

Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease

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ABSTRACT

One of the most overlooked of all serious complications of diabetes is cardiovascular autonomic neuropathy. There is now clear evidence that suggests activation of inflammatory cytokines in diabetic patients and that these correlate with abnormalities in sympathovagal balance. Dysfunction of the autonomic system predicts cardiovascular risk and sudden death in patients with type 2 diabetes. It also occurs in prediabetes, providing opportunities for early intervention. Simple tests that can be carried out at the bedside with real-time output of information – within the scope of the practicing physician – facilitate diagnosis and allow the application of sound strategies for management. The window of opportunity for aggressive control of all the traditional risk factors for cardiovascular events or sudden death with intensification of therapy is with short duration diabetes, the absence of cardiovascular disease and a history of severe hypoglycemic events. To this list we can now add autonomic dysfunction and neuropathy, which have become the most powerful predictors of risk for mortality. It seems prudent that practitioners should be encouraged to become familiar with this information and apply risk stratification in clinical practice. Several agents have become available for the correction of functional defects in the autonomic nervous system, and restoration of autonomic balance is now possible. (*J Diabetes Invest*, doi: 10.1111/jdi.12042, 2013)

KEY WORDS: Cardiac autonomic neuropathy, Inflammation, Pathogenesis

INTRODUCTION

Diabetic autonomic neuropathy (DAN) is among the least recognized and understood complications of diabetes, despite its significant negative impact on survival and quality of life in people with diabetes^{1–3}. It is also a major source of increased cost in caring for the diabetic patient. The metabolic disorders of diabetes lead to diffuse and widespread damage of peripheral and autonomic nerves, and small vessels. When diabetic neuropathy affects the autonomic nervous system, it can damage the cardiovascular, gastrointestinal, genitourinary and neurovascular systems, and impair metabolic functions such as glucose counter-regulation. Of these, cardiac autonomic neuropathy (CAN) encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics. CAN is a significant cause of morbidity and mortality associated with a high risk of cardiac arrhythmias and sudden death³. Important advances in technology during the past decade now make it possible to identify the early stages of autonomic dysfunction with the use of objective standardized measures, allowing earlier intervention when reversal of the condition is still possible.

The present review will go over the most important clinical manifestations of CAN, and will discuss recent findings on cardiovascular autonomic neuropathy pathogenesis, diagnosis and treatment; and its relationship with the inflammatory process.

PATHOGENESIS AND THE ROLE OF THE AUTONOMIC NERVOUS SYSTEM ON INFLAMMATION

Traditionally, the organization of physiological control of the autonomic nervous system functions has relied on the division into two main branches: the sympathetic and parasympathetic nervous system. Stimulation of the sympathetic nervous system mediates physiological responses of fight and flight that are manifested as increases in heart rate and blood pressure (BP), mobilization of required energy stores, and heightened arousal. The major neurotransmitters are epinephrine, norepinephrine and dopamine, and these neurotransmitters mediate cellular responses by interacting with G-protein coupled adrenergic receptors ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\beta 3$ and dopaminergic receptors D1, D2, D3). In contrast, stimulation of the parasympathetic nervous system tends to produce effects that are, in general, opposite to those of the sympathetic nervous system, such as slowing of the heart rate, cardiac contractility and enhanced digestive functions. The principal neurotransmitter, acetylcholine, interacts with G-protein coupled muscarinic acetylcholine receptors M1–M5 and nicotinic ligand-gated ion channels (termed neuronal type I–III and muscle type IV)⁴.

The systems are distinguished by unique anatomical features. Parasympathetic neurons reside in the brainstem medulla, and

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sacral portions of the spinal cord and sympathetic neuronal cell bodies in the thoracic and lumbar spinal cord. Presynaptic myelinated axons are projected through cranial nerves and/or spinal nerves to synapses located in ganglia from which post-synaptic unmyelinated fibers are derived, which target the innervated organ. It is also important to realize that although at first glance these systems appear to have opposing effects; for example, the sympathetic nervous system increases the heart rate and the parasympathetic nervous system slows the heart rate, stimulation of both results in a greater increase in cardiac output, because sympathetic stimulation increases the ejection fraction and parasympathetic stimulation slows the heart, allowing increased efficiency of cardiac filling. Similarly, baroreceptors, perceive stretch in the aorta with increasing BP, send signals through the sensory component of the vagus, terminating in the nucleus of the tractus solitarius in the medulla. From here, presynaptic fibers of the sympathetic nervous system project to the ventrolateral nucleus of the medulla and parasympathetic presynaptic neurons to nucleus ambiguus in the medulla. Coordinated firing of both arms of the autonomic nervous system is necessary to respond to changes in BP, and if damage occurs, this coordination is reset to accommodate new levels, which might be too high or too low for a particular individual. Thus, for any given situation, one needs to consider the system in its entirety, and that damage to one or other component might not have as devastating an effect as damage to the whole system. Figure 1 shows the various physiological functions of the autonomic nervous system.

There has been increasing awareness that inflammation is central to the pathogenesis of diabetes and its complications, including macro- and microvascular disease. Still, the origin for this inflammatory process has not been clearly delineated. Although inflammation can cause extensive tissue damage and death, it is also essential in the protection of the host, and is a defense mechanism against trauma, infection and stress amongst other provocative agents. What controls this response to trauma and infection has been the focus of energetic pursuits over the past decade, and some potential answers have been revealed. It is now believed that the inflammatory response is controlled, in part, by neural circuitry of the autonomic nervous system. The afferent arc consists of nerves that sense injury and infection, and this in turn activates a cholinergic anti-inflammatory pathway that modulates the response and is the potential target for future therapies of diabetes⁵⁻⁷. The inflammatory reflex is activated when increasing levels of inflammatory mediators from exogenous (e.g. pathogen-associated molecular pattern [PAMPs]) or endogenous (e.g. damage-associated molecular pattern [DAMPs]) molecules stimulate receptors in the vagus nerve and associated glomus cells. This leads to afferent action potentials being transmitted along the vagus nerve to the brainstem and other nuclei that control outgoing signals in the vagus nerve. Increased activity in the vagus nerve to the spleen and other organs reduces the innate immune system's response to DAMPs and PAMPs, decreases the release of

cytokines, and suppresses inflammation. Just as stimulating the vagus nerve can slow heart rate, so too, increasing vagus nerve activity slows innate immunity (Figure 2). It is important to note here that the inflammatory reflex circuit utilizes both the vagus nerve (classically referred to as "parasympathetic") and the splenic nerve (classically referred to as "sympathetic"), although this classical differentiation is currently under debate⁷. The lymphoid organs of the immune system are innervated by cholinergic, catecholaminergic and dopaminergic neurons, as well as peptidergic neurons, and the neurotransmitters can interact with immune cells and alter their level of function. Watkins *et al.* discovered that sensory neurons sense the presence of inflammation in tissues⁸. These responses to interleukin (IL)-1 induced inflammation are mediated by the vagus nerve, and could be abolished by vagotomy or a selective competitive antagonist of the IL-1 receptor^{9,10}. In cardiovascular physiology, oxygen, glucose and other metabolites are sensed by specialized glomus cells in association with the autonomic nervous system, and on activation release dopamine and norepinephrine. These cause depolarization of the sensory fibers in the vagus nerve, which propagate to the brainstem initiating a motor efferent arc. It appears that IL-1 binds to the glomus cells, initiating the afferent arc; however, there are a number of other ligands derived from macrophages, monocytes and dendritic cells (PAMPs and DAMPs described earlier), which activate Toll-like receptors and lead to increased expression of nuclear factor-kappa B (NF- κ B) and the consequent release of inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and IL-6. Thus, it seems that the autonomic nervous system is also capable of initiating responses to tissue injury and inflammation, and can per se initiate a pro-inflammatory response.

