Cardiovascular autonomic dysfunction impact on cardiovascular complications across glucose metabolism

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Thanks for all the fish.

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Papers in the dissertation

Study I

Jonas R. Schaarup, Lasse Bjerg, Christian S. Hansen, Signe T. Andersen, Marleen van Greevenbroek, Miranda T. Schram, Bastiaan E. de Galan, Coen Stehouwer, Daniel R. Witte (2025). Cardiovascular autonomic dysfunction is linked with arterial stiffness across glucose metabolism: The Maastricht Study. medRxiv 2024.12.03.24317865; doi: https://doi.org/10.1101/2024.12.03.24317865 (under peer-review at BMJ Open Diabetes Research & Care)

Study II

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Study III

Cardiovascular autonomic neuropathy and indices of heart failure in type 2 diabetes: The CANCAN Study - add pieces!!!!!

Additional publications

The 2 following original research studies and 2 preprints have been published during the PhD period, but have not been included in the dissertation.

Peer-reviewed

Schaarup JR, Christensen MS, Hulman A, Hansen CS, Vistisen D, Tabák AG, Witte DR, Bjerg L. Autonomic dysfunction is associated with the development of arterial stiffness: The Whitehall II cohort.GeroScience, 2023. https://doi.org/10.1007/s11357-023-00762-0

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Pre-prints

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Anders Aasted Isaksen, **Jonas R. Schaarup**, Lasse Bjerg, Adam Hulman. (2025). Changes in public perception of AI in healthcare after exposure to ChatGPT. medRxiv 2025.01.23.25321048; doi: https://doi.org/10.1101/2025.01.23.25321048 (under review at npj digital medicine)

Table of contents

List of Figures

List of Tables

Abbreviations

ACEI: Angiotensin-converting enzyme-inhibitors
APT: Antiplatelet therapy
ARB: Angiotensin receptor blockers
ATC: Anatomical Therapeutic Chemical (classification)
BMI: Body mass index
CAN: Cardiovascular autonomic neuropathy
CARTs: Cardiovascular autonomic reflex tests
CD: Carotid artery distensibility coefficient
cf-PWV: Carotid-femoral pulse wave velocity
CI: Confidence interval
CVD: Cardiovascular disease
DKD: Diabetic kidney disease
eGFR: Estimated glomerular filtration rate
FPG: Fasting plasma glucose
GLD: Glucose-lowering drugs
GLP1RA: Glucagon-like peptide-1 receptor agonists
HDL: High-density lipoprotein cholesterol
HRV: Heart rate variability
IR: Incidence rate
IRR: Incidence rate ratio
HbA1c: Haemoglobin-A1c
LDL-C: Low-density lipoprotein cholesterol
MACE: Three-point major adverse cardiovascular events
OGTT: Oral glucose tolerance test
OR: Odds ratio
PAEE: Physical activity energy expenditure
RPAQ: Recent Physical Activity Questionnaire
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
SGLT2i: Sodium glucose co-transporter type 2 inhibitors
SES: Socioeconomic status
SDNN: Standard deviation of NN intervals
SDANN: Standard deviation of the averages of NN intervals in 5-minute segments

SDNN index: Mean of the SDs of all NN intervals for all 5-minute segments
pNN50: Proportion of NN intervals differing by more than 50 ms
RMSSD: Root mean square of successive differences between NN intervals
TP: Total power (variance of NN intervals 0.4 Hz)
ULF: Ultra low-frequency range (0.003 Hz)
VLF: Very-low-frequency range (0.003–0.04 Hz)
LF: Low-frequency range (0.04–0.15 Hz)
HF: High-frequency range (0.15–0.4 Hz)
T2D: Type 2 diabetes mellitus
TC: Total cholesterol
TG: Triglycerides
UACR: Urine albumin-to-creatinine ratio

Abstract

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Make a new paragraph by leaving a blank line.

Table of contents

1. Introduction

Diabetes mellitus is a growing global health concern, posing pressing challenges for public health systems[1]. As prevalence rises, more individuals are exposed to an increased risk of premature mortality and cardiovascular disease (CVD)[1]. People live longer lives with diabetes they face longer periods under the burden of diabetes complications[2]. Despite advancements in cardiovascular care, many complications are still detected at more advanced stages. These include coronary artery disease, often identified through ischemia or major CVD events and symptomatic heart failure. Early detection of CVD risk and asymptomatic heart failure, such as increased atheroma burden or subclinical heart failure, is desirable[3][4].

