DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2011; **27:** 654–664.
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/dmrr.1224

REVIEW ARTICLE

Methods of investigation for cardiac autonomic dysfunction in human research studies

Luciano Bernardi[1]*
Vincenza Spallone[2]
Martin Stevens[3]
Jannik Hilsted[4]
Simona Frontoni[2]
Rodica Pop-Busui[5]
Dan Ziegler[6] Peter Kempler[7]
Roy Freeman[8] Phillip Low[9]
Solomon Tesfaye[10]
Paul Valensi[11] on behalf of The Toronto Consensus Panel on Diabetic Neuropathy†

[1]*Department of Internal Medicine, IRCCS S.Matteo and University of Pavia, Pavia, Italy* [2]*Department of Internal Medicine, University of Tor Vergata, Rome, Italy* [3]*Department of Medicine, University of Birmingham, Birmingham, UK* [4]*Copenhagen University Hospital, Copenhagen, Denmark* [5]*Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA* [6]*Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Heinrich Heine University, Düsseldorf, Germany* [7]*Department of Medicine, Semmelweis University, Budapest, Hungary* [8]*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA* [9]*Department of Neurology, Mayo Clinic, Rochester, MN, USA* [10]*Diabetes Research Unit, Sheffield Teaching Hospitals, Sheffield, UK* [11]*Service d'Endocrinologie-Diabétologie-Nutrition, Hôpital Jean Verdier, AP-HP, Université Paris Nord, CRNH-IdF, Bondy, France*

*Correspondence to: Luciano Bernardi, Department of Internal Medicine, IRCCS S. Matteo and University of Pavia, Pavia, Italy
E-mail: lbern1ps@unipv.it

†See Appendix for Members of The Toronto Consensus Panel on Diabetic Neuropathy.

Received: 12 April 2011
Accepted: 6 June 2011

Summary

This consensus document provides evidence-based guidelines regarding the evaluation of diabetic cardiovascular autonomic neuropathy (CAN) for human research studies; the guidelines are the result of the work of the CAN Subcommittee of the Toronto Diabetic Neuropathy Expert Group. The subcommittee critically reviewed the limitations and strengths of the available diagnostic approaches for CAN and the need for developing new tests for autonomic function.

It was concluded that the most sensitive and specific approaches currently available to evaluate CAN in clinical research are: (1) heart rate variability, (2) baroreflex sensitivity, (3) muscle sympathetic nerve activity, (4) plasma catecholamines, and (5) heart sympathetic imaging. It was also recommended that efforts should be undertaken to develop new non-invasive and safe CAN tests to be used in clinical research, with higher sensitivity and specificity, for studying the pathophysiology of CAN and evaluating new therapeutic approaches.

Keywords diabetic neuropathy; heart rate variability; baroreflex sensitivity; microneurography; catecholamines; cardiac imaging

Abbreviations: BRS – baroreflex sensitivity; CAN – cardiovascular autonomic neuropathy; CARTs – cardiovascular autonomic reflex tests; DHPG – 3, 4-dihydroxyphenylglycol; HED – [^{11}C]-metahydroxyephedrine; HRV – heart rate variability; MIBG – [^{123}I]-metaiodobenzylguanidine; MSNA – muscle sympathetic nerve activity.

Introduction

This consensus document provides evidence-based guidelines regarding the evaluation of diabetic cardiovascular autonomic neuropathy (CAN) for human research studies; the guidelines are the result of the work of the CAN Subcommittee of the Toronto Diabetic Neuropathy Expert Group.

The most sensitive and specific diagnostic tests currently available to evaluate CAN in clinical research are: (1) heart rate variability (HRV), (2) baroreflex sensitivity (BRS), (3) muscle sympathetic nerve activity (MSNA), (4) plasma catecholamines, and (5) heart sympathetic imaging.

This article briefly reports the rationale for each CAN diagnostic test, reviews critical evidence regarding the sensitivity and specificity of each test in diabetes, and provides succinctly final recommendations.

A detailed description of the technical methods, their use in diabetes, and additional references are reported in the online supplement attached to this article (see Supporting information).

The methodology adopted for rating the quality of evidence and strength of recommendations was that suggested by the American Academy of Neurology [1] for diagnostic studies.

Heart rate variability

Rationale

Heart rate is never completely stable. Continuous tonic, phasic, and transient external and internal stimuli of multiple origins affect heart rate to a variable but measurable extent. Five different mechanisms have been described: (1) sympathetic and parasympathetic efferences to the sinus node; (2) neurohumoral influences (e.g. catecholamines, thyroid hormones); (3) stretch of the sinus node; (4) changes in local temperature; and (5) ionic changes in the sinus node. Under resting conditions, it can be assumed that the short-term HRV is essentially determined by the first and third factors. The sympathetic and parasympathetic stimuli directly influence heart rate and are responsible for a physiologic variation in the heart rate, or HRV. The HRV can be evaluated in the time and frequency domains.

Time domain measures of the normal R-R intervals include the difference between the longest and shortest R-R intervals, the standard deviation of 5-min average of normal R-R intervals (SDANN), and the root-mean square of the difference of successive R-R intervals (rMSSD). Longer recordings (e.g. 24-h) allow the calculation of additional indices, as the number of instances per hour in which two consecutive R-R intervals differ by more than 50 ms over 24 h (pNN50). Essentially, all these indices explore the parasympathetic activity.

In the frequency domain, the use of spectral analysis of R-R interval (and other cardiovascular and respiratory signals) allows a precise description of the different fluctuations (see Supporting Information for technical details) of these signals. The components of the HRV obtained by spectral analysis provide information about both the sympathetic and parasympathetic influences on the heart [2,3].

It is traditionally accepted that the parasympathetic system affects the overall variability (e.g. variance, total power) and the sympathetic activity essentially influences a rather narrow band around 0.1 Hz (low frequencies) equivalent to a fluctuation of approximately 6 cycles/min.

However, the influence of these two factors on HRV is markedly different. While the parasympathetic activity influences the total amount of variability (the total power of the spectrum), the sympathetic activity rather than increasing or decreasing the fluctuations seems to act like a low-pass filter. When the sympathetic activity predominates (e.g. tilting, physical exercise), to a large extent only the fluctuations at lower frequency can influence the HRV, whereas the faster perturbations (e.g. those determined by respiration) cannot [4–8]. Accordingly, although respiration [which normally generates heart rate fluctuations at higher frequency, around 0.25 Hz, (high frequencies)] normally increases in depth and frequency during sympathetic activation, its influence on HRV is progressively smaller, and consequently the low frequencies predominates in the spectrum. However, the power in all frequency components is reduced, as an effect of the global reduction in HRV and in total power, induced by the parasympathetic withdrawal and the increase in heart rate. This explains the rather paradox phenomenon whereby the low frequencies predominates (in relative term) over the high frequencies during sympathetic activation, but the amplitude (or the 'power') in low- and high-frequency fluctuations actually decreases. With extreme sympathetic activation and parasympathetic withdrawal (that occurs in conditions like submaximal exercise and severe heart failure), the overall variability (or total power) is so small that the low-frequency component can no longer be measured. Accordingly, it is not surprising that the low-frequency power (when expressed in absolute values) neither correlates with direct measures of sympathetic activity (e.g. those provided by microneurography) nor reflects sympathetic changes, and it is now universally accepted that the low frequencies absolute power does not reflect the sympathetic activity.

Conversely, when measured in relative terms (i.e. as a percentage of the global HRV), the relative proportion of the low- over the high frequencies, provides a relative and approximate indication of the sympathetic modulation to the heart [9,10]. Thus, the sympathetic influences on HRV can only be evaluated on the relative proportion of HRV components [2]. Contributors to these low-frequency oscillations in blood pressure and heart rate include baroreflex activity and activity of an endogenous oscillator in the brainstem or spinal chord [2–5].

The respiratory component (normally at high frequency) is traditionally attributed to the parasympathetic activity (respiratory sinus arrhythmia, between 12 and 18 breaths/min, or approximately at an average of 0.25 Hz). Although the low-frequency fluctuations should not be influenced by respiration, respiration is highly variable and slow-breath-induced low frequencies are very frequent, particularly during spontaneous breathing. These spurious low frequencies explain the poor correlation between direct measures of sympathetic activity and the low frequencies, even when measured in relative terms, during spontaneous breathing (at rest or during different interventions) [11]. Conversely, by increasing or regularizing the breathing rate the correlation between the normalized low frequencies and the sympathetic activity increases [11] as a result of the elimination of the respiratory artefacts on HRV. The simultaneous analysis of respiration allows identification of periods without slow breaths during spontaneous breathing. Only the analysis performed on these data segments can be free from artefacts.

The direct stretch of the sinus node has a very small influence on the HRV of a healthy subject at rest (2–4% of HRV) [12], but accounts for nearly 100% of HRV in denervated hearts [13], severe CAN (due to a permanent loss of autonomic modulation), or also during transient withdrawal of autonomic modulation as it occurs during submaximal exercise. This can be seen

DOI: 10.1002/dmrr

as a small respiration-linked fluctuation that correlates positively with the increase in ventilation during physical exercise. During extreme sympathetic activation and the consequent reduced autonomic modulation of HRV, the direct stretch of the sinus node remains the only evident fluctuation [12]. This component should be taken into account in severe CAN or during submaximal exercise.

Other fluctuations in lower frequencies (e.g. very-low frequency components) are essentially caused by 'external' factors (changes in activity and posture of ambulant subjects [14]), and probably reflect parasympathetic activity, similar to the absolute power of the other frequency components, the total power, or the time-domain indices.

To avoid important bias in the interpretation of HRV it is recommended to perform spectral analysis with control for respiration; to include adequate beat editing capability to avoid the influence of artefacts and ectopic beats; to understand the different significance of absolute and relative components of HRV, to have clear indications as regards to the different methodological approaches and mathematical algorithms. Failing this, spectral analysis cannot provide additional information as compared with the simpler indices of global variability (standard deviation of R-R intervals, variance, total power) and results may be incorrect. This can occur when using some commercially available equipments developed for Holter 24-h electrocardiogram recordings for short-term and experimental data [15]. Conversely, when applied with appropriate methodology, the spectral analysis provides additional information to the time-domain indices, such as information about sympathetic activation (though in relative terms). Additionally, when beat-to-beat blood pressure recording is obtained simultaneously with HRV, it is possible to obtain an index of sympathetic activation from the low-frequency power of blood pressure [2,4–6,9]. The increase in low-frequency power of blood pressure is particularly evident during sympathetic activation induced by tilting [2,9] or physical exercise [6].

Based on studies using acceptable techniques, there is evidence of reduced parasympathetic modulation of heart rate in diabetes and also reduced modulation of systolic blood pressure in the low-frequency region [16–18] particularly after sympathetic stimulation in response to tilting, or in the microcirculation [19]. As most of the cardiovascular autonomic reflex tests (CARTs) essentially explore the parasympathetic activity (as strongly suggested in the other paper on CAN in this issue), there is no other simple test of sympathetic activity capable of identifying early (functional or anatomic) autonomic sympathetic abnormality [20]. CARTs are considered the gold standard for CAN testing. Impaired HRV time- and frequency-domain indices have been reported in diabetic patients before CARTs abnormalities arise. However, the few studies that assessed the diagnostic accuracy against the reference standard of CARTs found only fair results (see online supplement for details). Time- and frequency-domain analysis of 24-h electrocardiogram recordings has documented an abnormal nocturnal sympathetic predominance in diabetic patients that was linked to blood pressure non-dipping. In obese patients weight loss was associated with an improvement in global HRV and in parasympathetic HRV indices [21].

Highlights

- HRV testing is a clinically relevant measure in addition to CARTs and provides key information about autonomic – parasympathetic and sympathetic – modulation of the cardio-vascular system.
- Analysis of HRV can be done using statistical indices in the time and frequency domains.
- Time-domain indices of global HRV and total spectral power of HRV represent the index of parasympathetic activity, as well as the HRV spectral power in the high-frequency region, while the relative proportion (not the absolute power) in the low frequencies of HRV provides a relative measure of sympathetic modulation. This interpretation should be made with cautions if respiratory artifacts (slow breaths) cannot be excluded.
- The parasympathetic nervous system also modulates HRV in the high- and low-frequency regions, and low-frequency power decreases or does not change during sympathetic activation. Thus, the absolute power in the low-frequency region should not be used as an index of sympathetic activity.
- Application of the technique is critically dependant upon understanding of the underlying physiology, the mathematical analyses used, and the many confounders and possible technical artefacts.

Confounders

- Misinterpretation of power spectrum due to irregular respiratory pattern and verbalization during breathing, creating artefactual low frequencies and false 'sympathetic overactivity'.
- Lack of spectral decomposition algorithm when using autoregressive methodology.
- Use of the absolute power of R-R interval low-frequency spectral data as evidence of sympathetic activity.
- In case of very low HRV (2–4% of total variability found in healthy subjects) the interpretation of spectral components is affected by the presence of non-autonomic components in the respiratory range.
- Other confounding factors (such as drugs) similar as those reported for CARTs.

