The role of the autonomic nervous system in diabetes and cardiovascular disease : an epidemiological approach

Hillebrand, S.

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General Introduction and Outline

The autonomic nervous system (ANS) controls homeostasis and regulates visceral functions. ANS function is impaired in obesity, diabetes and cardiovascular disease. However, it is yet unclear whether this impairment is merely a consequence of underlying disease, or may also be involved in the development of disease. The main objective of this thesis was to study the role of ANS function in the development of diabetes and cardiovascular disease. This general introduction describes the current state of knowledge about ANS function and its role in health and disease. It furthermore describes the outline of this thesis and summarizes the studies and data that were used.

THE AUTONOMIC NERVOUS SYSTEM

Most functions of the ANS are not in conscious control, and are based on feedback loops and reflexes. The central part of the ANS is located in the medulla oblongata of the brainstem and the hypothalamus.[1] Afferent nerves convey information from organs, muscles, the limbic system, the circulatory system and sensory organs to the central part of the ANS. The efferent pathways consist of the parasympathetic and the sympathetic nervous system, which have mostly opposite effects on organs.[2] The functions of the parasympathetic and the sympathetic nervous system are illustrated in the Figure. The sympathetic nervous system is predominant in active situations, ranging from small actions such as posture changes to "flight-fight-or-fright" actions. The parasympathetic nervous system is predominant during periods of lower activity, in most individuals this is the larger part of the day. The sympathetic output originates from the ventrolateral medulla in the

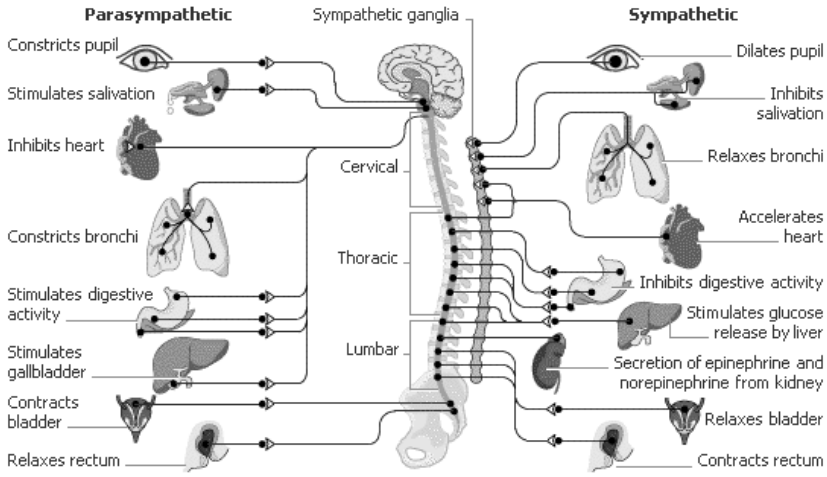

Figure. Functions of the parasympathetic and the sympathetic nervous system
Source: www.medicalterms.info

brain stem and is effectuated through sympathetic preganglionic neurons located in the spinal cord at the T1-L2 segments. The parasympathetic output also originates from the brainstem (nucleus ambiguus) and is effectuated through cranial nerves (CN3, CN7, CN9, CN10) and the spinal cord at the S2-4 segments.[2] The most important neurotransmitters in the efferent part of the ANS are norepinephrine for sympathetic transmission and acetylcholine for parasympathetic transmission.[2] The function of the ANS can be measured in several ways. Often, autonomic control of the cardiovascular system is used to estimate ANS function.

