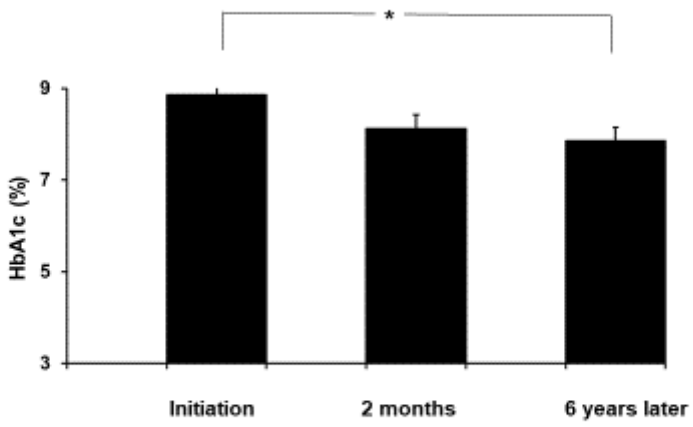


Figure 16. Systolic blood pressure response to standing at baseline and during the long-term follow up



* p<0,05, baseline vs 6 years later

Figure 17. HbA1c at baseline and during the long-term follow up

8 References

1. Boulton AJ, Kempler P, Ametov A, Ziegler D. Whither pathogenetic treatments for diabetic polyneuropathy? Diabetes Metab Res Rev. 2013; 29(5):327-33.

2. Kempler P, Keresztes K, Komoly S, Winkler G. The aetiology of neuropathies. In: Kempler P, Várkonyi T (editors) Neuropathies. A global clinical guide. Budapest: Zafír Press; 2012. p. 1–15.

3. Papanas N, Ziegler D. Risk factors and comorbidities in diabetic neuropathy: An update 2015. Rev Diabet Stud. 2015; 12:48-62.

4. Fleischer J, Yderstraede K, Gulichsen E, Lervang H, Eldrup E, Nygaard H, et al. Cardiovascular autonomic neuropathy is associated with macrovascular risk factors in type 2 diabetes: new technology used for routine large-scale screening adds new insight. J Diabetes Sci Technol. 2014; 8(4):874-880.

5. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, et al. Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. Diabet Med. 2002; 19:900-909.

6. Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in Type 2 diabetes. N Engl J Med. 2008; 358(6):580-591.

7. Putz Z, Tabák AG, Tóth N, Nóra I, Németh N, Gandhi RH, et al. Noninvasive evaluation of neural impairment in subjects with impaired glucose tolerance. Diabetes Care 2009; 32(1):181-183.

8. Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macroangiopathy. The MONICA/KORA Augsburg Surveys S2 and S3. Diabetes Care 2008; 31(3):464-469.

9. Grisold A, Callaghan BC, Feldman EL. Mediators of diabetic neuropathy - is hyperglycemia the only culprit? Curr Opin Endocrinol Diabetes Obes. 2017; 24(2):103–111.

10. Kim B, Feldman E. Insulin resistance in the nervous system. Trends Endocrinol Metab. 2012; 23(3):133–141.

11. Van de Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson AE. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. Am. J. Physiol. 1999; 276(1):178-183.

12. Madden K, Tedder G, Lockhart C, Meneilly GS. Euglycemic hyperinsulinemia alters the response to orthostatic stress in older adults with type 2 diabetes. Diabetes Care 2008; 31: 2203–2208.

13. Han L, Ji L, Chang J, Wen J, Zhao W, Shi H, et al. Peripheral neuropathy is associated with insulin resistance independent of metabolic syndrome. Diabetology & Metabolic Syndrome 2015; 7:14.

14. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012; 97(1): 28-38.

15. Ribeiro VB, Kogure GS, Reis RM Souza HCD, Ferriani RA, Reis RMD. Polycystic ovary syndrome presents higher sympathetic cardiac autonomic modulation that is not altered by strength training. Int J Exerc Sci. 2016; 9(5):554-566.

16. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al. Toronto Consensus Panel on Diabetic Neuropathy: Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011; 27(7):639-653.

17. Spallone V: Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: what is defined, what is new, and what is unmet. Diabetes Metab J. 2019; 43(1):3-30.

18. Pirart J: Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care 1978; 1(3):168-188.

19. The Diabetes Control and Complications Trial Research Group: The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). Diabetologia 1998; 41(4):416-423.

20. Martin CL, Albers JW, Pop-Busui R, and for the DCCT/EDIC Research Group: Neuropathy and related findings in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. Diabetes Care. 2014; 37(1):31–38.

21. Callaghan BC, Little AA, Feldman EL, Hughes RA: Enhanced glucose control for preventing and treating diabetic neuropathy. Cochrane Database Syst. Rev 6, CD007543 (2012).

22. Service FJ, Rizza RA, Daube JR, O'Brien PC, Dyck PJ: Near normoglycaemia improved nerve conduction and vibration sensation in diabetic neuropathy. Diabetologia 1985; 28(10):722-727.

23. Kitano Y, Kuwabara S, Misawa S, Ogawara K, Kanai K, Kikkawa Y, et al.: The acute effects of glycemic control on axonal excitability in human diabetics. Ann Neurol 2004; 56(4):462-467.

24. Fujita Y, Fukushima M, Suzuki H, Taniguchi A, Nakai Y, Kuroe A ,et al.: Short-term intensive glycemic control improves vibratory sensation in type 2 diabetes. Diabetes Res Clin Pract. 2008; 80(1):16-19.

25. Santini V, Ciampittiello G, Gigli F, Bracaglia D, Baroni A, Cicconetti E, et al. QTc and autonomic neuropathy in diabetes: effects of acute hyperglycaemia and n-3 PUFA. Nutr Metab Cardiovasc Dis. 2007;17(10):712–718.

26. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D: The effect of acute hyperglycaemia on QTc duration in healthy man. Diabetologia 2000; 43:571-575.

27. Yeap BB, Russo A, Fraser RJ, Wittert GA, Horowitz M: Hyperglycemia affects cardiovascular autonomic nerve function in normal subjects. Diabetes Care 1996; 19:880–882.

28. Lam WF, Muller ES, Souverijn JH, Lamers CB, Masclee AA: Effect of acute hyperglycaemia on basal and fat-induced exocrine pancreatic secretion in humans. Clin Sci (Lond) 1997; 93(6):573-80.

29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985, 28:412–419.

30. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19:41–47.

31. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J. 1982; 285:916-918.

32. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care 2017; 40(1):136-154.

33. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985; 8(5):491-498.

34. Lv SL, Fang C, Hu J, Huang Y, Yang B, Zou R, et al. Assessment of peripheral neuropathy using measurement of the current perception threshold with the Neurometer® in patients with type 1 diabetes mellitus. Diabetes Res Clin Pract. 2015; 109(1):130-134

35. Várkonyi T, Pető T, Dégi R, Keresztes K, Lengyel Cs, Janáky M, et al. Impairment of visual evoked potentials: An early central manifestation of diabetic neuropathy? Diabetes Care 2002; 25(9):1661-1662.

36. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. Am J Hypertens. 2001; 14:304S-309S.

37. Vollenweider P, Tappy L, Randin D, Schneiter P, Jéquier E, P Nicod, et al. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest. 1993; 92(1):147–154.

38. Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, Gude F, García F, Francisco AD, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocrine Disorders 2013; 13:47.

39. Pitei DL, Watkins PJ, Stevens MJ, Edmonds ME: The value of the Neurometer CPT in assessing diabetic neuropathy by measurement of the current perception threshold. Diabetic Med. 1994.11:872-876.

40. Vinik AI, Park TS, Stansberry KB, Pittenger GL: Diabetic neuropathies. Diabetologia, 2000; 43:957-973.

41. Kazamel M, Dyck PJ.: Sensory manifestations of diabetic neuropathies: Anatomical and clinical correlations. Prosthet Orthot Int 2015; 39(1):7-16.

42. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care. 2004; 27 (6):1458-86.

43. Keresztes K, Istenes I, Folhoffer A, Lakatos P, Horváth A, Csak T, et al. Autonomic and sensory nerve dysfunction in primary biliary cirrhosis. World J Gastroenterol. 2004; 10(20):3039-3043.

44. Hanewinckel R, Ikram MA, Franco OH, Hofman A, Drenthen J, van Doorn PA. High body mass and kidney dysfunction relate to worse nerve function, even in adults without neuropathy. J Periph Nerve Syst 2017; 22(2):112-120.

45. Oh TJ, Lee J-E, Choi SH, Jang HC. Association between body fat and diabetic peripheral neuropathy in middle-aged adults with Type 2 diabetes mellitus: A preliminary report. J Obes Metab Syndr. 2019; 28(2):112-117.

46. Kohner EM, Patel V, Rassam SM: Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. Diabetes 1995; 44:603-607.

47. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJL, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: Mechanisms, clinical significance, and treatment. J Am Soc Nephrol. 2017; 28(4):1023-1039.

48. Nyiraty S, Pesei F, Orosz A, Coluzzi S, Vági OE, Lengyel C, et al. Cardiovascular Autonomic neuropathy and glucose variability in patients with type 1 diabetes: Is there an association? Front Endocrinol (Lausanne) 2018; 9:174.

49. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, et al. Toronto Consensus Panel on Diabetic Neuropathy: Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev 2011; 27(7):654-64.

50. Bellavere F, Bosello G, Cardone C, Girardello L, Ferri M, Fedele D. Evidence of early impairment of parasympathetic reflexes in insulin dependent diabetics without autonomic symptoms. Diabete Metab. 1985; 11(3):152-156.

51. Kempler P, Váradi A, Tamás Gy. Which battery of cardiovascular autonomic function tests – suggestion for a rational diagnostic model. Diabetologia. 1990; 33 (10): 640.

52. Goldberger JJ, Arora R, Buckley U, Shivkumar K: Autonomic nervous system dysfunction. J Am Coll Cardiol 2019; 73(10):1189–1206.

53. Ang L, Jaiswal M, Martin C, Pop-Busui R: Glucose control and diabetic neuropathy: lessons from recent large clinical trials. Curr Diab Rep 2014; 14(9):528.

54. Ziegler D, Mayer P, Muhlen H, Gries FA: The natural history of somatosensory and autonomic nerve dysfunction in relation to glycaemic control during the first 5 years after diagnosis of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1991; 34:822–829.

55. Argente-Pla M, Pérez-Lázaro A, Martinez-Millana A, Del Olmo-García MI, Espí-Reig J, Beneyto-Castello I, et al. Simultaneous pancreas kidney transplantation improves cardiovascular autonomic neuropathy with improved valsalva ratio as the most precocious test. J Diabetes Res 2020; 2020:7574628

9 Acknowledgements

I would like to thank to Professor Tamás Várkonyi his guidance and encouragement during all these years and providing me with an indispensable help in writing this thesis.

I would like to express my gratitude to Professor Csaba Lengyel who gave me advice, help and inspiration writing this thesis.

I would like to thank Professor Peter Kempler all the support he gave me through these years.

I would like to say thanks to all of my colleagues who have been my co-workers and co-authors of the clinical studies described in this thesis.

I am deeply grateful to Professor Ferenc Szarvas and Professzor Janos Julesz, my former teachers who familiarised internal medicine and endocrinology with me and inspired and supported my works.

Thanks for my Family, Andi, Janka and Kristof to give inspiration for my work.

I.

Minerva Endocrinologica (New title: Minerva Endocrinology)
EDIZIONI MINERVA MEDICA

Peripheral sensory nerve hyperaesthesia in women with polycystic ovary syndrome

Sándor MAGONY, Szabolcs NYIRATY, Bettina TóTH, Fruzsina PESEI, Andrea OROSZ, György ÁBRAHáM, Péter KEMPLER, Csaba LENGYEL, Tamás VáRKONYI

Minerva Endocrinology 2021 Apr 21
DOI: 10.23736/S2724-6507.21.03418-7

Article type: Original Article

Article first published online: April 21, 2021
Manuscript accepted: April 1, 2021
Manuscript revised: March 26, 2021
Manuscript received: December 11, 2020

Sándor Magony[1], Szabolcs Nyiraty[1], Bettina Tóth[1], Fruzsina Pesei[1], Andrea Orosz[2], György Ábrahám[1], Peter Kempler[3], Csaba Lengyel[1], Tamás Várkonyi[1]

Peripheral sensory nerve hyperaesthesia in women with polycystic ovary syndrome

[1]Department of Medicine, [2]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary, [3]Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary

Correspondence: Sándor Magony, Department of Medicine, Kálvária str. 57. Szeged, 6720-Hungary, magonysandor@gmail.com, tel: +3662545189 fax: +3662545185

Abstract: Dysfunction of the nervous system is well-known in diabetes and also among patients with prediabetes, obesity and hypertension. However, there is only a limited amount of data available on the changes in neuronal function in polycystic ovary syndrome (PCOs), despite the fact that this condition is also accompanied by metabolic and vascular abnormalities. The aim of our study was to assess the cardiovascular autonomic and peripheral sensory function in patients with PCOs. The study involved 27 women with PCOs, and 24 healthy women as control subjects. Autonomic neuropathy (AN) was assessed using the four standard cardiovascular reflex tests. Peripheral sensory function was determined using the Neurometer. Electric stimulation was applied transcutaneously and the current perception threshold (CPT) values were determined on the median and peroneal nerves. No significant differences were found between the PCOs patients and the control group regarding the cardiovascular autonomic reflex tests and the AN scores. The CPT values of PCOs patients in the median and peroneal nerves were lower at all frequencies in comparison to controls. Conclusions: The cardiovascular autonomic nerve function was normal in the patients with PCOs. The current perception thresholds were consequently lower in the PCOs patients both in the upper and lower extremities at all frequencies, which serves as an early sign of neuropathy. As a novel observation, our results suggest that early neuronal damage manifests in the form of sensory hyperaesthesia in patients with PCOs.