In contrast, the efferent arc of this inflammatory response is termed the cholinergic anti-inflammatory pathway. The neurotransmitter acetylcholine interacts with the innate immune cells that express the nicotinic acetylcholine receptor subunit $\alpha 7$ ($\alpha 7$ nAChR). This has a tonic inhibitory role in the immune cells similar to the effects of the parasympathetic system on inhibition of heart rate. Exaggerated responses to inflammatory molecules are caused by vagotomy, and stimulation of the vagus downregulates the production of TNF- α , IL-1, IL-6 and IL-8, but does not alter the anti-inflammatory cytokine IL-10 and transforming growth factor- β (TGF- β). Thus, this is a defensive reflex protecting the organism from organ damage and death when exposed to syndromes of excessive cytokine release, such as infection, trauma and stress. One must therefore add a neuroregulatory role for the autonomic nervous system to the roles outlined above.

The traditional view of cardiac autonomic neuropathy is that there is an early phase of loss of parasympathetic function with increased resting heart rate and abnormalities in the expiration/inspiration ratio of heart rate variability. There might, however, be no parasympathetic denervation as such, but simply early augmentation of sympathetic tone. Early in the natural history of diabetes, there is impairment of

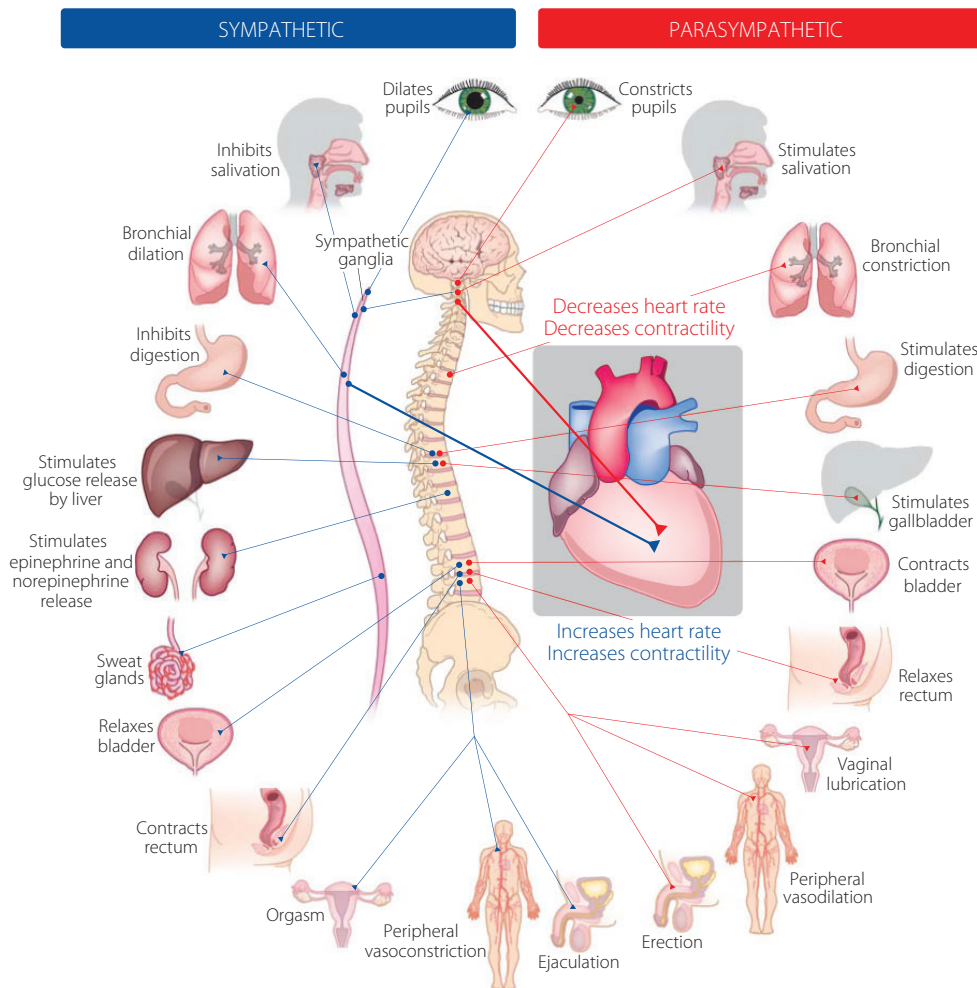


Figure 1 | Physiological functions of both the sympathetic and parasympathetic nervous system.

parasympathetic function, with a relative increase of sympathetic function causing an imbalance of the sympathetic/parasympathetic tone. Later, sympathetic denervation follows, beginning at the apex of the ventricles and progressing towards the base of the heart, leading to yet another imbalance, with an increase in propensity to arrhythmias¹¹. Even the prediabetic stage (i.e. impaired glucose tolerance) is associated with a decreased parasympathetic modulation of the heart and a shift toward augmented sympathetic tone. Thus, parasympathetic tone might decline with an autonomic imbalance shifting toward augmented sympathetic tone during the development from normal glucose tolerance to impaired glucose tolerance and finally diabetes¹².

The etiology of cardiovascular autonomic dysfunction seen early after the diagnosis of diabetes is not well understood. Hyperglycemia increases protein glycation and causes a gradual accumulation of advanced glycation end products (AGEs) in body tissues. These AGEs form on intra- and extracellular proteins, lipids and nucleic acids in complex arrangements

that lead to cross-linking. It has also been shown that an accumulation of AGEs in collagen tissue, such as skin, measured by skin autofluorescence correlates with the severity of peripheral and autonomic nerve abnormalities in diabetes, even before being clinically manifest¹³. This process is mediated through the pattern recognition receptor for AGE (RAGE). RAGE is constitutively expressed, but is induced by reactions known to initiate inflammation. Patients with diabetes often show elevated carboxymethyllysine (CML) levels that can bind and activate RAGE signaling¹⁴. Furthermore, AGE-RAGE interaction creates a chronic cascade of inflammation and tissue injury. Of further interest, RAGE knockout mice have a reduced capacity to develop neuropathy^{15,16}, and the soluble form of the AGE receptor, sRAGE, can act as a decoy molecule, thereby reducing the binding of ligands to RAGE and thereby inhibiting the inflammation cascade (Figure 3). Our own studies in Charcot neuroarthropathy have shown that the presence of severe somatic and autonomic neuropathy was related to reduced levels of sRAGE¹⁷.

