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Impairment in baroreflex sensitivity in recent-onset type 2 diabetes without progression over 5 years

Dissertation

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Widmung

Für meine Nichten Philine, Antonia, Marie und Mila,
für meine Freundinnen Franziska, Uta und Tamara,
und all die anderen fabelhaften, talentierten Mädchen und Frauen
denen noch mehr als zuvor die Zukunft offenstehen soll, von der sie träumen.

Zusammenfassung (deutsch)

Die diabetische Neuropathie ist eine der häufigsten Komplikation des Diabetes mellitus. Sie manifestiert sich am somatosensorischen und/oder autonomen Nervensystem und ist mit ungünstiger Prognose und reduzierter Lebensqualität assoziiert. Diese Arbeit beschäftigt sich mit der kardiovaskulären autonomen diabetischen Neuropathie (KADN), die insbesondere als Prädiktor für ein erhöhtes Mortalitätsrisiko und kardiovaskuläre Erkrankungen gilt. Obwohl die KADN bereits frühzeitig im Verlauf des Diabetes durch eine reduzierte Herzfrequenzvariabilität (HRV) nachweisbar sein kann, bleibt sie häufig undiagnostiziert oder wird erst im symptomatischen Spätstadium mit Ruhetachykardie, orthostatischer Hypotonie oder Belastungsintoleranz erkannt. Wichtige Risikofaktoren für die Entstehung der KADN sind Alter, Diabetesdauer, unzureichende Diabeteseinstellung, Insulinresistenz und arterielle Hypertonie. Der Baroreflex ist ein Mechanismus, der kurzzeitige Veränderungen des Blutdrucks über Barorezeptoren detektiert und über das zentrale Nervensystem reguliert. Die nicht-invasive Messung der spontanen Baroreflexsensitivität (BRS) ist relativ einfach durchführbar, wodurch Untersuchungen an größeren Kohorten ermöglicht werden. Bei Patienten mit Myokardinfarkt und chronischer Herzinsuffizienz besitzt eine reduzierte BRS eine prädiktive Aussagekraft für das nachfolgende kardiovaskuläre Mortalitätsrisiko. Bei länger bestehendem Diabetes ist die spontane BRS mit KADN assoziiert, wohingegen Patienten mit kürzlich diagnostiziertem Diabetes bisher nicht untersucht wurden. Die vorliegende Studie hatte daher zum Ziel zu untersuchen, 1.) inwieweit Parameter der BRS bereits kurzfristig nach Diagnose des Typ 1- bzw. Typ 2 Diabetes vermindert und mit Stoffwechselparametern assoziiert sind und 2.) wie die BRS über die nachfolgenden 5 Jahre verläuft. Die Erhebung der spontanen BRS erfolgte mit einer photoplethysmographischen Technik, die am Mittelfinger simultan spontane Beat-to-Beat-Fluktuationen von Blutdruck und Herzfrequenz aufnimmt, wobei drei verschiedene Methoden der BRS-Analyse zur Anwendung kamen. Die Insulinsensitivität (M-Wert) wurde mit Hilfe des hyperinsulinämischen-euglykämischen Clamps ermittelt, während der Glucagon-Stimulationstest der Bestimmung der Insulinsekretion diente. Untersucht wurden konsekutive Teilnehmer mit Typ 1 Diabetes (n=206) bzw. Typ 2 Diabetes (n=381) mit einer Diabetesdauer bis zu einem Jahr sowie zwei altersentsprechende Kontrollgruppen (n=65 bzw. n=83) aus der Baseline-Kohorte der Deutschen Diabetes Studie (DDS). Subgruppen von Teilnehmern mit Typ 1- (n=84) bzw. Typ 2 Diabetes (n=137) wurden 5 Jahre später nachuntersucht. Die vorliegende Studie zeigte, dass in der Gruppe mit Typ 1 Diabetes keine Veränderungen der BRS nachweisbar waren, während bei Teilnehmern mit Typ 2 Diabetes drei BRS-Indizes der Frequenzdomäne im Vergleich zu den entsprechenden Kontrollpersonen reduziert sowie positiv mit der Insulinsensitivität und invers mit Nüchternblutglucose und HbA1c assoziiert waren. Im Vergleich zu Baseline nahmen nach 5 Jahren ein BRS-Index bei Teilnehmern Typ 1- und vier Indizes bei denen mit Typ 2 Diabetes ab. Dies ließ sich jedoch durch den physiologischen Alterungsprozess erklären. Diese Ergebnisse weisen auf eine frühe Dysfunktion der BRS bei gut eingestellten Patienten mit kürzlich diagnostiziertem Typ 2 Diabetes hin. Diese Störung ist mit Hyperglykämie und Insulinresistenz assoziiert und über die nächsten 5 Jahre nicht progredient. Hingegen bleibt die spontane BRS innerhalb der ersten 5-6 Jahre nach Diagnose des Typ 1 Diabetes erhalten. Künftige Studien sollten klären, inwieweit eine günstige Baroreflexmodulation begleitet durch Verbesserung der Insulinsensitivität das Risiko für eine KADN und kardiovaskuläre Erkrankungen bei Typ 2 Diabetes senken könnten.

Abstract

Diabetic neuropathy is one of the most frequent complications of diabetes mellitus. It manifests in the somatosensory and autonomic nervous system and is associated with unfavorable prognosis and reduced quality of life. This thesis focuses on diabetic cardiovascular autonomic neuropathy (CAN) which is notably considered as a predictor of increased risk of mortality and cardiovascular disease. Although CAN may be detected early in the course of diabetes by reduced heart rate variability (HRV), it frequently remains undiagnosed or is detected only in late symptomatic stages presenting with resting tachycardia, orthostatic hypotension, or exercise intolerance. Important risk factors for the development of CAN include age, diabetes duration, poor glycemic control, insulin resistance, and arterial hypertension. The baroreflex is a mechanism that detects short-term fluctuations of arterial blood pressure via baroreceptors and regulates these changes through the central nervous system. The non-invasive measurement of spontaneous baroreflex sensitivity (BRS) is a relatively simple procedure, allowing for assessing larger cohorts. Reduced BRS is a predictor of subsequent cardiovascular mortality in patients with myocardial infarction and chronic heart failure. BRS is associated with CAN in patients with longer-term diabetes, but to date patients recently diagnosed with diabetes have not been examined. The present study aimed to investigate 1) whether indices of BRS are reduced as early as in recent-onset type 1 or type 2 diabetes and are associated with metabolic parameters and 2) the course of BRS over the next 5 years. Spontaneous BRS was assessed by a photoplethysmography technique simultaneously recording spontaneous beat-to-beat fluctuations of blood pressure and heart rate at the middle finger and was analyzed using three different methods. Insulin sensitivity (M-value) was assessed by a hyperinsulinemic-euglycemic clamp, while insulin secretion was determined using a glucagon stimulation test. Patients with recent-onset type 1 (n=206) or type 2 (n=381) diabetes (known diabetes duration ≤ 1 year) and corresponding age-matched individuals with normal glucose tolerance (Control 1/Control 2: n=65/83) from the baseline cohort of the German Diabetes Study (GDS) were studied consecutively. Subgroups of participants with type 1 (n=84) or type 2 diabetes (n=137) were examined prospectively after 5 years. The present study showed no differences in BRS indices in participants with type 1 diabetes compared with glucose-tolerant control individuals. In participants with type 2 diabetes, three frequency domain BRS indices were reduced compared with corresponding control individuals and were positively associated with measures of insulin sensitivity and inversely associated with fasting blood glucose and HbA1c levels. Compared to baseline, one BRS index declined in participants with type 1 diabetes, while four indices decreased in those with type 2 diabetes over 5 years. However, this was explained by the physiological age-dependent decline. These findings indicate an early baroreflex dysfunction in well controlled recent-onset type 2 diabetes individuals which is associated with both hyperglycemia and insulin resistance, but does not progress over the next five years. In contrast, spontaneous BRS remains preserved within the first 5-6 years after diagnosis of type 1 diabetes. Future studies should clarify to what extent a favorable modulation of BRS accompanied by improved insulin sensitivity may reduce the risk of CAN and cardiovascular disease in type 2 diabetes.

Abbreviations

ADA	American Diabetes Association
ANS	autonomic nervous system
BMI	body mass index
BP	blood pressure
BRS	baroreflex sensitivity
CAN	cardiovascular autonomic neuropathy
CARTs	cardiovascular reflex tests
CVD	cardiovascular disease
DN	diabetic neuropathy
DAN	diabetic autonomic neuropathy
DSPN	diabetic sensorimotor polyneuropathy
GAN	gastrointestinal autonomic neuropathy
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GDS	German Diabetes Study
HDL	high-density lipoprotein
HF	high frequency
HOMA	homeostasis model assessment
HRV	heart rate variability
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
MODY	maturity onset diabetes of the young
NGT	normal glucose tolerance
LF	low frequency
OGTT	oral glucose tolerance test
PNS	peripheral nervous system
RCTs	randomized controlled trials
SBP	systolic blood pressure
SMI	silent myocardial ischemia
UAN	urogenital autonomic neuropathy
VLF	very low frequency

Contents

List of figures

List of tables

1. Introduction

1.1 Diabetes mellitus

Diabetes mellitus is a widespread metabolic disease, the prevalence of which has reached epidemic proportions with an estimate of 9.3% of the global population aged between 20 and 79 years [1]. The most common forms are type 1 diabetes, type 2 diabetes, and gestational diabetes, among which type 2 diabetes accounts for the vast majority of the cases (90-95%). Other specific types of diabetes include monogenic forms (e.g. maturity-onset diabetes of the young, MODY) and secondary forms caused by other disorders (e.g. diseases of the exocrine pancreas, drug-induced diabetes) [1]. According to the American Diabetes Association (ADA), type 1 diabetes is characterized by insulin deficiency caused by autoimmune processes against pancreatic beta cells, while type 2 diabetes is primarily characterized by reduced insulin sensitivity in target tissue cells, while insulin secretion is usually preserved to a certain degree [2].

The current diagnostic criteria according to the ADA are based on plasma glucose and are defined by (1) the fasting plasma glucose (defined as no caloric intake for at least 8 h) or (2) the 2-h plasma glucose during a 75 g oral glucose tolerance test using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water (OGTT), or (3) hemoglobin A1c (HbA1c) criteria: fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l) or 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during OGTT or HbA1c ≥ 6.5% (48 mmol/mol) or, in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/l). In the absence of unequivocal hyperglycemia, results need to be confirmed by repeated testing [3].

1.2 Diabetic neuropathy

Diabetic neuropathies (DN) are the most frequent chronic microvascular complications of diabetes and can affect the somatic and autonomic parts of the peripheral nervous system (PNS). DN and its sequelae such as pain and foot ulcers are associated with a substantial morbidity, increased risk of mortality, and reduced quality of life [4]. Although being asymptomatic in up to 50% of cases, DN often manifests clinically with loss of sensation, neuropathic pain, motor impairments, and autonomic dysfunction. The most common forms are diffuse neuropathies with diabetic sensorimotor polyneuropathy (DSPN) and autonomic neuropathies such as cardiovascular (CAN), gastrointestinal (GAN), and

urogenital autonomic neuropathy (UAN) [5, 6], while rarer forms include mononeuropathies or radiculopathies [5].

The risk factors for the development of DN include the typical cardiovascular risk factors such as obesity, hypertension, dyslipidemia, and smoking as well as higher age, longer diabetes duration, poor glycemic control, height, alcohol abuse and the presence of other diabetic complications. In type 2 diabetes, especially higher body weight and hypoinsulinemia represent specific risk factors [7]. Most important comorbidities are vascular comorbidities such as peripheral arterial disease and cardiovascular disease, nephropathy, retinopathy, and depression [7].

1.2.1. Diabetic autonomic neuropathy

Diabetic autonomic neuropathy (DAN) is characterized by an impairment of nerve fibers regulating the physiological function of inner organs and glands [2]. Since all inner organs are innervated by the ANS, DAN can be the cause of a variety of symptoms affecting the cardiovascular, gastrointestinal, urogenital, pupillomotor, thermoregulatory, sudomotor, or neurovascular systems [3, 4]. Clinically most prominently affected are the cardiovascular, gastrointestinal, and genitourinary systems including conditions and symptoms such as resting tachycardia and orthostatic hypotension, gastroparesis with nausea, postprandial fullness, or vomiting, motility disorders of the esophagus, diabetic enteropathy with diarrhea, and colonic dysfunction resulting in constipation; erectile dysfunction and bladder emptying disorders; disturbed release of catecholamine in specific situations, anhidrosis, trophic disorders, heat intolerance or disorders of pupillomotor function [8, 9]. According to the Toronto consensus statement, DAN was defined as “a disorder of the autonomic nervous system (ANS) in the setting of diabetes or metabolic derangements of prediabetes after the exclusion of other causes” [10-12]. The consequences of DAN range from a restriction of daily life to potentially life-threatening complications.

