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Chapter 2

Autonomic Dysfunction in Cardiovascular Disease

A consequence of vascular damage

Submitted

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INTRODUCTION

The beat of our heart and our blood pressure are not constant. They vary with respiration, physical and mental stress and are controlled by the autonomic nervous system. In 1876, Mayer described periodical fluctuations in blood pressure with a cycle of once per 10 seconds. These ‘Mayer–waves’ are considered markers of sympathetic control of vasomotor tone. Heart rate variations with a period shorter than 3–5 seconds are reflections of respiration and vagal modulation, whereas variations of once per 10 seconds represent vagal as well as sympathetic cardiac modulation. After pharmacological ‘denervation’ of the heart with atropine and propranolol, nearly all variability of the heart rate is abolished and the intrinsic heart rate of 100 beats per minute reveals in humans.

The clinical relevance of a diminished heart rate variability was first recognized in 1965 when Hon and Lee perceived that fetal death was preceded by vanishing of heart rate variability. In the 70s, Ewing developed a set of bedside tests to diagnose diabetic cardiovascular autonomic neuropathy and showed that diminished blood pressure and heart rate responses to standard stimuli were predictive for mortality. Modern evaluation of cardiovascular autonomic function by analysis of heart rate variability (HRV) and baroreceptor reflex sensitivity (BRS) have proven their value for risk stratification in chronic heart failure and after myocardial infarction.

How should autonomic function be measured? A variety of tests is available, that depend on the integrity of different parts of the autonomic nervous system. The baroreceptors, located in the carotid artery that has its specific vascular properties, the afferent, central, and efferent pathways and the effector organs (heart, blood vessels and adrenal glands) all have their specific contribution to the autonomic function tests results, that reflect the function of different parts of the autonomic nervous system. Similarly, the factors that are responsible for the impaired autonomic function in cardiovascular disease may be multiple and may differ between diseases. Ewing attributed abnormal autonomic function tests to diabetic autonomic neuropathy. However, today’s evidence shows a strong association and probably dependence of autonomic function on structural and functional (cardio–) vascular properties. Not only in chronic heart failure, where impaired auto-
nomic control of heart rate is the result from both end–organ damage and neurohumoral activation. But also in diabetes the (subclinical) cardiac, micro– and macrovascular or endothelial dysfunction may be an important cause of a failing autonomic control. Viceversa, early sub-clinical autonomic neuropathy is strongly related to insulin resistance and probably precedes development of overt diabetes mellitus.

We will review the methods to assess autonomic function, the evidence on the predictive value of impaired autonomic function for mortality and the possible mechanisms involved, and summarize the determinants of autonomic function including recent reports on role of nitric oxide (NO) as a essential modulator of autonomic control.

**Blood pressure control**

Our blood pressure is kept between narrow limits. Figure 1 denotes the blood pressure controlling responses to a sudden change in blood pressure. Of the short–term systems, the baroreceptors respond within seconds. The baroreceptors have to long been thought to lose their effects after hours, after which the baroreflex is reset to a new blood pressure level\(^9\);\(^10\). However this has more recently been challenged by observations that damage to baroreceptors for example after bilateral carotid body tumor resection or after radiotherapy has long–term effects on heart rate or blood pressure control, especially variability\(^11\). More importantly, Lohmeier et al found that prolonged activation of the baroreflex in dogs led to a sustained fall in blood pressure, and heart rate, also associated with a fall in plasma norepinephrine levels\(^12\).

Ambulatory blood pressure monitoring has shown that blood pressure tends to be highest during the day and lowest during the night\(^13\);\(^14\). Individuals, whose blood pressure does not fall or falls scarcely at night, are called ‘non–dippers’\(^15\). The mechanism of the non–dipping nocturnal pattern is not well understood. An impairment in autonomic nervous activity may play an important role since under physiological conditions, the heart rate, cardiac output, peripheral resistance and plasma catecholamines are reduced during the night\(^16\);\(^17\). Withdrawal of sympathetic activity seems to play a pivotal role. Conditions with abnormal autonomic function, such as diabetes, heart failure and car-
diac transplantation are associated with a lack or attenuation of the day–night blood pressure changes\textsuperscript{18–20}. In hypertension, a non–dipping nocturnal blood pressure has been associated with increased cardiovascular morbidity, possibly resulting from the higher haemodynamic load throughout the 24 hours\textsuperscript{13;21–23}.