AUTONOMIC CONTROL OF THE CARDIOVASCULAR SYSTEM

The cardiovascular system consists of the heart and peripheral vasculature, and its purpose is to provide an adequate flow of blood and nutrients to the organs.[3] The heart is innervated by the parasympathetic nervous system via the vagal nerve and by the sympathetic nervous system via nerves arising from the upper thoracic region of the spinal cord. Baroreflexes buffer blood pressure, and thereby play a central role in the autonomic regulation of the cardiovascular system. Baroreceptors are not directly sensitive to blood pressure, but rather to the mechanical deformation of the nerve endings during stretching of the vascular wall. Two types of baroreceptors can be distinguished. Cardiopulmonary baroreceptors are localized in the heart, vena cava, and pulmonary vasculature and respond to changes in central venous pressure, whereas arterial baroreceptors in the aortic arch and carotid sinuses respond to changes in arterial pressure. When venous return and arterial blood pressure increase, the sympathetic outflow to the heart and peripheral vasculature is inhibited and the parasympathetic outflow is stimulated, resulting in a decreased peripheral resistance and decreased heart rate.[4] A decrease in venous return and arterial blood pressure results in more sympathetic outflow, decreased parasympathetic outflow, an increase in peripheral resistance and an increase in heart rate. Changes in blood pressure are caused by an adjustment in peripheral resistance rather than by changes in heart rate.

THE AUTONOMIC NERVOUS SYSTEM IN HEALTH AND DISEASE

In healthy individuals, there is modulation in the sympathetic and parasympathetic outflow influenced by the level of activity. This modulation is necessary for an optimal control of homeostasis and visceral functions and is a manifestation of adaptivity. Impaired ANS function is characterized by reduced modulation in sympathetic and parasympathetic outflow, chronic overactivation of the sympathetic nervous system

and chronic decreased activation of the parasympathetic nervous system. Impaired ANS function has been associated with obesity, diabetes and cardiovascular disease.

Obesity

Historically, two hypotheses on the association between ANS function and obesity were postulated. The first hypothesis stated that low activity of the sympathetic nervous system caused weight gain, and the second proposed that activation of the sympathetic nervous system and decreased activity of the parasympathetic nervous system were a result of obesity.[5, 6] The latter hypothesis was adopted by most contemporary researchers and is strongly supported by intervention studies on weight loss, showing that after a decrease in the amount of body fat by a hypocaloric diet or exercise training, activity of the sympathetic nervous system attenuated.[7-13]

Several epidemiological studies have shown inverse associations between body fat and ANS function, and positive associations between body fat and sympathetic nervous system activation.[14-22] However, only few studies were able to investigate the associations between various types of fat, for example visceral fat accumulation within the abdomen, and ANS function. A mechanism underlying of the association between visceral fat and activation of the sympathetic nervous system may be the production of adipocyte derived cytokines (adipokines). Adipocytes, or macrophages invading the adipose tissue, secrete several adipokines.[23] Visceral fat has a higher secretion rate of pro-inflammatory cytokines than subcutaneous fat and inhibits the secretion of anti-inflammatory factors.[24-27] These cytokines can either directly stimulate the central nervous system, by crossing the blood-brain-barrier, or induce low-grade inflammation, resulting in sympathetic activation.[23, 28] Previous studies have shown that visceral fat is stronger associated with cardiovascular risk factors and endpoints than other types of body fat.[29-32] It is, however, unknown whether visceral fat may also stronger influence sympathetic activation than other types of body fat.

Insulin resistance and type 2 diabetes mellitus

Impaired ANS function is a well-known complication of type 2 diabetes mellitus.[33, 34] High blood glucose and insulin levels and an abundance of advanced glycation endproducts can directly damage the nerves or interfere with chemical signalling. Impairment of the ANS worsens with the duration of type 2 diabetes, and is associated with an increased mortality risk.[35] However, several research findings have become known supporting the notion that impaired ANS function may not only be a complication of type 2 diabetes, but may also be involved in the development of the disease. The ANS innervates several organs involved in the glucose metabolism, such as the liver, pancreas, adrenal and skeletal muscles. The parasympathetic branch of the ANS seems responsible for the insulin

sensitivity of several organs.[36] The decreased parasympathetic outflow in impaired ANS function may therefore play a role in the development of type 2 diabetes. Moreover, non-diabetic offspring of type 2 diabetes patients, who are at high risk of developing type 2 diabetes, have a worse ANS function than individuals of the same age without a family history of type 2 diabetes, independent of other risk factors.[37] Together, these arguments suggest that impaired ANS function may not only be a complication of type 2 diabetes, but may also play a role in the development of the disease.