1.2.2. Diabetic cardiovascular autonomic neuropathy

1.2.2.1. Definition of CAN

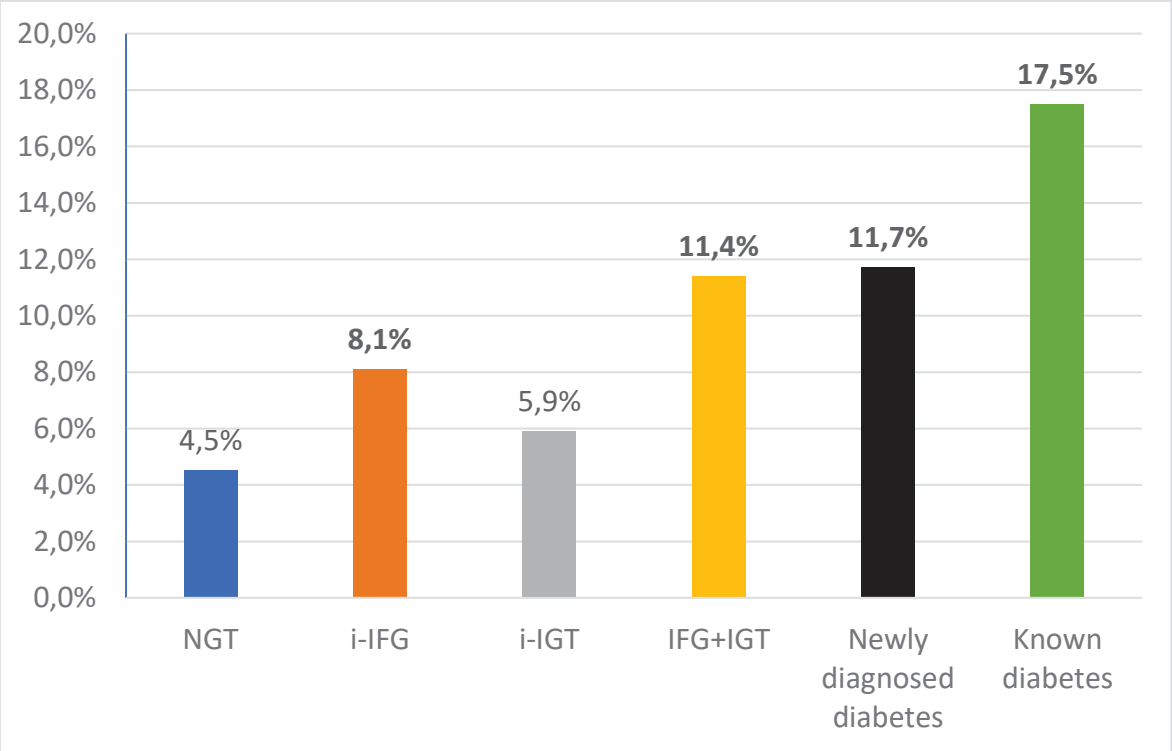
CAN represents one of the most relevant clinical manifestation of DAN [13-15] and is characterized by a functional and/or structural damage of the nerve fibers within the cardiovascular system leading to an imbalance of sympathetic and parasympathetic

regulation. Hence, the adaptability of the heart and blood vessels decreases and the physiological regulation of the circulation is impaired. CAN was defined as "the impairment of autonomic control of the cardiovascular system in the setting of diabetes after exclusion of other causes" [10-12]. The diagnosis of CAN is usually based on several cardiovascular reflex tests (CARTs) to assess heart rate variability (HRV). Clinical complications of CAN include resting tachycardia, orthostatic hypotension, exercise intolerance, silent myocardial infarction, and perioperative and intraoperative cardiovascular instability, which contribute to increased morbidity in patients with CAN [12, 15]. Major risk factors for CAN are age, diabetes duration, and poor glycemic control in both diabetes types, while hypertension, obesity, and dyslipidemia play an important role particularly in type 2 diabetes [12]. CAN commonly remains subclinical for a long period and is a frequently overlooked and neglected complication of diabetes [15]. However, a decline in autonomic function can occur early along the course of diabetes, even at the prediabetic stage [16, 17].

1.2.2.2. Epidemiology of CAN

The prevalence rates of CAN vary greatly in the literature due to different populations studied and diagnostic criteria used to define CAN [6, 12]. In clinic-based studies in unselected populations, prevalence rates between 16.6% and 20% have been reported in patients with type 1 or type 2 diabetes, when at least two abnormal CARTs were used to define confirmed CAN [12]. In population-based cohorts, prevalence rates of confirmed CAN between 17.5% and 31.2% have been reported in individuals with diabetes [17, 18]. Increasing evidence suggests that the prevalence of CAN is increased even in prediabetes and in early clinically manifest diabetes [6, 19]. Prevalence rates of confirmed CAN between 6% and 21% have been reported in prediabetes [6, 17, 19-21]. In the KORA S4 cohort the prevalence rates of confirmed CAN defined by at least two abnormal CARTs in a large population-based group of individuals aged between 55 and 75 years were 5.9% in individuals with isolated impaired fasting glucose (IFG), 8.1% in those with impaired glucose tolerance (IGT), 11.4% in individuals with both IFG and IGT, and 11.7% in patients with newly detected diabetes, compared with 4.5% in individuals with normal glucose tolerance (NGT) and 17.5% in patients with known diabetes (*Figure 1*) [17]. Longitudinal studies indicate an annual increase in prevalence of CAN of about 2% in type 1 diabetes and about 6% in type 2 diabetes [12].

***Figure 1**: Prevalence of CAN at different degrees of glucose intolerance.*



NGT: normal glucose tolerance, i-IFG: isolated impaired fasting glucose, i-IGT: isolated impaired glucose tolerance, IFG+IGT, impaired fasting glucose and impaired glucose tolerance. Bold indicates p<0.05 vs. NGT. Modified from Ziegler et al. 2015 [17].

DSPN and CAN tend to coexist frequently as shown by the DiaCAN-Study, in which more than 50% of patients with symptomatic DSPN showed evidence of CAN [22].

Notably, CAN contributes to an increased risk of cardiovascular events and mortality [13-15]. A meta-analysis of 15 longitudinal studies showed a relative risk for mortality of 3.65 (95% CI: 2.66-4.47) in patients with diabetes and CAN compared to those without CAN [15]. In the population-based MONICA/KORA study, participants with diabetes and reduced HRV showed a 9-year mortality rate of 37.5% compared to 23.9% in those with normal HRV [23]. In the population based, prospective Hoorn-Study, the risk of mortality over 9 years was increased in patients with diabetes when cardiac autonomic dysfunction was present [24].

1.2.2.3. Pathogenesis of CAN

Diabetic neuropathy

Evidence has accumulated suggesting that the pathogenesis of DN is multifactorial. The putative mechanisms thought to contribute to the development of DN include oxidative, carbonyl, and endoplasmic-reticulum stress, subclinical inflammation, altered intracellular pathways and gene expression, and autoimmune processes ultimately leading to nerve fiber loss [4, 9, 25]. Hyperglycemia leads to oxidative stress caused by an overproduction of superoxide anion by the mitochondrial electron transport chain and consecutive inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), culminating in an activation of four pathways contributing to the development of diabetic microvascular complications including neuropathy. First, due to disturbed insulin metabolism, nerve cells are no longer able to metabolize glucose. Although the alternative polyol pathway is insulin-independent, its activation results in sorbitol accumulation and deficits in endoneurial blood flow and nerve damage [4]. Second, increased activity of protein kinase C is considered to be responsible for excess vasoconstriction leading to hypoxic states, oxidative stress and reduced antioxidant mechanisms as well as higher nitric oxide production and endothelial damage [9]. Third, accumulation of advanced glycation end products leads to an impaired permeability and elasticity of vessels [4, 26]. Fourth, activation of the hexosamine pathway leads to changes in gene expression with overactivation of mediators that damage endothelial cells [4].

Diabetic cardiovascular autonomic neuropathy

The vagal nerve supplies a large area from the cranium to the left colon flexure fulfilling a variety of autonomic functions. Recent studies focused on the role of fat tissue and its components in the context of cardiovascular autonomic dysfunction and provided novel pathophysiological concepts including the interaction of inflammation, sympathetic activation and the specific function of adiponectin and leptin [27]. Inflammatory mediators are elevated in association with decreased HRV or sympathetic overactivation [28, 29]. Adiponectin and leptin, both regulating energy supply and maintenance of fat tissue, received particular attention in this context. In mouse models, hyperleptinemia and adiponectin deficiency could result both in a disrupted circadian blood pressure regulation and low leptin levels promoting the development diabetes, hypertension, and obesity [30]. There is evidence suggesting that adipose tissue may react to signals from the ANS resulting in changes of cytokine and fatty acid release and the development of oxidative stress [31]. Strong associations of impaired baroreflex sensitivity (BRS) and lower cardiac

parasympathetic activity with a higher percentage of hepatic fat were reported in patients with recent-onset type 2 diabetes [32]. However, it remains unclear, whether cardiac autonomic dysfunction is a consequence or cause of insulin resistance or liver fat content, and cause-effect relationships in both directions are possible [27].

1.2.2.4. Clinical complications of CAN

At an early stage, impairment due to CAN is often nonspecific and tends to remain subclinical for a longer period of time. The earliest sign and defining hallmark of CAN is typically a decrease in the physiological HRV or respiratory sinus arrhythmia [33, 34]. Later stages include resting tachycardia and completely suppressed heart rate fluctuations ("fixed heart rate") [6]. Clinical complications of advanced stages (*Table 1*) include orthostatic hypotension, exercise intolerance, intra- and perioperative hemodynamic instability, and a higher risk of silent myocardial ischemia. As a consequence, CAN contributes to substantial morbidity in patients with diabetes [12, 15].

Table 1: *Clinical features associated with CAN.*

Resting tachycardia and orthostatic hypotension Dizziness Visual impairment Faintness Weakness Syncope
Abnormal blood pressure regulation Nondipping Reverse dipping
Orthostatic tachycardia or bradycardia Dizziness Visual impairment Faintness Weakness Syncope
Left ventricular dysfunction and hypertrophy Exercise intolerance Impaired cardiorespiratory fitness
Silent myocardial ischemia Higher anginal perceptual threshold
Perioperative instability Higher rate of anesthetic accidents Increased intraoperative use of vasopressors

Obstructive sleep apnea

Adapted from Ziegler et al., 2017 [33], and Pop-Busui et al., 2017 [5].

Resting tachycardia is a characteristic finding in diabetes patients with advanced stages of CAN. While a more pronounced increase in resting heart rate is typically observed in patients with a predominant parasympathetic impairment, only modestly increased heart rate may be found in patients with combined parasympathetic and sympathetic damage [15, 35].

Orthostatic hypotension has been defined as a reduction of systolic blood pressure (SBP) of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg within 3 min after standing up [12]. It may become a disabling sequel of CAN [36] associated with presyncopal symptoms or postural tachycardia [6], but can also remain asymptomatic [15]. Up to one third of patients with diabetes report symptoms of orthostatic hypotension including dizziness, weakness, visual disturbances, cognitive impairments, nausea, nervousness, headache, and neck pain [37-39]. Both an inadequate cardiac output by cardiac autonomic dysregulation and a disturbed peripheral arterial resistance regulation caused by vasomotor denervation may contribute to the development of orthostatic hypotension in patients with CAN [35]. Drugs including several vasodilators, diuretics and antidepressants and comorbidities such as cardiovascular disease may aggravate orthostatic hypotension [15]. Postprandial hypotension is another possible complication, albeit reported rarely [37-39].

The disturbed adaption of heart rate and blood pressure to physical activity may cause exercise intolerance in patients with CAN [33]. Patients with CAN and exercise intolerance show diminished adaption of heart rate, blood pressure, and cardiac stroke volume in response to exercise, while the severity of CAN may inversely correlate with maximum heart rate during exercise [12, 15]. Therefore, careful exercise programs are indicated in those patients to avoid potentially hazardous levels of exercise intensity [15].

Moreover, CAN is associated with a two- to threefold increase in intra- and perioperative instability [12, 15]. A higher risk of complications during surgery has been reported in patients with diabetes. Autonomic dysregulation may lead to unexpected drops in blood pressure under general anesthesia as well as during tracheal intubation and extubation. CAN has been reported to be associated with more severe episodes of intraoperative hypothermia resulting in decreased drug metabolism as well as impaired wound healing

[15]. A disturbed respiratory drive may also occur requiring more intensive perioperative monitoring [8, 40].

Patients with CAN may present with impaired circadian blood pressure regulation. While healthy individuals show a parasympathetically controlled lower blood pressure at night, which rises again in the morning while the sympathetic tone increases, patients with CAN have an increased risk for a reversal of the physiological dipping in blood pressure, resulting in a nocturnal increase in blood pressure and a fall in the morning ("reverse dipping"). This reversal correlates with orthostatic hypotension in CAN as well as with the presence of neuropathic pain and diabetic nephropathy [41].

In patients with diabetes, CAN is an independent risk factor for silent myocardial ischemia (SMI) [5]. Although the risk of cardiovascular events in people with diabetes is generally elevated [42, 43], it seems to be highest when CAN and history of SMI are present [44]. A large meta-analysis revealed that the prevalence of SMI was 20% in patients with diabetes and CAN compared to 10% in patients with diabetes without CAN [12]. In the large Detection of Ischaemia in Asymptomatic Diabetics (DIAD) study, a CART used to diagnose CAN (Valsalva ratio) was the strongest determinant of SMI [45]. However, the mechanisms of CAN contributing to SMI remain not fully understood [15]. The perceptual threshold for angina pectoris may be increased in individuals with diabetes and CAN [46]. Comparing painful myocardial infarction with SMI, a higher proportion of individuals with diabetes has been observed in the SMI group [47]. Furthermore, SMI was detected in about one fifth of patients with diabetes without cardiac symptoms in association with reduced HRV next to diabetes duration and male sex [45]. An increased risk for fatal cardiovascular events or non-fatal myocardial infarction (hazard ratio: 4.3) over 5 years has been reported when CAN was present initially [48].

1.2.2.5. Diagnosis of CAN

According to the Toronto Consensus Panel on Diabetic Neuropathy, the diagnosis of CAN is based primarily on the assessment of HRV and blood pressure regulation using CARTs, while medical history may provide additional information about orthostatic hypotension and exercise intolerance [12]. While no unanimous criteria for the diagnosis of CAN have been adopted to date, a single abnormal CART result has been considered to detect possible or early CAN. However, it has been generally accepted that the presence of abnormalities in more than one test on several occasions was indicated as preferable for

diagnosis [49]. Several methods are available to assess HRV. Time domain analyses are used to calculate indices derived from statistical differences of R-R interval lengths, while frequency domain analyses assess the spectral power in different frequency bands from R-R interval recordings [49]. Using Fourier transformation as a common mathematical algorithm for spectral analysis, the resulting power spectrum can be assigned to the individual components of the ANS [50]. A distinction is made between the VLF band (very low frequency, 0.003-0.04 Hz, sympathetic arm and thermoregulation), the LF band (low frequency, 0.04-0.15 Hz, BRS with both sympathetic and parasympathetic components) and the HF band (high frequency, 0.15-0.4 Hz, respiratory arrhythmia). HRV is influenced by numerous individual factors including age, heart rate, arterial blood pressure, smoking, medication, physical activity, and the time of day [50]. Therefore, standardized test conditions are important. Before the start of the test, the patient should rest relaxed for 10 minutes in a supine position. Furthermore, acute illnesses, hypoglycemia, severe physical and emotional stress, and ketoacidotic metabolic disorders should be excluded prior to the examination [51].