Over the last decades, cardiovascular autonomic dysfunction has repeatedly gained attention as a risk factor for CVD[5]. Heart rate variability (HRV) is considered a reliable marker for measuring autonomic function, as it reflects the balance between sympathetic and parasympathetic modulation of heart rate intervals[6]. Despite its recognition as a CVD risk factor, cardiovascular autonomic dysfunction has not been implemented in healthcare practice. In diabetes, lower HRV is regarded as an early indicator of cardiovascular autonomic neuropathy (CAN), which is diagnosed using cardiovascular autonomic reflex tests (CARTs)[7]. Signs of autonomic dysfunction, may already be present in individuals with prediabetes[8].Despite rising prevalence and increased CVD risk, people with prediabetes often remain outside structured treatment pathways [9][10]. Although diabetes contributes to autonomic dysfunction, it is still unclear at what stage in the diabetes risk spectrum HRV and CARTs become clinically useful for assessing CVD risk.

In the past, measuring HRV needed special instruments like an electrocardiogram. Today, it's easy to track HRV with everyday devices like smartwatches[11][12]. This increased accessibility allows for continuous monitoring and a better understanding of HRV over extended periods and under various free-living conditions[13]. However, long-term HRV patterns and specific diurnal responses in relation to cardiovascular complications remain less well understood.

The overall aim of this dissertation is to understand how cardiovascular autonomic dysfunction/CAN affects cardiovascular disease risk (i.e. heart failure, stroke, myocardial infarction) and specific subclinical markers of CVD: carotid-femoral pulse wave velocity and carotid artery distensibility in populations covering the whole glycemic continuum, from healthy glucose metabolism to type 2 diabetes.

2. Background

I will strart introduce the concept of type 2 diabetes and its associated risk with CVD. Following, I will provide an overview of various cardiovascular complications, including arteriosclerosis, atherosclerosis, and heart failure. Lastly, I describe cardiovascular autonomic function and its potential to enhance our understanding of CVD.

2.1. Type 2 diabetes and prediabetes

The progression from normal glucose metabolism to type 2 diabetes is characterized by sustained elevations in blood glucose levels. Type 2 diabetes is characterized by a progressive decline in beta-cell function, most often as a consequence of chronic insulin resistance [[14]][15]. Insulin resistance occurs when certain tissues, such as muscle and liver tissues, lose their sensitivity to insulin. As a result, glucose is not effectively taken up by these tissues and remains in the blood circulation. Meanwhile, beta-cell function deteriorates, leading to a diminished insulin response to glucose intake. Years before diagnosis, these changes contribute to rising fasting and postprandial glucose levels[14].

The body regulates glucose through various mechanism to maintain glucose homeostasis. During fasting, pancreatic alpha cells secrete glucagon, which stimulates hepatic glucose production via glycogenolysis and gluconeogenesis. Meanwhile, glucose is endogenously produced by the liver and kidneys and utilized by body tissues. After a meal, rising blood glucose levels stimulate pancreatic beta cells to release insulin and trigger the secretion of incretins, such as glucagon-like peptide-1 (GLP-1) from the intestines. Insulin and incretins work together to suppress hepatic glucose production, while insulin promotes glucose uptake in muscle and adipose tissue. Excess glucose is primarily stored as glycogen in the liver and muscles, with some converted to triglycerides for long-term storage. Multiple organs, including the pancreas, liver, kidneys, intestines, muscle, and adipose tissue are involved in this coordinated process. The autonomic nervous system plays a supportive role in glucose homeostasis by modulating metabolic activity. Parasympathetic signals tend to reduce glucose production, while sympathetic signals enhance it, especially during hypoglycemia[15].