## CARDIOVASCULAR REFLEXES

Both arterial and cardiopulmonary baroreceptors exert a tonic inhibitory influence on centrally mediated sympathetic neural outflow. The arterial baroreceptors are located in all thoracic large arteries, but are strongly concentrated in the carotid artery and the aorta. Afferent nerves supply the cardiovascular control center in the medulla oblongata. Efferent nerves run to the atria and ventricles, peripheral arterioles and veins and adrenal glands\textsuperscript{24}.

The aim of the baroreflex is to keep blood pressure constant. A sudden rise in blood pressure will increase the inhibition of sympathetic outflow and stimulate vagal outflow, resulting in a decrease of heart rate, myocardial contractility and peripheral resistance (figure 2). The cardiopulmonary baroreceptors are located in the heart (atria and ventricles), lung, and great veins and are activated by increases in cardiac filling pressure, contractility, wall stress and the depth of breathing\textsuperscript{24}. The recent studies of Lohmeier et al have challenged some of our views on the function of the baroreflex: prolonged stimulation of the carotid baroreceptors was found to be sufficient to lead to a prolonged fall in blood pressure. The technique used to stimulate the carotid baroreceptors suggests that this effect goes beyond that of stretch of the vascular receptors in the wall of the carotid. Moreover, in the effector arm of the reflex indirect effects of the renal nerve on the renin angiotensin aldosterone system seem to be involved\textsuperscript{12}. In this review, we will focus on the arterial baroreceptor reflex.

## TECHNIQUES TO ASSESS THE AUTONOMIC FUNCTION

\textit{Ewing battery}. The ‘Ewing battery’ consists of five parameters derived
from four tests evaluating the heart rate responses to the Valsalva ma-
nuever, deep breathing and standing up from the supine position and
the blood pressure responses to standing up and sustained handgrip. These tests are still considered ‘golden standard’ to diagnose diabetic neuropa-thy and have proven their predictive value for mortality in diabetes (figure 3). However, the results are strongly dependent upon performance of the maneuvers and are age–dependent.

**Heart rate variability (HRV).** Analysis of heart rate variability of short (minutes) as well as long (24 to 48 hours) ECG recordings has been used as a measure of cardiac autonomic control. The time–domain approach consists of statistical analysis of the duration of the RR interval lengths and the differences of successive RR intervals. A number of time–domain variables is available, including the SDNN, the simplest variable, defined as the standard deviation of all normal RR intervals. Since it reflects all variance it is dependent on total recording length. A distinction has been made between variables that measure high and low frequency variation, representing the fast vagal and slower sympa-thetic modulations, respectively. For instance the RMSSD, the square root of the mean squared differences of successive RR intervals, is a high frequency parameter and the SDANN, the standard deviation of avarage RR interval length of 5 minute segments, is by definition a low frequency parameter (cycle >5 minutes).

An alternative approach is by means of spectral analysis of the RR interval lengths. The high frequency (HF, 0.15–0.40 Hz) components mainly represent vagal modulation and are influenced by respiration, whereas the low frequency (LF, 0.04–015 Hz) components are both sympathetically and vagally modulated. This was first shown by parasympathetic and total autonomic blockade with glycopyrro-late and propranolol in dogs and later reproduced in humans. Less is known about the physiological basis of the very low frequency (0.003–0.04 Hz) fluctuations, that may represent parasympathetic out-flow, thermoregulation and the renin–angiotensin–aldosteron system. The ratio between low and high frequency oscillations, the LF/HF ratio, is considered to reflect sympathovagal balance, although this has been under dispute.
Baroreceptor reflex sensitivity (BRS). The BRS can be assessed by measuring the changes in heart rate to mechanically or pharmacologically induced changes in blood pressure. Series of blood pressure measurements can be obtained invasively, as well as non–invasively. The non–invasive blood pressure measurements by a photoplethysmographic device (Finapres, Ohmeda, Englewood, Colo, USA) have been validated against intra–arterial recordings31. Classically, the BRS has been determined by measuring the changes in RR interval to intravenous injection of pressor agents (angiotensin II, phenylephrine)32 or depressor agents (nitroprusside). The BRS is then expressed in msec/mmHg. However, these drugs might influence the baroreflex itself. Similarly, RR interval length modulation after baroreceptor deactivation by a neck suction chamber can assess BRS33.