A standard test battery has been suggested including time domain, frequency domain, and vector analysis of HRV which includes (1) coefficient of variation or R-R intervals at rest, (2) spectral power in the VLF band, and (3) LF band, (4) HRV during deep breathing, (5) maximum 30:15 ratio, (6) Valsalva ratio, and (7) postural change in SBP. The specificity of this test battery for borderline and confirmed CAN has been reported to be excellent with 98% and 100%, respectively [49]. While efforts have been taken to further standardize and simplify the diagnosis and prediction of CAN, there is currently no consensus, which CART indices or metabolic parameters are suited best [17, 18]. There remains an unmet need to sensitively detect early cardiac autonomic dysfunction to diagnose or predict CAN in clinical practice.

1.2.2.6. Treatment of CAN

Preventive and therapeutic options in the management of CAN are (1) causal treatment including intensive glycemic control, lifestyle modification, and intensified multifactorial intervention; (2) pathogenetically oriented therapy including modulation of cardiac autonomic tone, and (3) symptomatic treatment of complications [33].

Causal treatment

Stringent glycemic control was found to be effective in the prevention and progression of CAN in individuals with type 1 diabetes. The Diabetes Control and Complications trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study

showed that the development of CAN was prevented, albeit not fully, by intensive insulin therapy compared to conventional insulin therapy in patients with type 1 diabetes. This effect was still measurable even more than ten years after the intervention, which was suggested to represent a “metabolic memory” effect [52]. In a prospective observational study, a complete prevention of the decline in autonomic nerve function from the diagnosis of type 1 diabetes over 24 years was observed during near-normoglycemia [53]. However, maintaining near-normoglycemia did not reverse manifest CAN [54].

In contrast, there is less compelling evidence that intensive diabetes therapy would exert similar effects on the development of CAN in type 2 diabetes, but most of the trials were not originally designed to evaluate endpoints specific for CAN [6]. In the Steno 2 Study, multifactorial intervention in type 2 diabetes patients including intensive diabetes treatment, adequate antihypertensive treatment, aspirin, and smoking cessation was associated with a lower incidence and progression of CAN over 14 years [55]. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, beneficial effects of intensive diabetes therapy on CAN were observed especially in participants with type 2 diabetes without cardiovascular disease (CVD) history at baseline, while intensive blood pressure control was effective especially in those who were ≥65 years old [56].

Lifestyle modification with increased physical activity and dietary changes is considered an essential part of the management strategies in diabetes patients and also shows positive effects on HRV [57]. However, while some uncontrolled studies suggested improved cardiac autonomic function in response to exercise in patients with diabetes, only a few randomized controlled trials (RCTs) studying the effects on exercise intervention programs on cardiac autonomic function in diabetes have been performed [58, 59]. The results of the RCTs suggest that exercise interventions may improve BRS rather than HRV, but more long-term RCTs are needed to study the effects of exercise on cardiac autonomic function in patients with type 1 and type 2 diabetes [6].

Pathogenetically oriented pharmacotherapy

While most pathogenesis-derived therapies have been studied patients with DSPN, limited data is available about the effects on cardiac autonomic function. α-Lipoic acid is an essential cofactor for mitochondrial beoenergetic enzymes with antioxidant properties [6]. In the Deutsche Kardiale Autonome Neuropathie (DEKAN) study, improved HRV indices were observed after oral administration of α-lipoic acid (800 mg/day) over 4 months compared with placebo in patients with type 2 diabetes [30]. A lower LF/HF ratio was

reported after 4 months of 600 mg/d vitamin E administration compared with placebo in patients with type 2 diabetes and CAN, suggesting an influence of antioxidative properties of vitamin E on cardiac autonomic function [60].

To improve the balance of sympathetic and vagal tone, drugs that diminish HRV should be avoided if possible in clinical practice, including class Ic antiarrhythmic agents (e.g. flecainide), β-blockers with intrinsic sympathomimetic activity (e.g. pindolol), clonidine, diltiazem, and tricyclic antidepressants (e.g. amitriptyline, imipramine, desipramine). Drugs that may increase HRV include cardioselective β adrenergic blocking agents without intrinsic sympathomimetic activity (e.g. bisoprolol, atenolol, metoprolol, celiprolol) [61], ACE inhibitors, angiotensin-II-receptor antagonists, the calcium channel blocker verapamil, and digoxin [49].

Symptomatic treatment

Symptomatic orthostatic hypotension and resting tachycardia are relatively infrequent but difficult to treat sequels of CAN. Initial approaches include non-pharmaceutical options which may already provide sufficient support against orthostatic hypotension. These include physical counter pressure maneuvers before anticipated position changes such as leg crossing, static handgrip, squatting, and general muscle tensing to increase cardiac output and systemic blood pressure [62]. Furthermore, the omission of concomitant drugs that trigger or promote orthostatic hypotension is recommended. These include diuretics, tricyclic antidepressants, and certain antihypertensive compounds (sympathetic blockers, α-adrenergic antagonists) [62]. Pharmacological treatment options for orthostatic hypotension include agents that aim to increase central blood volume and sympathomimetic agents (*Table 2*). The aim in the treatment of orthostatic hypotension is an increase in blood pressure while standing and concomitantly avoiding an increase in supine blood pressure [33]. Resting tachycardia may be treated by cardioselective ß-adrenoceptor blocking agents.

Table 2: *Overview of pharmacological treatment options and their mechanisms of action in orthostatic hypotension.*

Agent	***Mechanism of action***
Midodrine	Prodrug of desglymidodrine, an α1-adrenergic agonist, exerts pressor effect due to both arterial and venous constriction
Fludrocortisone	Increases blood volume by increased sodium reabsorption, increase of α-receptors

Erythropoetine	Increase of the erythrocyte mass
Droxidopa	Prodrug of norepinephrine, resulting in peripheral vasoconstriction

Modified from Ziegler, 2017 [33].

1.2.3. The baroreflex

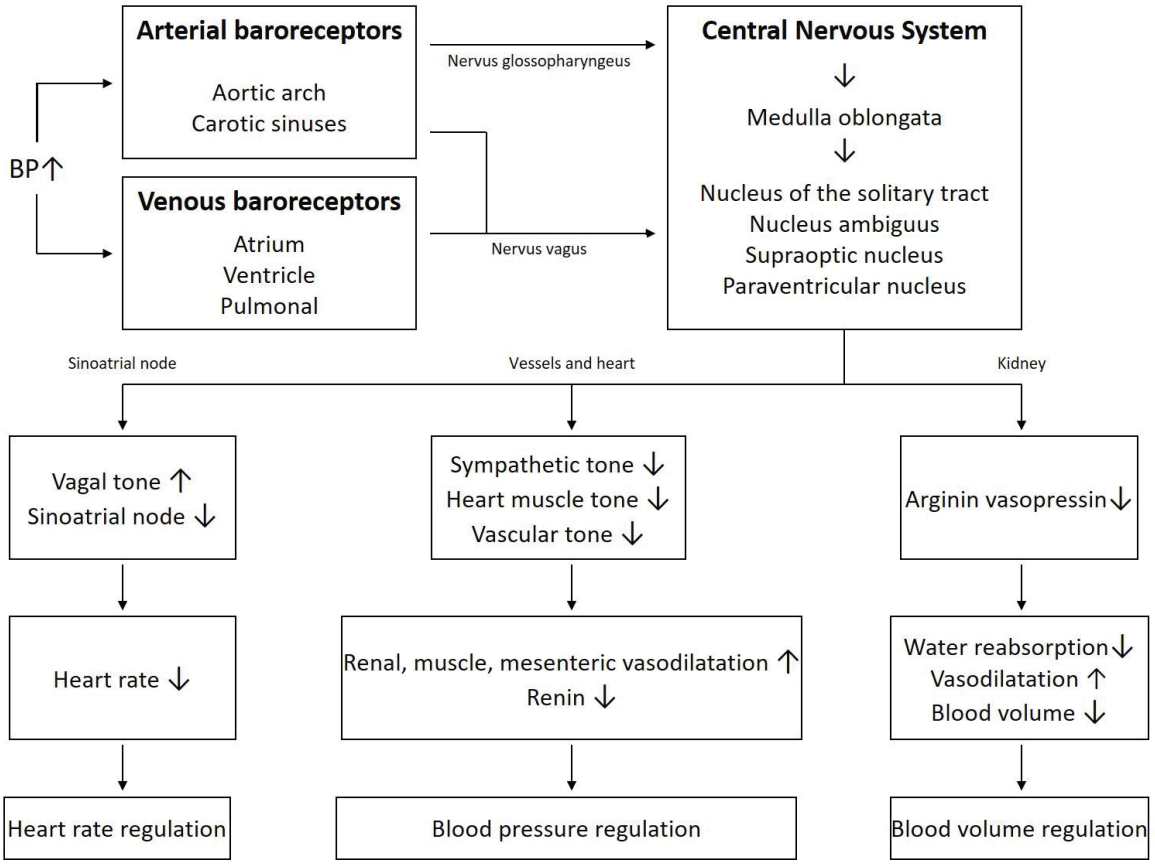
The baroreflex is an essential mechanism responsible for the short-term regulation of arterial blood pressure, heart rate, and cardiac output by neuronally influencing the sinus node and blood vessels via baroreceptors located in the aortic arch, carotic sinuses, in the heart, and in pulmonary veins. A rise in sensed blood pressure (BP) leads to an increase and decrease in the discharge of vagal and sympathetic neurons, respectively, resulting in decreased heart rate, cardiac contractility, and peripheral vascular resistance, whereas a fall in BP causes the opposite effects [63, 64]. Measurement of BRS is considered a meaningful tool to examine the function of the cardiovascular ANS. Furthermore, it has been shown that the BRS assessment can provide valuable information on the risk and prognosis of cardiovascular conditions [63].

1.2.3.1. Physiology of the baroreflex

The arterial baroreceptors are located in the aortic arch and carotic sinus, whereas the venous baroreceptors are located in thoracic veins and in the heart. The baroreceptors of the aortic arch are innervated both by the vagal and glossopharyngeal nerves, while those of the carotic sinus are innervated by the glossopharyngeal nerve only. In vessels, the nerve endings for the baroreceptors are located in the media and adventitia layers. The resting activity of the baroreceptors has an inhibiting effect on the sympathetic nervous system and a promoting effect on the parasympathetic nervous system, keeping blood pressure at a physiological level. The baroreceptors are stimulated by a change in blood pressure by registering a stretch in the vessel wall. The signal is transmitted via the cranial nerves to the central nervous system, more precisely to the circulation-controlling neurons of the medulla oblongata and the hypothalamus. In the central nervous system, various cranial nerve nuclei are involved in the processing of the signals including the solitary nucleus, nucleus ambiguus, supraoptic nucleus and the paraventricular nucleus. The efferents of the arterial baroreflex affect the sinus node, the vessels, and the heart muscle and thus influence the heart rate and blood pressure. When blood pressure increases, the stretch

results in an increased pulse frequency, which strengthens the inhibition of the sympathetic nervous system and simultaneously increases the activity of the parasympathetic nervous system. As a result, heart rate, total peripheral resistance, and the tone of the capacity vessels decrease. This leads to a drop in blood pressure and stroke volume [64-66] (*Figure 2*). These mechanisms contribute to a physiological HRV.

Figure 2: *Physiology of the baroreflex.*



BP, blood pressure. ↑ Indicates a rise; ↓ indicates a decrease. *Modified from Kaufmann* et al., *2020 [64].*

1.2.3.2. Clinical complications

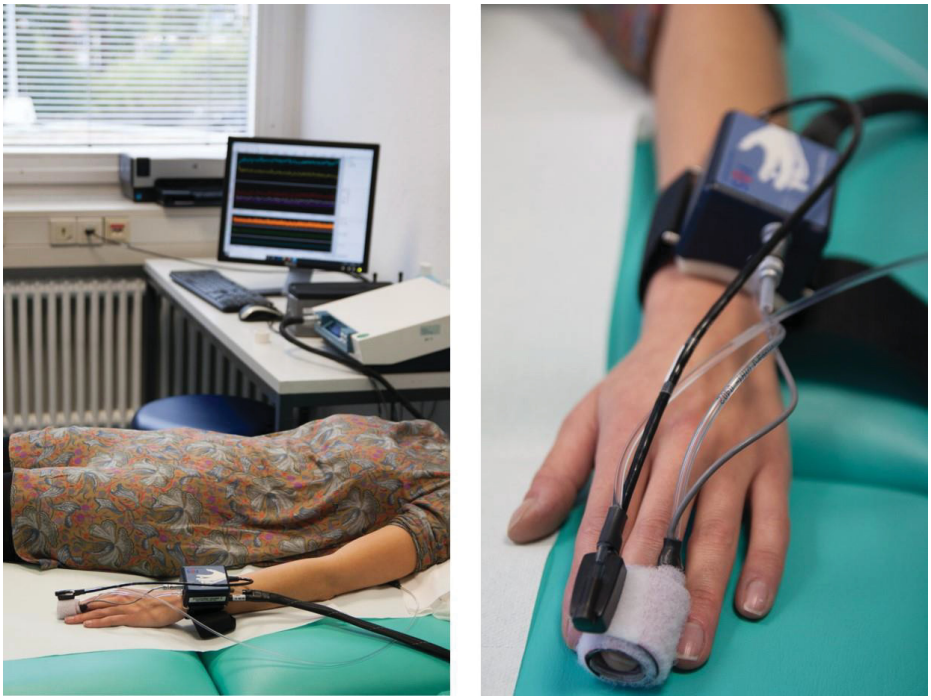
An impaired BRS function is characterized by a predominant chronic adrenergic activation causing an imbalance in the sympathovagal regulation of the HRV and is often accompanied by cardiovascular disease. Healthy baroreceptors maintain a continuous inhibition of the sympathetic signal. Consequently, damage to the baroreceptors results in sympathetic overactivation. The resulting imbalance in blood pressure regulation is predictive of an increased cardiovascular risk in patients with myocardial infarction and

heart failure [64, 65, 67, 68]. Moreover, there is evidence suggesting that the BRS could be an early marker of cardiovascular autonomic dysfunction [67, 69]. An impaired BRS may lead to a disturbed innervation of vessel walls, heart muscle cells and the sinoatrial node. Consequently, a disturbed cardiac electrical conduction increases the risk to develop cardiac arrhythmia [70].