Diabetes progression is a continuous process, with type 2 diabetes defined based on glucose thresholds associated with an increased risk of diabetes-specific microvascular complications, particularly retinopathy. The World Health Organization (WHO)[16] and American Diabetes Association (ADA)[17] diagnostic criteria for type 2 diabetes include fasting plasma glucose 7.0 mmol/L, 2-hour plasma glucose 11.1 mmol/L during an oral glucose tolerance test (OGTT), or hemoglobin A1c (HbA1c) 6.5% (48 mmol/mol). OGTT is defined by measuring glucose levels two hours after the ingestion of a standard 75-gram glucose load. Diabetes progression is a continuum, with type 2 diabetes defined based on glucose thresholds associated with an increased risk of diabetes-specific microvascular complications, particularly retinopathy. However, many complications of diabetes, such as macrovascular disease, neuropathy, cancer, and cognitive impairment, may develop at earlier stages of dysglycemia[[18]][1920]. This stage is referred to as prediabetes or high risk of diabetes and is defined by fasting plasma glucose levels between 6.1–6.9 mmol/L, 2-hour plasma glucose levels between 7.8–11.0 mmol/L (WHO criteria), and HbA1c levels between 5.7–6.4% (39–47 mmol/mol) (ADA criteria)[17]. In parallel with the growing prevalence of type 2 diabetes, the prevalence of prediabetes is also on the rise[9].

Risk factors for progression to type 2 diabetes and its complications range from genetic predisposition to lifestyle and socio-environmental factors. The most common precursor to diabetes is central obesity, characterized by excess body fat[21]. The accumulation of diabetes risk factors is linked with a combination of adverse changes in cardiometabolic markers, including increases in low-density lipoprotein (LDL) cholesterol, triglycerides, body mass index (BMI), and systolic blood pressure, along with decreases in high-density lipoprotein (HDL) cholesterol[22].

Diabetes increases the risk of both microvascular and macrovascular complications, which are major contributors to the morbidity and mortality associated with the disease[15]. Diabetes and cardiovascular disease (CVD) share common risk factors, including obesity, hypertension, and hypercholesterolemia, as well as lifestyle-related factors. Beyond these, chronic hyperglycemia promotes the formation of harmful byproducts such as reactive oxygen species and advanced glycation end products, which drive oxidative stress and inflammation[23]. These processes contribute to endothelial dysfunction and vascular damage[23]. As individuals progress toward diabetes, their cardiovascular risk increases, making them more susceptible to developing CVD[22]. However, the identification of preclinical stages of CVD and how CVD risk differs among individuals at high risk of diabetes and individuals with type 2 diabetes needs further definition.

2.2. Cardiovascular disease

Globally, CVD remains the leading cause of death. At the population level, CVD risk is primarily attributable to modifiable lifestyle behaviors such as chronic stress, physical inactivity, unhealthy diet, excessive alcohol consumption, and smoking, as well as socio-environmental factors like socio-economic status and air pollution[24]. At the individual level, these exposures often manifest through more proximal biological risk factors, including hypertension, hypercholesterolemia, diabetes, and obesity, which elevate the likelihood of developing CVD. Along the causal pathway, these intermediate conditions act as comorbidities that accelerate disease progression. These processes are underpinned by biomolecular mechanisms, including local and systemic inflammation, oxidative stress involving oxidized low-density lipoprotein (LDL), and dysregulated immune responses mediated by pro-inflammatory cytokines and signaling pathways. Risk factors across different types of CVD contribute to distinct pathophysiological mechanisms, involving structural, signaling, inflammatory, and hemodynamic changes within the cardiovascular system. Among these, cellular and molecular signaling pathways play a central role in regulating vascular tone, cardiac function, and inflammatory responses. These processes are closely modulated by the autonomic nervous system through sympathetic and parasympathetic nerve branches.

2.2.1. Arteriosclerosis

Hard CVD endpoints remain the primary focus of prevention strategies, emerging evidence emphasize the importance of vascular aging in early disease development[25]. Arteriosclerosis, commonly referred to as arterial stiffness, is a hallmark of this process. Biologically, the medial layer of large arteries consists of a structured network of vascular smooth muscle cells together with elastic and collagen fibers, forming functional musculoelastic sheets[26]. Arterial stiffness arises from progressive remodeling of the arterial wall [[25]][27]. This remodeling is driven by changes in the structural interactions between elastin and collagen fibers, along with functional alterations in vascular smooth muscle cells and the accumulation of calcium and advanced glycation end products[26]. Remodeling of the arterial wall increases systolic blood pressure and reduces coronary perfusion, thereby contributing to the development of hypertension and, eventually, cardiovascular disease[28]. Additionally, they elevate the pulsatile load on the microcirculation, promoting the progression of chronic kidney disease, vascular dementia, and Alzheimer's disease[25].

2.2.2. Atherosclerosis

Atherosclerosis is characterized by the accumulation of cholesterol, lipids, and other substances within the arterial walls, forming plaques that narrow the arteries and reduce blood flow (ref.). This chronic process can lead to progressive occlusion of the vessel, contributing to reduced oxygen supply to the heart (ref.).