ANS function has been extensively studied in relation with insulin resistance and glucose tolerance. Several cross-sectional studies have shown an inverse association between ANS function and insulin resistance and a positive association between ANS function and glucose tolerance. Insulin resistance, a subclinical stage of type 2 diabetes, is defined as an inadequate response by insulin target tissues to the physiologic effects of circulating insulin. The decreased sensitivity to insulin results in compensatory hyperinsulinaemia. Without this compensation, impaired glucose tolerance or type 2 diabetes mellitus may develop.[44, 45] Therefore, these previously mentioned studies suggest that ANS function is involved in the pathogenesis of type 2 diabetes.[38-43] However, because of the cross-sectional nature of these studies, the direction of the association remains undetermined and it is yet unclear whether impaired ANS function is indeed involved in the development of type 2 diabetes.

Cardiovascular disease

An association between ANS function and cardiovascular disease has frequently been reported. In 1978, an inverse association between variability in heart rate and mortality in patients admitted to the hospital with acute myocardial infarction was first reported.[46] Later studies showed that low baroreflex sensitivity and low heart rate variability were risk factors for cardiovascular events, including myocardial infarction,[47, 48] heart failure,[49] and atrial fibrillation[50] in populations with pre-existing cardiovascular disease. However, in these populations impaired ANS function may have been a result of the pre-existing cardiovascular abnormalities. Therefore, based on existing studies it remains unclear whether impaired ANS function is also a risk factor for the development of cardiovascular disease.

A mechanism underlying the association between impaired ANS function and cardiovascular disease may be an altered lipid metabolism. Experimental studies in rats showed that sympathetic denervation in the liver decreased both hepatic lipogenesis and hepatic secretion of very low density lipoprotein (VLDL).[51, 52] This indicates that sympathetic activation to the liver stimulates lipogenesis and the secretion of VLDL-triglycerides. Furthermore, in adipose tissue, stimulation of the sympathetic nervous

system induces lipolysis, thereby increasing the amount of glycerol and free fatty acids in the circulation.[53] Previous epidemiological studies have reported an association of sympathetic activation with fasting lipid levels in the circulation.[54-56] However, since most individuals are in a postprandial state for the larger part of the day, elevated postprandial serum triglyceride concentrations may be more important for cardiovascular risk.[57, 58] Furthermore, in addition to an increased concentration of lipids in the circulation, activation of the SNS may also result in accumulation of lipids in the liver.[59] The associations of sympathetic activation with postprandial triglyceride concentrations and intrahepatic triglyceride content have not been reported yet.

AIM AND OUTLINE OF THIS THESIS

Although the associations between impaired ANS function, obesity, diabetes and cardiovascular disease have been investigated in previous studies, it remains unclear whether an impaired ANS function is merely a consequence of pre-existing disease, or may also be involved in the development of diabetic and cardiovascular disease. The aim of this thesis was therefore to study the role of ANS function in the development of diabetes and cardiovascular disease.

In **chapter 2** we investigated the association between body fat and ANS function. Since the role of various fat depots has not been unravelled yet, we studied several types of body fat, including abdominal visceral and subcutaneous fat.

In **chapter 3** we investigated whether ANS function is a risk factor for type 2 diabetes. In order to gain more information on the direction of the association we used follow-up data of individuals without diabetes who had been followed until the occurrence of type 2 diabetes. In **chapter 4** we aimed to unravel the interrelationships between body fat, ANS function and insulin resistance.