Alternatively, cross–spectral analysis of spontaneous blood pressure and heart rate variation can be used to estimate BRS34. Similar to HRV, a spectrum of blood pressure variability (BPV) can be obtained by spectral analysis of blood pressure series. As a measure of BRS, the mean gain between BPV and HRV spectra in the LF and HF bands34 is calculated, see figure 3.35 Keeping in mind the goal of a stable blood pressure, a sensitive system responds to small changes in blood pressure with a powerful adaptation of the heart rate. Consequently, there is an expected inverse relationship between the sensitivity of the baroreceptor reflex and the short–term variability in blood pressure and a positive relationship between BRS and variability of heart rate.

Finally, the sequence method is based on identification of sequences of four or more consecutive heart beats characterized by a progressive rise in systolic blood pressure and RR interval lengthening or a progressive decrease in systolic blood pressure and RR interval shortening. The slope of regression line of the changes in systolic blood pressure and RR interval length is taken as index of BRS36. The different techniques to assess BRS are highly correlated35.

QTc. The QT interval reflects the total duration of ventricular myocardial depolarization and repolarization. Heart rate adjusted QT interval (QTc) prolongation is regarded as an indicator of an imbalanced distribution of autonomic nervous system activity to the heart37,38. Congenital of pharmacological perturbation of the autonomic nervous system may result in prolongation of the QTc interval39,40. Two mechanisms
have been suggested to explain the increased risk of a prolonged QTc. First, predominance of sympathetic nerve activity or parasympathetic damage results in dispersion of repolarization, resulting in a high risk of ventricular fibrillation. Second, disturbed myocardial membrane function may lead to electrical instability and consequently to a high risk of arrhythmia and sudden death.

**Predictive value of autonomic function parameters for mortality in health and cardiovascular disease**

**Healthy subjects.** The Framingham Heart Study has shown, in a 30 years follow-up, the prognostic value of a high heart rate for all-cause and cardiovascular mortality in subjects free of cardiovascular disease. Later, a diminished HRV from a 2-hour ambulatory ECG was predictive of cardiac events in the same study, independent from traditional cardiovascular disease risk factors. Similar results were seen from a low HRV in a 2-minute rhythm strip in the ARIC study. A high heart rate or a diminished HRV could be indicative of inappropriate autonomic arousal or may reflect subclinical cardiac disease or poor health. Prolonged QTc interval predicted cardiovascular and all-cause mortality in apparently healthy populations. One must be aware that alterations in QTc intervals may result from physiological manoeuvres and vasodilation in healthy subjects.

The predictive value of QTc could mean that it is an indicator of subclinical cardiovascular disease, since prolonged QTc is associated with traditional risk factors and with atherosclerotic disease as measured with intima media thickness.

**Post myocardial infarction (MI).** In the ATRAMI study, depressed autonomic function was a strong independent predictor of mortality; a low BRS (<3.0 ms per mmHg) or low HRV (SDNN <70 ms) carried a multivariate risk for cardiac mortality 3.2 and 2.8 while left ventricular ejection dysfunction and the presence of frequent ventricular premature complexes were included in the model. This study confirmed earlier studies of the predictive value of impaired HRV after myocardial infarction, both for the time-domain and frequency domain.
parameters\textsuperscript{53}. A possible explanation for this may be the increased susceptibility for the development of life-threatening arrhythmias in an altered state of autonomic balance (a relative sympathetic predominance and reduced vagal activity)\textsuperscript{54}. The association of a depressed BRS or reduced HRV with an increased incidence of arrhythmic events in postinfarction patients supports this hypothesis\textsuperscript{55}. Also, prolonged QTc, as a marker of autonomic disturbance, was predictive of sudden death in patients with myocardial infarction\textsuperscript{56}.