Recommendations

- The best approach to HRV testing involves the analysis of electrocardiogram recordings in

DOI: 10.1002/dmrr

conjunction with respiration and beat-to-beat blood pressure recordings (level C).
- When respiration cannot be recorded, breathing rate should be controlled (15 breaths/min), and hyperventilation or slow deep breathing avoided (level B).
- The subjects must not speak during recordings (level C).
- The optimal recording time is 4–5 min during well controlled rest. Longer times (7 min) may be preferable if fast Fourier transform methods are used and if frequent ectopics are to be edited. Long uncontrolled recording times should be avoided (level C).
- When testing is done under stable conditions, autoregressive or fast Fourier transform methods can be used.
- When fast changes are to be expected (e.g. during interventions) autoregressive algorithms are preferred, or alternatively special time-varying techniques.
- Age-related reference curve should be obtained for the healthy population in the same environment and using the methodology adopted, construct 95% confidence limits (level B).
- Other recommendations on confounding factors are similar as those reported for CARTs.
- Used with the appropriate methodology HRV has an increasingly important role in clinical research and therapeutic trials.

During 24-h recordings:

- If the goal is to define the circadian pattern of autonomic activity, long-duration spectra (e.g. 1 h) and autoregressive algorithms are preferable.
- If the goal is to define relatively faster modifications, shorter time windows (e.g. 5 min) are preferable. Special time-varying techniques can provide beat-to-beat autonomic changes.

Baroreflex sensitivity

Rationale

Continuous changes in blood pressure are sensed in different pressure-sensitive areas (particularly carotid bifurcations and aortic arch) of the arterial tree. The afferent fibres meet at the brainstem and elicit a double response, vagal and sympathetic. An increase in blood pressure reduces the firing of sympathetic vascular and cardiac efferents and increases the firing of vagal cardiac efferents, resulting in a rapid reduction in heart rate and in blood pressure. The reduction in blood pressure is due to both a reduction in cardiac output, which in turn is caused by bradycardia, and to a slower direct vasodilation secondary to sympathetic withdrawal. A reduction in blood pressure induces opposite responses. Thus, to correctly define the baroreflex function, one has to consider both the vagal efferent activity (evidenced by changes in heart rate in response to changes in blood pressure), and the sympathetic efferent activity (mainly directed to the arterial vessels). The latter response cannot be easily studied in clinical environment, but can be obtained for research purposes with simultaneous recordings of MSNA [22] or, indirectly, by neck suction [7]. In practice, the term 'baroreflex sensitivity' normally applies to the cardiac-vagal arm, and to methods measuring changes in heart rate in response to changes in (systolic) blood pressure. The BRS is an interesting approach as it combines information derived from both heart rate and blood pressure.

The measurement of the cardiac-vagal arm BRS can be done with several methods: drugs or physical manoeuvres can be applied to modify blood pressure; alternatively, spontaneous blood pressure variations can be used. In all cases the response in heart rate to the changes in blood pressure is quantified. These methods have been described in detail in the online supplement section of this article (see Supporting Information). None of the BRS tests available today – based on drug-induced or physically induced changes in blood pressure, spontaneous blood pressure fluctuations with the sequences technique or spectral analysis – have shown so far a definite advantage over the others, or a clinically relevant difference.

Longitudinal studies have demonstrated that BRS has important independent prognostic value in cardiac patients [23–25] and in diabetic patients [26].

Although some observations in diabetic patients support an early impairment of BRS before CARTs abnormalities [27,28], very few studies have evaluated so far the diagnostic accuracy of BRS measures as compared with the reference standard of CARTs with inconsistent results. Thus, no definite conclusion is possible on the diagnostic characteristics for CAN of BRS assessment, in particular on its sensitivity. In patients without CAN an early stage of functional BRS abnormalities [29] still responsive to life-style intervention – physical training [30] or dietary improvement and weight reduction [31] – has been documented. BRS assessment may warrant use for identifying subjects at risk for CAN and also in clinical trials.

Highlights

- Cardiac vagal BRS assessment is an important component of autonomic testing as it combines information derived from both heart rate and blood pressure.
- Cardiac vagal BRS is a widely recognised independent prognostic index for cardiovascular mortality and morbidity in the general – mainly cardiac – and the diabetic population (class II).
- No definite conclusion is possible on the diagnostic characteristics of BRS assessment (classes III–IV).
- The presence of early abnormalities with respect to CARTs and their reversibility with appropriate treatments warrant the clinical use of BRS in

DOI: 10.1002/dmrr

identifying subjects at risk for CAN and to test potential therapeutic approaches (classes II–III).
- Pharmacological methods allow assessment of BRS across a range of physiologically relevant blood pressure and – when used with microneurography – measurement of the sympathetic baroreflex. But this invasive technique is limited to research purposes.
- The methodology of BRS (in particular spontaneous BRS) is simple and fast
- All BRS techniques require a dedicated beat-to-beat non-invasive blood pressure monitor.
- None of the BRS tests today available have shown a definite advantage over the others, nor a clinically relevant difference (class II).

Confounders

- Fluctuations induced by drifts of the non-invasive blood pressure monitors.
- Most methods need a large number of arbitrary constraints imposed by the calculations that may affect the results.
- Respiratory pattern: although BRS measures in general do not need a strict control of respiratory pattern, slow breathing increases BRS and reduces sympathetic efferent drive [29,32]; therefore, some feedback from respiration is necessary to correctly interpret the results.
- Age-related reduction in BRS [33].
- Other confounding factors (e.g. drugs) are similar as those for CARTs.

Recommendations

- If the spontaneous approach is adopted, it is suggested to use a battery of methods based on the simplest single 5 min recording procedure (spontaneous BRS) and present the results in terms of a central measure (average or median) (level C).
- Recording should be performed during spontaneous breathing for 4–5 min, under monitored respiration, or during controlled breathing at 15 breaths/min (level C).
- Pre-filtering of the data improves the agreement between methods and provides a more robust estimate of BRS (level C).
- The recording time should be kept between 4 and 5 min of well-controlled rest. Avoid long uncontrolled recording times (level C).
- The subjects must not speak during recordings (level C).
- Age-related reference curves should be obtained for the healthy population of in the same environment and for the methodology adopted, and construct 95% confidence limits (level B).
- Other recommendations on confounding factors are similar as those reported for CARTs.

Muscle sympathetic nerve activity

Rationale

MSNA, i.e. bursts of efferent sympathetic activity in the skeletal muscle at rest or in response to various physiological perturbations, can be directly recorded and measured via microelectrodes inserted into a fascicle of a distal sympathetic nerve to the skin or muscle (microneurography) more commonly at the level of the peroneal nerve. MSNA bursts are related to an inhibitory effect of systole on the arterial baroreceptors, and the burst frequency increases during reductions in blood pressure and vice-versa.

Owing to its invasiveness and the time-consuming nature of the procedure, MSNA is not indicated for routine autonomic assessment. However, by being the most direct measure of sympathetic activity it is an essential research tool.

Increased resting MSNA and blunted responsiveness to physiological hyperinsulinaemia or glucose ingestion have been described in type 2 diabetic patients having neuroadrenergic autonomic dysfunction, and resembles insulin-resistant states and obesity. MSNA abnormalities in these conditions reverse with weight loss [20,34]. In contrast, type 1 diabetes is associated with a significant decrease in the number of bursts, by about half [35]. Although reproducibility is similar to non-diabetic subjects, obtaining good quality recordings is much more difficult in patients with diabetic polyneuropathy than in non-diabetic subjects [20,36], presumably as a result of a reduction in the conducting sympathetic nerve fibres.

Highlights

- The MSNA is the only method allowing direct and continuous measurement of sympathetic nerve traffic (class I).
- MSNA is the only method that can directly assess the sympathetic vascular arm of the arterial or cardiopulmonary baroreflex (class I).
- Type 1 diabetes appears to be associated with a reduction of MSNA (class IV).
- In early type 2 diabetes, resting MSNA might be increased, possibly due to hyperinsulinaemia (class IV).
- The technique is difficult, invasive, time-consuming, requires specialized trained operator and cannot be repeated often in the same subject (class II).

Confounders

- Blood pressure variation
- Large inter-individual variations
- Food intake
- Age
- Posture

DOI: 10.1002/dmrr

- Hypoxia
- Hydration
- Exercise
- Female reproductive hormones
- Arousal
- Sleep
- Mental stress
- Ethnicity

Recommendations

- MSNA should not be routinely employed for the diagnosis of CAN (level C).
- MSNA should be employed with standard CARTs or for specific tests aimed at measuring vascular sympathetic modifications (e.g. glycaemic clamps) (level C).

Catecholamine assessment and cardiovascular sympathetic tests

Rationale

The most important catecholamines in human plasma are norepinephrine and epinephrine, both reflecting sympathetic nervous activity: norepinephrine is released from sympathetic nerve endings by exocytosis, a small proportion reaching the systemic circulation [37]. Thus, circulating norepinephrine mirrors whole-body sympathetic activity when measured in systemic venous plasma. Epinephrine is derived from sympathetic (preganglionic) stimulation of the adrenal medulla and circulating epinephrine therefore reflects the degree of sympathetic activation of the adrenal medulla. Plasma norepinephrine and epinephrine levels can respond differentially in response to stressors; larger plasma norepinephrine responses than epinephrine responses are found upon exposure to cold, and larger plasma epinephrine responses are found in response to glucoprivation and fainting. Other catechols comprise the catecholamine precursor, 3,4-dihydroxy-L-phenylalanine and the main neuronal metabolite of norepinephrine, 3,4-dihydroxyphenylglycol (DHPG) [38].

Norepinephrine plasma appearance rate is in principle the biochemical equivalent of MSNA. Norepinephrine plasma appearance rate and clearance have been determined in idiopathic autonomic neuropathy as well as in diabetic CAN. While norepinephrine clearance is low in idiopathic autonomic neuropathy, this was not the case in CAN, and accordingly in diabetic CAN no additional diagnostic power was added by the inclusion of [^{3}H]-norepinephrine kinetic studies [39]. Thus, catecholamine kinetics is an interesting technique which may give more information about catecholamine production and clearance across different regions – but is unsuitable to be used as a diagnostic tool yet. Plasma DOPA is not related to sympathetic neuropathy and has a mixed neuronal and non-neuronal origin. Plasma DHPG may be a more sensitive marker of overall sympathetic innervation than supine plasma norepinephrine [40], and simultaneous measurement of norepinephrine and DHPG yields more information than measurement of either alone. Catecholamine assessment in diabetes showed in general lower than normal responses to postural changes [41], exercise [42,43], hypoglycaemia [44], and CARTs [45–47]. A subnormal orthostatic increment in plasma norepinephrine is a specific but not sensitive index of baroreflex–sympathoneural failure or sympathetic noradrenergic denervation.

Highlights

- Clinical investigations including catecholamine determinations have contributed significantly to the understanding of the pathophysiology of CAN (class III). In the diagnostic context, the significance has been less prominent, partly due to the limited inclusion of the assays in clinical evaluations.
- Plasma catecholamine concentrations can indicate sympathetic noradrenergic and adrenomedullary hormonal system activity. Because levels of catechols are extremely responsive to lifestyle factors such as posture, temperature, dietary intake, medications, distress, and comorbidities, the clinical diagnostic value of plasma levels of catechols depends importantly on controlling or monitoring these factors (class III).
- Whole-body plasma norepinephrine and epinephrine respond rather slowly (minutes) to different physiological manoeuvres.
- During turnover studies, different regional norepinephrine and epinephrine activities are 'diluted' into a large plasma pool, contributing to blunted responses. Standardization of experimental conditions is to a large extent prohibitive for clinical routine purposes. In general, there is no neurochemical index that specifically assesses cardiac sympathetic innervation or function. This requires measurement of rates of entry of norepinephrine into the venous drainage of the heart, in turn requiring right heart catheterization, measurement of coronary sinus blood flow, and infusion of tracer-labelled norepinephrine.

Confounders

- Plasma norepinephrine concentrations increase with age. Thus, age matching is mandatory for comparisons.
- Smoking increases sympathetic nervous activity and catecholamine concentrations – 24 h tobacco abstention is required for comparisons. Posture, emotional stress, and ambient temperature all affect catecholamine concentrations and should thus be standardized.

DOI: 10.1002/dmrr

Recommendations

- In a number of experimental conditions, plasma catecholamine measurements are mandatory. For clinical routine diagnosis and staging of CAN the usefulness of plasma catecholamine concentrations is less obvious (level C).
- Plasma norepinephrine, epinephrine, and DHPG concentrations should be measured when whole-body sympathetic activity is assessed together with other relevant physiological parameters (heart rate, blood pressure, cardiac output, hormonal and metabolic events).

Heart sympathetic imaging and heart function tests

Rationale

Direct assessment of cardiac sympathetic innervation is possible using radiolabelled catecholamines or sympathomimetic amines that are actively taken up by sympathetic nerve terminals. The attraction of this technique is that it allows direct characterization of the pattern of target organ dysinnervation in diabetes. It is unclear whether this modality can directly assess nerve terminal function. An important limitation is that the imaging depends on delivery of the agent by coronary perfusion. In patients with coronary arterial or arteriolar narrowing, decreased innervation can be difficult or impossible to distinguish from decreased perfusion, without concurrent perfusion imaging.

Although in principle, it is possible to directly assess the integrity of both the parasympathetic as well as the sympathetic nervous system, there has been a paucity of research on parasympathetic imaging of the heart. Cardiac sympathetic neuroimaging, before and after administration of particular pharmacologic probes, can assess specific aspects of neuronal function. This combination has rarely been used.