To investigate whether impaired ANS function is a risk factor for the development of cardiovascular disease, we performed a meta-analysis and meta-regression of prospective studies investigating this association in populations without pre-existing cardiovascular disease in **chapter 5**. We hypothesized that an altered lipid metabolism is a mechanism underlying the association between impaired ANS function and cardiovascular disease and investigated the association between impaired ANS function and alterations in lipid metabolism in **chapter 6**.

STUDY DESIGNS AND DATA USED IN THIS THESIS

The NEO study

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6673 individuals aged 45 to 65 years, with an oversampling of individuals with a BMI ≥ 27 kg/m^2, who were included between September 2008 and October 2012. At baseline, participants completed questionnaires for demographic, lifestyle, and clinical information and underwent an extensive physical examination. Random subsamples of the study population underwent magnetic resonance imaging (MRI) of the abdomen and magnetic resonance spectroscopy of the liver (n=2580), MRI of the heart (n=1207), or were equipped with an Actiheart®, a combined heart rate monitor and accelerometer (CamNtech Ltd, U.K.) for four days (n=955). To answer the research questions in **chapters 2, 4, and 6** of this thesis, we used the baseline measurements of the NEO study.

The Hoorn study

For the research question in **chapter 3** we used data from the Hoorn study, a prospective cohort study including 2484 adults aged 50 to 75 years from the town of Hoorn, the Netherlands. Baseline data were collected between 1989 and 1991 and included data on demographic characteristics, medical history, physical activity and a physical examination. In a subsample of participants (n=631) ANS function was measured. Follow-up examinations were performed in 1996-1998 and 2000-2001.

MEASUREMENT OF AUTONOMIC NERVOUS SYSTEM FUNCTION

Unfortunately, a gold standard for assessing activity of the ANS is lacking. Over the past decennia, several techniques have been developed. The main measures are described below and summarized in the Table.

Microneurography uses fine electrodes that are inserted percutaneously in the nerves to record bursts of activity. Often, the easily accessible common peroneal nerve is used to measure the efferent postganglionic muscle sympathetic nerve activity.[60] Muscle sympathetic nerve activity is a direct measure of sympathetic activity, but it is invasive and can only be used on superficial nerves as nerves to internal organs are not accessible.

Catecholamines are sympathetic neurotransmitters which can be measured to estimate sympathetic nervous system function.[61] Dopamine acts mainly in the central nervous system, while epinephrine and norepinephrine mostly have peripheral effects. Epinephrine is exclusively produced by the adrenal medulla, whereas norepinephrine is mainly produced

Table: Commonly used measures of autonomic nervous system function

Measure	Indicative of sympathetic nervous system	Indicative of parasympathetic nervous system	Validity	Advantages	Disadvantages	Used in this thesis (chapters)
Muscle sympathetic nerve activity	Yes	No	High	Direct measurement of nerve activity	Invasive Only peripheral nerves	No
Catecholamines (plasma, urine)	Yes	No	Very high	Direct measurement of neurotransmitter	Expensive	No
Heart rate and mean NN	Yes	Yes	High	Non-invasive Cheap Easy to measure	Influenced by many factors other than the ANS	2, 3, 4, 6
Heart rate variability – time domain (SDNN, RMSSD)	Yes	Yes	Intermediate	Non-invasive Cheap Easy to measure	Influenced by many factors other than the ANS	3, 4, 5, 6
Heart rate variability – frequency domain (HF, LF)	Yes	Yes	Intermediate	Non-invasive Cheap	Difficult to measure Strongly influenced by noise	3, 4, 5, 6
Baroreflex sensitivity	Yes	Yes	Intermediate	Combines RR interval and systolic blood pressure	Influenced by many factors other than the ANS	3
Ewing tests (RR max, RRmax/min, SBP difference, EI-difference)	Yes (EI-difference, RRmax)	Yes (all)	High	Standardized measurements	Time consuming	3
Electrocardiographic measures	Yes	No	Unknown	Non-invasive	No validation studies available	2