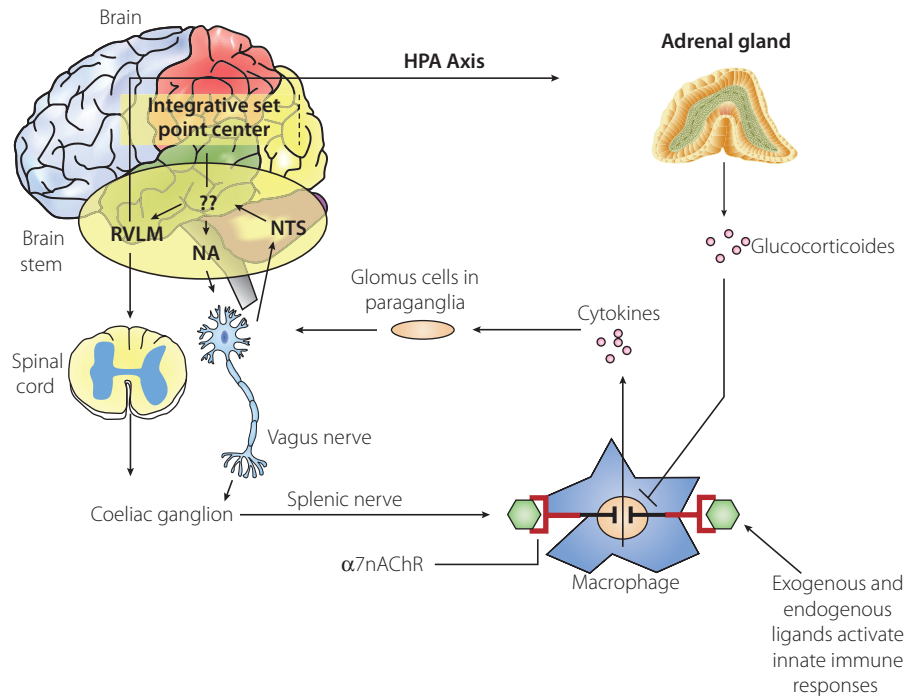


Figure 2 | Afferent sensory signals are transmitted by the vagus to the nucleus of the solitary tract (NTS) and polysynaptic receptors relay to the sympathetic through the rostral ventromedullary nucleus (RVLS), the parasympathetic nucleus ambiguus (NA) and the dorsal vagal nucleus. There is both sympathetic and parasympathetic output to the coeliac ganglion. The splenic nerve activates the inflammatory cascade in macrophages, which might be in the spleen, but occur diffusely throughout the body. Stimulation of the vagus inhibits this activation by acetylcholine binding to the nicotinic acetylcholine receptor subunit $\alpha 7$ receptor, which curtails the response. Also shown is the co-activation of the hypothalamic pituitary axis (HPA) with release of glucocorticoids, which also modulate the inflammatory response. It is unclear how this set point is determined in the body, but what is clear is that autonomic modulation of inflammation can be achieved by altering sympathetic/parasympathetic balance. Adapted from Tracey⁶.

In homeostasis, RAGE is expressed at low levels; but during biological stresses, such as inflammation and diabetes, RAGE expression is greatly upregulated as a direct result of AGE accumulation. Many studies have now shown that AGE-RAGE interactions alter intracellular signaling through pro-inflammatory cytokines, such as IL-6, TNF- α and free radicals that further propagate diabetic complications. We hypothesize that in metabolic syndrome and diabetes there is a constant increase in low-grade inflammation mediated by a large cadre of exogenous and endogenous ligands in combination with the central and autonomic nervous systems (see Figure 3). Thus, the loss of autonomic control with reduction of parasympathetic activity, which is the hallmark of loss of autonomic balance in diabetes, initiates a cascade of inflammatory responses that, if continued unabated, will culminate in considerable morbidity and mortality. Interestingly the two pathways, autonomic function and inflammation, might be linked.

We evaluated patients with newly diagnosed diabetes, established diabetes and healthy controls and measured a cadre of inflammatory markers in addition to time and frequency domain measures of autonomic function. Early in the development of autonomic dysfunction, there was loss of heart rate

variability (HRV), and this correlated with an increase in circulating markers of inflammation, such as C-reactive protein (CRP) and IL-6. Of great interest to us was the loss of HRV as well as the loss of sympathetic/parasympathetic balance, even before the advent of inflammation (Figure 4). Cardiac autonomic imbalance also correlated with markers of adipose tissue-derived inflammation (adiponectin-to-leptin ratio) and this was seen early in type 2 diabetes patients¹⁸. Analysis of 102 healthy middle aged individuals found an association between loss in HRV and IL-6-s levels¹⁹. Another study on 611 healthy adults showed an inverse correlation between HRV and CRP levels. The authors concluded that the effect could be attributed to low-grade inflammation as a result of a decrease in the cholinergic pathway of anti-inflammatory cascades with implications for addressing inflammation in the prevention and management of heart diseases²⁰. HRV, CRP and IL-6 levels were measured in 757 young adults, and HRV was significantly related to IL-6 and CRP levels. This was thought to be consistent with the notion that the cholinergic anti-inflammatory pathways exert a tonic inhibitor influence on innate immune responses and could be the potential target for future interventions. Furthermore, we found that newly diagnosed diabetic