Four tracers have been utilized to visualize the sympathetic nervous innervation of the heart: [^{123}I]-*meta*-iodobenzylguanidine (MIBG), [^{11}C]-*meta*-hydroxyephedrine (HED), 6-[^{18}F] dopamine, and [^{11}C]-epinephrine.

The washout rates from the myocardium of [^{11}C]-epinephrine or 6-[^{18}F]-dopamine can give information on vesicular integrity. In subjects with type 1 diabetes and CAN, the washout rates of [^{11}C]-epinephrine parallels those of [^{11}C]-HED, suggesting regional differences in vesicular uptake or retention [48]. Causes of defective tracer uptake or increased washout from the heart are a matter of current research.

The interpretation of findings using sympathetic neurotransmitter analogues is complicated by the fact that alterations in sympathetic nervous system tone may also affect the retention of these tracers, and this fact is often not considered as an explanation for the clinical findings. In the isolated rat heart model, elevated norepinephrine concentrations in the perfusion increased neuronal HED clearance rates consistent with the concept that neuronal 'recycling' of HED can be disrupted by increased synaptic norepinephrine levels [49]. Alternatively at high norepinephrine concentrations, non-neuronal uptake of HED into myocardial cells and impaired retention may be an interfering factor.

Additionally, interpretation of early myocardial [^{123}I]-MIBG retention is complicated by increased body mass index and diastolic blood pressure which have been reported to reduce myocardial MIBG uptake [50]. Moreover, difficulties and delays in acquisition of utilizable images can complicate the interpretation of the measurement obtained. The delivery of tracers is critically influenced by myocardial perfusion, so myocardial retention of tracers should be performed with a quantitative analysis of myocardial blood flow. This can be performed using positron emission tomography in order to derive a myocardial retention index [51]. However, although regional perfusion deficiencies can be excluded using single photon emission computed tomography, quantitative analysis of regional myocardial perfusion cannot be performed. Additionally, myocardial ischaemia or damage is also known to result in cardiac denervation which may occur in the absence of alterations in CARTs [52], whereas CAN is associated with impaired vasodilatory capacity in response to adenosine [53]. Anoxic ischaemia severely decreases the efficiency of vesicular sequestration and thus accelerates the loss of radioactivity, giving the false impression of denervation. Left ventricular dysfunction in diabetes has also been reported to reduce [^{123}I]-MIBG retention and increased washout rate [54].

Highlights

- Scintigraphic tracers directly assess the structural integrity of the sympathetic nervous system supply to the heart (class III).
- [^{123}I]-MIBG scanning and single photon emission computed tomography are widely used and available at most secondary care institutions; however, MIBG scanning is approved and reimbursed for evaluation of pheochromocytoma and so far not for evaluation of cardiac sympathetic innervation.
- Most data relate to the evaluation of cardiac sympathetic integrity; few studies evaluate the respiratory system.
- The relationships of deficits in tracer uptake/washout to sympathetic neuronal integrity and function are poorly understood: current tracers may not be the most optimum. Combined neuroimaging-pharmacologic approaches are required.
- Scintigraphic data correlates with HRV testing, but have greater sensitivity to detect changes in sympathetic neuronal structure and/or function [55,56] (class III).

DOI: 10.1002/dmrr

- Scintigraphic data correlate with indices of myocardial perfusion and left ventricular dysfunction in type 1 diabetes [57] (class III).
- Limited studies demonstrate that decreased 'uptake' and excessive 'washout' of MIBG-derived radioactivity is an adverse prognostic finding in a spectrum of conditions including diabetes and that scintigraphic data are affected by the quality of glucose control [58–60] (class III).
- Cost of scintigraphic studies is considerable.

Confounders

- Parasympathetic tracers are not yet generally available.
- [^{11}C]-HED and 6-[^{18}F]-dopamine positron emission tomography have limited availability and are not reimbursed.
- Damage to the myocardium and left ventricular dysfunction interferes with tracer uptake and washout independently of changes in CARTs.
- Regional myocardial [^{123}I]-MIBG 'uptake' is semi-quantitative and not a clean index of neuronal uptake, which occurs extremely rapidly.
- [^{123}I]-MIBG retention is affected by body mass index, diastolic blood pressure, and local factors which influence the tracer uptake and retention.
- Delivery of tracers is critically influenced by myocardial perfusion (myocardial retention of tracers should be performed with quantitative analysis of myocardial blood flow).
- The effects of the following on the kinetics of myocardial tracer retention are poorly understood:
  - age (*except for 6-[^{18}F]-dopamine*)
  - gender
  - glucose
  - insulin
  - dyslipidaemia
  - hypertension
  - vasoactive agents
- Methodology for the assessment of sympathetic integrity is not standardized.
- Normative values have not been developed.

Recommendations

- Scintigraphic studies should not be routinely employed for the diagnosis of CAN and should be utilized in concert with standard CARTs (level C).
- Scintigraphic studies are extremely valuable in the identification of sympathetic noradrenergic denervation as a mechanism of neurogenic orthostatic hypotension (level B).
- [^{123}I]-MIBG single photon emission computed tomography offers semi-quantitative assessment and [^{11}C]-HED, 6-[^{18}F]-dopamine, and [^{11}C]-epinephrine positron emission tomography offer quantitative assessment of cardiac sympathetic integrity (level B).
- There is no standardized methodology for scintigraphic assessment of cardiac sympathetic integrity and only limited data on the reproducibility exist (level C).
- Scintigraphic tracer uptake is affected by myocardial perfusion, and tracer retention is affected by available energy for the active neuronal and vesicular uptake transporters (level C).
- The results of scintigraphy should be compared with an appropriate control population (level C).
- Scintigraphic studies offer good sensitivity to detect sympathetic neuronal loss in the heart (level C).
- Scintigraphy is appropriate to explore the effects of sympathetic denervation on cardiac physiology, metabolism, and function (level C).
- Scintigraphy is useful as a marker of cardiac sympathetic denervation in cross-sectional and longitudinal research studies (level C).

Conclusions

Assessment of HRV and BRS are the most widely used and readily available diagnostic tests for CAN in clinical research. They offer the possibility to provide new information about the pathophysiology of autonomic dysfunction in diabetes, to clarify the natural history of CAN with regard to the early autonomic abnormalities observed in diabetes and pre-diabetes, and to obtain more sensitive and comprehensive end-points in clinical trials in CAN. They might also be used in clinical practice in secondary care institutions to provide additional early and prognostic information to current CARTs. To obtain meaningful results, however, they need control of confounding factors, strict standardization with regard to respiration and blood pressure recording and to comply with various technical requirements (in particular for HRV testing).

Scintigraphic investigations may be available at most secondary care institutions and are potentially useful in longitudinal research studies. The role of MSNA and catecholamine assessment as end-points in clinical trials, as already applied in life-style intervention trials in obesity, needs to be further elucidated. Conversely, these techniques are the gold reference for assessing the role of the sympathetic nervous system in quantitative terms.

Supporting information

Supporting information may be found in the online version of this article.

Conflict of interest

None declared.

DOI: 10.1002/dmrr

Appendix

The Toronto Consensus Panel on Diabetic Neuropathy

James W. Albers, MD, PhD, University of Michigan, Ann Arbor, MI, USA
Gérard Amarenco, MD, Service de Rééducation Neurologique et d'Explorations Périnéales, Hôpital Rothschild, AP-HP, Paris, France
Henning Anderson, MD, Department of Neurology, Aarhus University Hospital, Aarhus, Denmark
Joe Arezzo, PhD, Albert Einstein College of Medicine, New York, NY, USA
Misha-Miroslav Backonja, MD, Department of Neurology, University of Madison-Wisconsin, Madison, WI, USA
Luciano Bernardi, MD, Clinica Medica 1, Universita' di Pavia, Pavia, Italy
Geert-Jan Biessels, MD, Department of Neurology, Rudolf Magnus Institute, Utrecht, The Netherlands
Andrew J. M. Boulton, MD, Department of Medicine, University of Manchester, Manchester, UK
Vera Bril, MD, Department of Neurology, University of Toronto, Toronto, ON, Canada
Norman Cameron, PhD, University of Aberdeen, Aberdeen, UK
Mary Cotter, PhD, University of Aberdeen, Aberdeen, UK
Peter J. Dyck, MD, Department of Neurology, Mayo Clinic, Rochester MN, USA
John England, MD, Department of Neurology at Louisiana State University Health Sciences Center, New Orleans, LA, USA
Eva Feldman, MD, PhD, Department of Neurology, University of Michigan, Ann Arbor, MI, USA
Roy Freeman, MD, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
Simona Frontoni, MD, Department of Internal Medicine, University of Tor Vergata, Rome, Italy
Jannik Hilsted, MD, Copenhagen University Hospital, Copenhagen, Denmark
Michael Horowitz, MD, PhD, Department of Medicine, University of Adelaide, Adelaide, SA, Australia
Peter Kempler, MD, PhD, I Department of Medicine, Semmelweis University, Budapest, Hungary
Giuseppe Lauria, MD, Neuromuscular Diseases Unit, 'Carlo Besta' Neurological Institute, Milan, Italy
Philip Low, MD, Department of Neurology, Mayo Clinic, Rochester MN, USA
Rayaz Malik, MD, Division of Cardiovascular Medicine, University of Manchester, Manchester, UK
Peter C. O'Brien, PhD, Mayo Clinic College of Medicine, Rochester, MN, USA
Rodica Pop-Busui, MD, PhD, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA
Bruce Perkins, MD MPH, Division of Endocrinology, University of Toronto, Toronto, ON, Canada
Gerry Rayman, MD, Diabetes Centre, Ipswich Hospital, Ipswich, UK
James Russell, MD, Department of Neurology and Neurophysiology, University of Maryland, Baltimore, MD, USA
Søren Sindrup, MD, Department of Neurology, Odense University Hospital, Odense, Denmark
Gordon Smith, MD, Department of Neurology, University of Utah, Salt Lake City, UT, USA
Vincenza Spallone, MD, PhD, Department of Internal Medicine, University of Tor Vergata, Rome, Italy
Martin Stevens, MD, Department of Medicine, University of Birmingham, Birmingham, UK
Solomon Tesfaye, MD, Diabetes Research Unit, Sheffield Teaching Hospitals, Sheffield, UK
Paul Valensi, MD, Service d'Endocrinologie-Diabétologie-Nutrition, Hôpital Jean Verdier, Bondy, France
Tamás Várkonyi, MD, PhD, First Department of Medicine, University of Szeged, Szeged, Hungary
Aristides Veves, MD, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
Loretta Vileikyte, MD, PhD, Department of Medicine, University of Manchester, Manchester, UK
Aaron Vinik, MD, PhD, Strelitz Diabetes Research Institutes, Eastern Virginia Medical School, Norfolk, VA, USA
Dan Ziegler, MD, Institute for Clinical Diabetology, German Diabetes Center at the Heinrich Heine University, Leibniz Center for Diabetes Research, Leibniz, Germany; Department of Metabolic Diseases, University Hospital, Düsseldorf, Germany
Doug Zochodne, MD, Department of Clinical Neuroscience, University of Calgary, AB, Canada
NIDDK observer – Teresa Jones, MD, NIDDK, Bethesda, MD, USA

References

1. England JD, Gronseth GS, Franklin G, *et al*.; American Academy of Neurology. Practice parameter: evaluation of distal symmetric polyneuropathy: role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. *Neurology* 2009; **72:** 177–184.
2. Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation* 1998; **98**(23): 2640–2643.
3. Pomeranz B, Macaulay RJ, Caudill MA, *et al*. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985; **248:** H151–H153.
4. de Boer RW, Karemaker JM, Strackee J. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *Am J Physiol* 1987; **253:** H680–H689.
5. Sleight P, La Rovere MT, Mortara A, *et al*. Physiology and pathophysiology of heart rate and blood pressure variability in humans: is power spectral analysis largely an index of baroreflex gain? *Clin Sci (Lond)* 1995; **88**(1): 103–109.