Atherosclerotic plaques can be classified into stable and unstable types, each with distinct structural characteristics and clinical implications. Stable plaques typically have a thick fibrous cap composed of collagen, a small lipid core, and low levels of inflammation. These plaques are less likely to rupture and tend to remain intact over time due to internal remodeling. In contrast, unstable plaques, also known as vulnerable plaques, often contain a large lipid-rich necrotic core, a thin fibrous cap, and infiltration by inflammatory cells such as macrophages. A well-recognized subtype of unstable plaque is the thin-cap fibroatheroma, which is particularly prone to rupture. When rupture occurs, the necrotic core becomes exposed to the bloodstream, initiating the formation of a thrombus or blood clot. This acute event can abruptly obstruct the artery, resulting in myocardial infarction. In some cases, fragments of the thrombus may dislodge and travel further along the arterial tree, leading to thromboembolic complications such as stroke or peripheral ischemia[29]. Chronic ischemia due to reduced coronary perfusion can lead to myocardial remodeling, impaired contractility, and electrical instability, thereby increasing the risk of arrhythmias and heart failure[[30]][31].

Myocardial infarction

[Try to briefly mention the main advances that have powered this marked reduction in CVD events: much lower smoking prevalence, better management of hypertension and hyperlipidemia, but also thrombolysis, stents and CABGs becoming standard practice.]

Myocardial infarction (MI) occurs due to the rupture of an atherosclerotic plaque in the coronary arteries, triggering thrombus formation that blocks blood flow. This leads to oxygen deprivation (ischemia) and subsequent myocardial injury or necrosis. If untreated, this process can cause extensive cardiac damage and fatal arrhythmias. Over the past decades, the incidence of myocardial infarction (MI) has declined in high-income countries(ref.) with a marked reduction in MI-related mortality(ref.). These improvements are largely attributed to a combination of public health initiatives and medical advances. On the public health front, a substantial decrease in smoking prevalence has been the most important lifestyle-related factor contributing to the reduction in CVD [[32]][33]. Medically, the improved preventive management of hypertension and hyperlipidemia has reduced the burden of atherosclerotic disease. In acute care, the widespread adoption of evidence-based interventions such as thrombolytic therapy, percutaneous coronary interventions (including stenting), and coronary artery bypass grafting (CABG) has improved survival and outcomes following MI. In type 2 diabetes, the risk of MI is elevated by 72%, with an approximately threefold risk among patients under 60 years compared to age under 60 without type 2 diabetes[34]. Similar to the general population, its incidence and fatality have declined in diabetes.

Stroke

The majority of strokes are ischemic, caused by an obstruction in a cerebral artery, often due to an atherosclerotic plaque or embolism. The second main cause is hemorrhagic stroke, which is characterized as a hypertensive small-vessel disease, leading to small lipohyalinotic aneurysms that subsequently rupture, causing intracerebral bleeding[35]. Ischemic stroke remains one of the global leading contributor to mortality and disability[36]. The incidence, prevalence, and cause-specific mortality of stroke remain high but have stagnated, although some declines have been observed in high-income countries[37]. Stroke risk is already elevated at high levels of glucose (fasting plasma, OGTT, and HbA1c) among people in a pre-diabetic range where the the risk exceed of 26% higher risk compared to population without diabetes [[38]][39]. In type 2 diabetes, the ischemic stroke risk is elevated almost two-fold compared with individuals without diabetes[34].

2.2.3. Heart failure

Heart failure develops gradually with age and often accelerates with the progression of type 2 diabetes. As prevention and treatment of CVD have improved survival in recent years, the prevalence of heart failure has increased, while the incidence remains stable, but may rise with aging populations[40].

Heart failure is commonly classified as either ischemic or non-ischemic in origin. It may arise as a consequence of atherosclerosis, arteriosclerosis, or both, contributing to myocardial ischemia, pressure overload, and structural cardiac changes. Heart failure is a clinical condition characterized by symptoms of breathlessness, fatigue, and fluid retention, often accompanied by clinical signs such as pulmonary crepitations, jugular venous elevation, and peripheral edema. Heart failure can be defined hemodynamically as the inability to maintain adequate cardiac output at rest or during exertion, or the ability to do so only with elevated cardiac filling pressures. It is a complex cardiovascular disease caused by structural and functional changes in the heart musculature, affecting systolic and/or diastolic pumping function. Heart failure is generally classified into two subtypes: heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). Both subtypes involve cardiac remodeling but are defined by left ventricular ejection fraction (LVEF). HFrEF is defined by an LVEF $<$ 40%, while HFpEF is characterized by an LVEF   50% along with structural or functional cardiac abnormalities, as assessed by echocardiography. HFrEF is often a consequence of repeated, non-fatal myocardial infarctions. These events can leave behind scar tissue in the myocardium, impairing the heart's ability to contract effectively and leading to progressive systolic dysfunction.