Abbreviations: AN – autonomic neuropathy, BMI – body mass index, CPT – current perception threshold, CRT – cardiovascular reflex test, HOMA – Homeostasis Model Assessment, PCOs – polycystic ovary syndrome

Neuropathy is one of the most detrimental neurological conditions. It considerably impairs patients' quality of life and it is also associated with an increased morbidity and mortality (1). The underlying cause of polyneuropathy is typically found outside the nervous system (2). Preventing the manifestation or the progression of neuropathy is essential in high-risk patients. Therefore, it is very important to explore all possible conditions which might lead to this complication. In addition to high blood glucose, there are a number of pathological conditions which may potentially cause neuropathy. Common risk factors of distal symmetric sensorimotor polyneuropathy in diabetes and cardiovascular diseases include age, hypertension, dyslipidaemia, oxidative stress and obesity (3). Cardiovascular autonomic neuropathy (AN) also commonly associated with well-known macrovascular risk factors in type 1 and type 2 diabetic patients including high blood pressure, hypertriglyceridemia and smoking (4, 5). The complexity of the pathogenesis of neuropathy has been shown by the Steno 2 trial: in addition to close glucose control, intensified multifactorial intervention, the use of renin–angiotensin system blockers, aspirin, and lipid-lowering agents has been shown to slow the progression of neuronal complications in patients with type 2 diabetes (6). In accordance with the findings indicating that severe hyperglycaemia is not the exclusive cause of the neuronal damage, both autonomic and sensory neuropathies were documented in patients with prediabetes (7). The prevalence of polyneuropathy in prediabetes is associated with obesity, visceral fat and peripheral arterial disease (8). The decisive role of visceral fat is revealed by the fact that the incidence of neuropathy in obese patients – even with normal blood glucose levels – is higher compared to lean controls (9). Furthermore, "rubrometabolic syndrome", an interesting common manifestation of anaemia and metabolic diseases, also includes neuropathy among its components (10). Based on these findings, it is clear that insulin

resistance is the underlying common condition. Insulin resistance may develop in the neurons and results in injury to the peripheral and central nervous systems playing a role in the pathogenesis of neuropathy and Alzheimer's disease (11). Higher insulin levels exert a detrimental effect on the neuronal systems, as hyperinsulinemic/euglycemic clamps revealed increased muscle and cardiac sympathetic activity in healthy subjects (12), and an altered response to orthostatic stress in older adults with type 2 diabetes (13). In a cross-sectional study on 2,035 patients with metabolic syndrome, peripheral neuropathy was associated with insulin resistance independently of the additional components of metabolic syndrome (14). Insulin resistance has a decisive role in the pathogenesis of PCOs, and it is associated with a high number of risk factors for the development of neuropathy, including impaired glucose metabolism, hypertension, obesity and hyperlipidaemia (15). In addition, the excess of androgens in women with PCOs might be a contributing factor in the development of cardiovascular diseases and probably neuropathy as well. Although PCOs is the most common endocrine disorder in females, there is very limited amount of data available on the manifestation of neuronal dysfunctions in the patients. Higher sympathetic cardiac autonomic modulation during the evaluation of heart rate variability has been described in women with PCOs (16). Based on the lack of the previous characterization of the neuronal systems in PCOs, we designed a study with the aim to the assess the cardiovascular autonomic and peripheral sensory functions in women with this disease.

Patients and methods

Patients: 27 women with PCOs were involved in the study (age: 28.7±1.8 years, mean±SE). All of them were recruited from our outpatient department. The patients showed no symptoms of neuropathy. The mean BMI of the patient group was 29.7 kg/m^2,

which indicated that most of them were overweight or obese. None of the patients had been diagnosed with diabetes; their mean fasting blood glucose was 4.5±0.09 mmol/L.
24 healthy women with normal weight made up the control group. These individuals showed no significant differences in the descriptive parameters except for BMI (age: 28.1±1 years, fasting glucose: 4.1±0.07 mmol/L, BMI: 22.6±0.8 kg/m^2). The HOMA index of the PCOs group indicated insulin resistance (2.64±0.59 vs 1.92±0.33, $P<0.05$; PCOs vs control). PCOs was diagnosed following the Rotterdam consensus (17), based on the presence of at least two of the following criteria: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, ultrasound evidence of polycystic ovaries. Other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumours, Cushing's syndrome) were excluded. Patients with acute infection or chronic alcohol consumption were not involved in the study.

Study design

This study was a cross-sectional study. Cardiovascular AN and the peripheral sensory functions were determined in all patients and controls. Fasting blood glucose, fasting serum insulin, testosterone, androstenedione, body weight and height were measured in PCOs patients. These parameters were also assessed in controls with the exception of serum insulin levels. Homeostasis Model Assessment (HOMA, 18) was expressed from fasting insulin and glucose values in PCOs patients.

Assessment of AN

The presence and severity of AN was characterized using the four standard cardiovascular reflex tests (19). This approach provides a non-invasive, clinically relevant, reproducible and standardized gold-standard measurement (20). Three of these tests are based on

recording the changes in heart rate during specific manoeuvres, while the fourth test is designed to monitor blood pressure changes (21). Most of the tests aiming to detect heart rate changes are used primarily (but not exclusively) for the assessment of parasympathetic innervation, while the blood pressure response predominantly indicates the impairment of sympathetic functions (22). The following tests were applied:

Heart rate variation in response to deep breathing. Normally, the heart rate increases during inspiration and decreases during expiration. The patients were asked to breathe deeply at a rate of six breaths per minute (inhale for five seconds and exhale for five seconds). The result was expressed as the difference between maximum and minimum heart rates (bpm) during the six breathing cycles.

Heart rate response to the Valsalva manoeuvre

During the strain period of the Valsalva manoeuvre, the blood pressure drops, and the heart rate rises under physiologic conditions. Following the procedure, the blood pressure rises, and the heart rate slows. The patients were instructed to blow into a mouth-piece connected to a modified manometer and to hold it at a pressure of 40 mmHg for 15 seconds while an electrocardiogram was recorded continuously. The Valsalva ratio was calculated as the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre.

Heart rate response to standing (30:15 ratio)

Following the position change from lying to standing, the heart rate immediately increases and peaks around the 15^{th} beat after standing up. Then a relative bradycardia occurs in healthy subjects peaking at approximately the 30^{th} beat. At the start of the test, the patients were lying at rest while their heart rate was recorded continuously by an electrocardiogram. Then the patients stood up without interrupting the heart rate

monitoring. The 30/15 ratio was expressed as the ratio of the longest R-R interval at around the 30th beat and the shortest R-R interval at around the 15th beat after standing up.

Blood pressure response to standing (orthostatic hypotension)

In healthy subjects, pooling of blood in the lower extremities upon standing up is rapidly counterbalanced by peripheral vasoconstriction. Severe postural hypotension is a characteristic sign of AN. This test is based on blood pressure determinations in a lying position and after standing up. The postural fall is defined as the difference between systolic pressure after 10 minutes of lying and systolic pressures measured 1, 5 and 10 minutes after standing up. The largest difference from the systolic pressure in lying is defined as the blood pressure response to standing up.

Determination of peripheral sensory function

The peripheral sensory function was studied with a Neurometer (Neurotron Incorporated, Baltimore, MD, USA). This device is intended to quantify the function of different nerve fibres and provides a simple, non-invasive, and quantitative measure of peripheral sensory function (23). Low voltage electric sine wave stimulation was applied transcutaneously, and the current perception threshold (CPT) values were determined. Our study involved the median and peroneal nerves. The surface electrodes, 1 cm in diameter, were placed on the terminal phalanx of the index and the great toe. The electrodes were fixed only on intact skin surface, because wounds or scars would have disturbed the peripheral sensations. The amplitude of the delivered stimuli was between 0.01 and 9.99 mA. The stimulus was initially increased until a sensation was reported, then short stimuli (2 to 5 s) were applied at progressively lower amplitudes until a minimal threshold for consistent detection was determined. The CPT values of the upper and lower limbs were detected at three different stimulating frequencies (2 kHz, 250 Hz, and 5 Hz).

Statistical methods

Comparisons between PCOs and control patients were performed using the unpaired Student's t-test for normally distributed parameters. The possible associations between the measured values were analysed with the Spearmen correlation test. CRT-s, CPT-s and the descriptive parameters were expressed as mean values ± standard error (SE). A p value of <0.05 was regarded as statistically significant. The statistical analyses were performed using the SigmaStat 4.0 Systat Software and Statistica 12 packages.

This study was conducted in accordance with the ethical standards of the Helsinki Declaration and approved by the Human Research Ethics Committee of University of Szeged. All patients provided a written informed consent form before they were enrolled.

Results

The heart rate responses to deep breathing, the Valsalva manoeuvre and standing up, as well as the systolic blood pressure response to standing up from a lying position were obtained from both the PCOs and control patients and compared across the groups. Statistical evaluation revealed no significant differences in these test results reflecting the parasympathetic and sympathetic cardiovascular function (heart rate responses to deep breathing: 24.9±1.9 vs 24.5±1.6 beats/min; Valsalva ratio: 1.68±0.07 vs 1.86±0.06; 30/15 ratio: 1.4±0.06 vs 1.49± 0.06; orthostatic systolic blood pressure drop: 2.5±0.8 vs 2±0.8 mmHg, mean±SE, PCOs vs control; $p>0.05$ respectively, Figures 1-4).

The CPT levels in the median nerve at all three testing frequencies were significantly lower in the PCOs patients than in the controls (Table 1). Comparison of the CPT values in the peroneal nerve yielded similar findings, as these were significantly lower than in the

control group (Table 2). These observations provide clear evidence of peripheral sensory hyperaesthesia in PCOs patients compared to the healthy control subjects. To explore the possible role of insulin resistance in the abnormal sensory function, correlation tests were performed between HOMA indexes and the CPT values. No associations were observed between the CPT values measured in the median or the peroneal nerves, and the degree of insulin resistance expressed by the HOMA index (Table 3). Most of the analyses revealed no significant correlations between the BMI of PCOs patients and the CPT values measured in either extremity (Table 4). The only exception in this respect was a significant negative correlation between BMI and the CPT in the peroneal nerve at 5 Hz stimulation ($r = -0.39$; $p<0.05$). This observation may suggest that the higher BMI values were associated with lower sensory thresholds expressing the grade of hyperaesthesia. There was no statistical association between testosterone or androstenedione levels and the measured parameters of neuropathy. Multiple linear regression analysis yielded no predictors for sensory hyperaesthesia from the measured parameters (age, BMI, serum insulin, blood glucose, HOMA index, testosterone, androstenedione).

Discussion

Two important potential manifestations of neuropathy were analysed in our patients with PCOS. The cardiovascular autonomic function carries a very important predictive value regarding life expectancy. During the initial phase of AN, an alteration of the parasympathetic function is a characteristic finding (24). In prediabetes or diabetes, this has been shown by the early abnormalities in tests assessing heart rate responses to different procedures (14). Parasympathetic neuropathy at this phase is associated with a relative overdrive of sympathetic function. It is also well-known that increased sympathetic

tone is characteristic of insulin-resistant conditions (25). On the other hand, current hyperinsulinemia enhanced muscle sympathetic nerve activity in healthy subjects (19). Moreover, in a trial including women with PCOs, a spectral analysis of heart rate variability revealed a normal parasympathetic function with an increased sympathetic cardiac autonomic modulation (23).

Our data shows that heart rate and blood pressure responses in PCOs patients and healthy control subjects are similar. The putative harmful effect of insulin resistance, hyperandrogenism or obesity did not lead to a deficit in the parasympathetic or the sympathetic tone in patients with PCOs. As the mean age of the patients was 28 years, they were probably too young to be seriously exposed to the effects of insulin resistance exerted on autonomic function. It is very difficult to determine the duration of insulin resistance in PCOs. The degree of the insulin resistance may not have reached the threshold of sensitivity of the autonomic system, as the mean value of the HOMA index was slightly higher than the previously described normal range (26). This is the first study to evaluate data of the cardiovascular reflex tests of PCOs patients, and the findings indicate intact autonomic functions. This method is not suitable to estimate the hyperactivity of the parasympathetic or sympathetic systems, but its sensitivity, reproducibility and specificity ensures an accurate characterization of the potentially altered conditions. Our data provide further support for the hypothesis that in young PCOs patients, insulin resistance and the additional components of the syndrome without diabetes do not exert a negative effect on autonomic function.

The evaluation of the sensory function revealed characteristic findings. In both extremities and at all stimulating frequencies, the women with PCOs perceived the electric stimulation at lower intensities than the healthy controls. As their threshold of perception was lower,

they presumably became more sensitive to various stimuli than the healthy women. The Neurometer, our method of choice allows to selectively stimulate three types of sensory nerve fibres. Comparisons with conventional nerve function tests show that high frequency detection thresholds correlate best with tests of large fibre function, and low frequency detection thresholds indicate small fibre function (27). In our study, we tested the large and small myelinated fibres at 2000 Hz and 250 Hz, respectively. The sensory conduction of the small unmyelinated fibres were determined at a testing frequency of 5 Hz. The large fibres are responsible for the detection of tactile stimuli and vibration, while the small fibres convey the sensation of heat and pain (28). We found that in the large and small sensory fibres of the median as well as the peroneal nerves, hyperaesthesia was present in women with PCOs. Several aspects of these findings provide new approach to the pathophysiology of peripheral nerve damage. The described hyperaesthesia was also found in a minority of patients with prediabetes or diabetes at a very early stage of neuropathy, or it is associated with painful symptoms of neuropathy (14, 29). The nature of the progression of sensory neuropathy is still a subject of debate in the literature, but it is widely accepted that hyperaesthesia is a very early and mostly silent manifestation of neuropathy, which quickly turns into hypaesthesia as neuropathy worsens (30). Most of the patients are tested in the late phase of hypaesthesia, as this is the period when the symptoms appear, and patients seek medical aid. Hyperaesthesia is not a unique form of very early diabetic neuropathy. This neuronal complication has been proven to be present in diseases with other pathogenetic backgrounds. Using the Neurometer, Keresztes and co-workers found hyperaesthesia mainly in the peroneal nerve at all three frequencies in primary biliary cirrhosis with autoimmune pathogenesis (31). In our PCOs patients, lower perception thresholds were not associated with painful neuropathy, as our subjects did not

present with any symptoms. The pathogenesis of the observed sensory abnormality might be partially explained by obesity, as higher BMI correlated significantly with lower perception thresholds in the peroneal nerve at the 5 Hz stimulating frequency. This is supported by the fact that abdominal obesity was found to be associated with diabetic peripheral neuropathy, and previous intervention studies have shown that body weight loss decreases the incidence of neuropathy (32).