6. Spadacini G, Passino C, Leuzzi S, *et al*. Frequency-dependent baroreflex control of blood pressure and heart rate during physical exercise. *Int J Cardiol* 2006; **107**(2): 171–179.
7. Bernardi L, Hayoz D, Wenzel R, *et al*. Synchronous and baroceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. *Am J Physiol* 1997; **273:** H1867–H1878.
8. Saul JP, Berger RD, Albrecht P, Stein SP, Chen MH, Cohen RJ. Transfer function analysis of the circulation: unique insights into cardiovascular regulation. *Am J Physiol* 1991; **261:** H1231–H1245.
9. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 1994; **90**(4): 1826–1831.
10. Pagani M, Malliani A. Interpreting oscillations of muscle sympathetic nerve activity and heart rate variability. *J Hypertens* 2000; **18**(12): 1709–1719.
11. DeBeck LD, Petersen SR, Jones KE, Stickland MK. Heart rate variability and muscle sympathetic nerve activity response to acute stress: the effect of breathing. *Am J Physiol Regul Integr Comp Physiol* 2010; **299**(1): R80–R91.
12. Bernardi L, Bianchini B, Spadacini G, *et al*. Demonstrable cardiac reinnervation after human heart transplantation by carotid baroreflex modulation of RR interval. *Circulation* 1995; **92**(10): 2895–2903.
13. Bernardi L, Salvucci F, Suardi R, *et al*. Evidence for an intrinsic mechanism regulating heart rate variability in the transplanted and the intact heart during submaximal dynamic exercise? *Cardiovasc Res* 1990; **24**(12): 969–981.
14. Bernardi L, Valle F, Coco M, Calciati A, Sleight P. Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms. *Cardiovasc Res* 1996; **32**(2): 234–237.
15. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; **93**(5): 1043–1065.
16. Pagani M, Malfatto G, Pierini S, *et al*. Spectral analysis of heart rate variability in the assessment of autonomic diabetic neuropathy. *J Auton Nerv Syst* 1988; **23**(2): 143–153.
17. Gulli G, Fattor B, Marchesi M. Cross-spectral analysis of cardiovascular variables in supine diabetic patients. *Clin Auton Res* 2005; **15**(2): 92–98.
18. Zoppini G, Cacciatori V, Gemma ML, *et al*. Effect of moderate aerobic exercise on sympatho-vagal balance in Type 2 diabetic patients. *Diabet Med* 2007; **24**(4): 370–376.
19. Bernardi L, Rossi M, Leuzzi S, *et al*. Reduction of 0.1 Hz microcirculatory fluctuations as evidence of sympathetic dysfunction in insulin-dependent diabetes. *Cardiovasc Res* 1997; **34**(1): 185–191.
20. Bernardi L. Clinical evaluation of arterial baroreflex activity in diabetes. *Diabetes Nutr Metab* 2000; **13**(6): 331–340.
21. Maser RE, Lenhard MJ. An overview of the effect of weight loss on cardiovascular autonomic function. *Curr Diabetes Rev* 2007; **3:** 204–211.
22. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension* 1998; **31**(1): 68–72.
23. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998; **351**(9101): 478–484.
24. La Rovere MT, Pinna GD, Maestri R, *et al*. Prognostic implications of baroreflex sensitivity in heart failure patients in the beta-blocking era. *J Am Coll Cardiol* 2009; **53**(2): 193–199.
25. Johansson M, Gao SA, Friberg P, *et al*. Baroreflex effectiveness index and baroreflex sensitivity predict all-cause mortality and sudden death in hypertensive patients with chronic renal failure. *J Hypertens* 2007; **25**(1): 163–168.
26. Gerritsen J, Dekker JM, TenVoorde BJ, *et al*. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care* 2001; **24**(10): 1793–1798.
27. Frattola A, Parati G, Gamba P, *et al*. Time and frequency domain estimates of spontaneous baroreflex sensitivity provide early detection of autonomic dysfunction in diabetes mellitus. *Diabetologia* 1997; **40**(12): 1470–1475.
28. Weston PJ, James MA, Panerai RB, McNally PG, Potter JF, Thurston H. Evidence of defective cardiovascular regulation in insulin-dependent diabetic patients without clinical autonomic dysfunction. *Diabetes Res Clin Pract* 1998; **42**(3): 141–148.
29. Rosengård-Bärlund M, Bernardi L, Fagerudd J, *et al*.; FinnDiane Study Group. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia* 2009; **52**(6): 1164–1172.
30. Loimaala A, Huikuri HV, Kööbi T, Rinne M, Nenonen A, Vuori I. Exercise training improves baroreflex sensitivity in type 2 diabetes. *Diabetes* 2003; **52**(7): 1837–1842.
31. Alvarez GE, Davy BM, Ballard TP, Beske SD, Davy KP. Weight loss increases cardiovagal baroreflex function in obese young and older men. *Am J Physiol* 2005; **289**(4): E665–E669.
32. Goso Y, Asanoi H, Ishise H, *et al*. Respiratory modulation of muscle sympathetic nerve activity in patients with chronic heart failure. *Circulation* 2001; **104**(4): 418–423.
33. Monahan KD. Effect of aging on baroreflex function in humans. *Am J Physiol* 2007; **293**(1): R3–R12.
34. Huggett RJ, Scott EM, Gilbey SG, Bannister J, Mackintosh AF, Mary DA. Disparity of autonomic control in type 2 diabetes mellitus. *Diabetologia* 2005; **48**(1): 172–179.
35. Hoffman RP, Sinkey CA, Anderson EA. Microneurographically determined muscle sympathetic nerve activity levels are reproducible in insulin-dependent diabetes mellitus. *J Diabetes Complications* 1998; **12**(6): 307–310.
36. Fagius J, Wallin BG. Sympathetic reflex latencies and conduction velocities in patients with polyneuropathy. *J Neurol Sci* 1980; **47**(3): 449–461.
37. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med* 1980; **303**(8): 436–444.
38. Goldstein DS, Holmes C, Sharabi Y, Brentzel S, Eisenhofer G. Plasma levels of catechols and metanephrines in neurogenic orthostatic hypotension. *Neurology* 2003; **60**(8): 1327–1332.
39. Hilsted J. Catecholamines and diabetic autonomic neuropathy. *Diabet Med* 1995; **12**(4): 296–297.
40. Christensen NJ, Dejgaard A, Hilsted J. Plasma dihydroxyphenylglycol (DHPG) as an index of diabetic autonomic neuropathy. *Clin Physiol* 1988; **8**(6): 577–580.
41. Hilsted J, Parving HH, Christensen NJ, Benn J, Galbo H. Hemodynamics in diabetic orthostatic hypotension. *J Clin Invest.* 1981; **68**(6): 1427–1434.
42. Hilsted J, Galbo H, Christensen NJ. Impaired responses of catecholamines, growth hormone, and cortisol to graded exercise in diabetic autonomic neuropathy. *Diabetes* 1980; **29**(4): 257–262.
43. Bottini P, Tantucci C, Scionti L, *et al*. Cardiovascular response to exercise in diabetic patients: influence of autonomic neuropathy of different severity. *Diabetologia* 1995; **38**(2): 244–250.
44. Hepburn DA, MacLeod KM, Frier BM. Physiological, symptomatic and hormonal responses to acute hypoglycaemia in type 1 diabetic patients with autonomic neuropathy. *Diabet Med* 1993; **10**(10): 940–949.
45. Fanelli C, Pampanelli S, Lalli C, *et al*. Long-term intensive therapy of IDDM patients with clinically overt autonomic neuropathy: effects on hypoglycemia awareness and counterregulation. *Diabetes* 1997; **46**(7): 1172–1181.

46. Dessein PH, Joffe BI, Metz RM, Millar DL, Lawson M, Stanwix AE. Autonomic dysfunction in systemic sclerosis: sympathetic overactivity and instability. *Am J Med* 1992; **93**(2): 143–150.
47. Vaz M, Kumar MV, Kulkarni RN, Rodrigues D, Shetty PS. Variability of cardiovascular and plasma noradrenaline responses to sustained isometric contraction in normal human subjects. *Clin Sci (Lond)* 1993; **85**(1): 45–49.
48. Stevens MJ, Raffel DM, Allman K, *et al*. Cardiac sympathetic dysinnervation in diabetes – an explanation for enhanced cardiovascular risk? *Circulation* 1998; **98:** 961–968.
49. DeGrado TR, Hutchins GD, Toorongian SA, Wieland DM Schwaiger M. Myocardial kinetics of carbon-11-meta-hydroxyephedrine (HED): retention mechanisms and effects of norepinephrine. *J Nucl Med* 1993; **34:** 1287–1293.
50. Mantysaari M, Kuikka J, Mustonen J, *et al*. Measurement of myocardial accumulation of 123I-metaiodobenzylguanidine for studying cardiac autonomic neuropathy in diabetes mellitus. *Clin Autonom Res* 1996; **6**(3): 163–169.
51. Allman KC, Stevens MJ, Wieland DM, *et al*. Noninvasive assessment of cardiac diabetic neuropathy by C-11 hydroxyephedrine and positron emission tomography. *J Am Coll Cardiol* 1993; **22:** 1425–1432.
52. Nagamachi S, Jinnouchi S, Kurose T, *et al*. 123I-MIBG myocardial scintigraphy in diabetic patients: relationship with 201Tl uptake and cardiac autonomic function *Ann Nucl Med* 1998; **12**(6): 323–331.
53. Stevens MJ, Dayanikli F, Allman KC, *et al*.. Scintigraphic assessment of regionalized defects in myocardial sympathetic innervation and blood flow regulation in diabetic patients with autonomic neuropathy. *J Am Coll Cardiol* 1998; **31:** 1575–1584.
54. Sugiyama T, Kurata C, Tawarahara K, Nakano T. Is abnormal iodine-123-MIBG kinetics associated with left ventricular dysfunction in patients with diabetes mellitus? *J Nucl Cardiol* 2000; **7:** 562–568.
55. Giordano A, Calcagni ML, Verrillo A, *et al*. Assessment of sympathetic innervation of the heart in diabetes mellitus using 123I-MIBG. *Metab Clin Exp* 2000; **13**(6): 350–355.
56. Freeman MR, Newman D, Dorian P, Barr A, Langer A. Relation of direct assessment of cardiac autonomic function with metaiodobenzylguanidine imaging to heart rate variability in diabetes mellitus. *Am J Cardiol* 1987; **80**(2): 247–250.
57. Pop-Busui R, Kirkwood I, Schmid H, *et al*. Sympathetic dysfunction in type 1 diabetes: association with impaired myocardial blood flow reserves and diastolic dysfunction. *J Am Coll Cardiol* 2004; **44:** 2368–2374.
58. Stevens MJ, Raffel DM, Allman KC, Schwaiger M, Wieland DM. Regression and progression of cardiac sympathetic dysinnervation in diabetic patients with autonomic neuropathy. *Metabolism* 1999; **48:** 92–101.
59. Schnell O, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. Reduced myocardial ^{123}I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. *Diabetes* 1996; **45:** 801–805.
60. Ziegler D, Weise F, Langen K-J, *et al*. Effect of glycemic control on myocardial sympathetic innervation assessed by [^{123}]metaiodobenzylguanidine scintigraphy: a 4-year prospective study in IDDM patients. *Diabetologia* 1998; **41:** 443–451.

Investigation methods for cardiac autonomic function in human research studies

Luciano Bernardi, Vincenza Spallone, Martin Stevens, Jannik Hilsted, Simona Frontoni, Rodica Pop-Busui, Dan Ziegler, Peter Kempler, Roy Freeman, Phillip Low, Solomon Tesfaye, Paul Valensi

SUPPORTING INFORMATION

In this on-line supplement we include detailed technical aspects of the different investigation methods and detailed evidences of their applications in human diabetes research.

1. Heart rate variability (HRV) measures

1.1 Methodology of HRV measures

HRV provides important information about autonomic modulation of the cardio-vascular system, and can be identified by "time- " and "frequency-domain" measures.

The "time domain" measures are simple indexes of global variability. Time domain indexes, and similarly, the "total power" (derived from spectral analysis), all give information about global heart rate variability and represent - at large - an index of parasympathetic activity. Many of these indexes (eg variance= standard deviation^2 or total power) do not have a normal distribution). Therefore, either square root or logarithmic transformation should be performed, or indexes with a more normal distribution (eg standard deviation, SDNN) should be preferred for statistical evaluations.

Spectral analysis of HRV. The so-called "frequency domain" indexes identify specific components of HRV via spectral analysis of RR interval fluctuations and other signals. The components of variability are divided into:

- low frequency components (LF): typically from 0.03 to 0.15 Hz (but different studies use a larger or narrower band);
- respiratory components (HF): in theory these normally span from 0.15 to 0.40 Hz, but respiration should be checked in order to calculate the exact range of respiratory power.

In order to extract a sympatho-vagal balance the ratio LF/HF can be calculated, and, in addition the relative contribution of LF and HF can be obtained by the % of LF and HF over the sum of LF+HF power [1-3];

- the remaining components of HRV (particularly at lower frequencies) which at the moment have no specific value.

Because power data have by definition a non-normal distribution, the data should be used statistically after logarithmic transformation.

Information derived from spectral analysis of RR interval alone (ie without simultaneous recording of respiration and blood pressure) can be severely biased by lack of control of respiration, incorrect interpretation (absolute LF power does not represent sympathetic activity), but when applied appropriately (ie with control or recording of respiration, simultaneous recording of BP) the relative proportion of LF and HF, or when LF and HF power is measured in relative terms (normalized units) do provide an approximate proportion of sympathetic and parasympathetic modulation, respectively. With spectral analysis, a more reliable evaluation of sympathetic modulation can be obtained by the LF power in BP or microcirculatory blood flow. Amongst possible algorithms for spectral analysis, the autoregressive method is preferable due to its ability to directly identify individual oscillatory components, but if it is applied without a special spectral decomposition algorithm, this decisive advantage is nullified as compared to the Fast Fourier transform, while

however it still retains the ability to obtain a correct spectrum even with a limited number of data points.

HRV measures can be obtained from short-and long-term recordings.

Short-term recording

Short-term recordings are typical of laboratory setting. As such the information derived from the ECG should be completed with recording of at least respiration and beat-to-beat BP. Recording may last 4-5 minutes and obtained preferably under spontaneous breathing, but the subjects should be instructed as to maintain a regular breathing and avoid even occasional deep breathings (sighs). In case this still occurs, then observation of the respiratory recordings can allow selection of a subset of data with stable breathing. Recordings should be preferably performed in the supine position. Additionnally recordings can similarly be obtained for 4-5 minutes in the upright position .