NN, normal to normal RR intervals; SDNN, standard deviation of all normal intervals; LF, low frequency; HF, high frequency; EI, expiration-inspiration; SBP, systolic blood pressure; ANS, autonomic nervous system

by sympathetic postganglionic fibres and is therefore more specific to estimate activity of the sympathetic nervous system.[62] Norepinephrine can be measured both in urine or in plasma. All norepinephrine that is formed by the body is eventually excreted in the urine either unchanged or as a metabolite.[63] Therefore, total urinary excretion of norepinephrine or its metabolites can be used to estimate the synthesis rate of norepinephrine.[64, 65] Measurement of plasma norepinephrine is less straightforward, because it depends not only on the release of norepinephrine to plasma, but also on the clearance from the circulation.[66, 67] Spill-over techniques aim to solve this problem. Highly specific radiolabelled norepinephrine is infused into the plasma at a constant rate to reach steady-state conditions.[67] Then, the rate of release of endogenous norepinephrine into plasma and the rate of clearance from the plasma can be estimated using equations.[68] Unfortunately, laboratory measurements of norepinephrine in urine or plasma are expensive.

Because the measurement of muscle sympathetic nerve activity and catecholamines is invasive or expensive, these measurements are not easily employed in large epidemiological studies. In the population studies described in this thesis, we rather used the following measures of ANS function: resting heart rate, heart rate variability, baroreflex sensitivity, Ewing tests and electrocardiographic measures of sympathetic activation. Resting heart rate is a measure of the sympathovagal balance.[69] According to the Rosenblueth-Simeone model, heart rate is determined by the intrinsic heart rate multiplied by a sympathetic factor and a vagal factor.[70] Impaired ANS function with increased sympathetic nervous system activation is reflected in a higher resting heart rate. In 1981, the first findings on spectral analyses of heart rate fluctuations were published.[71] Heart rate variability is defined as the oscillations in the interval between consecutive heart beats.[72] Heart rate variability is a manifestation of activity of the baroreflex, which purpose is to buffer blood pressure by adaptions in sympathetic and parasympathetic outflow.[71, 73] Variability in heart rate can be expressed in several ways. In time domain analyses, a variety of statistical variables are calculated from intervals between adjacent normal R waves (NN intervals). The most commonly used time domain variables are the standard deviation of all NN intervals and the root mean square of successive differences between adjacent RR intervals.[74] The frequency domain analyses quantify cyclic fluctuations of NN intervals by the frequency of the fluctuation using Fourier transformation or autoregressive spectral analysis.[72] High frequency fluctuations, with a frequency range of 0.15-0.40 Hz, are caused by respiration (if breathing is at high frequency[75]) and are effectuated by parasympathetic outflow. Low frequency fluctuations, in the range between 0.04 and 0.15 Hz, are the result of a blood pressure resonance phenomenon mediated by the baroreflexes and are effectuated by both sympathetic and parasympathetic fluctuations in outflow.[73, 76] Impaired function of the ANS is reflected in lower heart rate variability. Heart rate and heart rate variability can be determined from electrocar-

diographic recordings. The total variance in heart rate increases with the duration of the recording, because long measurements (e.g. 24 hours) include more rhythms (e.g. circadian rhythm) than short measurements.[72] In this thesis we used heart rate and heart rate variability from recordings ranging from 10 seconds to 24 hours. In addition to standard electrocardiographic recordings we also used the Actiheart device, an accelerometer combined with a heart rate monitor (CamNtech Ltd, Cambridge, United Kingdom) to calculate heart rate variability. The Actiheart device uses two electrodes at the level of the second intercostal space to measure the electrocardiogram (ECG), after which detection of heart beats occurs and interbeat intervals are recorded. The ECG itself is not stored. After extensive data processing, this recording can be used to estimate heart rate variability over a 24 hour period. The correlation between a Holter ECG and the Actiheart for estimating heart rate (variability) is high (correlation coefficient for mean RR interval 0.998, 95% CI: 0.994-1.000).[77]

The changes in beat-to-beat heart rate are mediated by the baroreflexes. Therefore, also the quantification of baroreflex sensitivity is valuable in the assessment of ANS function.[78] Baroreflex sensitivity is estimated as the change in RR intervals divided by systolic blood pressure (ms/mmHg). Better ANS function is reflected in a higher baroreflex sensitivity.