The most common feature of HFpEF is left ventricular diastolic dysfunction, caused by impaired relaxation and increased stiffness, leading to elevated left atrial pressure and reduced diastolic reserve[41]. Over the past decades, the prevalence of HFpEF has increased with an aging population and more people living with conditions such as hypertension, diabetes, and obesity. It is diagnosed based on structural or functional abnormalities identified through echocardiographic measures, such as left ventricular hypertrophy, left atrial enlargement, or elevated filling pressures[42]. The diagnosis may seem straightforward, but it is often challenging in community settings, as patients frequently present without typical heart failure symptoms (e.g., shortness of breath) and are not routinely assessed with biomarkers like N-terminal pro-B-type natriuretic peptide (NT-proBNP) or brain-neuretic-peptide (BNP). As a result, HFpEF is commonly underdiagnosed and consequently detected at more severe stages, leading to hospitalization[42].

2.3. Cardiovascular autonomic dysfunction

The cardiovascular system is regulated by autonomic nervous system which influences heart rate and vasoconstrictuion through neurotransmitter release by the sympathetic and parasympathetic nerves. The primary neurotransmitter of the sympathetic nervous system is noradrenaline, while the parasympathetic nervous system primarily releases acetylcholine by stimulation through the Vagus nerve. Sympathetic activation increases heart rate and myocardial contractility by stimulating the sinoatrial (SA) node, atrioventricular (AV) node, and ventricular myocardium. In contrast, parasympathetic activation primarily reduces heart rate by directly modulating SA node activity through vagal stimulation. It also slows AV nodal conduction, predominantly via the left vagus nerve, thereby prolonging atrioventricular conduction time. Afferently nerves mainly carry sensory information (e.g., baroreceptor input from the carotid sinus and aortic arch) to the brain, which then adjusts efferent autonomic output to regulate arterial tone. Hence, the autonomic nervous system dynamically regulates heart rate and blood pressure to maintain homoeostasis in response to physiological demands, such as rest and physical activity.

[insert figure of brain heart and symphathtic nerves]

In youth, the autonomic nervous system is highly adaptive and responsive to living conditions, maintaining autonomic balance. However, with aging, there is a gradual decline in parasympathetic function and an increase in sympathetic activity. Additionally, metabolic-related conditions such as obesity and diabetes have been shown to further contribute to autonomic dysfunction. Autonomic dysfunction reflects a stressed cardiometabolic environment, as both dysfunction in lipid and glucose metabolism are associated with increased sympathetic activity[43]. This dysfunction may result from cumulative neural damage mediated by mechanisms such as hyperinsulinemia, insulin resistance, and elevated levels of adipokines. At the same time, autonomic dysfunction is known to disrupt lipid and glucose metabolism[43]. Therefore, the relationship between autonomic dysfunction and cardiometabolic factors is likely a vicious cycle[44]. The consequences can lead to cardiovascular autonomic dysfunction/neuropathy (CAN), resulting dysregulation in heart rate and vascular dynamics. In this thesis, we will use 'cardiovascular autonomic dysfunction' as the broader term, while 'CAN' will refer specifically to autonomic dysfunction resulting from neuropathy in type 2 diabetes.