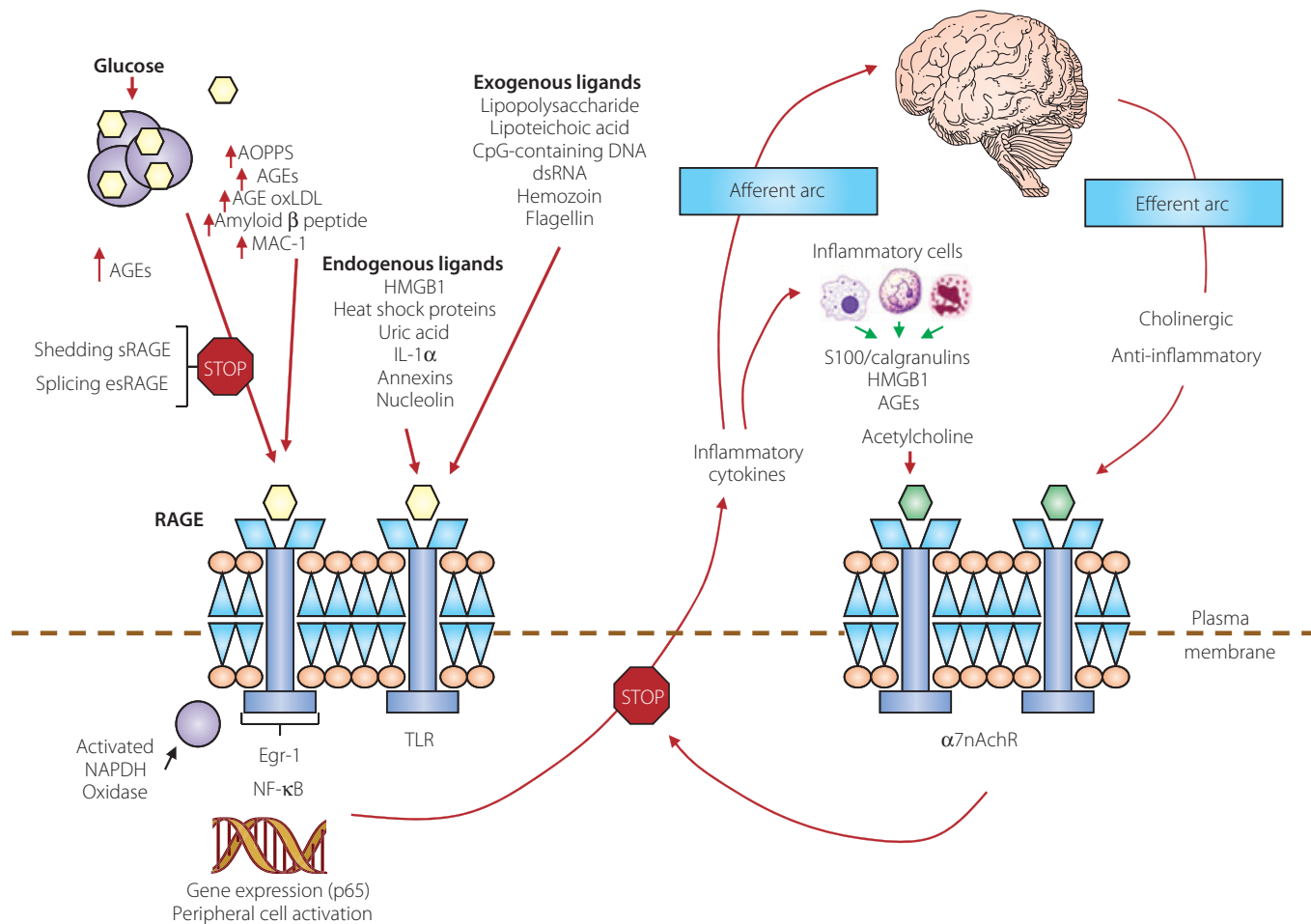


Figure 3 | The relationship between binding of ligands to the pattern recognition advance glycation end-product receptor (RAGE), and inflammation, gene expression, oxidative and nitrosative stress, and damage to the macro- and microvasculature. Elevated levels of glucose bind to proteins and form advance glycation end-products (AGEs), which bind to RAGEs. RAGE signaling activates nicotinamide adenine dinucleotide phosphate-oxidase and production of reactive oxygen species (ROS). Increased ROS increases advance oxidation protein products (AOPPs), more AGEs and AGE-modification of oxidized low-density lipoprotein (oxLDLs). Furthermore, increased ROS might deplete glutathione, thereby suppressing glyoxalase I activity, a mechanism favoring further AGE accumulation. AGEs, AOPPs, macrophage glycoprotein (MAC-1) and AGE-oxLDL ligands of RAGE sustain stimulation of RAGE, and these processes, together with increased ROS, activate key transcription factors, such as nuclear factor- κ B (NF- κ B) and Egr-1, which increase gene transcription factors and activate inflammatory mechanisms. The consequences include increased migration and activation of RAGE-expressing neutrophils, monocytes, macrophages, T-cells and dendritic cells. This results in the release of the pro-inflammatory RAGE ligands S100/calgranulins and high-mobility group protein box-1 (HMGB1). In this inflammatory environment, further AGEs might be formed as well. Through interaction with RAGE, these ligands magnify activation of NF- κ B, epidermal growth factor 1 (Egr-1) and other factors, thereby amplifying cellular stress and tissue damage leading to neurovascular dysfunction. Soluble RAGE (sRAGE) formed from cleavage of RAGE by disintegrins, such as ADAM 10, a metalloproteinase, and β - and γ -secretases. sRAGE or a spliced variant (esRAGE) compete for binding of ligands to RAGE, and a deficiency could theoretically initiate the sequence of events activating an inflammatory cascade with an increase in the expression of pro-inflammatory cytokines (E-selectin, endothelin-1 tissue factor, vascular endothelial growth factor, interleukin-6 and tumor necrosis factor- α) and damage to neurons, kidney, eye, the vasculature, and even bone. Increasing sRAGE or its administration could competitively reduce activation of the AGE/RAGE pathway and its consequences. In addition, endogenous and exogenous ligands bind to Toll-like receptors (TLR) also targeting NF- κ B, as well as inducing the secretin of pro-inflammatory cytokines, which activate afferent sensory neurons reaching the brainstem through axons in the vagus. This in turn activates the cholinergic anti-inflammatory efferent arc, which inhibits response in cytokine-producing immune cells and signals through the nicotinic acetylcholine receptor subunit α 7 (α 7nAChR). This in turn inhibits NF- κ B activation. Thus, the pathways of AGE/RAGE activation of the inflammatory cascade and the inflammatory ligands target activation of an inflammatory cascade that can be abrogated by either competing with the binding of ligands to RAGE or by vagal activation of the anti-inflammatory reflex. Thus, central to curtailing unbridled activation of the inflammatory cascade is the integrity of parasympathetic autonomic function or balance between the two arms of the autonomic nervous system. Reproduced from Vinik⁶⁵.

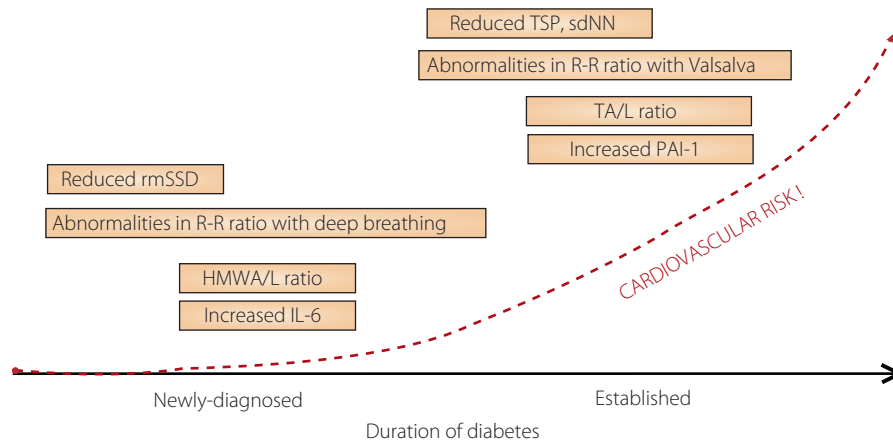


Figure 4 | The natural history of autonomic balance. HMWA/L, high molecular weight adiponectin/leptin ratio; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor 1; R-R, beat-to-beat interval; rmSSD, root mean square of the difference of successive beat-to-beat interval; sdNN, standard deviation of all normal beat-to-beat intervals; TA/L, total adiponectin/leptin ratio. Reproduced from Lieb *et al.*¹⁸.

patients had significantly lower HMW adiponectin/leptin ratios when compared with control subjects, and we saw significant correlations between the HMW adiponectin/leptin ratios and multiple measures of autonomic function¹⁸. Other studies have also shown interesting correlations between adiponectin and autonomic function. Wakabayashi and Aso studied 105 men and women with type 2 diabetes, and found that adiponectin concentrations correlated negatively with the low frequency/high frequency ratio²¹. These findings and the results in animal studies again suggest that the effects on adipose tissue cytokine release might be a consequence of autonomic dysfunction as opposed to the corollary. Clearly, a closer look at the relationship between adipose tissue cytokines, obesity, and the impact of weight reduction on the relationship between inflammation and HRV is warranted. With the explosive developments in global obesity and the proliferation of gastric bypass procedures on adults and even children, no doubt this will yield a fertile field for exploring these relationships and their potential for therapeutic intervention.

EPIDEMIOLOGY OF CAN

Establishing the prevalence of CAN has been hampered by heterogeneous and inadequate diagnostic criteria and population selection. The prevalence of CAN varies greatly and ranges from as low as 1.6–2.6% of the primary prevention cohort in the Diabetes Control and Complications Trial (DCCT)²², to as high as 90% of patients with longstanding type 1 diabetes who were potential candidates for a pancreas transplant²³. In a large cohort of patients with type 1 and type 2 diabetes, Ziegler *et al.*, using predefined criteria of HRV and spectral analysis of the beat-to-beat (R-R) intervals, found that 25.3% of patients with type 1 diabetes and 34.3% of those with type 2 diabetes had abnormal findings²⁴. A recent Consensus Panel on Diabetic Neuropathy, after extensive review of the literature, concluded that the prevalence of confirmed CAN in unselected people with

type 1 and type 2 diabetes is approximately 20%, but it can be as high as 65% with increasing age and diabetes duration. Clinical correlates or risk markers for CAN are age, diabetes duration, glycemic control, microvascular complications (peripheral polyneuropathy, retinopathy and nephropathy), hypertension and dyslipidemia. Established risk factors for CAN are glycemic control in type 1 diabetes, and a combination of hypertension, dyslipidemia, obesity and glycemic control in type 2 diabetes²⁵. Additional factors that have emerged as identifying susceptibility to cardiovascular events with intensification of glycemic control include: duration of diabetes >12–15 years, impaired renal function, coronary artery calcification, previous cardiovascular event, being African-American, being female, a history of neuropathy or numb feet and loss in HRV^{11,26,27}.

The Cardiovascular Autonomic Neuropathy Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy recommend CAN screening to all asymptomatic type 2 diabetic patients at diagnosis and all type 1 diabetic patients after 5 years of disease, in particular those at greater risk for CAN because of a history of poor glycemic control (hemoglobin A1c >7%) or the presence of one major cardiovascular risk factor (among hypertension, dyslipidemia and smoking), or the presence of macro- or microangiopathic complications (level of evidence B). They also suggested that CAN screening might be required in asymptomatic patients for preoperative risk assessment before major surgical procedures (level of evidence C)²⁵.