Subjects with special practices (eg yoga, martial arts) tend to naturally slow their breathing rate. If this very common occurrence is not recognised, then the interpretation of spectral peaks might be incorrect. Accordingly, strict definition of the HF band can lead to significant errors during special conditions if respiration is expected to vary beyond the imposed limits. In the absence of a respiratory signal the respiration should be carefully controlled. Controlled breathing at 15 breaths/minute is a simple procedure, but then care should be taken to avoid hyperpnoea (and the consequent hypocapnia).

Long-term (24 hour) data analysis.

In this case it is difficult to obtain additional signals like respiration or BP, and thus in most cases only R-R intervals are considered. Under this condition, important clinical information on the hourly changes and circadian rhythms in sympatho-vagal balance can be obtained by computing different spectra. However, if calculations are made over periods of 5 minutes and the results averaged (as suggested by the Task Force document [3]), this can be greatly influenced by continuous changes in sympatho-vagal balance and by occasional peaks of activation or artefacts. A more robust approach is to obtain one single spectrum for each hour of recording [4,5]. This is particularly helpful when using the autoregressive approach, as it extracts a smaller number of informations related to the most recurrent oscillations and provides a more robust estimate of the circadian changes of the autonomic modulation. Conversely, if faster changes are to be investigated (e.g. to test autonomic modifications caused by acute ischemic events), a data window of 5 minutes is typically adequate.

Other than spectral methods: non linear and time-varying methods

The two approaches described are simply the most widely used since at least 25 years for the study of the autonomic nervous system. However, newer additional spectral methods have been proposed. For example, time-varying spectral methods (eg Wigner-Ville transform, autoregressive time-varying algorithms, wavelet-based approaches and many others) can provide continuous evaluation of spectral data over time, often with very good time resolution. Alternatively, indexes derived from non-linear analysis are continuously growing and show a highly promising area of research. Unfortunately, none of these approaches has been so far used extensively enough to provide most of the basic information required to adopt a given methodology for a more or less routine clinical application. The greater complexity of these methods make a standardization even more difficult than with more traditional spectral methods, and, finally the physiologic meaning for most non-linear methods is still unknown. As such for the moment these approaches should remain useful research tool, particularly when fast changes in autonomic activity are to be monitored.

1.2 Evidences in diabetes for HRV measures

A large number of papers have used indexes derived from HRV and spectral analysis in diabetes. In summary, the most common result is a reduction in the global variability (a likely sign of parasympathetic dysfunction), and a specific reduction of the respiratory component of HRV (see [6,7] for recent comprehensive reviews). In diabetic neuropathy, unique information can be derived by applying this technique both in supine and upright (active or passive) position, in order to

observe the amount of change (or rather the lack of change) in sympatho-vagal modulation to the heart and to the arteries [8,9].

2. Baroreflex sensitivity (BRS)

2.1 Methodology of BRS measurement

The clinical approach is usually limited to the cardiac-vagal branch of the arterial baroreflex. More complex methodology (involving neck suction or microneurography, see section 3) is needed to test the sympathetic-vascular arm of the baroreflex.

The cardio-vagal BRS can be estimated with different methods which consist of measuring reflex change in HR (RR interval), in response to a change in systolic BP. The change in BP can be either spontaneous or provoked (e.g. by drugs or maneuvers like Valsalva).

This involves at least 1) a continuous measure of BP, and 2) a continuous and synchronised measure of HR (RR interval). The main drawback of BRS measurement is the cost of devices able to provide beat-to-beat blood arterial pressure. Noninvasive devices provide good estimates of BP fluctuations [10,11], and some of them contain software that provide BRS estimates using pulse interval as a surrogate for HR. While this is acceptable in healthy subjects, the measure is less precise in conditions of low HRV, as may occur in patients with CAN, and an estimate of HR from the electrocardiogram is much more reliable.

Three types of BRS methods are available.

1) Drug-induced changes in BP. This is the originally proposed technique [12]. The method requires a short infusion of a drug rapidly increasing (eg phenylephrine) or decreasing (eg amyl nitrite) BP. The reflex increase or decrease in RR interval is plotted against the transient rise or decrease in BP. Although the resulting relation is sigmoidal, there is a central part with good linearity that provides a linear slope, whose steepness measures the BRS (hence measured in ms/mmHg).

2) Physically-induced changes in BP. The most commonly used approach is the Valsalva manoeuvre: the changes in RR interval, particularly after the release of the manoeuvre, are plotted against the increase in BP and a linear slope (similar to that of the previous method) is obtained.

3) BRS from spontaneous BP fluctuations. Spontaneous fluctuations in BP are sensed by the arterial baroreceptors and thus the changes in HR are the result of the continuous action of the arterial baroreceptors. To obtain meaningful values, a recording of at least 2-4 minutes (depending on different methods and algorithms) of stable condition is sufficient. There are two more important and widely used approaches.

- The sequences technique [13] is based on the idea that whenever an increase in BP occurs with a lengthening in RR interval (or inversely a drop in BP occurring with a shortening in RR interval) this can be seen as the evidence of the intervention of arterial baroreflexes. When these sequences affect 3 or more consecutive heart beats then a linear slope is calculated. If the correlation coefficient is >0.85 then the slope is considered as significant. The baseline BRS is obtained by averaging the positive or negative slopes occurring during a stable period in the supine position.

- The methods based on spectral analysis [14] assume that a given fluctuation in BP is sensed by the arterial baroreceptors and results in a correspondent (i.e. at the same frequency) reflex fluctuation in RR interval. The fluctuations in RR and systolic BP are obtained by spectral analysis of the two signals. Different mathematical algorithms can be applied (the fast Fourier transform and the autoregressive ones are the most widely used). Accordingly, different indexes of BRS can be calculated by the ratio of the amplitudes of fluctuations in RR interval over the fluctuations at the same frequency in BP. A *low-frequency (LF) BRS* is obtained by the ratio of the amplitude of LF in RR interval over the amplitude of LF in systolic BP; similarly, a *high-frequency (HF) BRS* is obtained by the ratio amplitude of LF-RR interval/amplitude of LF-systolic BP. The *average of these two measures* is also calculated (also called *alpha coefficient or alpha BRS*). In order to ascertain that the fluctuations in RR interval are indeed related to the fluctuations in systolic BP a

coherence test is generally performed: if the coherence is >0.5 then it can be assumed that a given fluctuation is synchronous in both signals and can be used to calculate the baroreflex. A recent study however suggested that the coherence test may not be necessary [15]. The phase relationship could also provide useful information: if the RR interval fluctuation follows the fluctuation in BP this can be taken as an evidence of baroreflex-mediated change. Alternatively, the baroreflex index called "*transfer-function*" is the average of the combined spectrum obtained from RR interval and BP (cross-spectrum), divided by the spectrum of systolic BP, in the LF range (0.05-15Hz) [16]. - Combined variability. The above methods provide to some extent similar values, but different results can be obtained by slight modifications of each of the mathematical techniques adopted. Therefore, several previous studies have compared the different methods and obtained only a fair agreement between them [17-20]. On the other hand, none of the methods described have proved so far to have advantage over the others. Thus, although the clinical value of BRS is well established, and although all the methods proposed represent indeed valid measures of BRS, they in general provide different numerical results and numbers cannot be interchanged. However, a different clinical significance for the different methods does not clearly emerge so far.
Because none of the methods described appear to provide different clinical information, a practical approach is to perform a battery of the most common indexes that can be obtained from 4-5 minutes of spontaneous breathing, and report a central measure (average or median) of the tests performed, as recently suggested [21-22]. - Standard Deviations ratio. This new method estimates BRS by the simple ratio of the standard deviations of RR interval and systolic blood pressure. This approach was recently described and validated in 1409 subjects with different pathologies [22], and actually fits the average information provided by all other noninvasive spontaneous methods. The method is simple and easy to standardize, and thus appears very promising for clinical trials.
Normal BRS values with these methods range from 20-30 to 9-10 ms/mmHg, with a slightly non linear decrease as a function of ageing [23]. A more accurate general definition of normal reference ranges is difficult due to the scattering of results obtained with different methods.

2.2 Diagnostic accuracy of BRS measurement in diabetes

Among 23 studies on BRS in diabetes, only 2 studies were aimed at evaluating the diagnostic accuracy of BRS for CAN in comparison with the reference standard of cardiovascular tests (CARTs) [24-25]. One study (class III) in 108 mainly type 1 diabetic patients, using ROC analysis, found a low-moderate diagnostic accuracy of BRS in distinguishing between diabetic patients with or without CAN and control subjects (AUC 0.63-0.88 with CAN vs controls, 0.53-0.70 without CAN vs controls), HF gain of transfer analysis and slope of sequence method being the best discriminators between controls and patients without CAN [24]. Another study (class III-IV) failed to find in 53 type 2 patients a better sensitivity of BRS (sequence method) compared to CARTs (sensitivity 37% for CAN, sensitivity 83% for severe CAN) [25]. Most of the other studies (mainly class IV) reported lower mean values of BRS indexes in diabetic patients without CAN compared to control subjects as a demonstration of a higher sensitivity of BRS measurement compared to CARTs. Moreover, correlation between BRS and CARTs, association of impaired BRS with CAN, diabetic polyneuropathy, painful neuropathy [26] and microalbuminuria, and finally a predictive value of BRS - together with other autonomic indexes - on mortality [27] were documented in some studies.

3. Muscle sympathetic activity (MSNA)

3.1 Methodology and measures of MSNA

The measurement of MSNA is carried out by recording a sequence of bursts synchronous with the cardiac cycle. The bursts are integrated by suitable hardware or software, in order to obtain one burst present on each heart beat. The most frequently used method to extract sympathetic activity is

to count the bursts, either as bursts/beat or as bursts/minute. Because the latter does not consider heart rate, it could be influenced by higher or lower rates. More recently the spectral analysis has been applied also to these bursts and spectral bands similar to the HRV ones have been found. MSNA can be used to measure the sympathetic-vascular arm of the baroreflex [28].This is normally obtained by intravenous injections of sequential boluses of nitroprusside or phenylephrine. The vasoactive drugs induce changes of blood pressure that are counteracted by opposite changes of sympathetic activity. Less common methods consist of using steady-state infusions of the vasoactive substances or determining BRS from spontaneous BP variations. The spontaneous variations of resting BP and MSNA can also be quantified in a "threshold variability diagram" that defines the mean baroreflex setpoint and its variability. Another approach involves directly changing transmural pressure at the carotid sinus using pressure or suction at the neck.
When measured simultaneously, MSNA and norepinephrine spillover in the heart or the kidney in subjects at rest have shown in most studies significant positive correlations [29]. Thus, it appears that the inter-individual differences in resting sympathetic traffic are similar in nerves as in these organs. Similar results were obtained in recent studies in rabbits in which muscle, cardiac, and renal sympathetic activities were recorded simultaneously [30]. This parallelism is probably the main explanation of why the plasma concentrations of norepinephrine in forearm venous blood correlate with MSNA at rest: the norepinephrine concentration reflects spillover, not only from muscle nerves but also from nerves to other tissues that have similar inter-individual differences in sympathetic activity.

3.2 Evidence in diabetes for MSNA measures

The results from the relative small amount of studies in diabetic patients, with particular focus on autonomic neuropathy, suggest a different behaviour in type 1 vs. type 2 diabetic patients. Two studies [31,32] conducted in type 1 diabetic patients showed a reduced response of MSNA to OGTT or hyperinsulinaemic clamp study. One study [33] demonstrated in type 2 diabetic patients an increased mean frequency of sympathetic activity of s-MSNA (bursts/100beats). The significance of these studies is nevertheless hampered by the relatively small number of observations and the fact that this methodology is much better suited for measuring dynamic changes in sympathetic tone rather than its "resting" value, mainly because of the technical difficulties involved in obtaining a reproducible baseline recording.
Only one prospective study evaluated the prognostic role of MSNA in chronic heart failure patients [34], showing that only MSNA and forearm blood flow were significant independent predictors of mortality, in a multivariate analysis.

4. Catecholamine assessment

4.1 Methodological considerations of catecholamines assessment

Catecholamine assays for the measurement of plasma concentrations of norepinephrine (NE) and epinephrine (E) were developed in the early 1970's [35]. Resting supine plasma NE concentrations in antecubital venous plasma are 100-300 pg/ml in healthy subjects, and plasma E concentrations are tenfold lower. Under physiological conditions, plasma NE increases about tenfold during exercise, whereas plasma E increases 100 fold during hypoglycemia.
Catecholamine assays are double-isotope [35] or single isotope [36] techniques. They are comparatively expensive and may be acquired on a commercial basis, yet establishing catecholamine assays requires considerable technical expertise and, accordingly, reliable catecholamine assays are generally not available for routine purposes.Likewise, high pressure liquid chromatography (HPLC) with subsequent electrochemical detection requires expensive equipment and laboratory skills [37].The catecholamine level in blood is the resultant of NE release, re-uptake, regional blood flow and NE clearance rate. Estimates of NE release rate approximates sympathetic

nervous activity more accurately than net plasma concentrations of NE in conditions with altered NE clearance (e.g. damage to sympathetic nerves) [38]. However, the determination of NE release rate requires steady state infusion of ^{3}-H-norepinephrine, depends on regional extraction [39] and is suitable only for resting supine experiments.