Hyperaesthesia in our patients with insulin resistance might share a common pathogenesis with the well-known phenomenon of hyperfunctions, characteristic very early manifestations of retino- and nephropathy in diabetic patients. Increased microvascular circulation in the retina precedes the clinical manifestations of retinopathy (33), while hyperfiltration is an early characteristic sign of nephropathy (34). Hyperaesthesia in the early stage of neuropathy might be also considered to be an enhanced physiologic function in the course of the developing complication.

All three types of fibres in both the upper and lower extremities are targets in an extended pathogenetic process, because the manifestations of developing sensorimotor polyneuropathy generally appear first in the lower limb and mainly in large fibres (28). Our observations point to a more generalized early alteration without a selectivity to site or function.

The limitation of the study is its cross-sectional design and the relatively small number of patients.

Novel findings of our study include the fact that cardiovascular autonomic function is not altered in young women with PCOs, while a hypersensitive condition is proven in both extremities in PCOS patients. Similarly to previous observations in retinopathy and nephropathy, sensory hyperfunction may regarded as an early manifestation of diabetic

neuropathy. Follow-up of neural function in PCOs patients might reveal new insight into the nature of pathogenesis and progression of diabetic neuropathy.

Acknowledgements

A part of the original observations in this manuscript were presented at two conferences:

1. 26th Congress of the Hungarian Diabetes Association, 21 April 2018

2. 28th Annual Meeting of the Diabetic Neuropathy Study Group of the EASD, 6 September 2018.

The first presentation is cited in the PhD thesis of Szabolcs Nyiraty as an abstract, in the list of his scientific achievement as a co-author.

Frequency	**Controls**	**PCOs**	**p value**
2 kHz	2.88±2	1.64±1.7	<0.01
250 Hz	1.27± 0.12	0.73±0.08	<0.01
5 Hz	1± 0.18	0.49±0.06	<0.05

Table 1. Peripheral sensory function of the median nerve in women with PCOs and controls (CPT in mA, mean±SE)

Frequency	**Controls**	**PCOs**	**p value**
2 kHz	4.4±0.28	3.29±0.19	<0.05
250 Hz	2.02±0.56	1.34±0.49	<0.01
5 Hz	1.56±0.1	0.83±0.09	<0.01

Table 2. Peripheral sensory function of the peroneal nerve in women with PCOs and controls (CPT, mA, mean±SE)

Correlated parameters	**r**	**p value**
Median nerve		
HOMA-CPT at 2 kHz	0.30	0.11
HOMA-CPT at 250 Hz	0.15	0.44
HOMA-CPT at 5 Hz	0.01	0.93
Peroneal nerve		
HOMA-CPT at 2 kHz	0.08	0.65
HOMA-CPT at 250 Hz	0.06	0.73
HOMA-CPT at 5 Hz	0.33	0.08

Table 3. Correlation between the HOMA index and the peripheral sensory function of the patients

Correlated parameters	**r**	**p value**
Median nerve		
BMI-CPT at 2 kHz	-0.04	0.83
BMI-CPT at 250 Hz	-0.15	0.42
BMI -CPT at 5 Hz	-0.24	0.22
Peroneal nerve		
BMI-CPT at 2 kHz	-0.14	0.48
BMI-CPT at 250 Hz	-0.27	0.15
BMI-CPT at 5 Hz	-0.39	<0.05

Table 4. Correlation between BMI and the peripheral sensory function of the patients

References

1. Boulton AJ, Kempler P, Ametov A, Ziegler D. Whither pathogenetic treatments for diabetic polyneuropathy? Diabetes Metab Res Rev. 2013; 29(5):327-33.

2. Kempler P, Keresztes K, Komoly S, Winkler G. The aetiology of neuropathies. In: Kempler P, Várkonyi T (editors) Neuropathies. A global clinical guide. Budapest: Zafír Press; 2012. p. 1–15.

3. Papanas N, Ziegler D. Risk factors and comorbidities in diabetic neuropathy: An update 2015. Rev Diabet Stud. 2015; 12:48-62.

4. Fleischer J, Yderstraede K, Gulichsen E, Lervang H, Eldrup E, Nygaard H, et al. Cardiovascular autonomic neuropathy is associated with macrovascular risk factors in type 2 diabetes: new technology used for routine large-scale screening adds new insight. J Diabetes Sci Technol. 2014; 8(4): 874-80.

5. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, et al.: Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study., Diabet Med. 2002; 19:900-909.

6. Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in Type 2 diabetes. N Engl J Med. 2008; 358(6):580-91.

7. Putz Z, Tabák AG, Tóth N, Nóra I, Németh N, Gandhi RH, et al. Noninvasive evaluation of neural impairment in subjects with impaired glucose tolerance. Diabetes Care 2009; 32(1):181-3.

8. Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macroangiopathy The MONICA/KORA Augsburg Surveys S2 and S3. Diabetes Care 2008; 31(3):464-9.

9. Grisold A, Callaghan BC, Feldman EL. Mediators of diabetic neuropathy - is hyperglycemia the only culprit? Curr Opin Endocrinol Diabetes Obes. 2017; 24(2):103–111.

10. Kalra S, Coetzee A, Kalra PA, Saldaña JR, Kilov G. Rubrometabolic syndrome. Minerva Endocrinol. 2020. [Epub ahead of print]

11. Kim B, Feldman E. Insulin resistance in the nervous system. Trends Endocrinol Metab. 2012; 23(3):133–141.

12. Van de Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson AE. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. Am. J. Physiol. 1999; 276(1):178-83.

13. Madden K, Tedder G, Lockhart C, Meneilly GS. Euglycemic hyperinsulinemia alters the response to orthostatic stress in older adults with type 2 diabetes. Diabetes Care 2008; 31: 2203–2208.

14. Han L, Ji L, Chang J, Wen J , Zhao W, Shi H, et al. Peripheral neuropathy is associated with insulin resistance independent of metabolic syndrome. Diabetology & Metabolic Syndrome 2015; 7:14.

15. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012; 97(1): 28-38.

16. Ribeiro VB, Kogure GS, Reis RM Souza HCD, Ferriani RA, Reis RMD. Polycystic ovary syndrome presents higher sympathetic cardiac autonomic modulation that is not altered by strength training. Int J Exerc Sci. 2016; 9(5):554-566.

17. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19: 41–47.

18. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985, 28:412–419.

19. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J. 1982; 285:916-918.

20. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care 2017; 40(1):136-154.

21. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al.: Toronto Consensus Panel on Diabetic Neuropathy Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev. 2011; 27(7):639-53.

22. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985; 8(5):491-8.

23. Lv SL, Fang C, Hu J, Huang Y, Yang B, Zou R, et al. Assessment of peripheral neuropathy using measurement of the current perception threshold with the Neurometer® in patients with type 1 diabetes mellitus. Diabetes Res Clin Pract. 2015; 109(1):130-4

24. Várkonyi T; Pető T, Dégi R, Keresztes K, Lengyel Cs, Janáky M, et al. Impairment of visual evoked potentials: An early central manifestation of diabetic neuropathy? Diabetes Care 2002; 25(9):1661-1662.

25. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. Am J Hypertens. 2001; 14: 304S-309S.

26. Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, Gude F, García F, Francisco AD, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocrine Disorders 2013; 13:47.

27. Pitei DL, Watkins PJ, Stevens MJ, Edmonds ME: The value of the Neurometer CPT in assessing diabetic neuropathy by measurement of the current perception threshold. Diabetic Med. 1994.11:872-876.

28. Vinik AI, Park TS, Stansberry KB, Pittenger GL: Diabetic neuropathies. Diabetologia , 2000; 43:957-973.

29. Kazamel M, Dyck PJ.: Sensory manifestations of diabetic neuropathies: Anatomical and clinical correlations Prosthet Orthot Int 2015; 39(1):7-16.

30. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care. 2004; 27 (6):1458-86.

31. Keresztes K, Istenes I, Folhoffer A, Lakatos P, Horváth A, Csak T, et al. Autonomic and sensory nerve dysfunction in primary biliary cirrhosis. World J Gastroenterol. 2004; 10(20):3039-43.

32. Oh TJ, Lee J-E, Choi SH, Jang HC. Association between body fat and diabetic peripheral neuropathy in middle-aged adults with Type 2 diabetes mellitus: A preliminary report. J Obes Metab Syndr. 2019; 28(2):112-117.

33. Kohner EM, Patel V, Rassam SM: Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. Diabetes 1995; 44:603 –607.

34. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJL, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: Mechanisms, clinical significance, and treatment. J Am Soc Nephrol. 2017; 28 (4):1023-1039.

Notes:

Authors' contributions: S.M., S.N., B.T., F.P., A.O., performed the measurements. S.M., G.Á., P.K., C.L., T.V. were involved in planning and supervised the work. S.M., P.K.,T.V. processed the data, performed the analysis, drafted the manuscript, and designed the figures. All authors duscussed the results and commented on the manuscript.

All authors read and approved the final version of the manuscript.

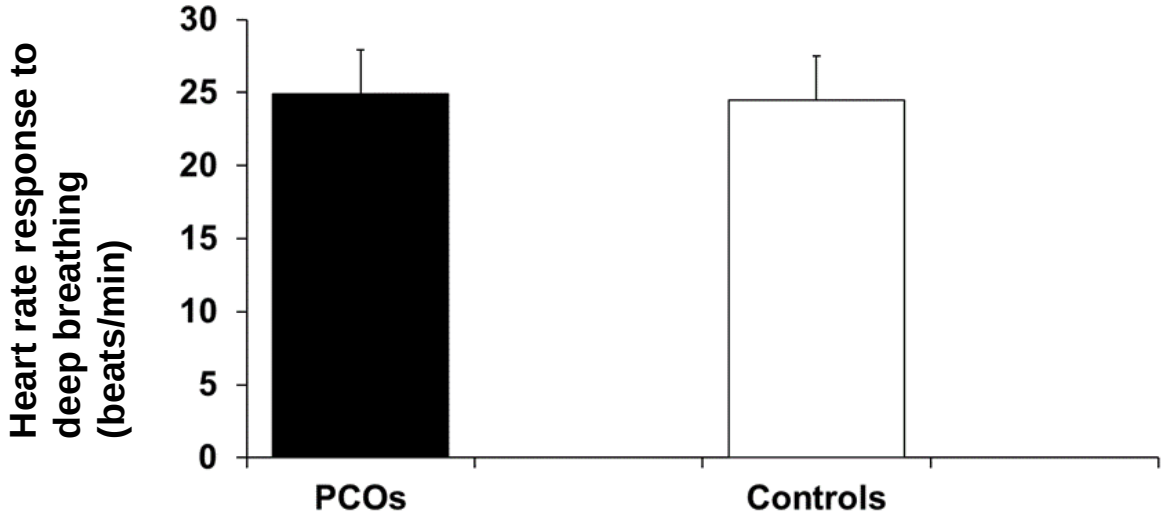


Figure 1. Heart rate response to deep breathing in women with PCOs and controls. $p>0.05$, PCOs vs controls.

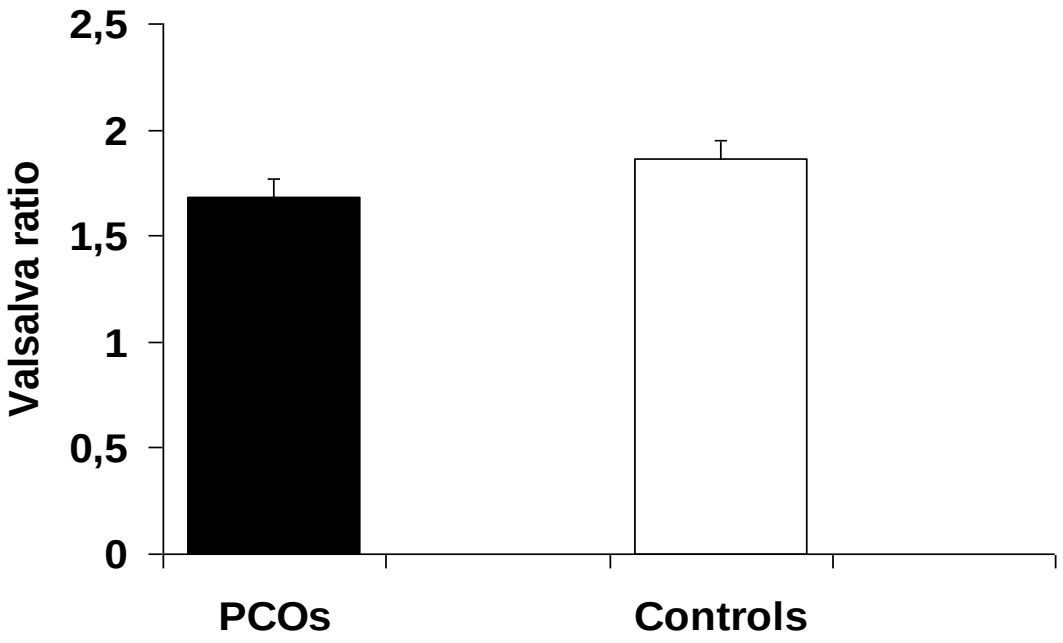


Figure 2. Heart rate response to Valsalva manoeuvre in women with PCOs and controls. $p>0.05$, PCOs vs controls