MORBIDITY AND MORTALITY IN CARDIAC AUTONOMIC NEUROPATHY

CAN is a significant cause of morbidity and mortality associated with a high risk of cardiac arrhythmias and sudden death, possibly related to silent myocardial ischemia. Cardiovascular disease remains the main cause of excess mortality among patients with type 1 and type 2 diabetes. Reduced HRV as a marker of autonomic dysfunction has been shown to have dire

consequences in terms of morbidity (e.g. progression of coronary atherosclerosis)²⁸ and mortality independent of cardiovascular risk factors in various populations, including those with prediabetes and diabetes^{2,29–33}. In type 1 diabetic patients, there is a fourfold increase risk of death³⁴. CAN is significantly associated with overall mortality^{2,35}, and in some – but not all – studies with morbidity, such as silent myocardial ischemia, coronary artery disease, stroke, diabetic nephropathy progression and perioperative morbidity.

In the Detection of Ischemia in Asymptomatic Diabetic Subjects (DIAD) study, a diminished Valsalva heart rate ratio (a measure of CAN) was strongly associated with silent myocardial ischemia, independent of more traditional risk factors including sex, age, hypertension and smoking³⁶. In the European Epidemiology and Prevention of Diabetes (EURODIAB) study, autonomic dysfunction was present in one-third of type 1 diabetic patients, and was strongly associated with coexisting cardiovascular disease (CVD) after adjustment for age, hemoglobin A1c and duration of diabetes³⁷. Results from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial again confirmed the association of CAN and mortality. These investigators showed that the individuals in this trial with baseline CAN were 1.55–2.14 times as likely to die as individuals without CAN²⁶. Furthermore, CAN in the presence of peripheral neuropathy was the highest predictor of CVD mortality (i.e. hazard ratio [HR] 2.95, $P < 0.008$). Indeed, combining indexes of autonomic dysfunction have been shown to be associated with the risk of mortality^{2,32,38}.

Thus, CAN assessment can be used for cardiovascular risk stratification in patients with and without established cardiovascular disease; as a marker for patients requiring more intensive monitoring during the perioperative period and other physiological stresses; and as an indicator for more intensive pharmacotherapeutic and lifestyle management of comorbid conditions.

CLINICAL MANIFESTATIONS

Traditionally, autonomic neuropathy has been considered a chronic complication that occurs only after long-term diabetes mellitus, but evidence now shows that neuropathic complications arise at least as early as the time of diagnosis of diabetes mellitus³⁹.

CAN is one of the most serious complications of diabetic autonomic neuropathy. As described earlier, it is found in one-quarter of type 1 and one-third of type 2 diabetic patients, is associated with increased mortality and silent myocardial ischemia, and might even predict the development of stroke. CAN might even have greater predictive power than traditional risk factors for cardiovascular events.

CAN has been linked to resting tachycardia, postural hypotension, exercise intolerance, enhanced intraoperative or perioperative cardiovascular liability, increased incidence of asymptomatic ischemia, myocardial infarction and decreased rate of survival after myocardial infarction. Cardiac autonomic

dysfunction can affect daily activities of individuals with diabetes, might affect their quality of life and might invoke potentially life-threatening outcomes.

Cardiovascular Symptoms and Signs

Resting Tachycardia

Whereas abnormalities in HRV are early findings of CAN, resting tachycardia and a fixed heart rate are characteristic late findings in diabetic patients with vagal impairment. Resting heart rates of 90–100 b.p.m. and occasional heart rate increments up to 130 b.p.m. occur. The highest resting heart rates have been found in patients with parasympathetic damage, occurring earlier in the course of CAN than sympathetic nerve dysfunction; in those with evidence for combined vagal and sympathetic involvement, the rate returns toward normal, but remains elevated. A fixed heart rate that is unresponsive to moderate exercise, stress or sleep indicates almost complete cardiac denervation. A blunted heart rate response to adenosine receptor agonists was described in both patients with diabetes and patients with metabolic syndrome, and attributed to earlier stages of CAN⁴⁰. Higher resting heart rates (>78 b.p.m.) compared with lower resting heart rates (<58 b.p.m.) and a rise in heart rate with time have been shown to be powerful, independent risk predictors for all-cause and CVD mortality in several prospective cohorts⁴¹. The prognostic value of resting HR is a useful tool for cardiovascular risk stratification and as a therapeutic target in high-risk patients^{11,25,35}.

Exercise Intolerance

Autonomic dysfunction impairs exercise tolerance, reduces response in heart rate and BP, and blunts increases in cardiac output in response to exercise. Diabetic patients who are likely to have CAN should be tested for cardiac stress before undertaking an exercise program³. Patients with CAN need to rely on their perceived exertion, not heart rate, to avoid hazardous levels of intensity of exercise.

Intraoperative Cardiovascular Liability

Perioperative cardiovascular morbidity and mortality are increased two- to threefold in patients with diabetes. Compared with non-diabetic subjects, diabetic patients undergoing general anesthesia might experience a greater degree of decline in heart rate and BP during induction of anesthesia, and less of an increase after tracheal intubation and extubation. Vasopressor support is required more often in diabetic individuals with CAN than in those without CAN⁴². The normal autonomic response of vasoconstriction and tachycardia does not completely compensate for the vasodilating effects of anesthesia. There is an association between CAN and more severe intraoperative hypothermia⁴³ that can result in decreased drug metabolism and impaired wound healing. Reduced hypoxic-induced ventilatory drive⁴⁴ requires preoperative CAN screening for loss of HRV. Preoperative cardiovascular autonomic screening might provide useful information for

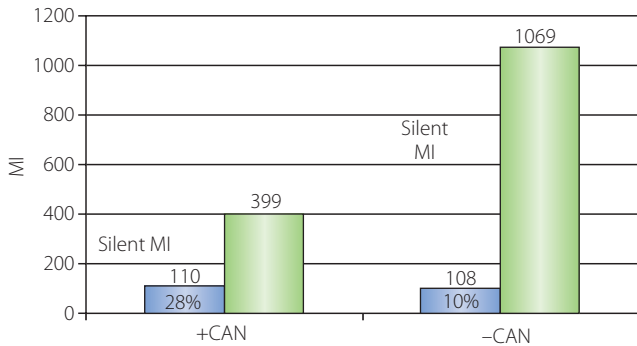


Figure 5 | Prevalence rate ratios and 95% confidence intervals for the association between cardiac autonomic neuropathy (CAN) and silent myocardial ischemia (MI) in 12 studies. Modified from Vinik *et al.*³.

anesthesiologists planning the anesthetic management of diabetic patients, and identify those at greater risk of intraoperative complications³.

Orthostatic Hypotension

Orthostatic hypotension is defined as a fall in BP (i.e. >20 mmHg for systolic or >10 mmHg for diastolic BP) in response to postural change, from supine to standing⁴⁵. In patients with diabetes, orthostatic hypotension is usually a result of damage to the efferent sympathetic vasomotor fibers, particularly in the splanchnic vasculature⁴⁶. Patients with orthostatic hypotension typically present with lightheadedness and presyncope symptoms. Symptoms, such as dizziness, weakness, fatigue, visual blurring, and neck pain, also might be a result of orthostatic hypotension. Many patients, however, remain asymptomatic despite significant falls in BP. Orthostatic symptoms can also be misjudged as hypoglycemia and can be aggravated by a number of drugs, including vasodilators, diuretics, phenothiazines, and particularly tricyclic antidepressants and insulin³.