1.2.3.3. Assessment of spontaneous baroreflex sensitivity

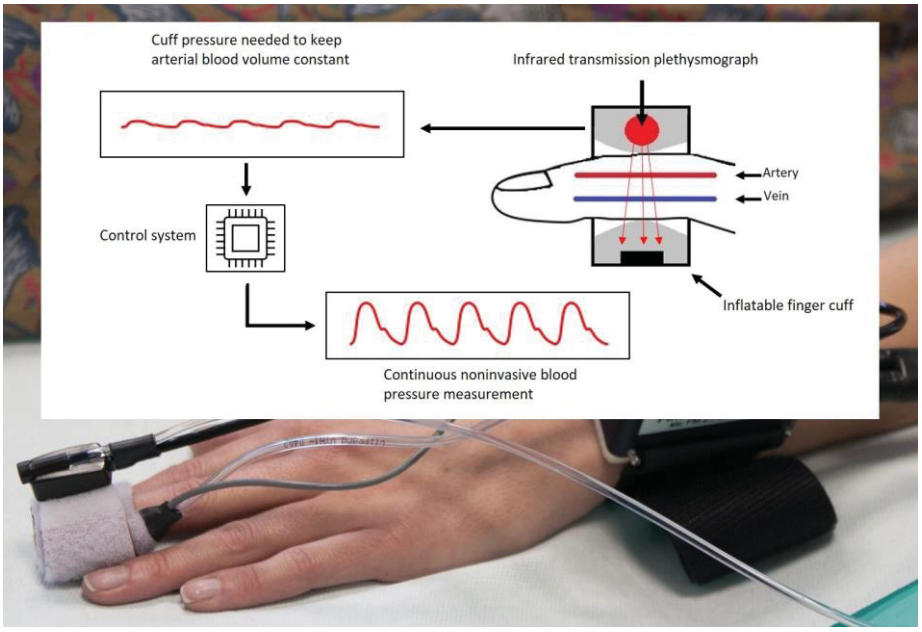
Traditionally, BRS has been assessed using manipulations such as injections of vasoactive drugs, Valsalva maneuver, and the neck chamber technique [63]. Baroreceptors seem to be not only activated by abrupt changes in arterial blood pressure but also by normal activity during daily life occurring continuously [66]. The measurement of these constant fluctuations without prior provocation by medication or maneuvers is known as the spontaneous BRS. More recently, noninvasive BRS assessment has allowed for examining larger cohorts by the relatively simple pulse photoplethysmography technique using simultaneous recordings of spontaneous beat-to-beat fluctuations of BP and heart rate measured on the middle finger (*Figure 3 and Figure 4*). The measurement in the present study performed in the supine position lasts 10 minutes, while the person investigated is breathing spontaneously (*Figure 3*). Several analysis tools exist to date, but no standardized procedure has yet been defined. The most frequently used methods to analyze these spontaneous changes in experimental and clinical studies include the sequence analysis, cross-spectrum analysis and spectral analysis [71-73] . In the present study, all three analyses were used to assess the BRS.

Figure 3: *Recording of arterial blood pressure and heart rate to assess BRS.*



Spontaneous fluctuations of beat-to-beat blood pressure and heart rate are recorded during spontaneous breathing in supine position using a Finometer Midi device. An inflatable finger cuff and an infrared transmission plethysmograph are attached to the middle finger with a finger clip.

Figure 4: *Illustration of bodyplethysmographic blood pressure measurement on the middle finger.*

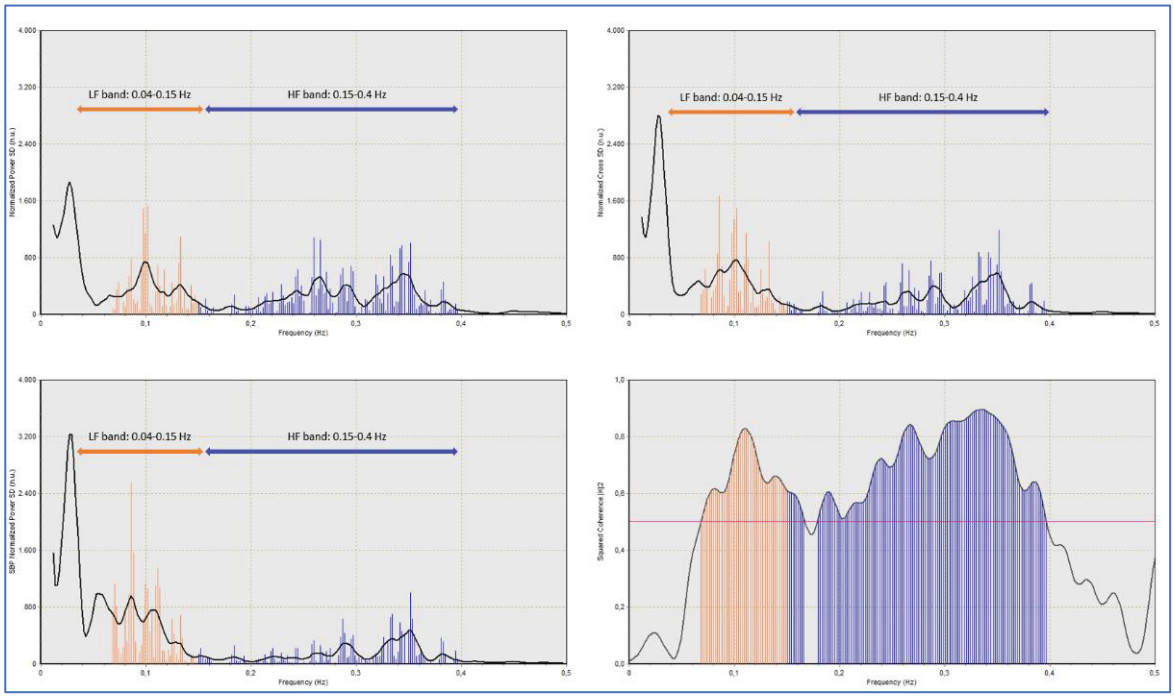


The inflatable finger cuff generates a pressure that keeps the arterial blood volume constant. The collected data is transferred to a control system attached to the wrist. Continuously recorded blood pressure and heart rate are transferred to a computer system for further analysis.

1.2.3.3.1. Spectral analysis

The baroreflex is a mechanism in which changes in blood pressure are linked to changes in pulse. Spectral analysis aims to detect and record oscillations in which pulse and blood pressure correlate. A squared coherence index of >0.5 is commonly used to define sufficient correlation between heart rate and blood pressure [114] and constitutes a prerequisite to compute BRS indices derived from spectral analysis. [71]. BRS spectral analysis allows to calculate the BRS in the different frequency bands (*Figure 5*) respective to HRV spectral analysis. These frequency bands are determined by computing the square root of the ratio between the spectral components of R-R intervals and systolic BP (α coefficient), whereby the following indices according to the respective frequency bands are obtained: BRS-αLF, BRS-αHF, and BRS-αMean (mean of BRS-αLF and BRS-αHF) [71].

***Figure 5**: Example for the calculation of parameters derived from spectral analysis in a patient with type 2 diabetes.*



Screenshot from Nevrokard BRS Analysis v6.3.0 software (Nevrokard, Izola, Slovenia). Horizontal arrows indicate the frequency ranges in the low frequency (LF, orange) and high frequency (HF, blue) bands. Spectral analysis is performed only when the squared coherence exceeds 0.5 (lower right spectrum, above pink line), representing sufficient linear correlation between SBP and R-R interval time series [71, 74]. Vertical bars (orange and blue) indicate time points with squared coherence >0.5 where BRS-αLF and BRS-αHF, respectively, could be calculated.

1.2.3.3.2. Cross-Spectral analysis

Cross-spectral analysis measures a defined number of values in a specified time window and outputs the largest recorded value per time window [72]. Cross-correlation BRS (xBRS) is computed by cross-correlation and regression between systolic BP and R-R intervals over 10-s sliding windows, a time span sufficient to fully accommodate a 10-s variability in rhythm or several cycles at ventilatory frequencies. The correlation coefficient is calculated six times per window. The highest value of all calculated cross-correlations is selected, and the corresponding regression slope is taken to determine xBRS, provided that it is positive and its probability of being a random regression is <0.1% [75].

1.2.3.3.3. Sequence analysis

The sequence method, described by Parati et al. [73], is used to calculate time domain BRS indices for positive, negative and cumulative sequences (BRS(+), BRS(-), BRS sequence all. Spontaneously occurring parallel trends of three or more RR intervals and systolic BPs are determined, while a change of 1 mmHg for consecutive systolic BPs and 5 ms for consecutive R-R intervals are required for a shift [73]. This method allows to evaluate the activity of the baroreflex arc.

1.2.3.3.4. Standard deviation analysis

The standard deviation analysis describes the ratio of the standard deviation of RR intervals and SBP. The BRS-to-SD ratio is used as a simplified index representing the ratio of the SD of R-Rs and the SD of SBP. Bernardi et al. [76] compared the BRS-to-SD ratio to six other BRS indices and reported that this parameter may provide an adequate estimate of BRS, while being easy to assess and comparably free from mathematical constraints.

1.3 Approval of the Ethics Committee

German Diabetes Study (GDS): Ethics committee of Heinrich Heine University; approval No. 4508 (16.12.2013)

1.4 Aims of the study

Our recent studies in recent-onset diabetes patients from the German Diabetes Study (GDS) baseline cohort demonstrated associations of lower vagus-mediated HRV with insulin resistance [32], and reduced cardiorespiratory fitness in both diabetes types [57] as well as hepatic steatosis in type 2 diabetes [77], suggesting that these factors may contribute to the early development of CAN [78]. Evidence has also emerged suggesting that BRS could be a marker of CAN in patients with longer-term diabetes [67, 69, 79-82]. However, the temporal sequence of BRS early in the course of both type 1 and type 2 diabetes remains unclear.

In the present study, we aimed to investigate 1) in a cross-sectional analysis whether various time domain and frequency domain measures of spontaneous BRS are altered in individuals with recent-onset type 1 and type 2 diabetes compared with glucose-tolerant

control groups and associated with metabolic parameters, such as insulin sensitivity or insulin secretion, and 2) in a prospective setting, the progress of BRS over the next 5 years.

We hypothesized that BRS may be reduced in recent-onset type 1 and type 2 diabetes representing an early marker of impaired cardiovascular autonomic function that could be differentially related to metabolic parameters including insulin secretion and sensitivity.

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Impairment in Baroreflex Sensitivity in Recent-Onset Type 2 Diabetes Without Progression Over 5 Years

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Impaired baroreflex sensitivity (BRS) predicts cardiovascular mortality and is prevalent in long-term diabetes. We determined spontaneous BRS in patients with recent-onset diabetes and its temporal sequence over 5 years by recording beat-to-beat blood pressure and R-R intervals over 10 min. Four time domain and four frequency domain BRS indices were computed in participants from the German Diabetes Study baseline cohort with recent-onset type 1/type 2 diabetes (n = 206/381) and age-matched glucose-tolerant control subjects (control 1/control 2: n = 65/83) and subsets of consecutive participants with type 1/type 2 diabetes who reached the 5-year follow-up (n = 84/137). Insulin sensitivity (M-value) was determined using a hyperinsulinemic-euglycemic clamp. After appropriate adjustment, three frequency domain BRS indices were reduced in type 2 diabetes compared with control 2 and were positively associated with the M-value and inversely associated with fasting glucose and HbA_{1c} ($P < 0.05$), whereas BRS was preserved in type 1 diabetes. After 5 years, a decrease in one and four BRS indices was observed in patients with type 1 and type 2 diabetes, respectively ($P < 0.05$), which was explained by the physiologic age-dependent decline. Unlike patients with well-controlled recent-onset type 1 diabetes, those with type 2 diabetes show early baroreflex dysfunction, likely due to insulin resistance and hyperglycemia, albeit without progression over 5 years.

Cardiovascular autonomic neuropathy (CAN) assessed by heart rate variability (HRV) affects ~20% of individuals with diabetes (1), 12–17% of patients with newly diagnosed type 2 diabetes (2,3), and 6–11% of those with prediabetes (2) and carries an increased risk of cardiovascular mortality (4–6). Clinical consequences, such as resting tachycardia, orthostatic hypotension, exercise intolerance, intra- and perioperative hemodynamic instability, and a higher risk of silent myocardial ischemia, contribute to substantial morbidity of patients with CAN (1,6). Established risk factors for CAN include poor glycemic control in type 1 diabetes and a combination of hypertension, dyslipidemia, obesity, and poor glycemic control in type 2 diabetes (1). In patients with recent-onset diabetes from the German Diabetes Study (GDS) baseline cohort, we demonstrated associations of lower vagus-mediated HRV with insulin resistance (7) and reduced cardiorespiratory fitness in both diabetes types (8) as well as hepatic steatosis (9) in type 2 diabetes, suggesting that these factors may contribute to the early development of CAN (10).

The baroreflex system plays an important role in regulating short-term fluctuations of arterial blood pressure (BP). Arterial baroreceptors in the carotid sinuses and aortic arch sense changes in BP and modulate efferent autonomic neural activity to the central nervous system accordingly. A rise in sensed BP leads to an increase and decrease in the discharge of vagal and sympathetic neurons, respectively, resulting in decreased heart rate, cardiac contractility, and peripheral vascular resistance, whereas a fall in BP causes the opposite effects (11). Traditionally, baroreflex sensitivity (BRS) has been assessed using manipulations such as injections of vasoactive drugs, Valsalva maneuver, and the neck chamber technique (11). More recently, noninvasive BRS assessment has allowed for examining larger cohorts by the relatively simple pulse photoplethysmography technique

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using simultaneous recordings of spontaneous beat-to-beat fluctuations of BP and heart rate, which can be analyzed in the time domain and frequency domain (11–15).