\textbf{Heart failure.} Chronic heart failure (CHF) is characterized by increased sympathetic activation, as mirrored by increased sympathetic nerve activity, and to some extent by high levels of norepinephrine and angiotensin II\textsuperscript{57,58}. Furthermore, CHF patients have a decreased vagal tone and severe baroreceptor impairment\textsuperscript{59}. This leads to cessation of the restraining influence on central sympathetic activity, and thus contributes to sympathetic excitation\textsuperscript{60}.

Neurohormonal activation, as reflected by plasma norepinephrine and plasma renin, is a predictor of mortality in CHF\textsuperscript{61–63}. Autonomic dysfunction, measured by BRS and HRV, is also a powerful predictor of mortality in CHF. A BRS <1.3 ms per mmHg (lowest quartile) created a relative risk of 2.7\textsuperscript{64} and the UK–Heart trial reported an annual mortality rate of 5.5\% in patients with a preserved HRV (SDNN >100 ms) compared to 12.7\% in patients with moderately impaired HRV (SDNN 50 to 100 ms) and 51.4\% with severely impaired HRV (SDNN <50 ms)\textsuperscript{65}. In both studies, the autonomic function was an independent predictor of known risk factors and only moderately correlated with left ventricular ejection fraction.
THE AUTONOMIC NERVOUS SYSTEM IN HYPERTENSION AND DIABETES MELLITUS.

Hypertension. The role of the sympathetic nervous system in the pathogenesis of hypertension has been acknowledged since decades. The evidence for increased sympathetic drive principally comes from studies of the early phases: borderline and mild hypertension. A hyperkinetic state (i.e. elevated cardiac output and heart rate) as a marker of increased sympathetic tone predicts later development of hypertension. Plasma catecholamine levels and norepinephrine spillover rates are increased in young hypertensive patients. Direct intraneural recordings of sympathetic nerves in borderline hypertensive patients show elevated activity. Also analysis of HRV shows sympathetic predominance in hypertension. Finally, In the Framingham Heart Study, lower HRV could predict the development of hypertension in normotensive men but not in women.

Whereas the evidence for the increased sympathetic tone in the development of hypertension is largely concordant, it is less clear whether the cardiovascular reflexes are changed very early on or whether the baroreflexes suffer as a result from structural and functional changes of the peripheral components of the baroreflex arch. Baroreflex function is in part genetically determined, as the presence of a family history of hypertension is the strongest predictor of BRS in hypertensive as well as in normotensive subjects. However, since the early work in this field of McCubbin et al demonstrated a marked resetting of the arterial baroreflex in chronic hypertension, skepticism has predominated about a primary role of the baroreflex in the long-term control of arterial blood pressure. Other studies also reported resetting of the baroreceptor reflex setpoint to a higher level and a reduction of the baroreceptor reflex sensitivity in hypertension. Other reasons that the concept of baroreceptor reflex dysfunction as a cause of hypertension was abandoned was that complete denervation of the baroreceptors does not result in sustained blood pressure elevation. Rather, following the short-term blood pressure stabilizing goal of the baroreceptor reflex, blood pressure variability was increased. An alternative view argues a resetting of the baroreceptor setpoint while the stimulus–response curve remains essentially the same, i.e. the baroreceptor reflex sensitivity is not changed.
Nonetheless, considering the importance of the baroreflex in the acute regulation of sympathetic activity and arterial pressure, there has been continued interest in the possibility that it plays a role in the pathogenesis of hypertension. Indeed, many have considered that the excessive sympathetic activation in hypertension might be accounted for by baroreflex dysfunction, an associated finding in some forms of experimental and clinical hypertension. However, whether impaired baroreflex suppression of sympathetic activity plays a role in the hypertensive process depends critically on whether baroreflexes completely reset when exposed to chronic changes in arterial pressure. If resetting is complete and baroreflexes do not chronically alter sympathetic activity, then they could not produce functional changes that influence the severity of hypertension. The fundamental question of whether baroreflexes completely reset and have the capacity to chronically alter sympathetic activity and arterial pressure remained unanswered up till recently due, in large part, to technical limitations that precluded the assessment of chronic changes in sympathetic nerve activity and the long–term effects of alterations in baroreflex activity on arterial pressure. Recently, however, a number of novel observations in chronically instrumented animals have indicated that the baroreflexes do not completely reset and are chronically activated in hypertension. These studies also support the hypothesis that in the effector path of the baroreflex suppression of renal sympathetic nerve activity and attendant increments in renal excretory function are key mediators of the antihypertensive response.