Silent Myocardial Ischemia/Cardiac Denervation Syndrome

The presence of both symptomatic and asymptomatic coronary artery disease (CAD) is increased in diabetic patients, and subclinical neuropathy is an important cause of silent ischemia in patients with diabetes. Figure 5 summarizes the results of 12 cross-sectional studies comparing the presence of silent myocardial ischemia, generally measured by exercise stress tests, between diabetic individuals with and without CAN. Five of the 12 studies showed a statistically significant increased frequency of silent myocardial ischemia in those with CAN compared with those without CAN. The point estimates for the prevalence rate ratios in these 12 studies ranged from 0.85 to 15.53. Mantel-Haenszel estimates for the pooled prevalence rate risk for silent myocardial ischemia in meta-analysis was 1.96, with a 95% confidence interval of 1.53–2.51 ($P < 0.001$; $n = 1468$ total subjects), showing a consistent association between CAN and the presence of silent myocardial ischemia³.

Silent ischemia in diabetic patients can either result from CAN, from autonomic dysfunction attributable to coronary artery disease itself, or from both. In the Framingham Study, the rates of unrecognized myocardial infarction (MI) were 39% in diabetic patients and 22% in non-diabetic subjects, but the difference was not significant⁴⁷. In a survey from the National Registry of Myocardial Infarction 2 (NRM-2), of 434,877 patients presenting with MI, 33% did not have chest pain. Among those presenting without chest pain, 32% had diabetes vs 25.4% in the group with chest pain⁴⁸. The mechanisms of painless myocardial ischemia are, however, complex and not fully understood. Altered pain thresholds, subthreshold ischemia not sufficient to induce pain and dysfunction of the afferent cardiac autonomic nerve fibers have all been suggested as possible mechanisms⁴⁹. In the DIAD study of 1,123 patients with type 2 diabetes, cardiac autonomic dysfunction was a strong predictor of ischemia³⁶.

Features of a MI in patients with CAN are silence, cough, nausea and vomiting, dyspnea, tiredness, and electrocardiogram (ECG) changes. Reduced appreciation for ischemic pain can impair timely recognition of myocardial ischemia or infarction, thereby delaying appropriate therapy. Thus, patients with CAN warrant more careful attention and cardiovascular autonomic function testing might be an important component in the risk assessment of diabetic patients with coronary artery disease³.

Autonomic Cardiomyopathy

CAN might be associated with abnormalities in left ventricle (LV) systolic and, particularly, diastolic functions in the absence of cardiac disease in diabetic patients. Studies have shown a significant correlation of the severity of CAN with reduced peak diastolic filling rate and with an augmented atrial contribution to diastolic filling as assessed by Doppler echocardiography. It is difficult to judge, however, whether CAN is an independent contributor to these abnormalities, because other factors, such as interstitial myocardial fibrosis and microangiopathic or metabolic changes, might also be responsible for LV dysfunction.

CAN has been associated with LV diastolic dysfunction (LVDD) at rest, both in patients with long-term type 2⁵⁰ or type 1 diabetes⁵¹. LVDD can progress to heart failure, mainly with preserved LV systolic function (diastolic heart failure), which is also related to high morbidity and mortality rates^{52,53}. The pathophysiology of LVDD includes delayed relaxation, impaired LV filling and/or increased stiffness⁵⁴. In patients with CAN, vagal impairment can lead to a relative predominance of sympathetic activity in the sympathovagal balance. Sympathetic overactivity stimulates the renin-angiotensin-aldosterone system and increases heart rate, stroke volume and peripheral vascular resistance, thus contributing to LV dysfunction⁵⁵.

DIAGNOSIS

Cardiovascular Autonomic Balance

Autonomic balance involves complex interactions with several physiological mechanisms that act to maintain heart rate and

BP within normal limits. Recent investigations have suggested that autonomic dysfunction (e.g. heightened activity of the sympathetic nervous system and suppressed activity of the parasympathetic nervous system) impairs the ability of the autonomic nervous system (ANS) to regulate the cardiovascular system. Thus, autonomic imbalance might be a key component involved in both the etiology and the clinical course of cardiovascular disease. What is also emerging is that one needs to distinguish the difference between autonomic imbalance and clear evidence of autonomic neuropathy. Autonomic imbalance produces a number of interesting and trying clinical situations, such as orthostatic tachycardia, orthostatic bradycardia and hypotension, and can be responsible for predisposition to arrhythmias and sudden death¹¹. The most interesting implication of loss of autonomic imbalance derives from the catastrophic increase of sudden death in the ACCORD study with intensive lowering of blood glucose in which the combination of numbness of the feet and loss of heart rate variability increased the susceptibility to an event, with a risk ratio (RR) of 4.43^{11,27}.

Diagnostic Tests

Methods of CAN assessment in clinical practice include assessment of symptoms and signs, cardiovascular autonomic reflex tests (CARTs) based on heart rate and BP, and ambulatory BP monitoring (ABPM)²⁵.

CARTs assess cardiovascular autonomic function through time-domain HR response to deep breathing, Valsalva maneuver and postural change, and by measuring the end-organ response; that is, heart rate and BP changes. Although indirect autonomic measures, they are considered the gold standard in autonomic testing. Heart rate variations during deep breathing, Valsalva maneuver and lying-to-standing (heart rate tests) are indices mainly of parasympathetic function; whereas the ortho-

static hypotension, the BP response to a Valsalva maneuver and sustained isometric muscular strain provide indices of sympathetic function. These tests are non-invasive, safe, clinically relevant (they correlate with tests of peripheral nervous system function), easy to carry out, sensitive, specific, reproducible and standardized, and therefore they are considered consolidated, gold-standard measures of autonomic function.

The Toronto Consensus Panel on Diabetic Neuropathy²⁵ have concluded the following regarding diagnosis of CAN (Table 1):

- The following CARTs are the gold standard for clinical autonomic testing: heart rate response to deep breathing, standing and Valsalva maneuver, and BP response to standing (class II evidence)
- These CARTs are sensitive, specific, reproducible, easy to carry out, safe and standardized (classes II and III)
- The Valsalva maneuver is not advisable in the presence of proliferative retinopathy and when there is an increased risk of retinal hemorrhage (class IV)
- CARTs are subject to a number of confounding or interfering factors (class III)
- Age is the most relevant factor affecting heart rate tests (class I)
- A definite diagnosis of CAN and CAN staging requires more than one heart rate test and the orthostatic hypotension test (class III).

CAN Diagnosis and Staging

- The presence of one abnormal cardiovagal test result identifies the condition of possible or early CAN, to be confirmed over time.
- At least two abnormal cardiovagal results are required for a definite or confirmed diagnosis of CAN.

Table 1 | Cardiovascular autonomic tests and suggested indications for their use

Test	Clinical diagnosis	Research	End-point in clinical trials
Heart rate cardiovascular tests	Yes	Yes	Yes
Orthostatic hypotension test	Yes	Yes	No (low sensitivity)
QT interval	Yes (additional information and risk stratification)	Yes	No (low sensitivity)
Ambulatory blood pressure monitoring for dipping status	Yes (risk stratification)	Yes	No (low sensitivity)
HRV time and frequency domain indices	Yes (additional information and risk stratification)	Yes	Yes
Baroreflex sensitivity measures	No (early additional information and risk stratification but low availability)	Yes	Yes
Scintigraphic studies	No (low availability, limited standardization)	Yes	Yes
Muscle sympathetic nerve activity	No (low availability, limited data in cardiovascular autonomic neuropathy)	Yes	Possible (used in lifestyle intervention trials in obesity)
Catecholamine assessment	No (low availability)	Yes	Possible (used in lifestyle intervention trials in obesity)

HRV, heart rate variability. Reproduced from Tesfaye *et al.*⁵⁷.