4.2 Application in diabetes of catecholamines assessment

Postural changes

Plasma catecholamines have been studied in numerous experiments in diabetic postural hypotension. Resting supine NE concentrations are normal or low with a subnormal increase upon standing up in diabetic postural hypotension [40,41]. Occurring comparatively late in the course of disease, postural hypotension and blunted NE responses are found only in patients with widespread sympathetic damage. Accordingly, many diabetic patients have abnormal scores in other autonomic (i.e. vagal) tests and normal blood pressure and NE responses to standing – a fact which classifies the NE response to standing [41] as a definitive but rather insensitive marker of sympathetic neuropathy.

Exercise

Dynamic exercise elicits a brisk increase in plasma NE and E concentrations. A number of studies have demonstrated blunted NE and – to a lesser extent – E responses to exercise in patients with diabetic neuropathy [42,43]. Correct interpretation of plasma NE and E exercise data requires maximal oxygen uptake determination [42] – a fact which leaves catecholamine determinations during exercise as a research instrument rather than a diagnostic tool.

Hypoglycemia

Plasma E increases up to 100 fold during hypoglycemia in healthy subjects. Several studies have investigated the influence of diabetic neuropathy on plasma E concentrations during hypoglycemia – the overall conclusion being a reduction in plasma E responses to hypoglycemia probably due to sympathetic neuropathy affecting the preganglionic fibers innervating the adrenal medulla [44]. However, antecedent hypoglycemia also profoundly affects the E response to hypoglycemia, making interpretation of data difficult in the absence of strict standardization of metabolic control [45]. Thus, catecholamine response to hypoglycemia is not suited as a routine test for sympathetic neuropathy.

Sustained handgrip

Sustained handgrip (isometric exercise) induces a 3-5 fold increase in plasma NE concentrations in healthy subjects. Decreased plasma NE responses to sustained handgrip have been reported in several neurological diseases, [46] both intra- and inter individual NE variation being considerable [47]. Studies in diabetic neuropathy have focused on the diastolic BP response rather than on plasma NE responses. Thus, plasma catecholamine responses to sustained handgrip may be of interest in the diagnosis of diabetic neuropathy, but documentation is sparse.

Cold pressor test, Valsalva maneuver and mental stress

Cold pressor test and Valsalva maneuver both increase plasma NE concentrations. Low NE responses have been identified in diabetic patients with severe neuropathy [48]. Likewise, mental stress induces significant catecholamine (mainly E) responses which are blunted in diabetic neuropathy. The literature is sparse, and the diagnostic power of catecholamine measurements in these experimental conditions is unsettled.

5. Heart sympathetic imaging ad heart function tests

5.1 Different techniques and definition of different scintigraphic indexes

Two tracers have been utilised to characterise the sympathetic nervous system: [^{123}I]-metaiodobenzylguanidine (MIBG) and [^{11}C]-metahydroxyephedrine (HED). These tracers share a number of characteristics: they are non-metabolised, taken up into the postganglionic presynaptic

sympathetic nerve terminals, stored in synaptic vesicles and continuously recycled into and out of the neuron [49]. The most widely utilized tracer (>95% of published reports) is [^{123}I]-MIBG [50-52], a guanethidine derivative, and its retention can be assessed by single photon emission computed tomography (SPECT). In the heart, early and delayed images are obtained and [^{123}I]-MIBG uptake is quantified in counts.min^{-1}.ml^{-1} tissue and normalized to injected dose and body weight. Regional myocardial [^{123}I]-MIBG uptake is calculated, normalized to the highest left ventricular pixel value and expressed as a % of this value. The analysis is semi-quantitative and involves blinded observers scoring images to obtain a "defect score" which is measured in the various cardiac segments and a value for total uptake is calculated. Myocardial uptake ratios of MIBG in different myocardial segments [53], heart-to-mediastinum (H/M) [54] or heart-to-liver (H/L) [55] count ratios are often calculated. A washout rate in the whole myocardium is also obtained [56].

[^{11}C]-HED has been utilized far less extensively than [^{123}I]-MIBG. Like MIBG, neuronal retention requires intact vesicular storage and its retention is also susceptible to changes in synaptic norepinephrine levels making its retention potentially affected by changes in sympathetic tone. HED retention is significantly correlated with myocardial NE content and NE density [57]. In the transplanted human heart, studies using [^{11}C]HED have demonstrated increased tracer retention in the proximal anterior wall which correlated with the presence of axons on histological assessment [58] and with increased myocardial perfusion on sympathetic activation [59] thus confirming the neuronal specificity of HED-PET and its ability to assess presynaptic sympathetic nerve integrity. The myocardial retention of [^{11}C]-HED is performed using positron emission tomography (PET) either semi-quantitatively or more quantitatively [60]. The heterogeneity of regional left ventricular (LV) [^{11}C]-HED retention is compared to a database of normal non-diabetic values, with values exceeding a predefined cut-off value being defined as abnormal. Increased heterogeneity has been proposed to reflect LV denervation [61,62]. A proposed advantage of the technique over the semi-quantitative analysis using MIBG is that changes in absolute regional [^{11}C]-HED retention can be quantified by calculating a "retention index" [60] which corrects tracer retention for myocardial tracer delivery. To date no direct clinical comparisons of [^{11}C]-HED and [^{123}I]-MIBG have been reported.

Few studies have addressed the reproducibility of scintigraphic techniques. The coefficient of variation for MIBG analysis in subjects with diabetes has been reported to be 4% [62]. In subjects with type 1 diabetes, repeat [^{11}C]-HED scans have been performed 7-8 days apart. The mean $\pm$ 1 SD of the LV retention index for these studies has been reported to be 0.008$\pm$0.008 mL blood/min/mL tissue [63]. Assuming [^{11}C]-HED as the gold standard measure of cardiac sympathetic integrity, the ability of reflex CAN testing to correctly classify subjects ad free of CAN (specificity) has been reported to be 0.86 and the sensitivity of these tests is 0.67 [61].

5.2 Applications of neuroimaging in diabetes

Many cross-sectional studies have compared the ability of [^{123}I]-MIBG and [^{11}C]-HED to detect cardiac sympathetic denervation compared to conventional HRV testing. In 42 subjects with type 2 diabetes, [^{123}I]-MIBG myocardial scintigraphy was compared with power spectral analysis [56]. In the delayed images, the retention and washout of [^{123}I]-MIBG was found to be significantly lower in subjects with CAN based upon HRV testing. The delayed image of [^{123}I]-MIBG showed the strongest correlation with power spectral analysis ($r = 0.55$, $p < 0.01$). In a cross-sectional study of 24 subjects with type 1 diabetes without coronary artery disease and 10 healthy control subjects the ability of [^{123}I] MIBG to detect CAN was compared with HRV testing. Only 6 subjects had normal scans. Of the 18 subjects with deficits of [^{123}I]-MIBG retention, only 7 exhibited abnormal HRV testing ($p<0.01$). Subjects with retention deficits had an impaired LV response to exercise [53].

In subjects with type 1 diabetes classified into those with and without CAN based upon HRV testing, deficits of [^{123}I]-MIBG retention were identified in 70% of subjects without CAN and in 100% of subjects with CAN [64]. In another study of type 1 diabetes, deficits of [^{123}I]-MIBG retention are present before the development of deficits of HRV [65]. In a study of 20 diabetic patients who underwent 24 h HRV testing and [^{123}I]-MIBG-SPECT, the parasympathetic components of HRV were found to negatively correlate with the sum of defect scores in late images ($r = -0.4$ $p < 0.05$) [66]. In a study of 30 subjects with type 2 diabetes (15 with HRV based CAN), only 1 subject was found to have normal (homogeneous) LV [^{123}I]-MIBG uptake whereas 14 subjects had reduced global tracer uptake. The uptake of [^{123}I]-MIBG was significantly lower in the posterior wall of the LV than in other segments which correlated with indexes of HRV at rest and during deep breathing. The posterior uptake was found to be associated with coefficients of variation and RMSSD of HRV at rest and coefficients of variation, RMSSD, E-I difference and ratio, MCR and 30-15 ratio during deep breathing [67].

In a study of 51 subjects with type 2 diabetes (36 with HRV based CAN), subjects with CAN had significantly larger [^{123}I]-MIBG deficits than those without CAN. A significant inverse relationship was detected between the deficit of MIBG uptake and the sympathetic component of HRV (area under curve of LF band and total power) and the area under the HF component but not with the LF/HF ratio. The MIBG mismatch was the only measure to independently identify CAN using multivariate logistic regression analysis [68]. In a study of insulin dependent diabetiabetic subjects with and without HRV based CAN, global retention [^{123}I]-MIBG was found to be reduced in the diabetic subject groups compared to healthy non diabetic controls, with a visual defect score being significantly higher in the CAN+ subjects than in those without CAN or healthy controls, but not between the latter 2 groups [69]. In a study of 31 subjects with diabetes and 12 healthy controls classified by sensory testing into those with and without neuropathy, uptake and washout of [^{123}I]-MIBG was significantly and maximally increased in the inferior LV wall in the subjects with neuropathy [54]. A study of 45 subjects with diabetes (14 type 1, 31 type 2) demonstrated respiratory sinus arrhythmia (RSA) abnormalites in 12/45 subjects whereas deficits of [^{123}I]-MIBG were identified in 26/45 subjects. No differences were detected between type 1 and type 2 diabetes, and abnormal MIBG-SPECT was correlated with vibration threshold and abnormal heart RSA tests but not with abnormality in QTc [70]. A study of 12 subjects with diabetes found that the late (6-h) H/L uptake ratio of [^{123}I]-MIBG was significantly ($p < 0.05$) lower in the diabetic patients with CAN compared with those without CAN [55].

A single study of subjects with type 1 diabetes, showed that deficits of [^{11}C]-HED retention affected up to ~10% of the LV and were identified in 40% of healthy subjects with diabetes without deficits of autonomic reflex testing [61]. These deficits began distally in the LV in the infero-lateral walls, and with progression of CAN, spread proximally and circumferentially. The presence of an abnormal Valsalva ratio, or the presence of symptomatic orthostasis predicted a deficit of LV tracer retention of greater than 40%. In contrast, a different pattern of reduced [^{11}C]-HED retention has been found in asymptomatic type 1 diabetic subjects [71] who exhibited extensive global deficits of LV [^{11}C]-HED retention, despite good glycaemic control.

Deficits of LV [^{123}I]-MIBG retention have been observed in metabolically compromised newly diagnosed type 1 subjects and these deficits were attenuated by intensive insulin therapy [72] consistent with acute neuronal dysfunction. Longer term prospective observational studies have utilised scintigraphic techniques to explore the effects of metabolic control on cardiac denervation. In a 3 yr study utilising [^{11}C]-HED in subjects with type 1 diabetes, the instigation of improved glycaemic control resulted in a significant reduction in the extent of tracer retention deficits, which contrasted with progression in the group of subjects with less good metabolic control [63]. In parallel, two other studies utilised [^{123}I]-MIBG to assess the natural history of scintigraphic

deficits. In a series of 22 subjects with type 1 diabetes and stable metabolic control, abnormalities of [^{123}I]-MIBG retention were stable being detected in 18 subjects at baseline and in 21 subjects after 3 years [73]. In a prospective 4 year study of subjects with type 1 diabetes, global myocardial [^{123}I]-MIBG retention increased in subjects who achieved better glycaemic control compared to subjects with poorer control. No differences were detected in HRV testing [74].

The effects of metabolic control were evaluated in a cohort of 20 subjects with type 2 diabetes followed over a period of 2.1 years (8 improved control, 12 unchanged diabetes control). No association was found between any parameter of glucose control and cardiac [^{123}I]-MIBG uptake. However the use of insulin was found to significant correlate with the H/M ratio [75].

There is little information about the ability of interventions other than glucose control to effect scintigraphic tracer retention. In a 1 yr prospective randomised placebo controlled trial of 19 subjects with diabetes[^{123}I]-MIBG -SPECT was able to identify a significant difference between acetyl cartinine and placebo treated subjects [62].

Limited data report the effects of diabetes and CAN on [^{123}I]-MIBG uptake and retention in the lung. A study of 20 type 1 diabetic subects (11 with HRV based CAN, 9 without) found that increased [^{123}I]-MIBG washout rate from the lung was associated with CAN [76]. In a 5 yr, prospective study of the same group of type 1 dabetes, the washout rate of [^{123}I]-MIBG paralleled the decline of respiratory function but did not correlate with HbA1c [77]. However another study of 38 subjects with diabetes, reported that lung uptake of [^{123}I]-MIBG was increased and clearance rate decreased in subjects with HRV based CAN [78]. Finally a study of 44 nondiabetic and diabetic subjects with and without coronary artery disease found that prolonged lung retention of [^{123}I]-MIBG was associated with decreased cardiac sympathetic innervation in diabetic coronary artery disease patients [79].

5.3 Prognostic value in the general and diabetic populations

The potential mechanisms whereby cardiac dysinnervation may increase the risk of myocardial instability have begun to be addressed using scintigraphic techniques. [^{123}I]-MIBG has been exclusively ultilised for this purpose. The retention of [^{123}I]-MIBG is reduced in the inferior and posterior LV in subjects with both silent [80,81] or symptomatic [81] myocardial ischemia. Scintigraphy has been used to explore myocardial blood flow/innervation relationships in type 1 diabetic subjects. Reduced myocardial blood flow reserve has been found in subjects with both type 2 diabetes [82,83] and type 1 diabetes [83-86] and has been associated with impaired glucose control [87]. Maximally impaired vasodilatory capacity in response to adenosine stress in advanced diabetic CAN and to cold pressor testing in diabetic subjects occurs in the proximal myocardial segments [84].