Cardiovascular autonomic function can be assessed using heart rate variability (HRV) indices, which measure the variation in successive normal RR intervals in milliseconds. HRV provides time- and frequency-domain estimates of the balance between sympathetic and parasympathetic activity. High HRV reflects an autonomic nervous system with strong adaptability to the body's demands, whereas low variation indicates poor adaptation to changing conditions. HRV changes in response to different physiological or environmental conditions (e.g., sleep, stress, posture, physical activity), and these changes can be observed in its natural 24-hour (diurnal) pattern[13]. Most studies have examined cardiovascular autonomic function using short-term ECG recordings at rest. However, extended HRV recordings across the circadian cycle may offer deeper insights into the influence of lower-frequency variability sources, such as very-low frequency (0.003–0.04 Hz) and ultra-low frequency (0.003 Hz)[reflecting what]. HRV has been applied across several research domains. For example, in psychology as a marker of mental stress, in exercise physiology as an indicator of recovery, in cardiovascular research as a marker of autonomic dysfunction due to cardiac complications, and in diabetes research as a marker of autonomic neuropathy(ref.,ref.ref.,ref.). Type 2 diabetes alters the expression of sympathetic bursts, as measured by resting muscle sympathetic nerve activity (MSNA). MSNA is elevated in individuals with both type 2 diabetes and hypertension, compared to those who are normotensive, regardless of whether they have diabetes or not[45]. Parasympathetic activity is also impaired in individuals with high cardiometabolic risk and type 2 diabetes, as reflected by reduced baroreflex sensitivity[46] and lower HF and RMSSD short-term HRV. Before onset of diabetes and during progression of diabetes long-term (24-hour) HRV has shown to be lower compare to those with normal glucose metabolism [44][8]. Cardiovascular autonomic reflex tests (CARTs) and orthostatic hypotension are considered the gold standard for assessing CAN[47]. The diagnosis includes assessing pulse rate ratio under test conditions, such as the deep breathing test, the lying-to-standing test, and the Valsalva maneuver[47]. Both HRV and CARTs have shown to be associated with cardiovascular disease, heart failure, and all-cause mortality, primarily in populations with type 2 diabetes or established cardiovascular disease. However, it remains unclear at which stage in the progression of diabetes risk to pre-diabetes to diabetes these measures begin to influence the risk of cardiovascular complications.

2.4. Risk-stratification

Current cardiopreventive guidelines place strong emphasis on prevent and treat type 2 diabetes. The 2022 ADA/EASD guidelines for the management of hyperglycemia in type 2 diabetes recommend, cardioprotective medication (GLP-1 receptor analogues and SGLT2-inhibitors) as first-line options for individuals at high cardiovascular risk. Due to their benefits in heart failure, SGLT2 inhibitors are specifically recommended for patients with documented HFrEF or HFpEF. High cardiovascular risk is defined as the presence of at least two risk factors at age >55 years, such as obesity, hypertension, smoking, dyslipidemia, or albuminuria. However, no additional preclinical markers are recommended to identify individuals at higher CVD or HF risk. Despite their increased risk of cardiovascular complications, individuals at high risk of developing diabetes remain outside structured treatment options, even though diabetes risk and cardiometabolic markers can be successfully modified through lifestyle interventions and medication such as GLP-1 analogoues [[48]][49]. During the progression and following the onset of type 2 diabetes, preclinical stages may be characterized by markers of elevated cardiovascular risk, highlighting the potential for early risk stratification. Risk stratification is the process of classifying or ranking individuals in increasing order of estimated risk, based on risk scores, biomarker levels, omic data (metabolomic, proteomics, and genomic) or preclinical conditions. This approach aids in identifying patients for prognostic or diagnostic purposes, identifying subgroups that require further evaluation, intensified treatment, or lifestyle modifications.

Cardiovascular autonomic dysfunction despite it's relationship with cardiovascular complication has not been used in clinical practice. Larger epidemiological cohort studies encompassing various stages of diabetes risk, from normal glucose metabolism to prediabetes, onset of type 2 diabetes, and longer term progression of type 2 diabetes, serve as valuable resources for identifying risk-stratification opportunities. Epidemiological studies provide a broad representation of the target population, allowing understand the relationship between cardiovascular autonomic dysfunction and cardiovascular complications. They also have potential to determine when, along the trajectory of diabetes progression and duration, autonomic function are meaningful for cardiovascular risk-stratification.