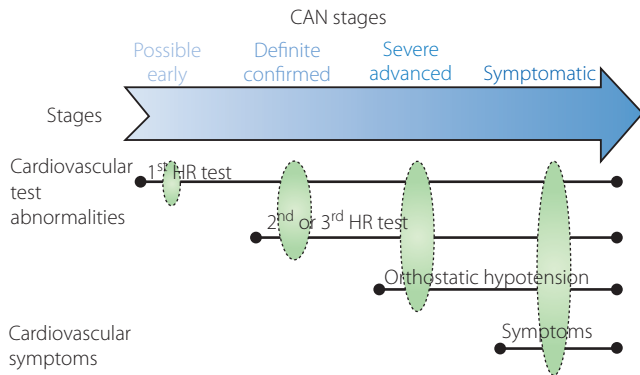


Figure 6 | Stages of cardiovascular autonomic neuropathy (reproduced from Spallone *et al.*²⁵).

- The presence of orthostatic hypotension in addition to heart rate test abnormalities identifies severe or advanced CAN (level B).
- CARTs allow CAN staging from early to advanced involvement (level C).
- Progressive stages of CAN are associated with increasingly worse prognosis (level B; Figure 6).

Analysis of HRV, with analysis of respiratory activity, independently and simultaneously measures parasympathetic and sympathetic activity⁵⁶, and thereby provides information with regard to autonomic balance of the cardiovascular system. Power spectral analysis of HRV can be carried out under resting conditions with demonstration of low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz) components. The LF component of the power spectrum of HRV primarily reflects sympathetic activity, whereas the HF component (also termed the respiratory-frequency [RF]) primarily reflects parasympathetic activity. LF/RF ratios are calculated and provide a measure of sympathetic/parasympathetic activity (Figure 7)³.

Diabetic patients with features of cardiac autonomic dysfunction, such as unexplained tachycardia, orthostatic hypotension and poor exercise tolerance, or with other symptoms of autonomic dysfunction should be evaluated for the presence of autonomic dysfunction. Screening for autonomic dysfunction should be carried out at the diagnosis of type 2 diabetes and 5 years after the diagnosis of type 1 diabetes, particularly in patients at greater risk because of a history of poor glycemic control, cardiovascular risk factors, diabetic peripheral neuropathy (DPN) and macro- or microangiopathic diabetic complications. Although a number of tests for autonomic dysfunction are in the research arena, some have been shown to be sufficiently robust to be incorporated into routine clinical care (Table 1)^{25,57}.

CAN Testing for Clinical Trials and Research

The “time-domain” measures are simple indexes of global HR 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 para-

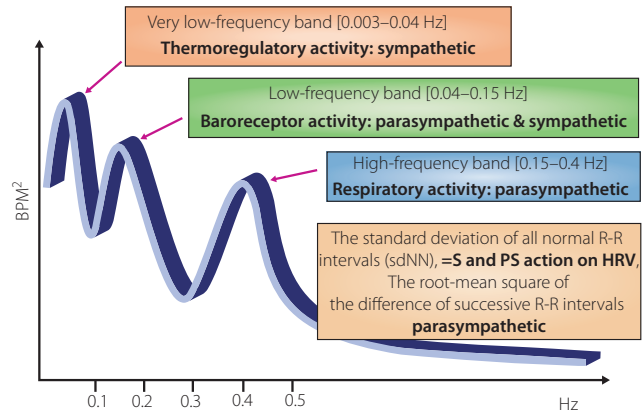


Figure 7 | Spectral analysis of heart rate variability. HRV, heart rate variability; PS, parasympathetic; S, sympathetic; sdNN, standard deviations of all normal beat-to-beat intervals (modified from Vinik *et al.*³).

sympathetic activity. The time-domain HR tests and the BP response to postural change have the reproducibility necessary for clinical trials. These tests were used as end-points in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT EDIC Research Group, 2005) and other clinical trials. The total spectral power (TSP) is calculated, as are the standard deviations of all normal R-R intervals (sdNN), a measure of both sympathetic and parasympathetic action on HRV. The root-mean square of the difference of successive R-R intervals (rmSSD) is a measure primarily of parasympathetic activity^{3,58}.

Frequency-domain indices obtained by applying spectral analysis to HR variability of short (5–7 min) and long (24-h) electrocardiogram recordings provide a measure of sympathetic and parasympathetic modulation of HR, as described earlier.

HRV testing is a clinically relevant measure in addition to CARTs, and provides key information about autonomic – parasympathetic and sympathetic – modulation of the cardiovascular system. The Toronto Consensus Panel on Diabetic Neuropathy have concluded that analysis of HRV can be used in clinical trials and research using statistical indices in the time and frequency domains. Nevertheless, application of the technique is critically dependent on an understanding of the underlying physiology, the mathematical analyses used, and the many confounders and possible technical artifacts⁵⁸.

TREATMENT OF AUTONOMIC DYSFUNCTION

Restoring Autonomic Balance and Potential for Reversal of CAN

Physiological health is characterized by organized variability in organ functions, which are controlled by neural networks that provide compensatory output to maintain homeostasis around set points, which are appropriate for the system in time, sex, age, bodyweight and BP among other variables, such as exercise, sedentary habits and smoking. Although these can theoretically

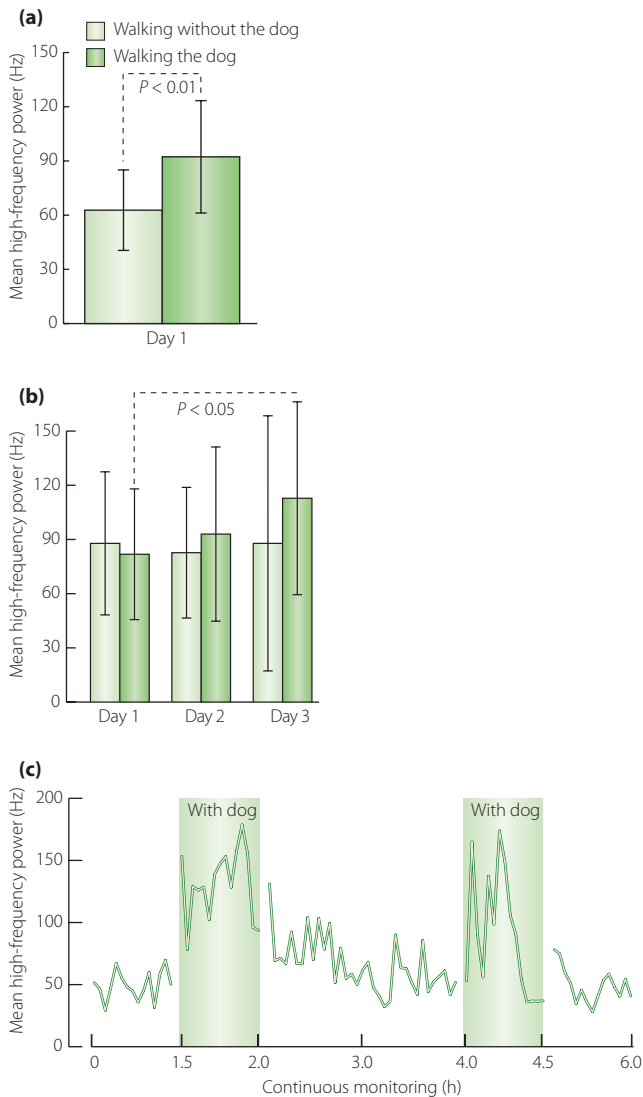


Figure 8 | The effects of walking with and without a pet dog on parasympathetic function (mean high frequency power). (a) The 80 min walking program including two 30 min walks without and with the dog ($n = 13$). (b) The 80 min walking program over three consecutive days ($n = 3$). (c) Interaction with the dog at home during 6 h of continuous monitoring ($n = 4$; reproduced from Motooka *et al.*⁵⁹. © Copyright 2006. The Medical Journal of Australia - reproduced with permission).