The afferent part of the baroreceptor reflex could be impaired secondary to changes in the vascular mechanical and endothelial functional properties at the site of the baroreceptors. The baroreceptor reflex sensitivity is strongly related to carotid artery elasticity, which is known to be diminished in hypertension. However, functional damage to the endothelium at the baroreceptor region may affect the integrity of the afferent pathway of the baroreceptor reflex as well. Impaired prostacyclin release, enhanced formation of oxygen free radicals and platelet aggregation suppress baroreceptor activity and could contribute to baroreceptor dysfunction in hypertension.

The efferent pathways of the baroreceptor reflex loop could suffer from adaptation to elevated blood pressure as well. Vascular hypertrophy may be responsible for increased adrenergic responsiveness to
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stress\textsuperscript{86}. However, one could also argue that the hypertrophied vascular tree loses compliance and thereby its buffering capacity. Furthermore, in animal models, development of left ventricular hypertrophy (LVH) impairs baroreflex control of heart rate, whereas treatment with an angiotensin–converting–enzyme (ACE) inhibitor could reverse these processes\textsuperscript{87}. In hypertensive patients, the reduction of LVH was confined to those with improved baroreceptor reflex control of heart rate as measured by HRV\textsuperscript{88}. Thus, the peripheral components of the baroreceptor reflex arch are vulnerable to adaptive changes of an sustained elevated blood pressure.

Further, evidence is emerging that in hypertension the renal nerves may be the critical efferent link for baroreceptor–induced suppression of central sympathetic output through which long–term compensatory reductions in arterial pressure are produced\textsuperscript{89}.

Irrespective whether autonomic imbalance and impaired baroreceptor reflex function precede or follow the development of hypertension, they are closely related to morbidity and mortality in hypertension. As clearly explained by Julius, elevated heart rate as marker of increased sympathetic tone is not only related to hypertension, but also to lipid disorders and insulin resistance, thus contributing to the increased coronary risk\textsuperscript{90,91}. Finally, in the Framingham Heart Study, heart rate was a predictor for cardiovascular and all–cause mortality in hypertensive men and women\textsuperscript{86}.

\textit{Diabetes mellitus.} Autonomic dysfunction is a common complication of diabetes mellitus. When assessed with Ewing's standard autonomic function tests, abnormal autonomic function is reported in 20–40% of unselected diabetic patients\textsuperscript{92}. The presence of cardiovascular autonomic dysfunction is associated with an increased risk for mortality in diabetes mellitus\textsuperscript{26,92–100}. The predictive value was first shown by Ewing \textit{et al.}, using the autonomic function tests that have become golden standard\textsuperscript{26}. However, in practice these tests are strongly dependent upon performance and suffer from poor reproducibility. Initially, autonomic dysfunction was thought to be a late complication, because it was diagnosed in patients with long–standing diabetes\textsuperscript{101}. With the development of more sensitive methods to diagnose autonomic dysfunction, it is earlier recognized. HRV and BRS can already be impaired, even when standard autonomic function tests are still normal\textsuperscript{102–105}. The
QT interval may also be prolonged already when diabetes mellitus is first diagnosed, and was found to be a better predictor of cardiac death than ankle brachial pressure index and autonomic function tests.\(^{106}\) The importance of early recognition is underscored by the predictive value of impaired autonomic function for mortality, even at a subclinical level.\(^{95,96}\)