Impairment of baroreflex mechanisms resulting in a chronic adrenergic activation often accompanies cardiovascular disease. A blunted baroreflex gain is predictive of increased cardiovascular risk in patients with myocardial infarction and heart failure (11,16). Evidence has also emerged suggesting that BRS could be a marker of CAN in patients with longer-term diabetes (17–22). However, the temporal sequence of BRS early in the course of both type 1 and type 2 diabetes remains unclear. We therefore sought to determine *1*) in a cross-sectional analysis whether various time domain and frequency domain measures of spontaneous BRS are altered in individuals with recent-onset type 1 and type 2 diabetes compared with control groups with normal glucose tolerance and associated with metabolic parameters, such as insulin sensitivity or insulin secretion, and *2*) in a prospective setting the progress of BRS over the next 5 years.

RESEARCH DESIGN AND METHODS

Study Participants

Patients recently diagnosed with diabetes (known diabetes duration ≤1 year) and glucose-tolerant control subjects were recruited consecutively from the baseline cohort of the GDS, a prospective observational study investigating the natural course of metabolic alterations and the development of chronic diabetic complications (ClinicalTrial.gov reg. no. NCT01055093). The study was approved by the local ethics committee of Heinrich Heine University, Düsseldorf, Germany, and informed written consent was obtained from all participants before participation. The study design and cohort profile of the GDS were described in detail previously (23). The present cross-sectional analysis included 206 consecutive participants with type 1 diabetes, 381 consecutive participants with type 2 diabetes, and two corresponding age- and sex-matched control groups with glucose-tolerant individuals (control 1, $n = 65$; control 2, $n = 83$). The prospective analysis included 84 individuals with type 1 diabetes and 137 participants with type 2 diabetes who were monitored for 5 years.

Spontaneous BRS

Continuous plethysmographic arterial measurements of spontaneous changes in systolic BP and R-R intervals (R-Rs), measured on the middle finger, were recorded using a Finometer MIDI device (Finapres Medical Systems, Enschede, the Netherlands) over 10 min in the supine position during spontaneous breathing. BRS parameters were calculated using the BeatScope Easy software (Nevrokard BRS Analysis v6.3.0.; Nevrokard, Izola, Slovenia). The sequence method was used to calculate time domain BRS indices for positive, negative, and all sequences to obtain BRS(+) slope, BRS(−) slope, and BRS sequence all, respectively. Spontaneously occurring parallel trends of three or more R-Rs and systolic BPs were determined, while a change of 1 mmHg for consecutive systolic BPs and 5 ms for consecutive R-Rs were required for a shift (24). Spectral analysis (autoregressive method) was used to determine frequency domain BRS parameters in the low-frequency (LF) (0.04–0.15 Hz) and high-frequency (HF) (0.15–0.4 Hz) bands by computing the square root of the ratio between R-R and systolic BP spectral components (α coefficient), i.e., BRS-αLF, BRS-αHF, and BRS-αMean (mean of BRS-αLF and BRS-αHF) (25). Because the correct interpretation of the α coefficient requires a high coherence for the phase link between R-R and BP variability signals (25), the α coefficient was computed only if coherence (K^2) was >0.5 (25), leaving the following group samples: control 1, $n = 55$; control 2, $n = 67$; type 1 diabetes, $n = 146$; and type 2 diabetes, $n = 223$. Cross-correlation BRS (xBRS) was computed by cross-correlation and regression between systolic BP and R-Rs over 10-s sliding windows, a time span sufficient to fully accommodate a 10-s variability in rhythm or several cycles at ventilatory frequencies. The correlation coefficient was calculated six times per window. The highest value of all calculated cross-correlations was selected, and the corresponding regression slope was taken to determine xBRS, provided that it was positive and its probability of being a random regression was <0.1% (26). The BRS-to-SD ratio was used as a simplified index representing the ratio of the SD of R-Rs and the SD of systolic BP, as previously described (27).

Heart Rate Variability

R-Rs were measured in the supine position during a hyperinsulinemic-euglycemic clamp over 3 h using a digital Spider View Holter recorder with seven electrodes to record three-channel electrocardiograms (Sorin Group, Munich, Germany), as previously described (7). Time domain HRV measures included the SD of differences between adjacent normal-to-normal (NN) intervals, SD of NN averages over 5 min (SDANN), SD of all NN intervals, the number of pairs of adjacent NN intervals differing by >50 ms in the entire recording divided by the total number of NN intervals, and the root mean square of successive differences. Frequency domain HRV indices included the very LF band (0.003–0.04 Hz), LF band (0.04–0.15 Hz), and HF band (0.15–0.4 Hz) and the LF-to-HF ratio.

Cardiovascular autonomic function tests were performed during spontaneous breathing over 5 min (coefficient of R-R variation, spectral analysis), at deep breathing (expiration-to-inspiration ratio), after standing up (maximum-to-minimum 30:15 ratio), and in response to a Valsalva maneuver (Valsalva ratio) using a VariaCardio TF5 system (MIE Ltd, Leeds, U.K.), as previously described (28). Age- and sex-dependent lower limits of normal for HRV tests were defined at the fifth percentile obtained from 218 (142 male, 76 female) glucose-tolerant individuals. Orthostatic hypotension was defined as a decrease in systolic or diastolic BP within 3 min after standing up of >28 or >15 mmHg in men and >27 or >11 mmHg in women, respectively. This definition differs from a commonly used one of ≥20 or ≥10 mmHg for systolic or diastolic BP, respectively (1). Borderline

CAN was assumed if the results of two of seven indices were abnormal, whereas definite CAN was diagnosed if three or more of seven indices were abnormal (28).

Bioelectrical Impedance

Participants were examined in the supine position in the morning after an overnight fast, with arms and legs abducted from their body. BIANOSTIC-AT double-size electrodes (Data Input, Pöcking, Germany) were fixed on the dorsum of the hand and foot of the dominant side of the body (29). Fat-free mass and fat mass were measured using Nutriguard-S (Data Input, Darmstadt, Germany) by determining resistance and reactance (30).

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed as previously described (8). In brief, all participants underwent an incremental exhaustive exercise test on an electronically braked cycle ergometer (Ergometrics 900; Ergoline, Bitz, Germany) at 60 rpm. Respiratory gas exchange measurements were determined by open-air spirometry (Masterscreen CPX; Jaeger/VIASYS, Hoechberg, Germany). Work rate was increased by 10 W/min, and exhaustion was reached on average after 12–15 min. Heart rate and a 12-lead electrocardiogram were recorded continuously, while arm BP was recorded every 2 min during the test. Cardiorespiratory fitness parameters included VO_{2max}, VO_2 at anaerobic threshold, VO_2 at the respiratory compensation point, and VCO_{2max}.

Hyperinsulinemic-Euglycemic Clamp

A modified Botnia clamp was performed, consisting of an intravenous glucose tolerance test, followed by a hyperinsulinemic-euglycemic clamp test with frequent measurements of blood glucose, C-peptide, and insulin to determine whole-body insulin sensitivity (M-value) (mg glucose * (body weight in kg)$^{-1}$ * min^{-1}), which was calculated from the difference between mean glucose infusion rates during steady state in the last 30 min of the clamp with glucose space correction. First-phase C-peptide secretion was calculated as the incremental area under the curve (iAUC) until 10 min (iAUC 0–10 min), second-phase C-peptide secretion as the iAUC between 10 and 60 min (iAUC 10–60 min), and the total C-peptide secretion as the sum of both (iAUC 0–60 min) (23).

Glucagon Stimulation Test

Blood samples were taken before injecting 1 mg glucagon (GlucaGen; Novo Nordisk, Mainz, Germany) intravenously and 6 min thereafter. Glucagon-stimulated insulin secretion was determined as the difference between the C-peptide concentrations at 6 min and 0 min (ΔC-peptide) (23).

Laboratory Analyses

Plasma glucose, cholesterol (total, HDLs, LDLs), serum triglycerides, and creatinine were measured on a Hitachi 912 analyzer (Roche Diagnostics, Mannheim, Germany) as previously described (23). Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (31).

Statistical Analysis

Data are presented as mean ± SD, median (first and third quartiles), or percentages. Categorical variables were compared using the χ^2 test and expressed as percentages of participants. Continuous data were assessed using the parametric *t* test or nonparametric Mann-Whitney *U* test for cross-sectional and the Wilcoxon test for prospective data. Correlations between two variables were determined using Spearman rank correlation for nonparametric data and Pearson correlation analyses for parametric data. For multiple linear regression analyses, dependent variables with skewed distribution were ln-transformed before analyses. The analyses were adjusted for sex, age, BMI, smoking, hypertension, fat mass, and VO_{2max}. All statistical tests were two-sided, and the level of significance was set at $\alpha = 0.05$. All analyses were performed using SPSS 22.0 software (IBM Corp, Armonk, NY).

Data and Resource Availability

The data that support the findings of this study are available from the GDS, but restrictions apply to the availability of these data, which were used under license for the current study and therefore are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of the GDS.

RESULTS

Cross-sectional Analysis

The demographic and clinical characteristics of the baseline cohort are given in Table 1. Compared with control 1 subjects, individuals with type 1 diabetes had higher HbA_{1c} and fasting glucose levels, while BMI, fat-free mass, M-value, fasting and stimulated C-peptide, ΔC-peptide, iAUC 0–10 min, iAUC 10–60 min, iAUC 0–60 min, and VO_{2max} were lower (all $P < 0.05$). Compared with control 2 subjects, participants with type 2 diabetes showed higher BMI, waist circumference, fat mass, heart rate, systolic BP, HbA_{1c}, fasting glucose, fasting C-peptide, triglycerides, hs-CRP, and albuminuria and were more frequently treated with antihypertensive agents, while HDL cholesterol, M-value, ΔC-peptide, iAUC 0–10 min, and VO_{2max} were lower (all $P < 0.05$). No differences between the corresponding diabetes and control groups were noted for the remaining variables.

The median levels of the time domain and frequency domain HRV indices in the four groups at baseline are reported in Table 2. After adjustment for age, sex, BMI, smoking, hypertension, fat mass, and VO_{2max}, SDANN and SD were lower in both diabetes groups compared with the corresponding control groups (all $P < 0.05$). No differences between the groups were noted for the remaining seven HRV indices.

The median levels of the BRS indices obtained in the four groups at baseline are presented in Table 3. No

Table 1—Demographic and clinical characteristics at baseline

Variable	Control 1	Type 1 diabetes	Control 2	Type 2 diabetes
n (% male)	65 (63)	206 (57)	83 (63)	381 (63)
Age (years)	34.3 ± 9.8	34.9 ± 10.8	49.8 ± 10.6	52.1 ± 10.1
BMI (kg/m^2)	26.4 ± 6.0	**24.5 ± 4.1**	28.2 ± 5.6	**31.6 ± 6.3**
Waist circumference (cm)	88 ± 17	85 ± 13	96 ± 16	**105 ± 16**
Fat-free mass (kg)	60.6 ± 13.0	**57.0 ± 10.8**	59.4 ± 12.7	61.4 ± 11.9
Fat mass (%)	25.5 ± 7.7	24.5 ± 8.1	31.2 ± 7.8	**34.7 ± 8.2**
Current smoking status (% yes)	21.5	21.8	20.5	24.4
Heart rate (bpm)	66.3 ± 8.4	68.0 ± 10.6	67.5 ± 9.0	**70.8 ± 10.2**
Systolic BP (mmHg)	116 ± 13	116 ± 12	122 ± 14	**129 ± 15**
Diastolic BP (mmHg)	65.1 ± 8.9	65.3 ± 9.6	70.5 ± 9.5	72.2 ± 9.1
Triglycerides (mmol/L)	1.12 ± 0.77	1.02 ± 0.68	1.35 ± 0.80	**1.91 ± 1.84**
Cholesterol (mmol/L)	4.66 ± 0.79	4.80 ± 0.92	5.31 ± 0.99	5.29 ± 1.07
HDL cholesterol (mmol/L)	1.64 ± 0.53	1.61 ± 0.45	1.56 ± 0.44	**1.24 ± 0.38**
LDL cholesterol (mmol/L)	2.79 ± 0.82	2.84 ± 0.81	3.41 ± 0.93	3.38 ± 0.92
Creatinine (nmol/L)	79.0 ± 13.4	77.8 ± 14.9	78.3 ± 13.6	77.4 ± 17.0
hs-CRP (nmol/L)	15 ± 25	55 ± 515	16 ± 16	**84 ± 574**
HbA_{1c} (%)	5.12 ± 0.29	**6.57 ± 1.17**	5.27 ± 0.28	**6.44 ± 0.84**
HbA_{1c} (mmol/mol)	32.5 ± 3.2	**48.4 ± 12.8**	34.0 ± 3.1	**46.9 ± 9.2**
Fasting glucose (mmol/L)	4.79 ± 0.42	**7.52 ± 2.73**	4.96 ± 0.39	**7.25 ± 2.33**
M-value (mmol × min^{-1} × kg^{-1})	11.3 ± 3.7	**8.8 ± 3.3**	10.0 ± 3.2	**6.3 ± 2.7**
Fasting C-peptide (nmol/L)	0.55 ± 0.28	**0.33 ± 0.24**	0.63 ± 0.31	**1.08 ± 0.52**
Stimulated C-peptide (nmol/L)	1.72 ± 0.72	**0.61 ± 0.48**	2.06 ± 0.97	2.11 ± 0.96
ΔC-peptide (nmol/L)	1.18 ± 0.55	**0.27 ± 0.28**	1.43 ± 0.80	**1.03 ± 0.57**
iAUC 0–10 min (nmol/L)	15.9 ± 6.2	**4.0 ± 3.1**	18.1 ± 7.5	**12.8 ± 6.4**
iAUC 10–60 min (nmol/L)	76.1 ± 31.0	**27.3 ± 22.0**	85.0 ± 33.1	90.0 ± 43.3
iAUC 0–60 min (nmol/L)	92 ± 36	**31 ± 25**	103 ± 39	103 ± 50
Diabetes duration (days)	—	197 ± 97	—	186 ± 94
VO_{2max} (mL × kg body wt^{-1} × min^{-1})	30.7 ± 8.3	**28.3 ± 7.8**	26.1 ± 7.6	**19.9 ± 5.5**
Insulin treatment (%)	—	95.1	—	9.7
Antidiabetic drugs (%)	—	10.2	—	66.9
Antihypertensive drugs (%)	6.2	5.8	14.5	**48.8**
Albuminuria (mg/L)	4.7 ± 6.7	13.2 ± 83.0	3.0 ± 4.2	**14.5 ± 31.6**
eGFR (mL/min × 1.73 m^2)	101 ± 15	101 ± 17	91 ± 13	91 ± 16
Retinopathy (%)	0	1.7	0	2.0
Subclinical/borderline CAN (%)	0	4.1	0	6.5
Definite CAN (%)	0	3.6	0	4.5
Orthostatic hypotension (%)	0	3.4	1.3	6.3

Data are % or mean ± SD. Boldface type indicates $P < 0.05$ vs. corresponding controls. eGFR, estimated glomerular filtration rate.

differences were observed between participants with type 1 diabetes compared with control 1 subjects. In the group with type 2 diabetes, all three spectral analysis indices (BRS-αLF, BRS-αHF, and BRS-αMean) were lower compared with control 2 subjects after adjustment for age, sex, BMI, smoking, hypertension, fat mass, and VO_{2max} (all $P <$ 0.05). Further adjustment for definite CAN did not alter these results. Table 4 reports the full multiple linear regression models including CAN for the three frequency domain BRS indices in control 2 participants and patients with type 2 diabetes at baseline. No differences between the group with type 2 diabetes and control 2 were found for the remaining five BRS indices.