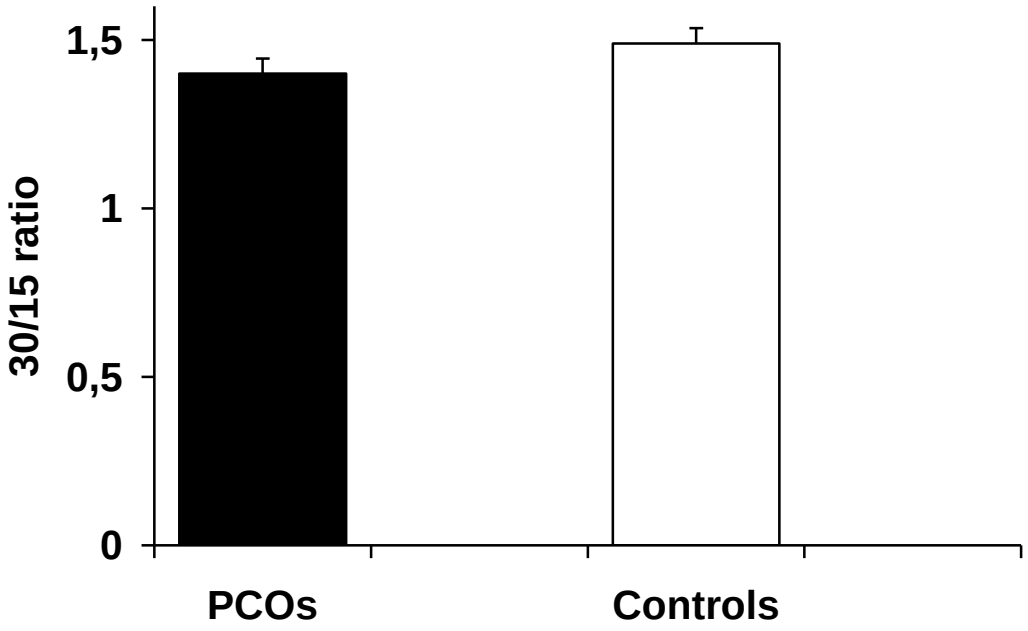


Figure 3. Heart rate response to standing (30/15 ratio) in women with PCOs and controls $p>0.05$, PCOs vs controls

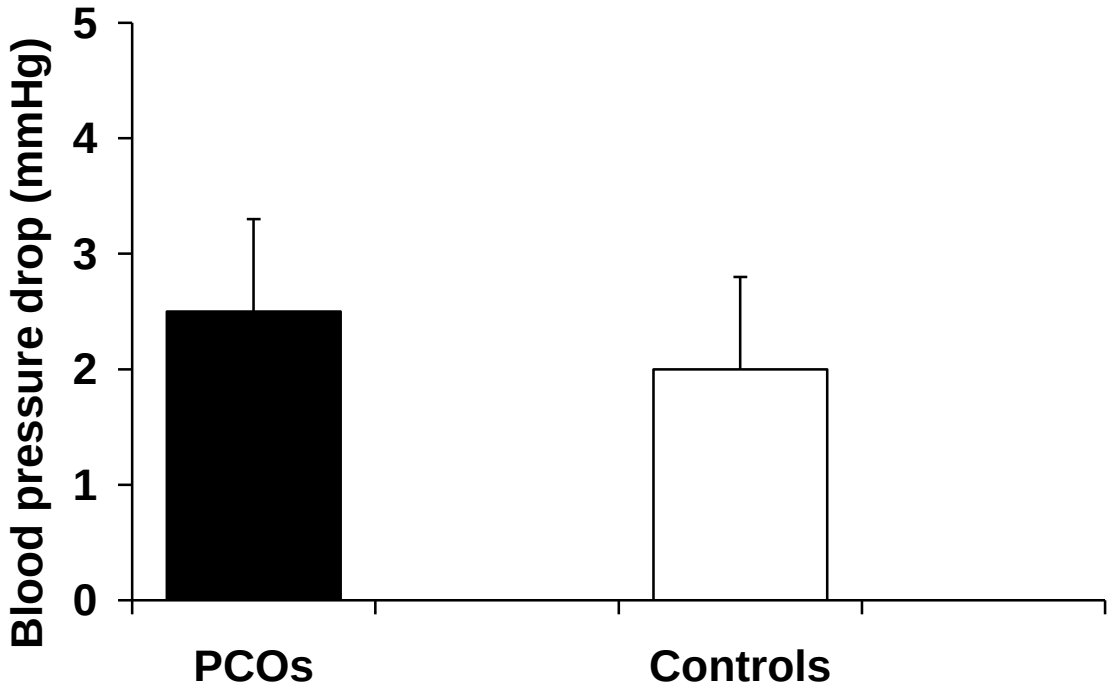


Figure 4. Orthostatic systolic blood pressure response to standing in women with PCOs and controls. $p>0.05$, PCOs vs controls

II.

Szegedi Tudományegyetem, Belgyógyászati Klinika, Szeged,[1] Szegedi Tudományegyetem, Farmakológiai és Farmakoterápiai Intézet, Szeged,[2] Semmelweis Egyetem, Belgyógyászati és Onkológiai Klinika, Budapest[3]

Inzulinpumpa-kezelést igénylő, kedvezőtlen anyagcsere-állapotú 1-es típusú diabeteses betegek autonóm idegrendszeri funkciójának jellemzői

Magony Sándor dr.,[(1)] Nyiraty Szabolcs dr.,[(1)] Fehértemplomi Katalin dr.,[(1)] Tóth Bettina dr.,[(1)] Pesei Fruzsina dr.,[(1)] Orosz Andrea dr.,[(2)] Lengyel Csaba dr.,[(1)] Kempler Péter dr.,[(3)] Várkonyi Tamás dr.[(1)]

Összefoglalás

Bevezetés: *A szénhidrát-anyagcsere súlyos instabilitása csökken inzulinpumpa bevezetése után 1-es típusú diabetesben. A glükózanyagcsere zavara autonóm neuropathiát okozhat, azonban az autonóm károsodás is felelős lehet az anyagcsere romlásáért. Felmerül a kérdés, hogy milyen mértékű az autonóm idegrendszer érintettsége a pumpakezelés indikációjának megfelelő rossz anyagcsere mellett, továbbá a neuropathia javulása akár rövid időn belül bekövetkezik-e a pumpakezelés megkezdése után.* ***Célkitűzés:*** *Vizsgálataink célja a kardiovaszkuláris autonóm funkció jellemzése volt pumpakezelés megkezdésekor, majd a kezelés bevezetése után 2 hónappal.* ***Betegek, módszerek:*** *Vizsgálatainkba 1-es típusú diabeteses beteget vontunk be (38 beteg, 23 nő, 15 férfi, életkor: 29,5±1,3 év, betegségtartam: 13,8±1,5 év; BMI: 23,2±0,6; átlag±SE). A kontrollcsoportba 10 nem diabeteses személy került (életkor: 27,8±2 év). Az autonóm neuropathia (AN) vizsgálatát az inzulinpumpa felhelyezése előtt és azt követően 2 hónappal kardiovaszkuláris reflextesztek (CRT) segítségével végeztük el.* ***Eredmények:*** *A betegcsoportban a legérzékenyebb paraszimpatikus teszt, a belégzésre bekövetkező szívfrekvencia-változás az egészséges kontrollhoz képest szignifikánsan kórosnak bizonyult a kezelés kezdetén (belégzés: 32,6±3,8 vs. 18,6±1,5 ütés/min., p<0,001). A paraszimpatikus károsodás mértéke annál kifejezettebb volt, minél hosszabb volt a bólus-bázis inzulinkezelés időtartama az inzulinpumpa felhelyezése előtt a betegekben (AN score–tartam: r=0,51, p<0,05; belégzés–tartam: r=–0,63, p<0,001). A két hónapos pumpakezelés mellett az AN összesített súlyossága csökkent (AN score: 2,2±0,2 vs. 1,5±0,2, p<0,05), a belégzésre bekövetkező szívfrekvencia-változás pedig szignifikánsan javult (belégzés: 18,6±2,1 vs. 22,4±2 ütés/min., p<0,05). Az átlagos HbA_{1c} a két hónapos kezelés alatt 0,7%-ot csökkent (8,7±0,2% vs. 8,0±0,3%, p=0,125).* ***Következtetés:*** *A pumpakezelés megkezdésekor a fiatal 1-es típusú diabeteses betegekben a paraszimpatikus idegrendszeri diszfunkció dominált, ami már két hónapos pumpakezelés mellett javulást mutatott. Az idegrendszeri érintettség súlyossága szoros összefüggést mutatott a pumpakezelést megelőző bólus-bázis inzulinkezelés időtartamával. Adataink 1-es típusú diabetes mellitusban megerősítik azt a megfigyelést, hogy kóros szénhidrát-anyagcsere esetén a paraszimpatikus idegrendszer károsodik elsőként, ami eredményeink szerint a pumpakezelés elkezdését követően már rövid időn belül is javulhat.*

Kulcsszavak: ***1-es típusú diabetes, inzulinpumpa-kezelés, autonóm neuropathia***

Közlésre érkezett: 2020. október 12. • Közlésre elfogadva: 2020. november 30.

A levelezésért felelős szerző: Dr. Magony Sándor

SZTE, Belgyógyászati Klinika
6725 Szeged, Kálvária sgt. 57.
E-mail: magony.sandor@med.u-szeged.hu

Characteristics of autonomic neuronal function of type 1 diabetic patients with poor glycemic control at the initiation of insulin pump treatment

Summary: *Introduction: Insulin pump treatment is introduced due to the severe instability of glycemic control in type 1 diabetes (DM). Unstable glucose metabolism leads to the development and progression of autonomic neuropathy (AN) in these patients, while AN may improve even within a short period of better glycemic control.* ***Objectives:*** *The aim of our study was to describe the characteristics of the cardiovascular autonomic function at initiation of insulin pump treatment and 2 months later.* ***Methods:*** *38 type 1 DM patients were involved (23 women, 15 men, age: 29.5±1.3 years, duration of DM: 13.8±1.5 years; BMI: 23.2±0.6; mean±SE). 10 non diabetic subjects were enrolled as a control group (age: 27.8±2 years). Autonomic neuropathy (AN) was assessed at the first application of insulin pump and 2 months later by cardiovascular reflex tests (CRT).* ***Results:*** *The most sensitive parasympathetic test, the heart rate response to breathing was significantly impaired in type 1 DM patients compared to healthy controls (breathing: 32.6±3.8 vs. 18.6±1.5 beats/min, p<0.001). Longer duration of bolus-basal insulin treatment correlated with more severe parasympathetic dysfunction (AN score-duration: r=0.51, p<0.05; breathing-duration: r=–0.63, p<0.001). The overall severity of AN reduced (AN score: 2.2±0.2 vs. 1.5±0.2, p<0.05) and the heart rate response to breathing significantly improved two months after the initiation of insulin pump treatment (breathing: 18.6±2.1 vs. 22.4±2 beats/min, p<0.05). The mean HbA_{1c} decreased by 0.7% during the two-month treatment period (8.7±0.2% vs. 8.0±0.3%, p=0.125).* ***Conclusion:*** *Moderate to severe autonomic neuropathy was found in type 1 diabetic patients at the initiation of insulin pump treatment which improved after two months of treatment. The severity of the parasympathetic involvement strictly correlated with the duration of the bolus-basal insulin treatment. Our data confirm the theory that in type 1 diabetes the parasympathetic function is impaired earlier during the progression of the metabolic disease and might improve after a short-term insulin pump treatment.*

Key words: ***type 1 diabetes, insulin pump treatment, autonomic neuropathy***

DIABETOLOGIA HUNGARICA **28** (№5) **289–296.** 2020. december DOI: **10.24121/dh.2020.21**