Studies in diabetic patients have shown that altered myocardial [^{123}I]-MIBG uptake has been shown to correlate with alterations of the QT interval [69] and QT dispersion [87-90].

In subjects with diabetes, LV dysfunction has been shown to correlate with abnormal cardiac [^{123}I]-MIBG retention [53, 91, 92]. An abnormal LV response to exercise has been reported to correlate with defects of [^{123}I]-MIBG uptake in subjects with type 1 diabetes [93] and in 40 subjects with type 2 diabetes [94].

The relationships of early diabetic microangiopathy (manifested as background diabetic retinopathy or microalbuminuria) to alterations of cardiac sympathetic tone and LV function have been reported in healthy subjects with stable type 1 diabetes [71]. Deficits of LV [^{11}C]-HED retention were extensive and global in the subjects with preclinical microangiopathy despite preservation of cardiovascular autonomic reflex tests. Diastolic dysfunction was detected by 2-D echocardiography in 5/8 of these subjects.

The prognostic value of [^{123}I]-MIBG to predict cardiovascular events and death has been explored retrospectively over a period of 7.2 years in a cohort of 144 subjects with type 2 diabetes [95] using a H/M ratio. A reduced uptake ratio was found to be an independent predictor of overall mortality but not cardiac mortality. In a prospective 35 month study of a cohort of 205 nondiabetic and diabetic subjects with impaired LV function (LV ejection fraction <50%), diabetes as well as reduced [^{123}I]-MIBG uptake were independent predictors of cardiac death [96]. In a cohort of 82 subjects with type 2 diabetes and 11 healthy controls without known cardiovascular disease except for hypertension, differences in the washout rate for [^{123}I]-MIBG were found to predict mortality over a period of 18 months [97].

Abnormal retention of LV [^{123}I]-MIBG in subjects with diabetes has also been found to be predictive of sudden death [98].

References

1
Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation* 1998; **98**: 2640-2643.

2
Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol*. 1985; **248:** H151-153.

3
Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996; **93**: 1043-1065.

4
Bernardi L, Ricordi L, Lazzari P, Soldá P, Calciati A, Ferrari MR, Vandea I, Finardi G, Fratino P. Impaired circadian modulation of sympathovagal activity in diabetes. A possible explanation for altered temporal onset of cardiovascular disease. *Circulation* 1992; **86**: 1443-1452.

5
Spallone V, Bernardi L, Ricordi L, Soldà P, Maiello MR, Calciati A, Gambardella S, Fratino P, Menzinger G. Relationship between the circadian rhythms of blood pressure and sympathovagal balance in diabetic autonomic neuropathy. *Diabetes* 1993; **42**: 1745-1752.

6
Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation*. 2007; **115**: 387-397.

7
Schönauer M, Thomas A, Morbach S, Niebauer J, Schönauer U, Thiele H. Cardiac autonomic diabetic neuropathy. *Diab Vasc Dis Res*. 2008; **5**: 336-344.

8
Pagani M, Malfatto G, Pierini S, Casati R, Masu AM, Poli M, Guzzetti S, Lombardi F, Cerutti S, Malliani A. Spectral analysis of heart rate variability in the assessment of autonomic diabetic neuropathy. *J Auton Nerv Syst*. 1988; **23**: 143-153.

9
Gulli G, Fattor B, Marchesi M. Cross-spectral analysis of cardiovascular variables in supine diabetic patients. *Clin Auton Res*. 2005; **15:** 92-98.

10
Kemmotsu O, Ohno M, Takita K, Sugimoto H, Otsuka H, Morimoto Y, Mayumi T. Noninvasive, continuous blood pressure measurement by arterial tonometry during anesthesia in children. *Anesthesiology* 1994; **81**: 1162-1168.

11

Castiglioni P, Parati G, Omboni S, Mancia G, Imholz BP, Wesseling KH, Di Rienzo M. Broad-band spectral analysis of 24 h continuous finger blood pressure: comparison with intra-arterial recordings. *Clin Sci (Lond)* 1999; **97**: 129-139.

12
Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res* 1969; **24**: 109-121.

13
Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. A new approach to analysis of the arterial baroreflex. *J Hypertens Suppl* 1985; **3**: S79-S81.

14
Pagani M, Somers V, Furlan R, Dell'Orto S, Conway J, Baselli G, Cerutti S, Sleight P, Malliani A. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension* 1988; **12**: 600-610.

15
Pinna GD, Maestri R. New criteria for estimating baroreflex sensitivity using the transfer function method. *Med Biol Eng Comput* 2002; **40**: 79-84.

16
Pinna GD, Maestri R. Reliability of transfer function estimates in cardiovascular variability analysis. *Med Biol Eng Comput* 2001; **39**: 338-347.

17
Laude D, Elghozi JL, Girard A, Bellard E, Bouhaddi M, Castiglioni P, Cerutti C, Cividjian A, Di Rienzo M, Fortrat JO, Janssen B, Karemaker JM, Lefthériotis G, Parati G, Persson PB, Porta A, Quintin L, Regnard J, Rüdiger H, Stauss HM. Comparison of various techniques used to estimate spontaneous baroreflex sensitivity (the EuroBaVar study). *Am J Physiol* 2004; **286**: R226-R231.

18
Pitzalis MV, Mastropasqua F, Passantino A, Massari F, Ligurgo L, Forleo C, Balducci C, Lombardi F, Rizzon P. Comparison between noninvasive indices of baroreceptor sensitivity and the phenylephrine method in post-myocardial infarction patients. *Circulation* 1998; **97**: 1362-1367.

19
Maestri R, Pinna GD, Mortara A, La Rovere MT, Tavazzi L. Assessing baroreflex sensitivity in post-myocardial infarction patients: comparison of spectral and phenylephrine techniques. *J Am Coll Cardiol* 1998; **31**: 344-351.

20
Colombo R, Mazzuero G, Spinatonda G, Lanfranchi P, Giannuzzi P, Ponikowski P, Coats AJ, Minuco G. Comparison between spectral analysis and the phenylephrine method for the assessment of baroreflex sensitivity in chronic heart failure. *Clin Sci (Lond)* 1999; **97**:503-513.

21
Rosengård-Bärlund M, Bernardi L, Fagerudd J, Mäntysaari M, Af Björkesten CG, Lindholm H, Forsblom C, Wadén J, Groop PH; FinnDiane Study Group. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia*. 2009; **52**: 1164-1172.

22
Bernardi L, De Barbieri G, Rosengård-Bärlund M, Mäkinen VP, Porta C, Groop PH. New method to measure and improve consistency of baroreflex sensitivity values. *Clin Auton Res* 2010; **20**: 353-361.

23
Monahan KD. Effect of aging on baroreflex function in humans. *Am J Physiol*. 2007; **293**: R3-R12.

24
Ziegler D, Laude D, Akila F, Elghozi JL. Time- and frequency-domain estimation of early diabetic cardiovascular autonomic neuropathy. *ClinAuton Res* 2001; **11**: 369-376.

25
Tank J, Neuke A, Mölle A, Jordan J, Weck M. Spontaneous baroreflex sensitivity and heart rate variability are not superior to classic autonomic testing in older patients with type 2 diabetes. *Am J Med Sci* 2001; **322**: 24-30.

26
Gandhi RA, Marques JL, Selvarajah D, Emery CJ, Tesfaye S. Painful diabetic neuropathy is associated with greater autonomic dysfunction than painless diabetic neuropathy. *Diabetes Care*. 2010**; 33**: 1585-1590.

27
Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, Heethaar RM, Stehouwer CD. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care*. 2001; **24**: 1793-1798.

28
Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension*. 1998; **31**: 68-72.

29
Wallin BG, Thompson JM, Jennings GL, Esler MD. Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *J Physiol (Lond)* 1996; **491**: 881– 887.

30
Kamiya A, Kawada T, Yamamoto K, Michikami D, Ariumi H, Miyamoto T, et al. Dynamic and static baroreflex control of muscle sympathetic nerve activity (SNA) parallels that of renal and cardiac SNA during physiological change in pressure. *Am J Physiol* 2005; **289**: H2641–2648

31
Fagius J, Berne C. The increase in sympathetic nerve activity after glucose ingestion is reduced in type I diabetes. *Clin Sci (Lond)* 2000; **98**: 627-632.

32
Hoffman RP, Sinkey CA, Kienzle MG, Anderson EA. Muscle sympathetic nerve activity is reduced in IDDM before overt autonomic neuropathy. *Diabetes* 1993; **42**: 375-380.

33

Huggett RJ, Scott EM, Gilbey SG, Bannister J, Mackintosh AF, Mary DA. Disparity of autonomic control in type 2 diabetes mellitus. *Diabetologia*. 2005; **48**: 172-179.

34
Barretto AC, Santos AC, Munhoz R, Rondon MU, Franco FG, Trombetta IC, Roveda F, de Matos LN, Braga AM, Middlekauff HR, Negrão CE. Increased muscle sympathetic nerve activity predicts mortality in heart failure patients. *Int J Cardiol* 2009; **135**: 302-307.

35
Christensen NJ. Plasma catecholamines in juvenile diabetics. *Scand J Clin Lab Invest* 1971; **27**: 227-231.

36
Cryer PE. Isotope-derivative measurements of plasma norepinephrine and epinephrine in man. *Diabetes* 1976; **25**: 1071-1082.

37
Eldrup E, Christensen JN, Andersen J, Hilsted J. Plasma dihydroxyphenylalanine (DOPA) is independent of sympathetic activity in humans, *Eur J Clin Invest* 1989;**19**: 514-517.

38
Esler M, Jackman G, Bobik A, Kelleher D, Jennings G, Leonard P, Skews H, Korner P. Determination of norepinephrine apparent release rate and clearance in humans. *Life Sci* 1979; **25**: 1461-1470.

39
Hilsted J, Christensen NJ, Madsbad S. Whole body clearance of norepinephrine. The significance of arterial sampling and of surgical stress. *J Clin Invest* 1983; **71**: 500-505.

40
Hoeldtke RD, Cilmi KM. Norepinephrine secretion and production in diabetic autonomic neuropathy. *J Clin Endocrinol Metab* 1984; **59**: 246-252.

41
Hilsted J, Parving HH, Christensen NJ, Benn J, Galbo H. Hemodynamics in diabetic orthostatic hypotension. *J Clin Invest*. 1981; **68**: 1427-1434.

42
Hilsted J, Galbo H, Christensen NJ. Impaired responses of catecholamines, growth hormone, and cortisol to graded exercise in diabetic autonomic neuropathy. *Diabetes*. 1980; **29**: 257-262.

43
Bottini P, Tantucci C, Scionti L, Dottorini ML, Puxeddu E, Reboldi G, Bolli GB, Casucci G, Santeusanio F, Sorbini CA, et al. Cardiovascular response to exercise in diabetic patients: influence of autonomic neuropathy of different severity. *Diabetologia*. 1995; **38**: 244-250.

44
Hepburn DA, MacLeod KM, Frier BM. Physiological, symptomatic and hormonal responses to acute hypoglycaemia in type 1 diabetic patients with autonomic neuropathy. *Diabet Med*. 1993; **10**: 940-949.

45
Fanelli C, Pampanelli S, Lalli C, Del Sindaco P, Ciofetta M, Lepore M, Porcellati F, Bottini P, Di Vincenzo A, Brunetti P, Bolli GB. Long-term intensive therapy of IDDM patients with clinically overt autonomic neuropathy: effects on hypoglycemia awareness and counterregulation. *Diabetes*. 1997; **46**: 1172-1181.

46
Dessein PH, Joffe BI, Metz RM, Millar DL, Lawson M, Stanwix AE. Autonomic dysfunction in systemic sclerosis: sympathetic overactivity and instability. *Am J Med*. 1992; **93**: 143-150.

47
Vaz M, Kumar MV, Kulkarni RN, Rodrigues D, Shetty PS. Variability of cardiovascular and plasma noradrenaline responses to sustained isometric contraction in normal human subjects. *Clin Sci (Lond)*. 1993; **85**: 45-49.

48
Luft D, Maisch C, Hofmann-Krück V, Radjaipour M, Häring HU. Correlates of venous catecholamine concentrations in patients with type 1 diabetes during a cold pressor test. *Clin Auton Res* 2000; **10**: 131-137.

49
Raffel DM, Corbett JR, Schwaiger M, Wieland DM. Mechanism-based strategies for mapping heart sympathetic function. *Nucl. Med. Biol*. 1995; **22:** 1019-1026.

50
Wieland DM, Brown LE, Tobes MC, Rogers WL, Marsh DD, Mangner TJ, Swanson DP, Beierwaltes WH. Imaging the primate adrenal medulla with [I-^{123}] and [I-^{131}] metaiodobenzylguanidine: concise communication. *J. Nucl. Med*. 1981; **22:** 358-364.

51
Sisson JC, Wieland DM, Mangner TJ, Tobes MC, Jacques S. Metaiodobenzylguanidine as an index of the adrenergic system integrity and function. *J. Nucl. Med*.1987; **28:** 1620-1624.