be corrected by relatively simple maneuvers, they are in general difficult to obtain and maintain. However, this is possible. As shown by Motooka *et al.*, there are simple maneuvers that improve HRV⁵⁹. Little old ladies walking the dog have enhanced parasympathetic function. Removing the dog is enough to incite sympathetic overactivity (Figure 8). Our interest in falling and fractures in older diabetics has taught us that much of this is a result of the loss of the organized variability, and loss of strength and reaction times. With very simple strength and balance training, falls risk can be significantly reduced⁶⁰. We have shown that

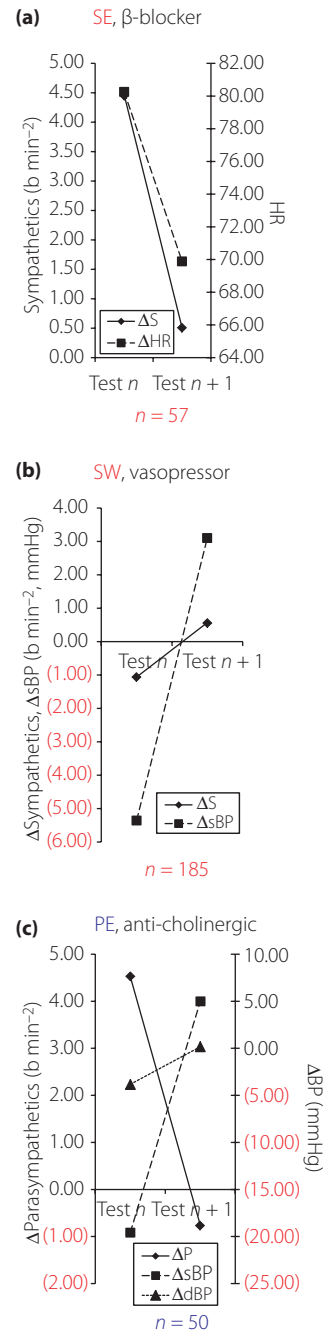


Figure 9 | The ability to correct sympathetic excess with (a) a β -adrenergic blocking agent, (b) sympathetic withdrawal with an adrenergic agonist and (c) parasympathetic excess with an anti-cholinergic agent. BP, blood pressure; HR, heart rate; PE, parasympathetic excess; SE, sympathetic excess; SW, sympathetic withdrawal. Reproduced from Vinik *et al.*¹¹.

balance can be adjusted with agents that act on one or another arm of the autonomic nervous system (Figure 9)¹¹. For the abnormalities in autonomic balance, adrenergic excess can be addressed with beta-blockers and parasympathetic excess with anticholinergic drugs (Table 2)⁶¹.

Table 2 | Diagnosis and management of autonomic dysfunction

Symptoms	Assessment modalities	Management
Resting tachycardia, exercise intolerance, early fatigue and weakness with exercise	HRV, respiratory HRV, MUGA thallium scan, 123I MIBG scan	Graded supervised exercise, beta blockers, ACE-inhibitors
Postural hypotension, dizziness, lightheadedness, weakness, fatigue, syncope, tachycardia/bradycardia	HRV, blood pressure measurement lying and standing	Mechanical measures, clonidine, midodrine, octreotide, erythropoietin, pyridostigmine
Hyperhidrosis	Sympathetic/parasympathetic balance	Clonidine, amitriptylline, trihexyphenidyle, propantheline, or scopolamine, botox, glycopyrrolate

ACE, angiotensin-converting enzyme; HRV, heart rate variability; MIBG, metaiodobenzylguanidine; MUGA, multi-gated acquisition. Reproduced from Vinik *et al.*⁶⁶.

We have reported that simple pharmacological manipulation of situations of parasympathetic and sympathetic excess or deficiencies cause important changes in the symptom complex and quality of life of the afflicted individuals¹¹. Intervention studies have documented the protective effects of glycemic control on autonomic function in type 1 diabetic patients (DCCT trial). In the Steno memorial trial, Gaede *et al.*^{62,63} showed that a multifactorial strategy aimed at lifestyle change with pharmacological correction of hyperglycemia, hypertension, dyslipidemia and microalbuminuria in type 2 diabetic patients reduces abnormalities in autonomic function by 68%. Ziegler *et al.*⁶⁴ reported that alpha lipoic acid improves autonomic function. In fact, it is one of the only drugs targeting the nervous system to be endorsed by the Toronto Consensus⁵⁷. In contrast, failure to identify loss of parasympathetic integrity is accompanied by dire consequences, as witnessed by the 22% increase in sudden death in the ACCORD study with intensification of glucose control^{26,27}.

The Toronto Consensus Panel on Diabetic Neuropathy concluded the following in relation to CAN treatment:

- Intensive diabetes therapy retards the development of CAN in type 1 diabetes (level A).
- Intensive multifactorial cardiovascular risk intervention retards the development and progression of CAN in type 2 diabetes (level B).
- Lifestyle intervention might improve HRV in prediabetes (level B) and diabetes (level B).
- Symptomatic orthostatic hypotension might be improved by non-pharmacological measures (level B), and by midodrine (level A) and/or fludrocortisone (level B).

The recommendations from the Toronto Consensus Panel on Diabetic Neuropathy are as follows:

- Diabetes therapy in patients with type 1 and type 2 diabetes should consider the individual risk profile and comorbidities (class I).
- Lifestyle intervention should be offered as a basic preventive measure (class I).
- Given the limited evidence from very few large-scale randomized clinical trials, recommendations cannot be given for

pharmacological and non-pharmacological treatments of CAN.

- Drugs that might reduce HRV should be avoided in patients with CAN (class III)
- Resting tachycardia associated with CAN can be treated with cardioselective beta-blockers (class I).
- The first therapeutic approach in symptomatic orthostatic hypotension should consider the exclusion of drugs exacerbating orthostatic hypotension, correction of volume depletion (class I) and other non-pharmacological measures (class IIa).
- Pharmacotherapy of symptomatic orthostatic hypotension should include midodrine (class I) or fludrocortisone, or a combination of both in non-responders to monotherapy (class IIa).
- Because of the limited evidence, the potential risk of any pharmacological treatment should be thoroughly weighed against its possible benefit (class I).
- CARTs should be used as end-points in prospective observational and clinical trials.

CONCLUSION

Diabetic CAN is a serious complication found in one-quarter of type 1 and one-third of type 2 diabetic patients. It is associated with increased mortality and silent myocardial ischemia, and with a poor prognosis. Autonomic dysfunction has been shown to be a predictor of cardiovascular risk and sudden death in patients with type 2 diabetes. It might be attributable to a functional abnormality or to organic structural damage to the different components of the autonomic nervous system. There is now strong evidence of inflammation with activation of inflammatory cytokines in patients with diabetes. These changes correlate with abnormalities in sympathetic-vagal balance. Several investigations have shown the neuroregulatory role for the autonomic nervous system, being a key instrument in the inflammatory process.

Patients suffer from severe orthostasis, postural hypotension, exercise intolerance, enhanced intraoperative instability, and an increased incidence of silent MI and ischemia. There are simple bedside tests to diagnose CAN using HRV, responses to

breathing, the Valsalva maneuver and standing. Functional abnormalities and imbalance between the sympathetic and parasympathetic nervous system are discerned with respiratory modulation of different-frequency oscillations in HRV.

Several agents have become available for the correction of functional defects in the autonomic nervous system. Restoration of autonomic balance is possible and has been shown with therapeutic lifestyle changes, increased physical activity, diabetes treatment, β -adrenergic blockers and potent anti-oxidants, such as α -lipoic acid. There are exciting new prospects for pathogenesis-oriented intervention.

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