Az autonóm neuropathia a mortalitás önálló rizikófaktora 1-es típusú diabetesben, aminek oka többek között a szimpatikus idegrendszer fokozott aktivitása, a kardiovaszkuláris adaptáció zavara, a diastolés diszfunkció létrejötte, valamint a fokozott aritmiakészség lehet, amelyek a hirtelen szívhalál vagy légzésleállás kialakulásának fontos etiológiai tényezői.[1] Ezek az adatok felhívják a figyelmet arra, hogy minden terápiás lehetőséget igénybe kell vennünk ahhoz, hogy megelőzzük a szív-érrendszer állapotát döntően meghatározó autonóm neuropathia kialakulását vagy progresszióját, biztosítva ezzel a korai mortalitás csökkentését. 1-es típusú diabetesben ennek leghatékonyabb eszköze minden kétséget kizáróan a minél inkább hatékony anyagcsere-beállítás, aminek elérése után várható, hogy a neuropathia kisebb kockázattal fejti ki hátrányos hatását. Nem ismert, hogy az anyagcsere-optimalizálás milyen időtartamon belül fejti ki előnyös hatását az autonóm idegrendszer működésére, jóllehet a betegellátásban segítséget jelentene számunkra, hogy betegünk szövődménystátuszát pontosabban tudjuk megítélni. A hosszú távú glykaemiás kontroll és a neuropathia gyakorisága közötti összefüggésre számos korábbi vizsgálat rávilágított már. E kérdés egyik úttörője, *Pirart* több mint 40 éve, 1977-ben közölte megfigyeléseit, amelyek során 25 éven keresztül követett mintegy 4400 cukorbeteget, dokumentálva a rendszeres ellenőrzések során talált éhomi és posztprandiális vércukorértékeket, a glucosuria mértékét, valamint az ezen idő alatt megfigyelt ketoacidosisos epizódokat és a betegek panaszait. Eredményei igazolták, hogy a retinopathia, a nephropathia és a neuropathia kialakulásának gyakorisága nem(csak) a diabetes tartamától, hanem az anyagcsere-beállítás minőségétől is függ.[2] A DCCT-EDIC követéses vizsgálat eredményei igazolták a korai intenzív kezelés hosszú távú jótékony hatását a neuropathia incidenciájára és progressziójára. A mintegy 20 éves követés során azt találták, hogy a szívfrekvencia variabilitása, amelyet az EKG R-R hullámainak elemzése révén rögzítettek, kisebb mértékű paraszimpatikus károsodást igazolt az intenzív inzulinkezelésben korán részesülő betegekben 1-es típusú diabetesben. A felhasznált metodika a legkorábban jelzi a paraszimpatikus funkció esetleges romlását.[3,4] Az EURODIAB-IDDM

mérföldkő fontosságú vizsgálat az autonóm neuropathia számos rizikófaktorát igazolta 1-es típusú diabetesben, amelyek közé a hosszú távú glykaemiás kontroll és a diabetestartam is tartozik.[5] A UKPDS pedig 2-es típusú diabetesben tette egyértelművé, hogy az intenzív anyagcserekezelés mellett a neuropathiás szövődmények relatív kockázata (biothesiometerrel mérve) csökkent a megfigyelés 9., illetve 15. évére.[6] Néhány vizsgálati adat, jóval kisebb betegszámú tanulmányokban, mint az előbbiekben említett vizsgálatokban, amellett szól, hogy már rövid távú glükózszintváltozás is befolyásolhatja az idegrendszeri funkciókat. 8 hónappal a subcutan pumpakezelés megkezdése után a konzervatív inzulinkezelésben részesülőkhöz képest a perifériás idegvezetéses eredmények már javulást mutattak.[7] 4 héttel az intenzív inzulinkezelés megkezdését követően az idegvezetési paraméterek és az axonális szintű ingerületvezetés javulását találták japán cukorbetegekben.[8] 20 napos konzekvensen fenntartott normoglykaemia mellett 2-es típusú diabetesben a vibrációérzet kétféle metodikával vizsgálva is javulást mutatott az alsó és a felső végtagokon.[9] Experimentális körülmények között clamp vizsgálatban létrehozott 2 órás hyperglykaemia alatt az EKG-t monitorizálva a QT idő aktuális növekedését észlelték 2-es típusú diabeteses betegekben, amihez az autonóm idegrendszer instabilitását tükröző fokozott szimpatikus aktivitást is rögzítettek.[10] Egészséges személyekben is megfigyelték a 2 órás, átmeneti 15 mmol/l-es vércukorérték kamrai repolariációt rontó és katecholaminszintet emelő hatását.[11] Egészséges emberekben 150 percen keresztül létrehozott arteficiális hyperglykaemia esetén a nyugalmi szívfrekvencia fokozódását és a felállásra bekövetkező szívfrekvencia-válasz kórossá válását igazolták, ami a magas vércukorszint paraszimpatikus rendszerre kifejtett akut hatását igazolja.[12] Egészséges személyekben a pancreas-polipeptid bazális és stimulált szekréciója egyaránt csökkent kísérletes körülmények közötti hyperglykaemia esetén, ami szintén a vagális cholinerg működés romlását jelzi akkor, ha a vércukor aktuálisan magas.[13] Az irodalmi adatok egyértelműen arra utalnak, hogy a tartósan ideális anyagcsere hosszú távon előnyös lehet az autonóm neuropathia prevenciójában és a progresszió csökkentésében. A rövid távú, pár napos-hetes normoglykaemia, illetve a hyperglykaemia idegrendszerre kifejtett aktuális hatásáról szóló adatok felvetik a kérdést, hogy az anyagcsere-stabilizálás mennyi idő eltelte után hoz eredményt ebből a szempontból. 1-es típusú diabetesben a legintenzívebb kezelés és az optimalizálás leghatékonyabb formája a subcutan pumpakezelés bevezetése. A fentiek ismeretében elvégeztünk egy vizsgálatot, amelyben az inzulinpumpa-kezelés előtt, majd azt követően rövid időn belül megvizsgáltuk a kardiovaszkuláris autonóm idegrendszer funkcióját fiatal, 1-es típusú diabeteses betegekben. Vizsgálatunk célja annak felderítése volt, hogy pumpafelhelyezés kezdetén melyek a kardiovaszkuláris autonóm idegrendszeri működés jellemzői, és ezek hogyan változnak 2 hónapos pumpakezelést követően. Mindezek mellett összefüggést kerestünk a diabetest jellemző paraméterek és az autonóm idegrendszer funkciója között.

Betegek és módszerek

Vizsgálatainkban 38 (23 nő és 15 férfi) 1-es típusú diabeteses beteg és 10 egészséges kontrollszemély (6 nő és 4 férfi) vett részt. A betegek a vizsgálatok elvégzésekor átlagban fiatal felnőtt korúak voltak (29,5±1,3 év, átlag±SE), betegségük gyermek- vagy pubertáskorban kezdődött (diabetestartam: 13,8±1,5 év), valamennyi beteg a diagnózist követően rögtön inzulinkezelésben részesült. Testtömegindexük szerint a normális tartományba tartoztak, a BMI-értékük 23,2±0,6 volt. Az első autonóm idegrendszeri vizsgálatra minden beteg esetében akkor került sor, amikor megkezdődött inzulinpumpa-kezelésük. Vércukor-ingadozásuk jelentős volt, gyakran haladta meg a napi 10 mmol/l-t a változás mértéke (24/38 beteg), illetve a HbA_{1c}-értékük minden beteg esetében magasabb volt, mint 8% (38/38 beteg), a vizsgálat kezdetekor az átlagos HbA_{1c} 8,7%±0,2 volt a betegcsoportban. 6 beteg esetében a szövődmények gyors progresszióját észleltük, 2 nőbeteg esetében a pumpakezelés fentieken túli indikációja mellett fertilitási igény is volt. A diabeteshez társuló szövődmények előfordulási gyakorisága a vizsgálatba bevont betegek körében: retinopathia 16, nephropathia 10, igazolt diabeteses neuropathia 10 beteg esetében állt fenn. Az anamnézis szerint 1-1 betegnek volt korábban myocardialis infarctusa, illetve agyi éreredetű eseménye. Az autonóm funkciót felmérő második vizsgálat 2 hónappal a pumpa

felhelyezése után történt. A betegeknél mind a két vizsgálat alkalmával történt HbA_{1c}-meghatározás.

A kontrollcsoport tagjai a leíró paraméterek vonatkozásában nem különböztek szignifikánsan a betegcsoporttól. Az átlagéletkor 27,8±2 év, a BMI-érték pedig 24,1±0,4 volt.

A vizsgálathoz használt módszerek

Az autonóm neuropathia jelenlétét és súlyosságát a *Ewing* és munkatársai által leírt 4 standard kardiovaszkuláris reflexteszttel jellemeztük.[14]

Az általunk alkalmazott reflexteszteket, valamint azok normális és kóros értékeit az *1. táblázat* foglalja össze.

A szívfrekvencia-változást vizsgáló tesztek

1. Mély légzést kísérő szívfrekvencia-változások: Fiziológiásan a szívfrekvenciának a légzéssel szinkron változásai figyelhetők meg, amit légzési aritmiaként ismerünk. Belégzés során nő, kilégzés alatt csökken a szívfrekvencia. Diabetesben kardiovaszkuláris autonóm neuropathia fennállása esetén ez a légzésre bekövetkező szívfrekvencia-variabilitás csökken, vagy meg is szűnik. A szívfrekvencia maximális változása egészséges egyénekben akkor figyelhető meg, ha a légzési periódus (be- és kilégzés együttvéve) 6/perc körül van. Vizsgálata: A vizsgált személy fekvő helyzetben 6/perc légzési frekvenciával mély be- és kilégzést végez 30 másodpercig (5-5 másodpercig tartó be- és kilégzés), miközben folyamatos EKG-vizsgálat készül. Ezáltal meghatározhatjuk a belégzés alatti legrövidebb, valamint a kilégzés alatti leghosszabb RR távolság közötti különbséget. A 15/perc feletti frekvencia-különbség normálisnak, a 11–14/perc közötti határesetnek, a 10/perc alatti pedig kórosnak számít.
2. A Valsalva-manővert kísérő szívfrekvencia-változások: A Valsalva-manőver zárt epiglottis melletti erőltetett kilégzés, amely közben a hasűri és mellüregi nyomás emelkedik, és ez a vagus által közvetített paraszimpatikus funkción keresztül hat a szív működésére. Fiziológiás esetben a manőver végzése közben a vérnyomás csökken, a szívfrekvencia pedig nő. Közvetlenül a manőver után teljesen ellentétes állapot, vérnyomás-emelkedés figyelhető meg – ami eléri, majd meghaladja a manőver előtti értéket –, miközben a szívfrekvencia csökken a vagális baroreflex miatt. Vizsgálata: A vizsgált személynek 40 Hgmm-es nyomást tartva 15 másodpercig kell fújnia egy klasszikus vérnyomásmérővel összekötött gumicsőbe. A manőver alatt folyamatosan, majd utána 1 percen keresztül EKG-felvétel készül. Az erőltetett kilégzést követő leghosszabb és a manőver alatti lerövidebb RR távolság hányadosa adja a Valsalva-hányadost. Normálértéknek az 1,21 vagy nagyobb, határértéknek az 1,20–1,11 közötti, míg kórosnak az 1,10 alatti érték tekinthető.
3. Felállás után bekövetkező szívfrekvencia-változások (30:15 hányados): Egészséges egyénekben fekvésből történő felállást követően a szívfrekvencia átmenetileg nő, majd ezután csökkenni kezd. A maximális szívfrekvencia a felállást követő 15. szívverésnél, a legalacsonyabb pedig a 30. környékén észlelhető.

1. táblázat. *A kardiovaszkuláris reflexteszek normális értékei Ewing és munkatársai nyomán (forrás: Kempler Péter, Várkonyi Tamás: Neuropathiák a klinikai gyakorlatban, 98. o. ISBN 978-615-50052-2-0)*

Módszer	Mért érték	Normális érték	Határérték	Káros érték
Paraszimpatikus funkciót vizsgáló tesztek				
Mély be- és kilégzés	Beat-to-beat variáció (ütés/perc)	≥15	11–14	≤10
Valsalva-manőver	Valsalva-hányados	≥1,21	1,11–1,20	≤1,10
Felállás	30:15 hányados	≥1,04	1,01–1,03	≤1,00
Szimpatikus funkciót vizsgáló tesztek				
Felállás	Systolés vérnyomáscsökkenés (Hgmm)	<10	11–29	≥30
Handgrip teszt	Diastolés vérnyomáscsökkenés (Hgmm)	>16	11–15	≤10

Vizsgálata: A vizsgált személy 5 percig nyugalomban fekszik, majd feláll úgy, hogy a karjait a teste mellett lazán lógatva tartja. Ezt a testhelyzetet 1 percig tartja, miközben folyamatosan EKG-felvétel készül a fekvéstől kezdődően, amelyből meghatározzuk a felkeléstől számított 15. szívverés körüli legrövidebb RR távolság (maximális frekvencia-növekedés) és a 30. ütés körüli leghosszabb RR távolság (maximális frekvencia-csökkenés) hányadosát. Normálérték az 1,04 vagy nagyobb hányados, határérték az 1,01–1,03 közötti, míg kóros az 1,00 alatti érték.

Vérnyomásváltozást vizsgáló teszt

Felállást követő systolés vérnyomáscsökkenés: Normál körülmények között a felállást követően különböző reflexmechanizmusok lépnek működésbe a gravitáció következtében az alsó végtagokba kerülő vérnek a test felsőbb részeibe pumpálása érdekében. A szívfrekvencia növekedésén kívül a vérnyomás is hirtelen, rövid ideig nő, majd gyorsan csökkenni kezd. Az autonóm neuropathia következtében a splanchnikus erek vasoconstrictiója elmarad, aminek következményeként felállás után hirtelen vérnyomásesés, orthostaticus hypotonia alakul ki, ami akár ájulást is okozhat.

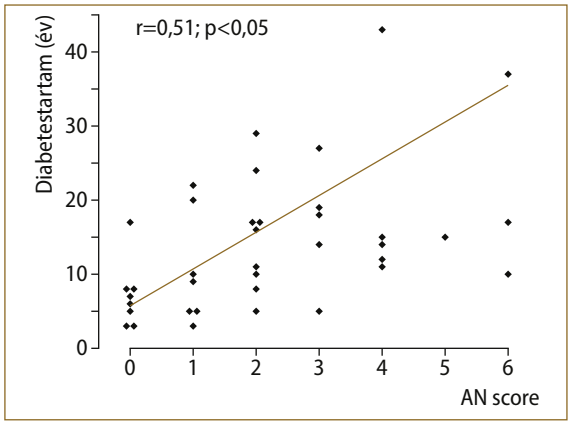
Vizsgálata: A vizsgált személy 10 percig nyugalomban fekszik, majd ezt követően feláll. A felállást követő 1., 5. és 10. percben vérnyomásmérés történik. Normális, nem károsodott autonóm funkciót jelez, ha a systolés vérnyomásérték kevesebb mint 10 Hgmm-rel csökken a felállás után. Ha a vérnyomásesés 10 Hgmm és 30 Hgmm közötti, az határértéknek számít, ha a vérnyomásesés meghaladja a 30 Hgmm-t, akkor a teszt kóros, és az a szimpatikus beidegzés súlyos zavarára utal.