52
Glowniak JV. Cardiac studies with metaiodobenzylguanidine: a critique of methods and interpretation of results. *J. Nucl. Med*. 1995; **36:** 2133-2137.

53
Kreiner G, Wolzt M, Fasching P, Leitha T, Edlmayer A, Korn A, Waldhausl W, Dudczak R. Myocardial m-[123I]iodobenzylguanidine scintigraphy for the assessment of adrenergic cardiac innervation in patients with IDDM. Comparison with cardiovascular reflex tests and relationship to left ventricular function. *Diabetes* 1995*;* **44:** 543-549.

54
Hattori N, Tamaki N, Hayashi T, Masuda I, Kudoh T, Tateno M, Tadamura E, Yonekura Y, Nakao K, Konishi J. Regional abnormality of iodine-123-MIBG in diabetic hearts. *J. Nucl Med* 1996; **37:** 1965-1990.

55

Mantysaari M, Kuikka J, Mustonen J, Tahvanainen K, Vanninen E, Lansimies E, Uusitupa M. Measurement of myocardial accumulation of 123I-metaiodobenzylguanidine for studying cardiac autonomic neuropathy in diabetes mellitus. *Clin Autonom Res* 1996; **6:**163-169.

56
Murata K, Sumida Y, Murashima S, Matsumura K, Takeda H, Nakagawa T, Shima T. A novel method for the assessment of autonomic neuropathy in type 2 diabetic patients: a comparative evaluation of 123I-MIBG myocardial scintigraphy and power spectral analysis of heart rate variability. *Diabet Med* 1996; **13:**266-272.

57
Ungerer M, Hartmann F, Karoglan M, Chlistalla A, Ziegler S, Richardt G, Overbeck M, Meisner H, Schömig A, Schwaiger M. Regional in vivo and in vitro characterization of autonomic innervation in cardiomyopathic human heart. *Circulation* 1998; **97:** 174-180.

58
Schwaiger M, Hutchins GD, Kalff V, Rosenspire K, Haka MS, Mallette S, Deeb GM, Abrams GD, Wieland D. Evidence for regional catecholamine uptake and storage sites in the transplanted human heart by positron emission tomography. *J Clin Invest* 1991; **87:** 1681-1690.

59
Di Carli MF, Tobes MC, Mangner T, Levine AB, Muzik O, Chakroborty P, Levine TB. Effects of cardiac sympathetic innervation on coronary blood flow. *New Engl J Med* 1997; **336:** 1208-1215.

60
Allman KC, Stevens MJ, Wieland DM., Hutchins GD, Wolfe ER, Greene DA, Schwaiger M. Noninvasive assessment of cardiac diabetic neuropathy by C-11 hydroxyephedrine and positron emission tomography. *J Am Coll Cardiol* 1993; **22,** 1425-1432.

61
Stevens MJ, Raffel DM, Allman K, Dayanikli F, Ficaro E., Sandford T, Weiland D, Pfeifer MA, Schwaiger M. Cardiac sympathetic dysinnervation in diabetes- an explanation for enhanced cardiovascular risk? *Circulation* 1998; **98:** 961-968.

62
Turpeinen, A K. Kuikka, J T. Vanninen, E. Yang, J. Uusitupa, M I. Long-term effect of acetyl-L-carnitine on myocardial 123I-MIBG uptake in patients with diabetes. *Clin Auton Res* 2000; **10:**13-16.

63
Stevens MJ, Raffel DM, Allman KC, Schwaiger M, Wieland DM. Regression and progression of cardiac sympathetic dysinnervation in diabetic patients with autonomic neuropathy. *Metabolism* 1999; **48,** 92-101.

64
Giordano A, Calcagni ML, Verrillo A, Pellegrinotti M, Frontoni S, Spallone V, Gambardella S. Assessment of sympathetic innervation of the heart in diabetes mellitus using 123I-MIBG. *Metab Clin Exp* 2000; **13:**350-355.

65

Schnell O, Kirsch C-M, Stemplinger J, Haslbeck M, Standl E. Scintigraphic evidence for cardiac sympathetic dysinnervation in long-term IDDM patients with and without ECG-based autonomic neuropathy. *Diabetologia* 1995**; 38:** 1345-1352.

66
Uehara A, Kurata C, Sugi T, Mikami T, Shouda S. Diabetic cardiac autonomic dysfunction: parasympathetic versus sympathetic Ann Nucl Med. 1999;13:95-100.

67
Schnell O. Hammer K. Muhr-Becker D. Ziegler A. Weiss M. Tatsch K. Standl E. Cardiac sympathetic dysinnervation in Type 2 diabetes mellitus with and without ECG-based cardiac autonomic neuropathy. *J Diabet Comp* 2002; **16**: 220-227.

68
Freeman MR, Newman D, Dorian P, Barr A, Langer A. Relation of direct assessment of cardiac autonomic function with metaiodobenzylguanidine imaging to heart rate variability in diabetes mellitus. Am J Cardiol 1987; **80:** 247-250.

69
Langen K-J, Ziegler D, Weise F, Piolot R, Boy C, Hubinger A, Gries FA, Muller-Gartner,H.-W. Evaluation of QT interval length, QT dispersion and myocardial m-iodobenzylguanidine uptake in insulin-dependent diabetic patients with autonomic neuropathy. *Clin. Sci.* 1997; **92:**325-333.

70
Claus D, Meudt O, Rozeik C, Engelmann-Kempe K, Huppert PE, Wietholtz H. Prospective investigation of autonomic cardiac neuropathy in diabetes mellitus**.** *Clin Auton Res*. 2002; **12:**373-378.

71
Pop-Busui R, Kirkwood I, Schmid H, Marinescu V, Schroeder J, Larkin D, Yamada E, Raffel D, Stevens MJ. Sympathetic dysfunction in type 1 diabetes: association with impaired myocardial blood flow reserves and diastolic dysfunction. *J. Am Coll. Cardiol***.** 2004; **44,** 2368-2374.

72
Schnell O, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. (1996) Reduced myocardial ^{123}I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. *Diabetes* 1996; **45:** 801-805.

73
Schnell O, Muhr D. Weiss M, Kirsch CM, Haslbeck M, Tatsch K, Standl E. Three-year follow-up on scintigraphically assessed cardiac sympathetic denervation in patients with long-term insulin-dependent (type I) diabetes mellitus. *J Diabet Comp* 1997; **11:**307-313.

74
Ziegler D, Weise F, Langen K-J, Piolot R, Boy C, Hubinger A, Muller-Gartner H-W, Gries FA. Effect of glycemic control on myocardial sympathetic innervation assessed by [^{123}]metaiodobenzylguanidine scintigraphy: a 4-year prospective study in IDDM patients. *Diabetologia* 1998; **41:** 443-451.

75

Nagamachi S, Jinnouchi S, Kurose T, Nishii R, Kawai K, Futami S, Tamura S, Matsukura S. Serial change in 123I-MIBG myocardial scintigraphy in non-insulin-dependent diabetes mellitus. *Ann Nucl Med* 2002; **16:**33-38.

76
Antonelli Incalzi R, Fuso L, Giordano A, Pitocco D, Maiolo C, Calcagni ML, Ghirlanda G. Neuroadrenergic denervation of the lung in type I diabetes mellitus complicated by autonomic neuropathy. Chest 2002; **121:**443-451.

77
Antonelli Incalzi R, Fuso L, Pitocco D, Basso S, Trové A, Longobardi A, Calcagni ML, Giordano A, Ghirlanda G. Decline of neuroadrenergic bronchial innervation and respiratory function in type 1 diabetes mellitus: a longitudinal study. Diabet MetabRes Rev 2007; **23:** 311-316.

78
Murashima S, Takeda K, Matsumura K, Yamakado K, Sakuma H, Kitano T, Nakagawa T, Ichihara T, Yamakado T, Murata K. Increased lung uptake of iodine-123-MIBG in diabetics with sympathetic nervous dysfunction. *J Nucl Med* 1998; **39:** 334-338.

79
Unlu M, Inanir S. Prolonged lung retention of iodine-123-MIBG in diabetic patients. *J Nucl Med* 1998; **39:**116-118.

80
Matsuo S, Takahashi M, Yoshida S, Inoue T, Nakamura Y, Mitsunami K, Kinoshita M. Characteristics of regional sympathetic innervation in diabetic patients with silent myocardial ischemia assessed by 123I-metaiodobenzylguanidine imaging. *Jap J Nucl Med* 1996; **33 :** 493-499.

81
Koistinen M J, Airaksinen K E, Huikuri H V, Linnaluoto M M, Heikkila J, Torniainen P. Ahonen A. No difference in cardiac innervation of diabetic patients with painful and asymptomatic coronary artery disease. *Diabet Care* 1996; **19:** 231-233.

82
Nitenberg A, Valensi P, Sachs R, Dali M, Aptecar E, Attali JR. Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes* 1993*;* **42:** 1017-1025.

83
Di Carli MF, Janisse J, Grunberger G, Ager J. Role of chornic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. *J Am Coll Cardiol* 2003; **41:** 1387-1393**.**

84
Stevens MJ, Dayanikli F, Allman KC, Sandford T, Wieland DM, Corbett J, Schwaiger M: Scintigraphic assessment of regionalized defects in myocardial sympathetic innervation and blood flow regulation in diabetic patients with autonomic neuropathy. *J Am Coll Cardiol* 1998; **31:** 1575-1584.

85

Pitkanen OP, Nuutila P, Raitakari OT, Ronnemaa T, Koskinen PJ, Iida H, Lehtimaki TJ, Laine HK, Takala T, Viikari JS, Knuuti J. Coronary flow reserve is reduced in young men with IDDM. *Diabetes* 1998; **47:** 248-254.

86
Di Carli MF, Bianco-Batlles D, Landa ME, Kazmers A, Groehn H, Muzik O, Grunberger G. Effects of autonomic neuropathy on coronary blood flow in patients with diabetes mellitus. *Circulation* 1999; **100:** 813-819.

87
Yokoyama I, Ohtake T, Momomura S, Yonekura K, Woo-Soo S, Nishikawa J, Sasaki Y, Omata M Hyperglycemia rather than insulin resistance is related to reduced coronary flow reserve in NIDDM. Diabetes 1998; **47:**119-124.

88
Shimabukuro M, Chibana T, Yoshida H, Nagamine F, Komiya I, Takasu N. Increased QT disperson and cardiac adrenergic dysinnervation in diabetic patients with autonomic neuropathy. *Am J Cardiol* 1996; **78:** 1057-1059.

89
Hara M, Sakino H, Katsuragi I, Tanaka K, Yoshimatsu H. Regulation of QT indices mediated by autonomic nervous function in patients with type 2 diabetes. *Int. J. Cardiol*. 2004; **96:** 375-379.

90
Takahashi N, Nakagawa M, Saikawa T, Watanabe M, Ooie T, Yufu K, Shigematsu S, Hara M, Sakino H., Katsuragi I, Tanaka K, Yoshimatsu H. Regulation of QT indices mediated by autonomic nervous function in patients with type 2 diabetes. *Int J Cardiol* 2004; **96**: 375-379.

91
Mustonen J, Mäntysaari M, Kuikka J, Vanninen E, Vainio P, Länsimies E, Uusitupa M. Decreased myocardial 123I-metaiodobenzylguanidine uptake is associated with disturbed left ventricular diastolic filling in diabetes. *Am Heart J* 1992; **123:**804-805.

92
Sugiyama T, Kurata C, Tawarahara K, Nakano T. Is abnormal iodine-123-MIBG kinetics associated with left ventricular dysfunction in patients with diabetes mellitus? *J Nucl Cardiol* 2000; **7**:562-568.

93
Scognamiglio R, Casara D, Avogaro, A. Myocardial dysfunction and adrenergic innervation in patients with Type 1 diabetes mellitus. *Metab Clin Exp* 2000; **13:** 346-349.

94
Nagaoka H, Iizuka T, Kubota S, Kato N, Suzuki T, Inoue T, Endo K, Nagai R. Depressed contractile response to exercise in diabetic patients in the absence of cardiovascular disease: relationship to adrenergic cardiac dysinnervation. *Nucl Med Comm* 1997; **18:** 761-770.

95
Nagamachi S, Fujita S, Nishii R, Futami S, Tamura S, Mizuta M, Nakazato M, Kurose T, Wakamatsu H Prognostic value of cardiac I-123 metaiodobenzylguanidine imaging in patients with non-insulin-dependent diabetes mellitus *J Nucl Cardiol* 2006; **13:** 34-42.

96
Nakata T, Wakabayashi T,. Kyuma M, Takahashi T, Hashimoto A, Ogata H, Tsuch K, Shimamoto K Prognostic implications of an initial loss of cardiac metaiodobenzylguanidine uptake and diabetes mellitus in patients with left ventricular dysfunction. *J Cardiac Failure*. 2003; **9:** 113-121.

97
Tamura K, Utsunomiya K, Nakatani Y, Saika Y, Onishi S, Iwasaka T. Use of iodine-123 metaiodobenzylguanidine scintigraphy to assess cardiac sympathetic denervation and the impact of hypertension in patients with non-insulin-dependent diabetes mellitus. *Eur J Nucl Med* 1999;. **26:** 1310-1316.

98
Kahn JK, Sisson JC, Vinik AI. Prediction of sudden cardiac death in diabetic autonomic neuropathy. *J Nucl Med* 1988; **29:** 1605-1606.