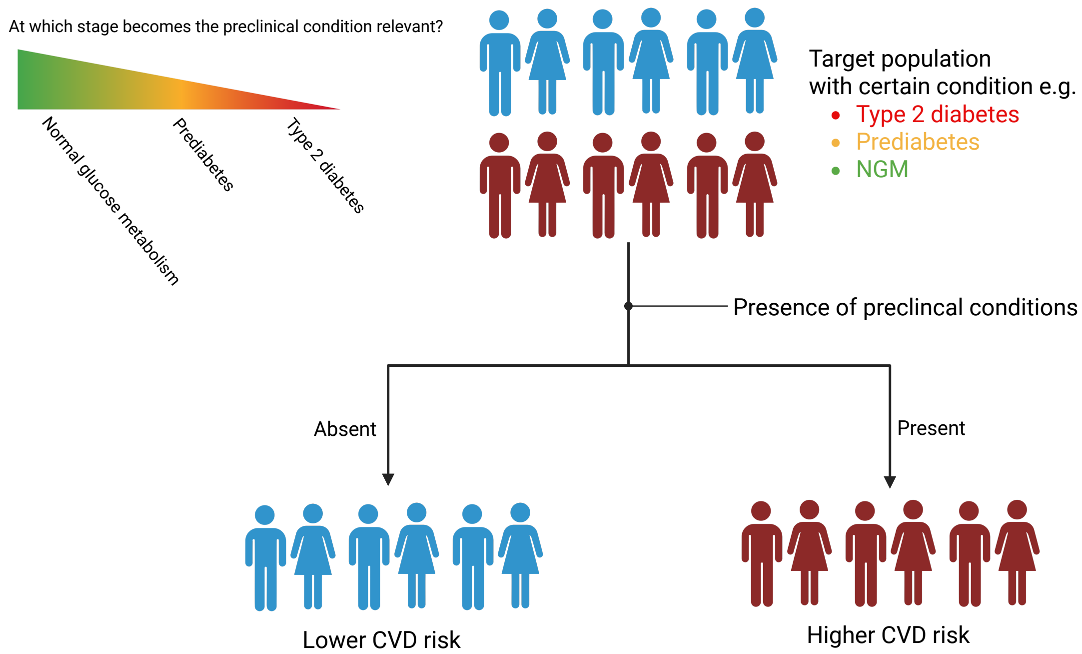

Figure 2.1.: Risk-stratification based on preclinical disease. (Source: Author)

2.5. Aetiological research [Kortes ned eller fjern]

Aetiology seeks to identify the causes and contributing factors of disease, forming the foundation for understanding its development and underlying mechanisms. Ideally, to determine causal effects, we would compare outcomes in individuals who were exposed to a risk factor with what would have happened if they had not been exposed. Since this counterfactual scenario is impossible to observe directly, we rely on study designs, such as randomized controlled trials when feasible, and apply statistical methods to data from observational cohort studies to approximate these comparisons.

Cardiovascular autonomic dysfunction is linked to CVD and all-cause mortality. However, many questions remain regarding the underlying causal mechanisms. Furthermore, as dysglycemia is known to be a primary driver of autonomic dysfunction[50], the questions is to which extent it modulates the relationship between cardiovascular autonomic dysfunction and CVD? This relationship remains unclear, highlighting the need for a deeper understanding of this interplay in target populations representing different stages of glucose metabolism.

3. Aim and hypothesis

The hypotheses of this dissertation are:

CAN and autonomic dysfunction is associated with CVD and acts as an early risk factor for heart failure and other cardiovascular complications, including stroke, and myocardial infarction in patients with prediabetes and/or type 2 diabetes. In addition autonomic dysfunction is associated with higher levels of sub-clinical meassures such as carotid-femoral pulse wave velocity and carotid artery distensibility.

The overall aim of this dissertation is to understand how cardiovascular autonomic dysfunction/neuropathy (CAN) affects cardiovascular disease risk (i.e. heart failure, stroke, myocardial infarction) and specific subclinical markers of CVD: carotid-femoral pulse wave velocity and carotid artery distensibility in populations covering the whole glycemic continuum, from healthy glucose metabolism to type 2 diabetes.

Study I: Quantify the cross-sectional association between 24-hour HRV and subclinical markers of cardiovascular complications: carotid-femoral pulse wave velocity and carotid artery distensibility, in a participants with normal glucose metabolism, prediabetes or type 2 diabetes.

Study II: Quantify the longitudinal association of week-long and hourly HRV with incidence ischemic-CVD, heart failure, and all-cause mortality in a population with high-risk of diabetes.

Study III: Quantify the cross-sectional association between CAN and heart failure. Heart failure will be defined by clinical measures i.e. N-terminal-pro-BNP (Pro-BNP), WATCH-DM risk, and New York Heart Association (NYHA) scores among individuals with type 2 diabetes.