Autonóm score

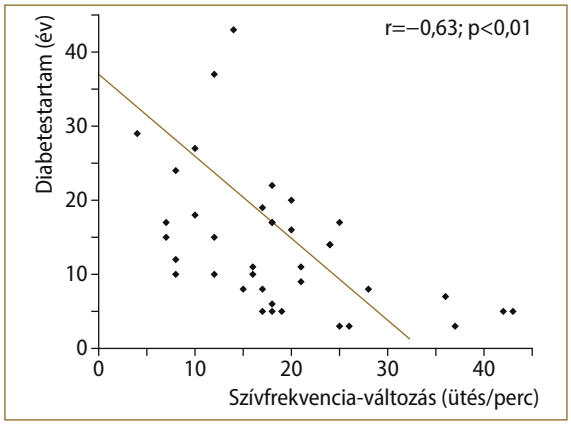
Az egyes kardiovaszkuláris reflextesztek után kapott értékeket összesítettük, és 0-tól 8-ig terjedő skálával jellemeztük az autonóm neuropathia súlyosságát, amit autonóm score-ként fejeztünk ki. Az egyes tesztek esetében a normális tartományba eső érték 0 pont, a határérték tartományba eső 1 pont, míg a kóros tartományba eső 2 pontot jelent. Az 4 reflexteszt alapján összesen 0–1 pont felel meg a szövődménymentes kategóriának. 2–3 pont esetén már az autonóm neuropathia enyhe jelei mutatkoznak. A kifejezett, nem súlyos neuropathia 4–6 pont közötti, a súlyos neuropathia pedig 7 pont feletti értékkel jellemezhető.

Eredmények

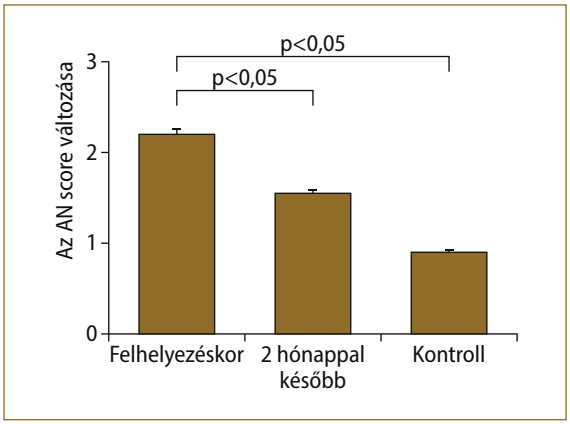
Az 1-es típusú diabetes tartama a pumpafelhelyezés idejéig erős pozitív, szignifikáns korrelációt



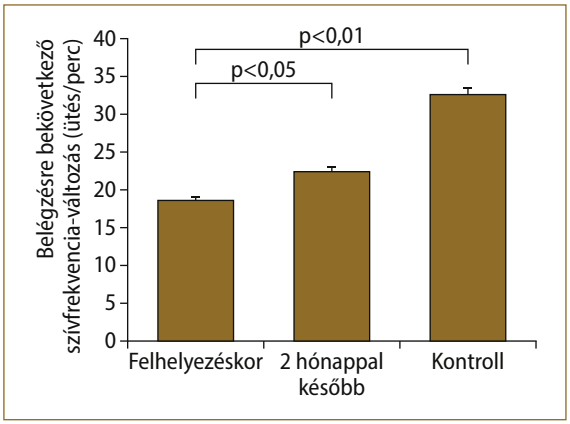
1. ábra. Összefüggés a diabetestartam és az autonóm neuropathia (AN) score között 1-es típusú diabeteses betegekben inzulinpumpa felhelyezésekor



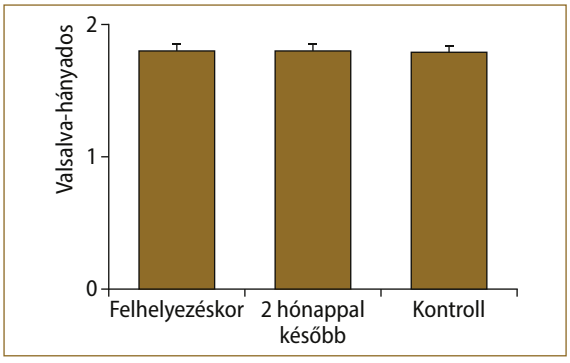
2. ábra. Összefüggés a diabetestartam és a légzésre bekövetkező szívfrekvencia-változás között 1-es típusú diabeteses betegekben inzulinpumpa felhelyezésekor



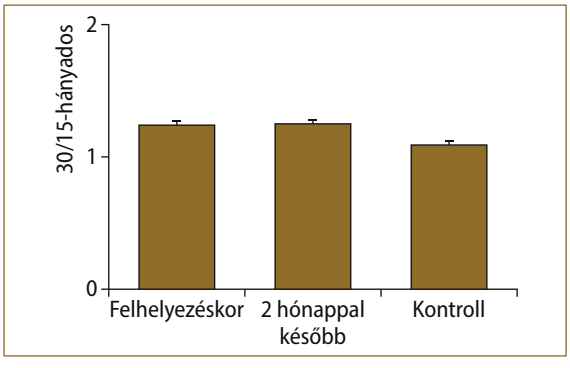
3. ábra. Az autonóm neuropathia (AN) score változása a diabeteses betegekben és a kontrollcsoport értéke



4. ábra. A belégzésre létrejövő szívfrekvencia-válasz változása inzulinpumpával kezelt diabeteses betegekben és a kontrollcsoport értéke



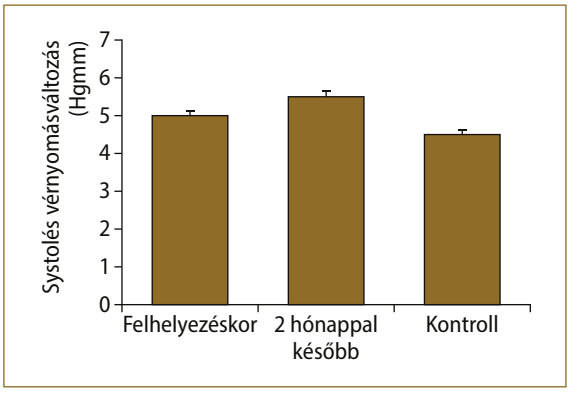
5. ábra. A Valsalva-hányados változása inzulinpumpával kezelt diabeteses betegekben és a kontrollcsoport értéke



6. ábra. A 30/15 hányados változása inzulinpumpával kezelt diabeteses betegekben és a kontrollcsoport értéke

mutatott az AN összesített súlyosságát kifejező score értékkel *(1. ábra)*. A statisztikai eredmény arra utal, hogy a hosszabb, rossz anyagcsere-körülmények között töltött diabetestartam esetén súlyosabb volt az AN összesített mértéke.

A diabetestartam és a legérzékenyebb paraszimpatikus teszt eredménye, a légzésre bekövetkező szívfrekvencia-válasz is szignifikáns összefüggésben volt egymással a pumpa felhelyezésének idejében *(2. ábra)*. A korreláció negatív, ami arra utal, hogy minél alacsonyabb volt a szívfrekvencia-változás mértéke, annál hosszabb volt a diabetes fennállása.



7. ábra. A felállásra bekövetkező systolés vérnyomásváltozás inzulinpumpával kezelt diabeteses betegekben és a kontrollcsoport értéke

A követés során azt észleltük, hogy az összesített AN score a pumpafelhelyezés után két hónappal kismértékű, de szignifikáns csökkenést mutatott *(3. ábra)*. A betegek kiinduláskor és a követés időpontjában mért értékeit az egészséges kontrollal összehasonlítva megállapítható, hogy mindkét időpontban mérsékelt súlyosságú autonóm neuropathia volt detektálható, amely csökkenő tendenciát mutatott 2 hónapos pumpakezelés után.

A belégzésre bekövetkező szívfrekvencia-válasz követésekor azt találtuk, hogy ennek értéke szignifikánsan javult a két hónapos pumpakezelés során *(4. ábra),* ami a paraszimpatikus működés javulására utal ezen időszak alatt. Mind a kiinduláskor, mind két hónap múlva a teszt eredményei elmaradtak az egészséges személyek értékeitől.

A további reflextesztek esetében konzekvensen azt figyeltük meg, hogy a Valsalva-hányadosra és a felállásra bekövetkező szívfrekvencia-változás, továbbá a felállásra létrejövő systolés vérnyomásesés nem változott szignifikánsan a 2 hónap alatt, és az egészséges kontrollhoz képest sem bizonyultak kórosabbnak *(5., 6. és 7. ábra)*.

A betegek HbA_{1c}-értéke a 2 hónapos követés során 8,7%±0,2-ról 8,1±0,2%-ra csökkent, de ez a változás nem volt szignifikáns.

Megbeszélés

A kedvezőtlen anyagcserehelyzet az autonóm neuropathia kialakulásának kockázatát növeli. A 3004 beteg adatait feldolgozó EURODIAB IDDM vizsgálatban az autonóm neuropathia előfordulása 36%-nak bizonyult, 2 kóros teszt 6%-ban, 1 kóros teszt 30%-ban fordult elő.[5] A paraszimpatikus autonóm neuropathia prevalenciája nem csak a HbA_{1c}-vel, de számos egyéb paraméterrel is szignifikáns összefüggést mutatott, amelyek közé a diabetestartam is tartozott. A vizsgálatunkban részt vevő 1-es típusú diabeteses betegek mindegyikének esetében a korábbi terápiás törekvések ellenére sem sikerült stabil anyagcsere-állapotot elérni, ezért vált szükségessé az inzulinpumpa-kezelés bevezetése. Valamennyi betegünk fiatal volt. Az általunk végzett vizsgálatban az években kifejezett betegségtartam és az autonóm neuropathia súlyossága között derült ki szignifikáns összefüggés, ami az irodalmi adatokkal egybevetve arra utal, hogy mind a prevalencia, mind a neuropathia súlyossága összefügg a krónikus anyagcserezavar hosszával. A nagy glükózvariabilitás mértékének és a kardiovaszkuláris reflextesztek kórosságának pozitív összefüggése 1-es típusú diabetesben korábban már igazolást nyert.[15] Az autonóm neuropathia súlyosságának abszolút értékét illetően vizsgálatunkban a több mint 10 éves diabetestartam és a pumpakezelést szükségessé tevő anyagcsere mellett mérsékelt súlyosságú idegkárosodás jött létre. Az egyes reflexteszteket szeparáltan vizsgálva megállapítottuk, hogy a mély belégzésre bekövetkező szívfrekvencia-válasz szignifikáns negatív összefüggést mutatott a betegségtartammal. A mély be- és kilégzésre bekövetkező szívfrekvencia-változás a paraszimpatikus innervatio vizsgálatára szolgál, és ez tekinthető a legszenzitívebb metodikának ezen funkciózavar megítélése szempontjából. A nagyfokú szenzitivitás miatt a legkorábban kórossá váló tesztnek bizonyul az AN vizsgálata során.[16] Egyes vélemények szerint ez a teszt önmagában is alkalmas lehet az autonóm neuropathia detektálására, a többi procedúra egyidejű elvégzése inkább a súlyosság mértékének meghatározásában nyújthat segítséget.[17,18] A negatív korreláció azt mutatja, hogy minél hosszabb ideje tartott a diabetes, annál kisebb volt a légzésre bekövetkező szívfrekvencia-változás mértéke. A többi reflexteszt és a betegségtartam között nem találtunk összefüggést. Az adatok arra utalnak, hogy ebben a betegcsoportban a pumpafelhelyezésig tartó diabetesexpozíció mérsékelt fokú AN-t okozott, amelynek első manifesztációjaként a paraszimpatikus működés károsodott. A paraszimpatikus károsodás mielőbbi csökkentése a kardiovaszkuláris kockázat mérséklését is eredményezi, mivel ilyenkor relatív szimpatikotónia is fennáll, ami a szív-érrendszer szempontjából kifejezetten rossz prognózist jelent.[19]

Vizsgálatunk követéses részében 2 hónapos pumpakezelést követően az autonóm score szignifikáns csökkenését észleltük. Ez az adat arra utal, hogy a mérsékelt autonóm károsodás globálisan csökkent. Az egyes reflexteszteket vizsgálva pedig megállapítható, hogy a paraszimpatikus működés mutatott leginkább javulást 60 nap alatt. Azok az adatok, amelyek azt mutatják, hogy az egyes perifériás vagy autonóm idegrendszerben működő neuronok funkciói akár párórás anyagcserezavar esetén is romlanak, illetve a krónikus hyperglykaemia

elhárítása után napokon vagy heteken belül is javulnak, koherensek megfigyeléseinkkel. A 2 hónap alatt a hyperglykaemiára legérzékenyebb paraszimpatikus működés már javulást mutat, ami az anyagcserezavar 1-es típusú diabetesben leghatékonyabb javítását biztosító pumpakezelés révén valósult meg. A HbA_{1c} nem szignifikáns mértékben csökkenő tendenciát mutatott, ami támogatja az anyagcserezavar javulásának szerepét. A glykaemiás állapot változását leginkább a glükózvariabilitás követésével tudtuk volna megjeleníteni, azonban ez nem történt meg a jelenlegi vizsgálatban, ami a vizsgálat limitációjának is tekinthető. A dolgozat további korlátja a pumpakezelés rövid időtartama, ugyanakkor a két hónap során bekövetkező változások dokumentálása érdemesnek tűnt.

Összefoglalásként megállapíthatjuk, hogy mérsékelt súlyosságú autonóm neuropathia jellemezte az 1-es típusú diabeteses betegeket pumpakezelésük kezdetekor, amikor anyagcseréjük terápiaváltoztatást tett szükségessé. A diabetestartam konzekvens összefüggést mutatott a paraszimpatikus neuropathia súlyosságával, amely a korai autonóm idegrendszeri károsodás manifesztációjaként alakul ki. A két hónapos pumpakezelés alatt az autonóm funkció javult. Eredményeink szerint az anyagcsere-állapot pumpakezelés során bekövetkező viszonylag gyors javulása már rövid idő alatt mérsékelheti az autonóm idegrendszer működészavarát, hosszabb távon pedig feltételezhetően lassíthatja a neuropathia progresszióját. A szénhidrát-anyagcsere stabilizálása tehát az egyik eszköz lehet az autonóm egyensúly felborulásával létrejövő kardiovaszkuláris kockázat – akár rövid időn belül bekövetkező – csökkentésének 1-es típusú diabetesben.