Ewing tests have been important for many years in the detection of diabetic autonomic neuropathy.[79] In **chapter 3**, we used four measures from the Ewing test battery. Three measures are obtained during an active change in position from lying to standing: the difference between the mean RR interval during one minute of rest prior to standing up and the minimum RR interval within 15 seconds after standing up (RRmax), the maximum RR interval between 15 and 30 seconds after standing up divided by the minimum RR interval 15 seconds after standing up (RRmax/min), and the systolic blood pressure difference (SBP difference) after standing up defined as the mean SBP over 30 seconds during 90-120 seconds after standing up, minus the mean over 30 seconds prior to standing up. Impaired ANS function is reflected in lower RRmax and RRmax/min and a larger (more negative) SBP difference. We also assessed the difference in maximum and minimum RR interval during inspiration and expiration averaged over six breaths (EI-difference) during deep breathing. Lower EI-difference is a reflection of impaired autonomic nervous system function.

Finally, three recent experimental studies showed that activation of the sympathetic nervous system is also reflected in changes on the standard ECG.[80-82] These changes include alterations in conduction times and ventricular repolarization. In **chapter 2** of this thesis, we introduced these novel measures to estimate activity of the sympathetic nervous system.

EPIDEMIOLOGICAL APPROACH

The aim of this thesis was to investigate the role of the ANS in the development of diabetes and cardiovascular disease by using observational population studies. To this end, we used observational data from large cohort studies for causal inference, the process of drawing a conclusion about a causal connection. Causal inferences are most straightforward from randomized controlled trials. However, for the study of ANS function, randomized controlled trials are neither possible nor ethical. Observational studies may be used for causal inferences, after sources of bias have been taken into account, such as confounding and reverse causation.[83]

Confounding may occur in observational studies when the exposure and the outcome variable share a common cause that may obscure the causal association and explain the observed crude association.[84] By adjusting for, or stratifying on, a confounding variable, the effect of the common cause may be eliminated from the association under study. In this thesis, we aimed to adjust our analyses for all known and measured confounding variables of the associations under study.

Reverse causation may occur especially in cross-sectional studies. When the exposure and outcome variables are measured at the same time, the observed exposure may be a consequence of the outcome variable rather than a cause. For example, when glucose tolerance and ANS function are associated in a cross-sectional study, it remains unclear whether glucose tolerances influences ANS function or vice versa. Longitudinal studies are less likely to suffer from reverse causation, as these studies provide information on the temporal relation between the exposure and outcome. However, in longitudinal studies reverse causation may also play a role when the outcome or disease of interest is already (subclinically) prevalent at baseline. For example, when ANS function is studied as a risk factor for the occurrence of cardiovascular disease after a myocardial infarction, we do not know whether impaired ANS function is merely a result of the myocardial infarction, or also a risk factor for cardiovascular disease in general.

In this thesis we used several approaches to overcome an effect of pre-existing diabetes and cardiovascular disease on ANS function in our studies. First, we excluded or adjusted for individuals with prevalent disease in our studies. Second, we restricted our analyses to populations without subclinical cardiovascular disease or diabetes at baseline; and third, we studied associations of ANS function with incident diabetes and cardiovascular disease using prospective follow-up studies. By studying first events of diabetes and cardiovascular disease during follow-up of individuals without pre-existing disease at baseline, we aimed to minimize a potential influence of underlying disease.

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