What mechanisms are responsible for the autonomic dysfunction in diabetes and how do they contribute to increased mortality? The abnormal cardiovascular autonomic function in the initial reports of Ewing was ascribed to the presence of neuropathy, originating from the observation of a lower nerve conduction velocity and longer terminal latency in patients with abnormal heart rate response to the Valsalva maneuver.\(^{107}\) Indeed, cardiovascular autonomic dysfunction is often seen in patients with diabetic peripheral neuropathy, but there is a lower than expected concordance and a variable relationship between them.\(^{108–110}\) A number of non–neural abnormalities, like increased intima media thickness at the site of the baroreceptors, reduced distensibility, impaired cardiac vagal function, left ventricular hypertrophy and endothelial dysfunction could contribute to the autonomic dysfunction in diabetes mellitus. Interestingly, these abnormalities are closely associated with the presence of (micro–)albuminuria, which is seen as a reflection of endothelial dysfunction or vascular damage in diabetes mellitus.\(^{111–115}\) Furthermore, autonomic dysfunction is particularly pronounced when diabetes is complicated by (micro–)albuminuria in both type 1 and type 2 diabetic patients. In fact, autonomic dysfunction may explain part of the increased mortality that is associated with (micro–)albuminuria in diabetes mellitus.\(^{96,119}\) Conversely, since autonomic dysfunction is a predictor of deterioration of renal function in diabetes mellitus, renal disease may partly account for the increased mortality rate in patients with diabetes mellitus complicated by autonomic neuropathy.\(^{120,121}\)

Another mechanism by which autonomic dysfunction may deteriorate prognosis is the increased vulnerability to lethal arrhythmias, by analogy with post myocardial infarction patients. This could explain the high number of unexplained deaths in diabetic patients.\(^{114}\) Finally, the notion that autonomic neuropathy is a result of longstanding hyperglycaemia is incompatible with the recent report of a higher incidence
in non–diabetic offspring of type 2 diabetic patients compared with non–diabetic offspring of nondiabetic subjects. Clearly, these subjects had not been exposed to longstanding hyperglycaemia. Rather, in this study autonomic neuropathy was associated with features of the metabolic syndrome and microvascular damage (increased urinary albumin excretion).

In summary, widespread vascular damage at the endothelial, vascular and cardiac level is an important determinant of autonomic dysfunction. For diabetes mellitus, the impaired cardiovascular reflexes, as assessed by bedside autonomic function tests, in the diabetic patients in the original paper from Ewing may very well have been related to and partly caused by vascular damage, rather than neuropathy per se. With the availability of modern, sensitive measures of autonomic function, the stratification of diabetic patients at risk may improve. Until now, the predictive value of BRS and spectral and time–domain parameters of HRV has yet to be proven in diabetes mellitus.

Similar to impaired autonomic function as measured by Ewing’s tests, HRV and BRS, prolonged QTc duration is a predictor of cardiovascular mortality in type 1 and type 2 diabetes mellitus. Prolonged QTc is associated with traditional risk factors in type 1 diabetic patients, underscoring the relation between (subclinical) cardiovascular damage and autonomic dysfunction.

**The interplay between autonomic control and vascular function.**

*Cardiovascular determinants of autonomic function.* As discussed earlier, a number of structural and functional changes of the cardiovascular system can contribute to autonomic dysfunction in cardiovascular disease, by modifying the afferent or effector systems.

First, the effects of structural damage to the baroreceptors after for example radiotherapy have been discussed earlier. A reduction in arterial compliance may reduce the baroreflex mediated control of cardiac output and blood pressure. An attenuated baroreflex control of cardiac output and arterial blood pressure has been shown after an acute increase in arterial compliance in rats. In humans, a diminished BRS
and reduced carotid arterial compliance were found in hypertensive subjects compared to normotensive subjects. However, although a significant correlation of $r=0.53$ was found between BRS and arterial compliance in the complete group, this could not be demonstrated in the hypertensive subjects only, suggesting that other factors also contributed to baroreflex dysfunction in these individuals. In a study of 19 healthy young subjects, BRS was significantly related to carotid artery distensibility with $r=0.78$. However, other reports have shown that changes in baroreceptor properties do not simply depend on a decreased distensibility of the carotid sinus arterial wall.