The three frequency domain BRS indices showing reduction in patients with type 2 diabetes compared with control 2 participants were assessed further with respect to their possible associations with metabolic parameters (Table 5). After adjustment for sex, age, BMI, smoking, and

Table 2—Time domain and frequency domain parameters of HRV at baseline

	Control 1	Type 1 diabetes	Control 2	Type 2 diabetes
Time domain HRV indices				
pNN50 (%)	18.1 (4.5, 36.4)	14.5 (4.7, 27.5)	5.2 (1.4, 17.6)	4.6 (1.2, 12.7)
RMSSD (ms)	43.6 (26.2, 67.0)	38.4 (26.4, 57.9)	28.2 (20.1, 43.4)	27.0 (19.3, 37.7)
SDNN (ms)	75.3 (52.5, 98.3)	70.7 (52.7, 88.9)	55.9 (44.1, 73.4)	50.0 (39.0, 64.7)
SDANN (ms)	54.1 (37.2, 67.5)	**39.7 (27.7, 48.8)**	39.8 (31.7, 54.4)	**30.7 (22.8, 40.7)**
SD (ms)	93.6 (72.8, 130.8)	**85.2 (65.9, 107.0)**	73.9 (59.8, 91.2)	**63.3 (50.1, 79.4)**
Frequency domain HRV indices				
VLF power (ms^2)	3,040 (1,870, 5,815)	2,576 (1,596, 4,152)	1,929 (1,253, 2,866)	1,458 (884, 2,374)
LF power (ms^2)	1,296 (716, 1,946)	1,259 (695, 2,060)	637 (393, 1,340)	552 (317, 1,006)
HF power (ms^2)	444 (180, 918)	397 (169, 833)	195 (82, 442)	159 (76, 311)
LF-to-HF ratio	3.03 (2.02, 4.04)	3.17 (2.18, 4.97)	3.49 (2.46, 5.25)	3.59 (2.26, 5.77)

Data are median (first, third quartiles). Boldface type indicates $P < 0.05$ vs. corresponding control subjects. All analyses were adjusted for sex, age, BMI, smoking, hypertension, fat mass, and VO_{2max}. pNN50, the number of pairs of NN >50 ms/number of all NN intervals; RMSSD, root mean square of successive differences; SD, SD of differences between adjacent NN intervals; SDANN, SD of the average NN intervals; SDNN, SD of NN intervals; VLF, very-low-frequency band.

antihypertensive medication, BRS-αLF, BRS-αHF, and BRS-αMean were positively associated with the M-value and inversely associated with fasting blood glucose and HbA_{1c} (all $P < 0.05$). There were no associations of BRS indices with fasting insulin, fasting C-peptide, and glucagon-stimulated incremental C-peptide or with first-phase insulin secretion (intravenous glucose tolerance test) in patients with type 2 diabetes or with any metabolic parameter in control 2 subjects (data not shown).

All BRS indices were inversely correlated with age in both diabetes and control groups (control 1: $r = -0.48$ to -0.71, control 2: $r = -0.38$ to -0.57, type 1 diabetes: $r = -0.30$ to -0.45, type 2 diabetes: $r = -0.20$ to -0.33). Figure 1 shows representative plots of the correlations between xBRS and age in the groups with type 1 and type 2 diabetes and their corresponding control groups.

Prospective Analysis

In patients with type 1 diabetes, HbA_{1c} increased from baseline to 5 years from 6.4 ± 1.1 to 6.9 ± 1.0% and in those with type 2 diabetes from 6.3 ± 0.8 to 7.0 ± 1.1% (both $P < 0.05$). Furthermore, hs-CRP decreased from 22.3 ± 16.3 to 15.4 ± 21.8 nmol/L in participants with type 1 diabetes and from 105 ± 738 to 26.5 ± 24.2 nmol/L in those with type 2 diabetes (both $P < 0.05$). Table 6 reports the follow-up of BRS indices over 5 years in the diabetes groups. In patients with type 1 diabetes, only the BRS-to-SD ratio declined after 5 years ($P < 0.05$), while the remaining seven indices did not change. In participants with type 2 diabetes, xBRS, BRS(+) slope, BRS-αHF, and BRS-αMean decreased after 5 years (all $P < 0.05$ vs. baseline). However, after adjustment for the 5-year follow-up period using age-dependent equations for the BRS indices obtained from glucose-tolerant control subjects, statistical significance was lost for each of the five BRS indices that showed an unadjusted significant decline.

DISCUSSION

The results of this study point to an early baroreflex dysfunction, detected by frequency domain rather than time domain BRS indices, which is associated with both hyperglycemia and insulin resistance rather than insulin secretion in patients with well-controlled recent-onset type 2 diabetes. Four of eight BRS indices decreased further over the next 5 years in participants with type 2 diabetes, but this could be explained by an aging effect rather than by diabetes, because the decline was no longer

Table 3—Indices of BRS at baseline

BRS variable (ms/mmHg)	Control 1	Type 1 diabetes	Control 2	Type 2 diabetes
BRS-to-SD ratio	4.82 (3.51, 6.77)	5.12 (3.30, 6.75)	3.86 (2.82, 6.00)	3.53 (2.23, 5.81)
Sequence analysis				
BRS(+) slope	16.3 (9.2, 23.5)	14.9 (10.3, 22.3)	9.4 (6.5, 13.6)	7.9 (5.5, 11.7)
BRS(−) slope	17.3 (10.7, 25.5)	16.0 (11.1, 22.5)	11.2 (8.1, 17.3)	9.3 (6.2, 13.6)
BRS sequence all	17.6 (10.1, 26.1)	15.7 (10.8, 23.3)	10.5 (7.5, 15.6)	8.7 (6.1, 12.6)
Spectral analysis				
BRS-αLF	12.8 (8.6, 16.6)	11.8 (8.5, 16.8)	9.7 (7.6, 13.6)	**7.5 (4.8, 11.1)**
BRS-αHF	17.7 (10.9, 30.8)	17.4 (12.8, 26.2)	11.8 (7.5, 16.7)	**8.6 (5.4, 13.2)**
BRS-αMean	15.2 (9.8, 24.1)	15.8 (10.7, 21.5)	10.9 (7.5, 15.4)	**8.5 (5.3, 12.7)**
Cross-spectral analysis				
xBRS	13.9 (7.7, 19.5)	11.7 (7.5, 16.7)	8.2 (5.5, 12.0)	6.7 (4.4, 9.8)

Data are median (first, third quartile). Boldface type indicates $P < 0.05$ vs. control 2 subjects after adjustment for sex, age, BMI, smoking, hypertension, fat mass, and VO_{2max}.

Table 4—Multiple linear regression analysis (full model) of frequency domain BRS indices in control 2 subjects and patients with type 2 diabetes at baseline

	BRS-αLF		BRS-αHF		BRS-αMean	
	β	*P*	β	*P*	β	*P*
Sex	−0.074	0.532	0.041	0.731	−0.004	0.973
Age	**−0.217**	**0.003**	**−0.225**	**0.003**	**−0.226**	**0.003**
BMI	0.017	0.910	−0.013	0.931	0.007	0.965
Smoking	0.071	0.248	0.032	0.602	0.046	0.456
VO_{2max}	0.085	0.323	0.067	0.439	0.075	0.384
Fat mass	−0.012	0.949	−0.101	0.594	−0.081	0.668
Hypertension	−0.092	0.166	−0.040	0.546	−0.062	0.350
CAN	−0.042	0.500	−0.032	0.616	−0.041	0.514
Type 2 diabetes	**−0.161**	**0.017**	**−0.139**	**0.040**	**−0.154**	**0.022**

Boldface type indicates statistical significance ($P < 0.05$).

statistically significant after adjustment for the 5-year follow-up period. By contrast, spontaneous BRS is not disturbed within the 1st year after diagnosis of type 1 diabetes and remains unaltered over the next 5 years.

There are no published data available that would allow a direct comparison with ours, since previous studies neither focused on BRS comparing recent-onset type 1 diabetes and type 2 diabetes nor did they include multiple BRS indices, measures of insulin sensitivity and secretion, or a prospective follow-up. Moreover, most of the published studies included relatively small sample sizes and focused on longer-term type 1 diabetes (19–21) or mixed groups of patients with type 1 and type 2 diabetes with a mean diabetes duration ranging from 9 to 28 years (18,22). On one hand, a decline in BRS with increasing diabetes duration was reported in individuals with longer-term type 2 diabetes, but no control group was included for comparison (32). On the other hand, although blunted BRS could be demonstrated in patients with type 2 diabetes compared with age-matched lean control subjects, a similar impairment was present in obese individuals without diabetes, suggesting that BRS dysfunction may also be due to the coexistence of obesity per se rather than type 2 diabetes (33). In fact, evidence has emerged suggesting that spontaneous BRS may also be impaired in persons with the metabolic syndrome (12) and prediabetes (14). Collectively, these studies suggest that diminished BRS may be detected not only in patients with longer-term type 1 and type 2 diabetes but also in individuals with the metabolic syndrome and obesity.

Only a few studies have assessed spontaneous BRS in patients with recently diagnosed type 2 diabetes. Michel-Chávez et al. (34) found no BRS alterations in the supine position in 30 patients with recently diagnosed type 2 diabetes whose diabetes duration was <2 years. In contrast, Gerritsen et al. (35) and Wu et al. (14) demonstrated reduced BRS using a single BRS index in individuals with newly diagnosed type 2 diabetes detected by screening, but measures of insulin sensitivity were not reported. Since in our study baseline mean HbA_{1c} was 6.5% in participants with type 2 diabetes, we hypothesize that even a slight degree of hyperglycemia is sufficient to induce alterations in BRS, particularly in view of the unknown duration of unrecognized hyperglycemia preceding the diabetes diagnosis. Our results in the baseline GDS cohort with type 2 diabetes are in line with some previous studies (14,35) and extend on these data by adding insulin resistance, assessed by the gold standard measure (M-value), as a risk factor for early BRS impairment in type 2 diabetes. Likewise, in obese individuals without diabetes, lower BRS was associated with the HOMA of insulin resistance (36), a surrogate measure of insulin resistance, and healthy individuals with higher HOMA of insulin resistance also showed reduced BRS (37). Of note, the relationship between insulin resistance and BRS was statistically mediated by cerebral blood flow in central autonomic regions, including the insula and cingulate cortex. Thus, activity within the central autonomic network may link insulin resistance to blunted BRS (37). However, experimental data obtained from a chronic hyperinsulinemic model

Table 5—Associations of frequency domain BRS indices with metabolic parameters in participants with type 2 diabetes at baseline

	BRS-αLF		BRS-αHF		BRS-αMean	
	r	*P*	*r*	*P*	*r*	*P*
M-value	0.196	**0.005**	0.290	**<0.0001**	0.237	**0.0002**
Fasting blood glucose	−0.164	**0.015**	−0.154	**0.022**	−0.188	**0.005**
HbA_{1c}	−0.197	**0.003**	−0.202	**0.002**	−0.228	**0.001**

Boldface type indicates $P < 0.01$ after adjustment for age, sex, BMI, smoking, and antihypertensive medication.

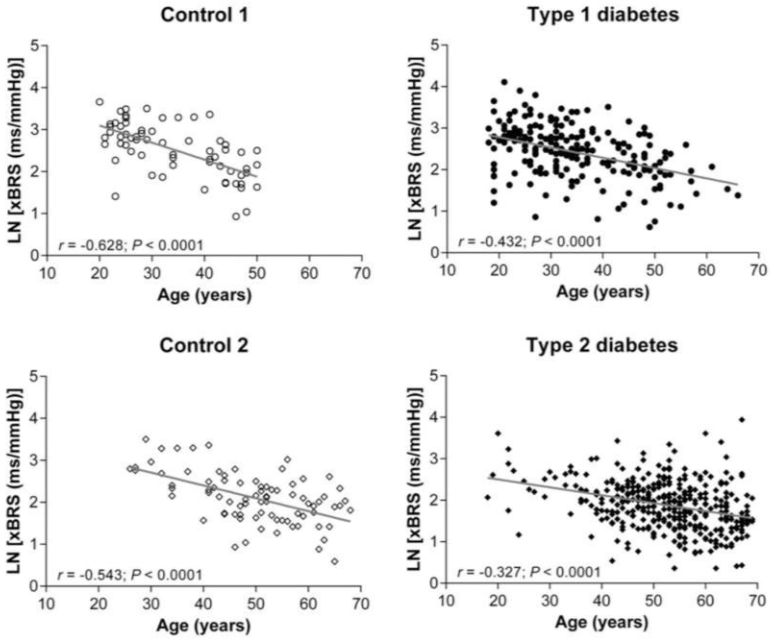

Figure 1—Correlations of xBRS with age in the groups with type 1 and type 2 diabetes and their corresponding control groups.

suggest that chronic hyperinsulinemia rather than insulin resistance could contribute to baroreflex dysfunction, possibly due to insulin-mediated central effects of sympathoexcitation and vagal withdrawal (38).