Irodalom

1. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al.: Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011; 27(7): 639-653. DOI:10.1002/dmrr.1239
2. Pirart J: Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabete Metab 1977; 3(2): 97-107.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). Diabetologia 1998; 41(4): 416-423. DOI:10.1007/s001250050924
4. Pop-Busui R, Low PA, Waberski BH, Martin CL, Albers JW, Feldman EL, et al.: Effects of prior intensive insulin therapy on cardiac autonomic nervous system function in type 1 diabetes mellitus: the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC). Circulation 2009; 119; 2886-2893. DOI:10.1161/CIRCULATIONAHA.108.837369
5. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, et al.: Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. Diabet Med 2002; 19(11): 900-909. DOI:10.1046/j.1464-5491.2002.00821.x
6. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998; 352(9131): 837-853. DOI:10.1016/S0140-6736(98)07019-6
7. Service FJ, Rizza RA, Daube JR, O'Brien PC, Dyck PJ: Near normoglycaemia improved nerve conduction and vibration sensation in diabetic neuropathy. Diabetologia 1985; 28(10): 722-727. DOI:10.1007/BF00265018.
8. Kitano Y, Kuwabara S, Misawa S, Ogawara K, Kanai K, Kikkawa Y, et al.: The acute effects of glycemic control on axonal excitability in human diabetics. Ann Neurol 2004; 56(4): 462-467. DOI:10.1002/ana.20232
9. Fujita Y, Fukushima M, Suzuki H, Taniguchi A, Nakai Y, Kuroe A, et al.: Short-term intensive glycemic control improves vibratory sensation in type 2 diabetes. Diabetes Res Clin Pract 2008; 80(1): e16-19. DOI:10.1016/j.diabres.2007.12.011
10. Santini V, Ciampittiello G, Gigli F, Bracaglia D, Baroni A, Cicconetti E, et al.: QTc and autonomic neuropathy in diabetes: effects of acute hyperglycaemia and n-3 PUFA. Nutr Metab Cardiovasc Dis 2007; 17(10): 712-718. DOI:10.1016/j.numecd.2006.09.006
11. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D: The effect of acute hyperglycaemia on QTc duration in healthy man. Diabetologia 2000; 43(5): 571-575. DOI:10.1007/s001250051345
12. Yeap BB, Russo A, Fraser RJ, Wittert GA, Horowitz M: Hyperglycemia affects cardiovascular autonomic nerve function in normal subjects. Diabetes Care 1996; 19(8): 880-882. DOI:10.2337/diacare.19.8.880
13. Lam WF, Muller ES, Souverijn JH, Lamers CB, Masclee AA: Effect of acute hyperglycaemia on basal and fat-induced exocrine pancreatic secretion in humans. Clin Sci (Lond) 1997; 93(6): 573-580. DOI:10.1042/cs0930573
14. Ewing DJ, Clarke BF: Diagnosis and management of diabetic autonomic neuropathy. Br Med J (Clin Res Ed) 1982; 285(6346): 916-918. DOI:10.1136/bmj.285.6346.916
15. Nyiraty S, Pesei F, Orosz A, Coluzzi S, Vági OE, Lengyel C, et al.: Cardiovascular Autonomic neuropathy and glucose variability in patients with type 1 diabetes: Is there an association? Front Endocrinol (Lausanne) 2018; 9: 174. DOI: 10.3389/fendo.2018.00174
16. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, et al.: Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev 2011; 27(7): 654-664. DOI:10.1002/dmrr.1224
17. Bellavere F, Bosello G, Cardone C, Girardello L, Ferri M, Fedele D: Evidence of early impairment of parasympathetic reflexes in insulin dependent diabetics without autonomic symptoms. Diabete Metab 1985; 11(3): 152-156.
18. Kempler P, Váradi A, Tamás Gy: Which battery of cardiovascular autonomic function tests – suggestion for a rational diagnostic model. Diabetologia 1990; 33(10): 640. DOI:10.1007/BF00400214
19. Spallone V: Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: what is defined, what is new, and what is unmet. Diabetes Metab J 2019; 43(1): 3-30. DOI:10.4093/dmj.2018.0259

III.

CEDA diabetes
Zentraleuropäische Diabetesgesellschaft
Central European Diabetes Association
Föderation der Internationalen Donau-Symposia über Diabetes mellitus
Federation of International Danube-Symposia on Diabetes mellitus

Long-Term Follow-up of the Autonomic Function Among Patients with Type 1 Diabetes Treated with Insulin Pump

S. Magony[1], S. Nyiraty[1], K. Fehértemplomi[1], B. Tóth[1], F. Pesei[1], A. Orosz[2], C. Lengyel[1], P. Kempler[3], V. Horváth[3,*], T. Várkonyi[1,*]

Introduction

Autonomic neuropathy (AN) is an independent risk factor for mortality in type 1 diabetes, particularly explained by the relatively increased activity of the sympathetic nervous system due to an attenuated parasympathetic function [Spallone 2011]. Further etiological factors of the poor life expectancy in patients with AN are the impaired cardiovascular adaptation, the development of diastolic dysfunction and the increased rate of arrhythmias that may be associated with sudden cardiac death or respiratory arrest [Spallone 2019]. As AN is a critical determinant of the cardiovascular integrity, it is mandatory to apply all possible therapeutic options to prevent the development or to reduce the progression of this diabetic complication.

In type 1 diabetes, one of the most important tools to achieve these aims is to keep the glycaemic control strictly in the target range. It is a well-known fact, that there is an association between long-term glycaemic control and the prevalence of neuropathy, as it was shown in several previous studies including large numbers of patients. Pirart's early results in the 25-year long follow-up of 4 400 patients showed that the incidence of neuropathy depends on the quality of metabolic control and the duration of diabetes [Pirart 1978]. At the closeout of the Diabetes Control and Complications Trial (DCCT) after 6.5 years of follow-up of 1 441 patients, the investigators reported that intensive insulin treatment significantly reduced the incidence of diabetic neuropathy, similarly to findings for diabetic retinopathy and nephropathy [The Diabetes Control and Complications Trial Research Group 1998]. By the end of the DCCT the prevalence of AN remained the same in the intensively treated group, while it almost doubled in participants on conservative treatment. The progression of AN was reduced by 45 % with intensive treatment compared to the conventionally treated group during the course of the DCCT [The Diabetes Control and Complications Trial Research Group 1998]. The observational Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up was established to monitor the long-term effects of the prior intensive treatment in the DCCT cohort on the development and progression of neuropathy. All of the study participants in EDIC were advised to follow intensive treatment regimens after the conclusion of the DCCT. At 13 – 14 years of EDIC the groups receiving previous intensive or conservative insulin treatment differed primarily in their R-R variation to deep breathing. This cardiovascular test remained significantly higher in the group with intensive treatment in the original DCCT, compared with the group applying conservative treatment in that period of the trial. The R-R variation to deep breathing is a sensitive marker of the parasympathetic function and becomes abnormal early during the progression of AN. This observation underlines the importance of the role of the early glycaemic control in the long-term progression of AN and provides evidence for the dominance of the parasympathetic impairment in the initial phase of AN [Martin 2014]. The EURODIAB IDDM Complications Study identified a number of risk factors for AN in type 1 diabetes, including long-term glycaemic control and duration of diabetes [Kempler 2002]. A meta-analysis of 17 randomized trials revealed, as part of the Cochrane database, that effective glucose control significantly prevented the development of clinical neuropathy in type 1 diabetes mellitus, whereas this association in type 2 diabetes is less evident [Callaghan 2012].

There is no doubt that the long-term glycaemic control plays an important role in the development of neuropathy in type 1 diabetes, but some remark-

CEDA Science

Unter CEDA Science veröffentlicht die Zentraleuropäische Diabetes-Gesellschaft (CEDA/FID) wissenschaftliche Artikel ihrer Mitglieder.

1) Department of Medicine, University of Szeged, Szeged, Hungary
2) Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary
3) Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary
* Viktor Horváth and Tamás Várkonyi contributed equally to the study as last authors.

able observations in small numbers of patients suggest that even short-term changes of glucose levels may also affect the nervous system function in both types of diabetes or even in healthy subjects. Eight months after starting subcutaneous pump therapy, peripheral nerve conduction measurements and vibratory sensory threshold improved compared to those receiving conservative insulin therapy in patients with type 1 diabetes [Service 1985]. Four weeks following initiation of intensive insulin therapy, improvements in vibratory sensation were observed in Japanese patients with type 2 diabetes [Kitano 2004]. Only 2 hours of hyperglycaemia increased the corrected QT interval at electrocardiogramm (ECG) in patients with type 2 diabetes [Santini 2007] and in healthy men as well [Marfella 2000]. In addition, a 150 minute-long hyperglycaemia increased the supine heart rate and altered a parasympathetic reflex test in healthy individuals [Yeap 1996]. Moreover, acute hyperglycaemia inhibited basal and stimulated pancreatic polypeptide secretion in non-diabetic participants suggesting an actual vagal inhibition induced by high glucose levels [Lam 1997]. These latter observations point to a reversable decreased parasympathetic activity and a relatively increased sympathetic activity in the case of currently high glucose. The data from the literature clearly suggest that well-treated glucose metabolism is the only way to prevent or to reduce the progression of neuropathy. The most intensive form of the treatment and optimisation of the glycaemic control in type 1 diabetes is the introduction of subcutaneous pump therapy which ensures the continuous insulin supply by the basal rate of the administration supplemented with bolus doses before main meals.

Analysing the previous papers, it is not clear when a better glycaemic condition should be achieved to improve neuronal function. The available data from small studies range from hours to years defining the glycaemic condition affecting the parameters of neuropathy.

The aim of our study was to explore the characteristics of autonomic cardiovascular function among patients with type 1 diabetes whose glycaemic control necessitated insulin pump therapy. Our further aim was to have a short-term and a long-term follow-up of the autonomic function during the treatment. In accordance with these aims cardiovascular autonomic function was detected at the initiation of the insulin pump treatment and was followed after 2 months and 6 years of pump treatment. We assessed the nature of the change in the tests and seeked for possible correlations between diabetes-specific parameters and autonomic nervous system function.

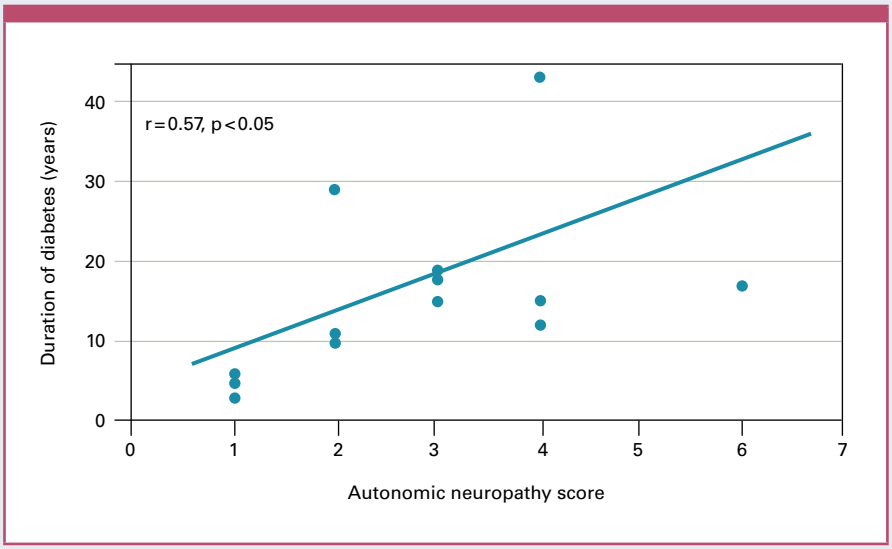


Fig. 1: Association between the duration of diabetes and the autonomic neuropathy score in patients with type 1 diabetes at the initiation of insulin pump treatment.

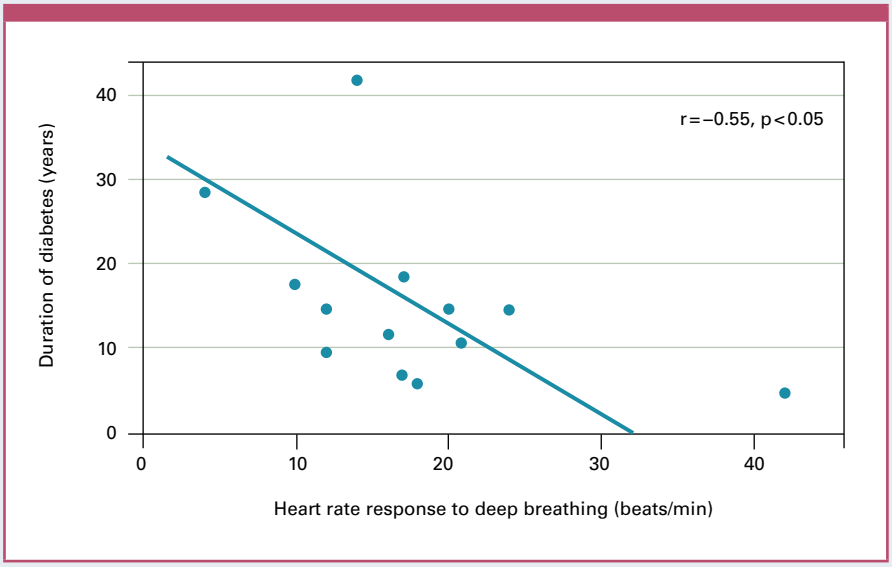


Fig. 2: Association between duration of diabetes and the heart rate response to deep breathing in patients with type 1 diabetes at the initiation of insulin pump treatment.