Second, the effects of a defective endothelium at the site of the baroreceptor in the carotid arterial wall on baroreflex function have been reported. Local, paracrine and endocrine effects may play a role. An impaired formation of PGI$_2$ was partly responsible for decreased baroreceptor sensitivity in hypertension and atherosclerosis. Furthermore, aggregating platelets at the carotid sinus decreased baroreceptor activity in an animal model, without altering the carotid pressure–diameter relation, suggesting a direct inhibitory effect on the baroreceptors. The role endothelial–derived NO is discussed in some more detail below. Also in an animal model, oxidative stress contributed to baroreceptor dysfunction. Possibly, such mechanisms plays a role in atherosclerosis.

Third, cardiac changes can reduce effective baroreflex control of heart rate and cardiac output in pathological states, as discussed in more detail above for myocardial infarction and heart failure. However, the mechanism of baroreflex dysfunction in CHF is probably multifactorial and is not limited to cardiac changes but may be located in all components of the reflex arc.

The interaction between nitric oxide and the autonomic nervous system. The interaction between nitric oxide, either endothelium–derived or not, and baroreflex mediated sympathetic and cholinergic signaling deserves some special attention. This subject has recently been extensively reviewed by Sartori et al. The relationship is complex, as illustrated by the differential dose–related effects of modulating NO synthesis for example by l–NMMA infusions: at high doses, l–NMMA and phenylephrine similarly suppress sympathetic nerve activity, whereas when infused at lower doses, these drugs have differential
sympathetic effects. Sartori proposed that these differential sympathetic effects of low- and high-dose l-NMMA infusion in humans may be partly due to differences in IC50 doses for l-NMMA–induced blockade of neural vs. endothelial NO synthesis. It also seems as if inhibition of NO synthase has central sympathoexcitatory effects that are masked by an inhibitory effect of the baroreflexes.

Insulin is another player in the interaction between NO and the autonomic nervous system which is relevant in cardiovascular disease. Insulin has a role in the regulation of peripheral vascular tone and arterial blood pressure in humans. In lean, healthy subjects, insulin infusion increases, as a marker of its sympathetic vasoconstrictor action, sympathetic neural outflow to skeletal muscle tissue. On the other hand however, insulin has also been reported to stimulate blood flow and decrease vascular resistance in skeletal muscle. These effects are not due to beta-adrenergic or cholinergic mechanisms, because propranolol and atropine do not attenuate insulin’s vasodilation. There is now abundant evidence, that NO release accounts for insulin’s vasodilator action. In line with this concept, insulin–induced vasodilation in humans is abolished by l-NMMA. Thus, insulin–induced vasodilation is mediated by stimulation of endothelial and sympathetically mediated NO release. Studies in patients with sympathectomy and autonomic failure indicate that the sympathetic vasoconstrictor tone restricts this response, thereby preventing exaggerated insulin–induced vasodilation and hypotension.

In normal subjects, the cholinergic system plays a major, hitherto unrecognised role in offsetting pressor effects caused by NO synthase inhibition. Lepori et al. examined the effects of cholinergic blockade on the blood pressure and peripheral vasoconstrictor responses to systemic l-NMMA infusion in normal subjects. Cholinergic blockade had a dramatic effect on the pressor response to NO synthase inhibition in healthy subjects: the l-NMMA–induced increase in mean arterial pressure was roughly 3 times larger in the presence than in the absence of atropine infusion. This potentiation is specific for NO–dependent vasoconstriction, because atropine did not alter the responses to phenylephrine infusion. As for effects of NO on heart rate, the role of neuronal NO seems to predominate above that of endothelial NO. Studies in intact neuronal NO synthase knockout mice, as well as isolated atria harvested from such animals, suggest that parasym-
pathetic control of heart rate is impaired in the absence of neuronal NO synthase\textsuperscript{150,151}. Interestingly, the exercise training–induced bradycardia in mice appears also to be related to upregulation of neuronal NO synthase\textsuperscript{152}. Studies by Brunner \textit{et al.} support that endothelial NO synthase–derived NO appears to have little role in the peripheral autonomic regulation of heart rate\textsuperscript{153}. Chowdhary found in human studies, using heart rate variability analysis suggest that NO augments cardiac vagal control in healthy subjects and patients with heart failure\textsuperscript{154;155}.