The only noninterventional study that assessed BRS prospectively is the FinnDiane Study including 80 patients with type 1 diabetes with a mean diabetes duration of 8.8 years. After a 5-year follow-up, only BRS-αHF of six time and frequency domain indices deteriorated after adjustment for time of follow-up. When the six parameters were averaged, BRS declined after 5 years, but statistical significance for the change was lost when adjusted for time of follow-up. Low BRS at baseline did not progress to CAN but predicted an increase in the nighttime systolic BP (13). We extend on these data by demonstrating that BRS remains preserved over the first 5–6 years after the diagnosis of type 1 diabetes and that the early BRS alterations observed in recent-onset type 2 diabetes do not progress over 5 years in excess of the physiologic decline associated with the aging process.

The putative mechanisms underlying the BRS alterations observed herein deserve comment. Under physiological conditions, an evoked rise in arterial pressure reduces

Table 6—Follow-up of spontaneous BRS over 5 years

	Type 1 diabetes (n = 84)		Type 2 diabetes (n = 137)	
BRS variable (ms/mmHg)	Baseline	5 years	Baseline	5 years
BRS-to-SD ratio	5.33 (3.31, 7.37)	**4.41 (3.00, 6.25)**	3.50 (1.92, 5.70)	3.31 (2.10, 5.96)
Sequence analysis				
BRS(+) slope	14.85 (9.82, 20.75)	13.61 (7.41, 17.64)	8.02 (5.50, 12.71)	**6.70 (4.33, 8.85)**
BRS(−) slope	15.48 (10.8, 21.98)	12.56 (9.30, 16.56)	8.69 (6.24, 13.82)	7.73 (5.39, 13.53)
BRS sequence all	16.07 (10.56, 22.8)	12.92 (8.63, 17.03)	8.93 (6.21, 13.86)	7.17 (5.37, 11.3)
Spectral analysis				
BRS-αLF	12.90 (8.25, 18.34)	10.82 (7.53, 16.99)	7.22 (4.28, 11.48)	7.30 (4.40, 9.35)
BRS-αHF	17.61 (13.59, 28.57)	16.17 (9.81, 28.34)	8.95 (5.23, 14.69)	**6.89 (4.75, 10.39)**
BRS-αMean	15.86 (8.51, 23.21)	14.01 (8.51, 23.21)	9.21 (5.20, 13.24)	**6.44 (4.95, 9.94)**
Cross-spectral analysis				
xBRS	11.10 (6.76, 16.53)	9.59 (6.84, 13.85)	6.52 (4.66, 10.68)	**5.42 (3.86, 7.79)**

Data are median (first, third quartile). Boldface indicates $P < 0.05$ vs. baseline (Wilcoxon signed-rank test) and $P > 0.05$ after adjustment for the 5-year follow-up period.

heart rate via excitation of arterial baroreceptor afferent nerves that activate nucleus tractus solitarius (NTS) neurons in the brainstem, which in turn excite cholinergic neurons in nucleus ambiguus to activate cardiac parasympathetic efferent nerves and neurons that produce γ-aminobutyric acid in the caudal ventrolateral medulla to inhibit presympathetic neurons in the rostral ventrolateral medulla (39). In obese Zucker rats, treatment with metformin or pioglitazone enhanced baroreflex control of heart rate by improving the NTS response to raising arterial pressure, suggesting that impaired glucose homeostasis in prediabetic, insulin-resistant male obese Zucker rats contributes to reduced baroreceptor-mediated NTS activation and bradycardia even before the onset of overt type 2 diabetes (39). Moreover, the attenuation of arterial BRS in diabetes and cardiovascular disease has also been attributed to changes in the arterial vascular walls, mechanosensitive ion channels, and voltage-gated ion channels. Some endogenous factors (such as angiotensin II and superoxide anion) can modulate these morphological and functional alterations through intracellular signaling pathways in impaired arterial baroreceptors. It has been suggested that arterial baroreceptors could serve as a potential therapeutic target to improve the prognosis of patients with diabetes and cardiovascular diseases (16).

The therapeutic consequences of BRS impairment in the early phase of type 2 diabetes can be derived from experimental and clinical studies suggesting favorable effects of nonpharmacological and pharmacological interventions on BRS function. In previously sedentary, otherwise healthy, middle-aged adults, 2 years of high-intensity exercise training improved integrated cardiovascular regulation by enhancing the BRS and dynamic Starling mechanism (40). The only randomized clinical trial in individuals with type 2 diabetes demonstrated improved BRS after 52 weeks of exercise training, which was confirmed by several nonrandomized trials over 12–24 weeks (41). Among the pharmacological interventions, pioglitazone treatment for 12 weeks augmented arterial BRS in relation to decreased muscle sympathetic nerve activity in patients with type 2 diabetes and acute myocardial infarction (42), while treatment with the calcium channel antagonist lacidipine for 4 weeks improved 24-h BRS in hypertensive patients with type 2 diabetes (43). From the therapeutic perspective, it is noteworthy that interventions known to enhance insulin sensitivity, such as exercise training, insulin sensitizers, or drugs targeting the renin-angiotensin system (44), also enhance BRS.

The strengths of this study are its prospective design, the relatively large baseline sample size of individuals with well-controlled type 1 and type 2 diabetes, the comprehensive phenotyping, including gold standard measurement of insulin sensitivity, and the inclusion of multiple frequency and time domain techniques to determine BRS.

Limitations of this study are the relatively small prospective 5-year cohorts of individuals with type 1 or type 2 diabetes and lack of prospective analysis of the glucose-tolerant control subjects. To account for this limitation, we used the equations for the physiologic age-dependent decline in BRS indices over 5 years in glucose-tolerant control subjects to compute age-adjusted BRS values, which were added to those obtained at 5 years.

Another limitation is that the group with type 2 diabetes and the corresponding control subjects were not matched for BMI and systolic BP. However, rigorous adjustment for these and other potential confounders, such as fat mass or VO_{2max}, still unveiled baroreflex dysfunction in recent-onset type 2 diabetes.

In conclusion, this study demonstrates an early baroreflex dysfunction in individuals with well-controlled recent-onset type 2 diabetes, which is associated with both hyperglycemia and insulin resistance rather than insulin secretion and does not progress over the next 5 years in excess of aging. Moreover, frequency domain BRS indices appear to be more sensitive than time domain BRS indices in detecting early baroreflex dysfunction in type 2 diabetes. In contrast, spontaneous BRS remains preserved within the first 5–6 years after the diagnosis of type 1 diabetes. It is conceivable that favorable modulation of BRS accompanied by improved insulin sensitivity can be translated into a reduction of cardiovascular end points and improved prognosis in people with type 2 diabetes, but this remains to be demonstrated in large-scale controlled clinical trials.

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Appendix

The GDS group consists of A.E. Buyken, J. Eckel, G. Geerling, H. Al-Hasani, C. Herder, A. Icks, J. Kotzka, O. Kuss, E. Lammert, D. Markgraf, K. Müssig, W. Rathmann, J. Szendroedi, D. Ziegler, and M. Roden (speaker).

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3. Discussion

Damage to the ANS caused by DN is a known, albeit currently underestimated diabetic complication, has numerous consequences especially for the cardiovascular system, and can be measured early in the course of diabetes even in the absence of clinical symptoms. The aim of the present study was to assess and compare the state of baroreflex sensitivity in a larger cohort of individuals from the GDS cohort with type 1 and type 2 diabetes diagnosed within the last 12 months. We measured several indices of spontaneous BRS derived from sequence analysis, cross-spectrum analysis, spectral analysis, and time domain analysis at baseline and after 5 years in subsets of consecutive participants. The results of this study point to an early baroreflex dysfunction, detected by frequency domain rather than time domain BRS indices, which was associated with both hyperglycemia and insulin resistance rather than insulin secretion in patients with well controlled recent-onset type 2 diabetes. Four out of eight BRS indices decreased further over the next 5 years in type 2 diabetes participants, but this could be explained by an aging effect rather than diabetes, since the decline was no longer statistically significant after adjustment for the 5-year follow-up period. By contrast, spontaneous BRS was not disturbed within the first year after diagnosis of type 1 diabetes and remained unaltered over the next 5 years. Spectral analysis proved to be the most sensitive method to detect early baroreflex dysfunction. Furthermore, spectral analysis of BRS appeared to detect cardiovascular autonomic dysfunction earlier than that of HRV.

3.1 Cross-sectional analysis

3.1.1 Early baroreflex dysfunction in recent-onset type 2 diabetes

In the present study, three BRS indices derived from spectral analysis were decreased in patients recently diagnosed with type 2 diabetes, whereas no reduction in indices of BRS were observed in those with recent-onset type 1 diabetes.

To date, no published data are available that would allow for a direct comparison with the present study. Previous studies neither focused on BRS comparing recent-onset type 1 diabetes and type 2 diabetes nor did they include multiple BRS indices as well as measures of insulin sensitivity and secretion. Moreover, most of the published studies included a relatively small sample size and focused on longer-term type 1 diabetes [79-81] or mixed groups of patients with type 1 and type 2 diabetes with a mean diabetes duration ranging from 9 to 28 years [69, 82]. Only a few studies are available which assessed BRS in

patients with recent-onset type 2 diabetes. In a study by Wu et al. [83], BRS derived from spectral analysis was reduced in participants with newly diagnosed diabetes (type not defined) or IGT compared with healthy controls, while no such reduction was observed in those with IFG. In a smaller study by Michel-Chávez et al. [84], 30 participants with recent-onset type 2 diabetes whose diabetes duration was <2 years were compared with healthy individuals, but no differences in BRS were observed under resting condition, whereas slightly decreased BRS was detected in response to postural change in participants with recent-onset type 2 diabetes. However, no measures of insulin sensitivity were reported. In the population-based Hoorn study, BRS calculated using cross-spectral analysis was reduced in individuals with newly diagnosed unspecified diabetes, but not in those with IGT, compared with individuals with NGT [85].

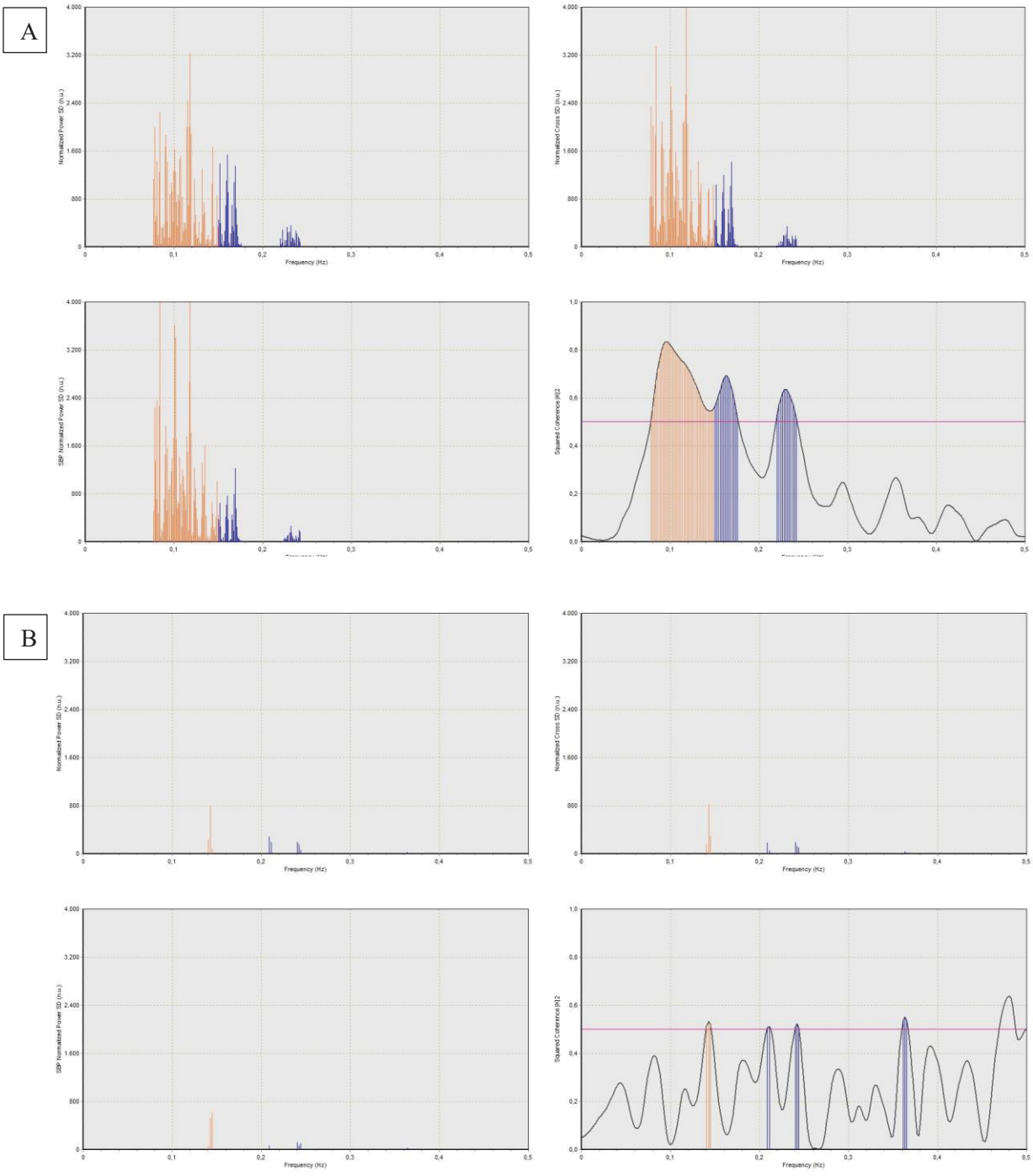
In the present study, four analysis methods were used to assess the BRS, namely sequence analysis, cross-spectrum analysis, spectral analysis using time and frequency domain, and a time-domain derived index. After adjustment, three frequency domain BRS indices of spectral analysis (BRS-αLF, BRS-αHF, BRS-αMean) were reduced in type 2 diabetes compared with the control 2 group, while no differences between the group with type 2 diabetes and the corresponding controls were found for the remaining five BRS indices. No differences were observed between participants with type 1 diabetes compared with the corresponding control group.