Study design

The first cardiovascular reflex tests (CRTs) were performed in all patients within 1 week before starting subcutaneous insulin pump therapy. The assessments of the cardiovascular autonomic function were performed 2 months and 6 years after the initiation of pump treatment. HbA_{1c} levels were determined at the time of all the three tests.

Patients and methods

13 patients (7 women and 6 men) with type 1 diabetes were included in the study. The patients were young adults at the time of the study initi-

ation (30.4 ± 2.7 years, mean ± SE). Their disease started in childhood or puberty (duration of diabetes at baseline: 16.5 ± 2.7 years, age at baseline: 27.8 ± 2 years). All patients received intensive insulin treatment immediately after their diagnosis until the start of insulin pump therapy. The mean body mass index (BMI) of the patient group at the initiation of the pump was 24.2 ± 1.0 kg/m^2, while 8.85 ± 0.2 % was the mean HbA_{1c} at the same time.

The quantitative characteristics of AN were determined by four standard CRTs [Ewing 1982]. These procedures ensure a non-invasive, clinically applicable, reproducible and standardized measurement of the autonomic control [Pop-Busui 2017]. Three of these tests evaluate the change of the heart rate during specific manoeuvres while the 4th test follows blood pressure changes [Spallone 2011]. The heart rate tests mainly (but not exclusively) assess the parasympathetic function while the blood pressure response predominantly reflects the impairment of sympathetic functions [Ewing 1985].

The following tests were applied:

1. Heart rate variation to deep breathing. The difference between maximum and minimum heart rates (beats/min) during the 6 breathing cycles was analysed.
2. Heart rate response to Valsalva manoeuvre. The Valsava ratio was calculated as the ratio of the longest R-R interval after the procedure to the shortest R-R interval during the manoeuvre.
3. Heart rate response to standing (30:15 ratio) The 30:15 ratio was defined as the ratio of the longest R-R interval at around the 30th beat after standing up to the shortest R-R interval at around the 15th beat.
4. Blood pressure response to standing up as a detection of orthostatic hypotension. The largest difference from the systolic blood pressure from lying position to standing up was evaluated.

A final score was created from the results of the four separate tests expressing the overall severity of the cardiovascular autonomic function. This autonomic score rated the severity of AN from 0–8 points.

Cardiovascular test	Baseline	After 2 months	After 6 years
Deep breathing (beats/min)	18.6 ± 0.5	23.2 ± 2.2	19.0 ± 0.7
30:15 ratio	1.14 ± 0.1	1.25 ± 0.1	1.12 ± 0.1
Valsalva manoeuvre (beats/min)	2.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
Orthostatic blood pressure (mmHg)	5.2 ± 1.9	2.3 ± 0.7	5.1 ± 1.1

Tab. 1: Cardiovascular reflex tests at baseline and during the follow-up (mean ± SE). All changes were non-significant.

Results

At the baseline tests, when the pump treatment was initiated, the autonomic score that defines a sum of the autonomic dysfunction correlated positively with the duration of type 1 diabetes (Fig. 1). The baseline disease duration also correlated with a cardiovascular test, the heart rate response to deep breathing. It was a significant negative correlation meaning that the longer duration of diabetes was associated with a less physiologic change of the heart rate to the procedure of deep breathing reflecting a parasympathetic impairment (Fig. 2). At baseline a moderate to severe AN was revealed (Fig. 3). During the follow-up an improvement of the total AN score was detected 2 months after the implementation of pump (2.85 ± 0.3 vs. 1.23 ± 0.3, $p < 0.01$). The AN score measured 6 years later was similar to the initial value (Fig. 3). Heart rate responses to deep breathing, to Valsalva manoeuvre and to standing up, as well as the blood pressure response to standing up did not differ significantly during the follow-up (Tab. 1). In 3 of the 4 tests there was a non-significant tendency of an improvement by the 2nd month (Tab. 1), while a progression was not revealed by the 6th year in comparison to the initial values at all of the tests (Tab. 1). Regarding the glycaemic control, the HbA_{1c} decreased by 0.7 % after 2 months as a mean (8.85 ± 0.2 % vs. 8.12 ± 0.3 %, $p = 0.07$) and it was significantly lower by the end of the follow-up (8.85 ± 0.2 % vs. 7.85 ± 0.3 %, $p < 0.05$) (Fig. 4).

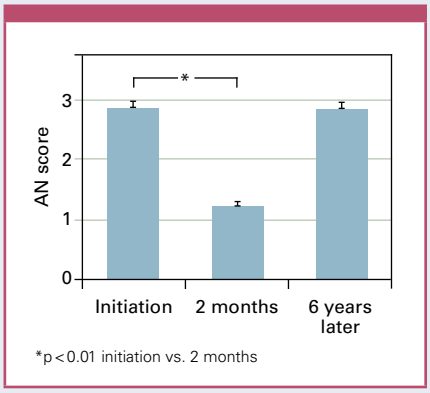


Fig. 3: Autonomic neuropathy (AN) score at baseline and during the follow-up (mean ± SE).

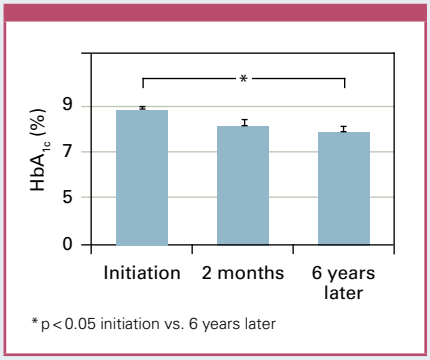


Fig. 4: HbA_{1c} at initiation and during the follow-up (mean ± SE).

Abbreviations

AN	autonomic neuropathy
BMI	body mass index
CRT	cardiovascular reflex test
DCCT	Diabetes Control and Complications Trial
ECG	electrocardiogramm
EDIC	Epidemiology of Diabetes Interventions and Complications
SE	standard error

Discussion

We had the opportunity to follow patients with type 1 diabetes whose glycaemic control necessitated the application of a subcutaneous insulin pump treatment. The observations at baseline

provide data on the characteristics of AN in a patient group who had a seriously bad glycaemic control. The overall grade of AN was not high, as the mean AN score was 2.85, but a strong relationship was found between the duration of diabetes and the severity of the autonomic dysfunction. In the presence of longer disease duration a more severe AN was documented. The patients had at least a 10-year long duration of type 1 diabetes and before the pump treatment several unsuccessful therapeutic efforts were performed due to their unstable glucose metabolism. These data are in accordance with our previous findings that the variability of the glucose levels is in close relationship with the severity of AN [Nyiraty 2018]. The detailed analysis of the possible correlation between each of the four CRTs and the duration of diabetes revealed that the results of the most sensitive parasympathetic test [Bernardi 2011], the heart rate response to deep breathing, are less physiologic in the presence of a longer metabolic dysregulation. This observation leads to the conclusion that mainly parasympathetic impairment is expected in patients with type 1 diabetes at the initiation of pump treatment. Moreover, this condition is frequently associated with a relative dominance of the sympathetic function resulting in an increased cardiovascular risk for these patients [Goldberger 2019].

A significant short-term improvement was found in the overall cardiovascular autonomic function during the follow-up of the pump treatment. Two months after the pump application, a significantly lower overall autonomic score was found. This parameter is accumulated from the scores of CRTs, thus it ensures a general characterisation of the autonomic function. The analysis of the separate tests did not reveal a significant change in the results by the 2nd month, however, 3 of the 4 tests suggested a tendency of improving the cardiovascular function. The significant reduction of the autonomic score might be a cumulative result of partial improvement in the parasympathetic and sympathetic functions. A beneficial effect during such a short period of intensified glycaemic control on the cardiovascular autonomic function was not observed earlier in type 1 diabetic patients. Our data might support the hypothesis that the moderate impairment of autonomic regulation might be sensitive for the short-term changes of the metabolic conditions and the pathogenetic process is particularly reversable. The results of the follow-up after 6 years reflected the same severity of neuropathy as it was recorded at the baseline tests. This means that the autonomic function was preserved during a 6-year period with the most intensive insulin treatment. The degree of glycaemic control characterized by HbA_{1c} did not change by the 2nd month but became significantly lower by the 6th year, although the mean value did not reach the glycaemic target. As HbA_{1c} did not decrease markedly during the follow-up despite of the preserved autonomic function might rise a hypothesis that the global stability of the glycaemic control has a more important role in the prevention than the average glycaemia characterized by HbA_{1c}. The initial moderate tendency of improvement in autonomic function seemed to be temporarily by the 6th year. There is no evidence from the literature about the shortest time interval with good glycaemic control which has a beneficial effect on autonomic function in diabetic patients. In the DCCT the prevalence of AN almost doubled in the conventional group during 6.5 years, while remained the same in the intensive group [Ang 2014]. Simultaneous pancreas-kidney transplantation improved Valsalva ratio in patients with type 1 diabetes by the 3rd year of follow-up [Ziegler 1991]. In a smaller study, heart rate variation was significantly less impaired within 24 months in a group of patients with type 1 diabetes with an HbA_{1c} of less than 8.3 % as compared to those having higher HbA_{1c} [Argente-Pla 2020]. Our data suggest that the beneficial effect might begin as early as some months after the start of the intensive treatment and lasts up to 6 years. The main limitation of our study is that only HbA_{1c} values were used to characterise glycaemic control. Data on glucose variability might also provide important information on the metabolic state of these patients. Moreover, the number of patients was relatively small. All patients were followed until the end of the study.

Conclusion

In summary, a moderate, initial improvement was followed by a preservation of autonomic function after 6 years of insulin pump treatment in patients with type 1 diabetes.

References

1. Ang L, Jaiswal M, Martin C, Pop-Busui R: Glucose control and diabetic neuropathy: lessons from recent large clinical trials. Curr Diab Rep 2014; 14: 528
2. Argente-Pla M, Pérez-Lázaro A, Martinez-Millana A, Del Olmo-García MI, Espí-Reig J, Beneyto-Castello I, López-Andújar R, Merino-Torres JF: Simultaneous pancreas kidney transplantation improves cardiovascular autonomic neuropathy with improved valsalva ratio as the most precocious test. J Diabetes Res 2020; 2020: 7574628
3. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, Ziegler D, Kempler P, Freeman R, Low P, Tesfaye S, Valensi P, Toronto Consensus Panel on Diabetic Neuropathy: Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev 2011; 27: 654-664
4. Callaghan BC, Little AA, Feldman EL, Hughes RA: Enhanced glucose control for preventing and treating diabetic neuropathy. Cochrane Database Syst Rev 2012; 6: CD007543
5. Ewing DJ, Clarke BF: Diagnosis and management of diabetic autonomic neuropathy. Br Med J 1982; 285: 916-918
6. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985; 8: 491-498
7. Goldberger JJ, Arora R, Buckley U, Shivkumar K: Autonomic nervous system dysfunction. J Am Coll Cardiol 2019; 73: 1189-1206
8. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerényi Zs, Tamás Gy, Ward JD, Fuller JH, EURODIAB IDDM Complications Study Group: Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. Diabet Med 2002; 19: 900-909
9. Kitano Y, Kuwabara S, Misawa S, Ogawara K, Kanai K, Kikkawa Y, Yagui K, Hattori T: The acute effects of glycemic control on axonal excitability in human diabetics. Ann Neurol 2004; 56: 462-467
10. Lam WF, Muller ES, Souverijn JH, Lamers CB, Masclee AA: Effect of acute hyperglycaemia on basal and fat-induced exocrine pancreatic secretion in humans. Clin Sci (Lond) 1997; 93: 573-580
11. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D: The effect of acute hyperglycaemia on QTc duration in healthy man. Diabetologia 2000; 43: 571-575
12. Martin CL, Albers JW, Pop-Busui R, and for the DCCT/EDIC Research Group: Neuropathy and related findings in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. Diabetes Care 2014; 37: 31–38

13. Nyiraty S, Pesei F, Orosz A, Coluzzi S, Vági OE, Lengyel C, Ábrahám G, Frontoni S, Kempler P, Várkonyi T: Cardiovascular autonomic neuropathy and glucose variability in patients with type 1 diabetes: is there an association? Front Endocrinol (Lausanne) 2018; 9: 174
14. Pirart J: Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care 1978; 1: 168-188
15. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D: Diabetic neuropathy: a position statement by the American Diabetes Association. Diabetes Care 2017; 40: 136-154
16. Santini V, Ciampittiello G, Gigli F, Bracaglia D, Baroni A, Cicconetti E, Verri C, Gambardella S, Frontoni S: QTc and autonomic neuropathy in diabetes: effects of acute hyperglycaemia and n-3 PUFA. Nutr Metab Cardiovasc Dis 2007; 17: 712-718
17. Service FJ, Rizza RA, Daube JR, O'Brien PC, Dyck PJ: Near normoglycaemia improved nerve conduction and vibration sensation in diabetic neuropathy. Diabetologia 1985; 28: 722-727
18. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S, Low P, Valensi P, Toronto Consensus Panel on Diabetic Neuropathy: Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011; 27: 639-653
19. Spallone V: Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: what is defined, what is new, and what is unmet. Diabetes Metab J 2019; 43: 3-30
20. The Diabetes Control and Complications Trial Research Group: The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). Diabetologia 1998; 41: 416-423
21. Yeap BB, Russo A, Fraser RJ, Wittert GA, Horowitz M: Hyperglycemia affects cardiovascular autonomic nerve function in normal subjects. Diabetes Care 1996; 19: 880-882
22. Ziegler D, Mayer P, Muhlen H, Gries FA: The natural history of somatosensory and autonomic nerve dysfunction in relation to glycaemic control during the first 5 years after diagnosis of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1991; 34: 822-829

Adress for correspondence
Sándor Magony
Department of Medicine
University of Szeged
Kálvária str. 57
Szeged
6720-Hungary
Tel.: +36-62 54 51 89
Fax: +36-62 54 51 85
E-mail: magonysandor@gmail.com