In the earlier discussed study by Lepori \textit{et al}, cholinergic blockade had not only marked vasoconstrictive effects in the vascular responses to NO synthase inhibition in humans, but also alters the reflex decrease in heart rate to l–NMMA infusion alone to a sympathetically mediated increase in heart rate (could be blocked by propranolol)\textsuperscript{149}.

\textit{The triangle of sympathetic activation, insulin resistance and metabolic syndrome (crossroads of hypertension and diabetes).} The metabolic syndrome carries an increase in cardiovascular risk and its occurrence is associated with alterations which might include sympathetic hyperactivity, because the metabolic syndrome is associated with insulin resistance and hyperinsulinemia, which has sympathostimulating effects\textsuperscript{156}. Sympathetic activation has been considered as a link between insulin resistance, hyperinsulinemia, and hypertension. Hypertension may, however, also be considered as a confounder, because essential hypertension without components of the metabolic syndrome is also associated with sympathetic hyperactivity. However, a recent study by Grassi \textit{et al} using MSNA recordings in persons with NCEP–ATP III criteria metabolic syndrome patients provided direct evidence that sympathetic activation is not limited to individuals in whom the metabolic syndrome is accompanied by hypertension\textsuperscript{157}. Sympathetic nerve traffic was also greater than in control subjects when the metabolic syndrome was associated with normal BP values, and thus its diagnosis depended on criteria other than the BP elevation. So, a hyperadrenergic state represents an intrinsic feature of this condition. Epidemiologically, in the Framingham Heart Study, an impaired fasting glucose level was also associated with lower HRV, independent of blood pressure levels\textsuperscript{158}. In the Grassi study, subjects with metabolic syndrome had an attenuation of the sensitivity of baroreceptor sympathetic control, which suggests that impairment of the ability of the baroreflex to restrain adrenergic
tone is also involved, besides factors like the degree of insulin resistance. Endothelial NO synthesis is defective in insulin resistant states, and, as discussed above, may contribute to the neuronal abnormalities of the metabolic syndrome. Autonomic dysfunction may be an early defect, before the metabolic syndrome has become manifest, as suggested by an increase in sympathovagal balance measured by HRV in response to hyperinsulinemia in nondiabetic offspring of type 2 diabetic patients, compared to controls\textsuperscript{159}. As for the transitional area between metabolic syndrome and hypertension, the HRV abnormalities in a group of hypertensive patients were particularly pronounced in those with metabolic features of the insulin–resistance syndrome\textsuperscript{160}.

**CONCLUSION**

Analysis of (variations of) heart rate and blood pressure in rest and during stimuli can assess cardiovascular autonomic function. Ewing’s tests and analysis of heart variability are now accompanied by modern techniques as baroreceptor reflex sensitivity, QTc interval length, muscle sympathetic nerve activity and paracrine and endocrine markers. The predictive value for morbidity and mortality of these autonomic function parameters has been and is established in health and cardiovascular disease, like heart failure, post myocardial infarction and diabetes mellitus.

The cardiovascular autonomic function should be considered as the result of the interplay between many components, consisting of vascular, neural, cardiac and paracrine and endocrine entities. The autonomic dysfunction in cardiovascular disease is therefore most likely the result of defects at multiple sites. Furthermore, cardiovascular risk factors and abnormalities may cluster in individuals and populations and the cardiovascular autonomic dysfunction must be seen as the outcome of damage at different sites, resulting in a compromised integrity of the cardiovascular reflexes.
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