Several previous studies indicate that an impaired BRS may allow for an earlier and eventually more sensitive detection of cardiac autonomic dysfunction than CARTs [69, 82, 86] and may be better feasible in clinical practice. The suitability of BRS to assess the risk for cardiovascular complications such as arterial hypertension [87, 88], myocardial infarction [63, 89, 90] or cardiac insufficiency [91, 92] has been subject of prior studies. Decreased BRS showed a predictive value for cardiovascular risk similar to reduced HRV in patients with myocardial infarction and heart failure [66, 93], while the ATRAMI study demonstrated that an impairment of BRS is associated with increased mortality after myocardial infarction [63]. However, it remains unclear which method to calculate BRS is best to detect early signs of cardiac autonomic dysfunction to improve the early detection of CAN in patients with diabetes and which method of BRS assessment is the most appropriate [94]. In the present study, differences in BRS were detected only with BRS indices computed by spectral analysis, while no differences were observed for those computed by sequence, cross-spectrum, and time-domain analyses. Therefore, it is tempting to assume that spectral analysis BRS might be superior to detect early alterations

in autonomic function, even in a group of well controlled patients with recent-onset diabetes.

Spectral analysis BRS indices were only calculated when squared coherence as an indicator of a relationship between heart rate and blood pressure was >0.5 (*Figure 6*). In the present study, calculation of spectral indices was possible for between 59% and 85% of participants from the different groups studied. While on one hand missing results are disappointing, it may corroborate the validity of the results and foster the detection of early cardiac autonomic dysfunction, when flawed results are excluded automatically.

Figure 6: *Comparison of spectral analysis in a healthy individual and a patient with type 2 diabetes.*



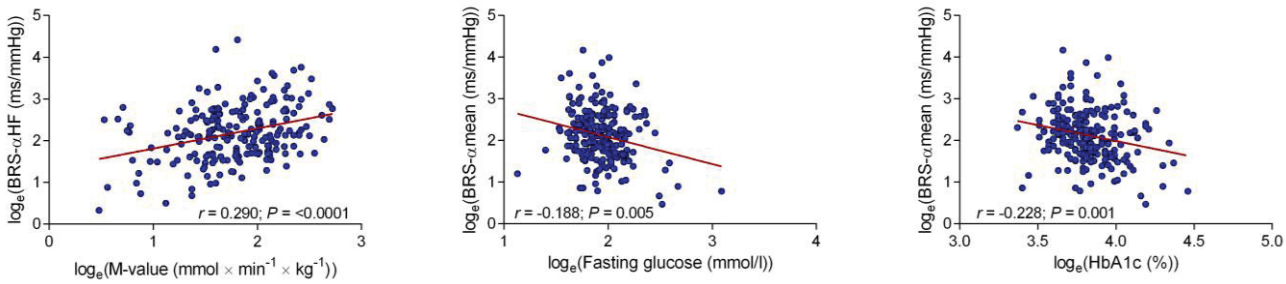
Spectral analysis in a healthy individual (A) and in a participant with type 2 diabetes (B) with insufficient coherence to calculate spectral analysis derived BRS.

Furthermore, BRS assessment by spectral analysis may add diagnostic value to existing HRV measurements. First, BRS contains information about both heart rate and blood pressure regulation. In contrast, most HRV indices are based on heart rate analysis alone. Second, frequency domain BRS indices taking blood pressure into account were more sensitive in detecting abnormality than longer-term frequency domain HRV indices computed over 3 hours during the hyperinsulinemic-euglycemic clamp. By contrast, BRS indices are calculated from single short-term ECG recordings over 10 minutes without any orthostatic or respiratory maneuvers, which could facilitate the implementation in clinical practice.

3.1.2 Associations of baroreflex indices with metabolic parameters

In the type 2 diabetes baseline cohort, the three reduced frequency domain BRS indices (BRS-αLF, BRS-αHF, BRS-αMean) were positively associated with insulin sensitivity (M-value) and inversely associated with parameters of glycemia (fasting blood glucose, HbA1c) (*Figure 7).*

Figure 7: *Associations between M-value, fasting blood glucose, HbA1c and selected BRS indices in the type 2 diabetes cohort at baseline.*



Multiple linear regression revealed that higher age and the presence of type 2 diabetes were the strongest confounders for the frequency domain BRS indices in participants with recent-onset type 2 diabetes and corresponding control individuals combined. Although cross-sectional observations do not allow to draw conclusions about functional relationships, these associations suggest that insulin resistance and mild hyperglycemia (mean HbA1c 6.5% in the recent-onset type 2 diabetes group) are sufficient to induce alterations in BRS in patients with early type 2 diabetes. In a previous study by Indumathy and colleagues, lower BRS was associated with a higher HOMA (homeostasis model assessment) index of insulin resistance [95], a surrogate measure of insulin resistance, in a group of obese individuals (body mass index (BMI) ≥27.5 kg/m²). In a study by Ryan and

colleagues [96], a higher HOMA index representing greater insulin resistance was associated with reduced BRS in healthy individuals, while the relationship was statistically mediated by cerebral blood flow in central autonomic regions, including the insula and cingulate cortex. Thus, activity within the central autonomic network may link insulin resistance to blunted BRS [96]. However, experimental data obtained from a chronic hyperinsulinemic Sprague-Dawley rat model suggested that chronic hyperinsulinemia rather than insulin resistance could contribute to baroreflex dysfunction possibly due to insulin-mediated central effects of sympathoexcitation and vagal withdrawal [97]. Although the exact mechanisms how hyperglycemia affects baroreflex sensitivity remain not fully understood, there are several studies that demonstrated, how glycemic fluctuations affect neurons of autonomic pathways up the nucleus of the solitary tract and the hypothalamus [98-100]. Glucose influenced the rate of action potentials emanating from the vagal nerve, thus reducing the parasympathetic control of the circulatory system resulting in an impaired baroreflex with an increase in heart rate and decrease in HRV [101, 102]. There are also indications that glycemic variability may play a pivotal role in the development of baroreflex dysfunction. An inverse correlation between glycemic variability and BRS was reported by Matsutani et al. [103] in patients with type 2 diabetes, whereas no correlation with acute blood glucose levels found. Glycemic variability was also identified as a predictor of an impaired BRS, independent of age, sex, hypertension and other factors. On the other hand, there is evidence to suggest that hypoglycemia may also induce diminished BRS [43, 104]. Therefore, blood glucose fluctuations ranging from hypo- to hyperglycemia could be a significant contributor to baroreflex dysfunction in diabetes.

In the present study, in contrast to type 2 diabetes, no reduction in BRS indices were observed in recent-onset type 1 diabetes compared with corresponding control individuals after adjustment for sex, age, BMI, smoking, arterial hypertension, fat mass, and cardiorespiratory fitness, suggesting different processes contributing to the development of cardiovascular autonomic dysfunction in type 1 and type 2 diabetes. While in type 2 diabetes the exact time of onset is admittedly not as certain as in type 1 diabetes, previous studies reporting reduced BRS in participants with IGT before the onset of overt type 2 diabetes as well as our prospective results over 5 years corroborate the differences between both diabetes types. Recent evidence points to a stronger heterogeneity in the pathogenesis of type 2 diabetes than previously assumed and that attributing hyperglycemia to relative insulin resistance alone does not sufficiently address the complexity of type 2 diabetes.

Thus, different clinical phenotypes of type 2 diabetes could lead to different manifestation patterns of diabetic complications including CAN [105] [106]. In a study by Holwerda et al. [107], blunted BRS could be demonstrated in patients with type 2 diabetes compared with age-matched lean control subjects, but a similar impairment was also present in obese individuals without diabetes, suggesting that BRS dysfunction may also be influenced by components of the metabolic syndrome such as obesity. In the GDS baseline cohort, the prevalence of CAN differed between subgroups of diabetes with the highest prevalence observed in a subtype of type 2 diabetes characterized by severe insulin deficiency, with a similar prevalence in a subtype characterized by severe insulin resistance, compared with lower prevalence rates in severe autoimmune, moderate age-related and moderate obesity-related diabetes subtypes [106]. Therefore, it should be subject of further analyses, whether BRS is differently associated especially with various subtypes of type 2 diabetes.

3.2. Prospective baroreflex analysis over 5 years

The prospective analyses of the present study demonstrate that BRS remained preserved in recent-onset type 1 diabetes, while the impairment of BRS in recent-onset type 2 diabetes showed no further progression in excess of a physiologic age-dependent decline over the first 5-6 years from diabetes diagnosis onward. Early BRS impairment of BRS in recently diagnosed type 2 diabetes patients was most likely explained by insulin resistance and hyperglycemia.

The only non-interventional study that assessed BRS prospectively is the FinnDiane study that investigated the development of BRS over 5 years in 80 type 1 diabetes patients [108]. In that study, patients with type 1 diabetes initially presented with one impaired BRS index out of six parameters studied, but after adjustment, the mean BRS declined over 5 years to a similar degree as in healthy individuals due to aging. However, impaired BRS at baseline predicted an increase in blood pressure, but did not predict CAN. Of note, only type 1 diabetes patients were examined, had a longer duration of diabetes (initially 8.8 years on average) and higher HbA1c, whereas diabetes duration was ≤1 year in the present study, and participants showed excellent glycemic control. It is likely that in the early phase of diabetes, observation periods longer than 5 years are required to observe progression in BRS dysfunction especially in well controlled patients. Thus, longer-term follow-up of the GDS cohort has to be awaited in this regard.

3.3 Strategies to improve baroreflex function

Several experimental and clinical studies suggest favorable effects of nonpharmalogical and pharmacological approaches to improve baroreflex function. Hieda et al. [109] examined healthy adults undergoing a 2-year high and low intensity exercise training with 4-6 hours per week and found an improvement of cardiovascular regulation with better BRS and improved dynamic Starling mechanism, but no conclusions about the underlying mechanisms could be drawn. Various studies examining the influence of physical activity on autonomic function in patients with diabetes have been performed, but the majority of them suggesting an improvement in BRS due to physical activity had significant flaws [110]. Only one RCT was conducted, in which BRS improved in male participants with type 2 diabetes after a 12-month exercise training [111]. However, the exact mechanisms leading to a meaningful improvement in baroreflex function are not yet fully understood [110]. The question remains to what extent physical activity may affect BRS at an early diabetes stage and the exact mechanisms behind the effect would be of considerable interest for future research.

In a study by Rosengård-Bärlund and colleagues [81], slow deep breathing maneuvers were associated with improved baroreflex function in type 1 diabetes patients with a diabetes duration between 6 and 12 years, comparable to metabolically healthy individuals, while patients with denervated hearts after transplantation did not show this effect. Hence, the reduction in BRS, especially in early disease stages, may be largely functional und thus reversible. In a more recent study by the same group, reduced BRS was still potentially reversible even after 30 years of type 1 diabetes as long as CARTs were within the normal range [112]. Therefore, an increased oxygen supply, be it through improved respiration or an increase in the oxygen content of the breathing air, may improve parasympathetic control of the baroreflex, and impaired parasympathetic baroreflex function may be linked primarily to hyperglycemia [112, 113]. These findings support the concept that baroreflex function may be improved by exercise.

Among the pharmacological interventions, pioglitazone treatment for 12 weeks augmented arterial BRS in relation to decreased muscle sympathetic nerve activity in patients with type 2 diabetes and acute myocardial infarction [114], while treatment with the calcium channel antagonist lacidipine for 4 weeks improved 24-h BRS in hypertensive patients with type 2 diabetes [115]. From a therapeutic perspective, it is noteworthy that interventions known to enhance insulin sensitivity, such as exercise training, insulin sensitizers, or drugs targeting the renin-angiotensin system [116] may also enhance BRS.

Therefore, sensitive assessment of baroreflex function is necessary to evaluate the possible therapeutic benefits of improved baroreflex and cardiac autonomic function in the future.

3.4 Strengths and limitations

The strengths of this study are its prospective design, the relatively large baseline sample size of individuals with well controlled recent-onset type 1 and type 2 diabetes, the comprehensive phenotyping, including gold standard measurement of insulin sensitivity, and the inclusion of multiple frequency and time domain techniques to determine BRS.

Limitations of this study are the relatively small prospective 5-year cohorts of individuals with type 1 or type 2 diabetes and lack of prospective analysis of the glucose-tolerant control individuals. To account for this limitation, we used equations for the physiologic age-dependent decline in BRS indices over 5 years in glucose-tolerant control subjects to compute age-adjusted BRS values, which were added to those obtained at 5 years. Another limitation is that the group with type 2 diabetes and the corresponding control subjects were not matched for BMI and SBP. However, rigorous adjustment for these and other potential confounders, such as fat mass or cardiorespiratory fitness still unveiled early baroreflex dysfunction in recent-onset type 2 diabetes.

3.5 Conclusions

In conclusion, the present study demonstrates an early baroreflex dysfunction in individuals with well controlled recent-onset type 2 diabetes, which was associated with both hyperglycemia and insulin resistance rather than insulin secretion and did not progress over the next 5 years in excess of aging. These associations suggest that poor glycemic control and increasing insulin resistance promote an impairment in BRS early in type 2 diabetes. In contrast, in type 1 diabetes no such effects were found, and spontaneous BRS remained preserved even within the first 5–6 years after diabetes diagnosis. Frequency domain BRS indices were more sensitive than time domain BRS indices in detecting baroreflex dysfunction early in type 2 diabetes. Longer-term follow-up examinations of the GDS cohort will demonstrate the precise temporal sequence in the progression of BRS dysfunction in both diabetes types. Ultimately, it is conceivable that favorable BRS modulation accompanied by improved insulin sensitivity can be translated into a reduction

of cardiovascular endpoints and improved prognosis in people with type 2 diabetes, but this remains to be demonstrated in large-scale controlled clinical trials.

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