4. Materials and methods

4.1. Overview of the studies

Table 4.1.: Table 1: Overview of studies

	Study I	Study II	Study III
Title	Cardiovascular autonomic dysfunction is linked with arterial stiffness across glucose metabolism: The Maastricht Study	Cardiovascular autonomic dysfunction precedes cardiovascular disease and all-cause mortality: 11-year follow-up in the ADDITION-PRO study	Cardiovascular autonomic neuropathy and subclinical heart failure in type 2 diabetes: The CANCAN study
Design	Aetiological cross-sectional study	Aetiological prospective cohort study	Descriptive cross-sectional study
Cohort	Maastricht study	ADDITION-PRO study	CANCAN study
Study population	3673 people with normal glucose metabolism, prediabetes, and type 2 diabetes	2082 people with high risk of diabetes	173 patients with type 2 diabetes visiting outpatients clinics
Data sources	Population-based cohort from The Maastricht Study in the Netherlands	Cohort study of selected people based on having high risk of diabetes	Clinical cohort study
Determinant	24-hour HRV	multiday and hourly HRV	Cardiovascular autonomic reflex test
Primary outcome	Arterial stiffness	Major adverse cardiovascular events, heart failure, and all-cause mortality	NT-proBNP and NYHA classification?
Statistical analysis	Linear regression	Poisson regression	Logistic regression

	Study I	Study II	Study III
Missing data	Complete case analysis	Multiple imputation of chained equations for confounders	Complete case analysis and multiple imputation of chained equations for CART and confounders

4.1.1. Study population

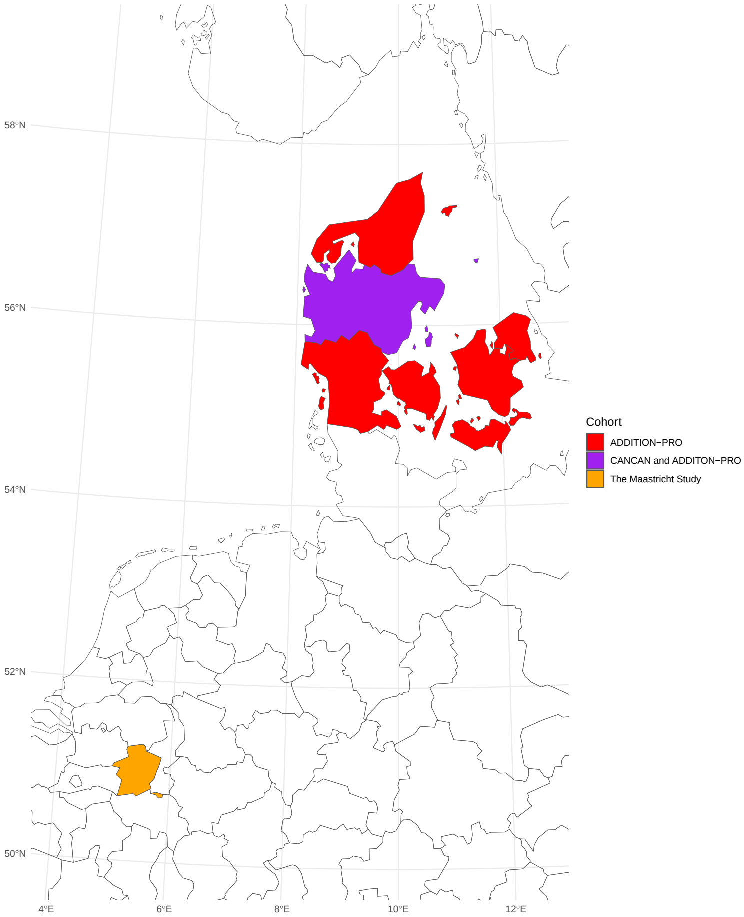

Figure 4.1.: Study populations

4.1.1.1. Study I - The Maastricht Study

The Maastricht Study is a prospective observational population-based study of the general population of the province of Limburg, in the southern part of the Netherlands. The study emphasized the recruitment of people with type 2 diabetes, through the regional Diabetes Patient Registry, to extensively phenotype individuals with type 2 diabetes and those in intermediate stages of the disease. The eligibility criteria included an age range of 40–70 years. Participants were recruited through mass media campaigns and mailings from municipal registries (Gemeentelijke Basis Administratie; GBA). In the analysis of Study I, the study among 7449 people included participants with measurements of 24-hour HRV and at least one measure of arterial stiffness (carotid-femoral pulse wave velocity or carotid artery distensibility), both of which were completed within a three-month period between November 2010 and December 2020. The study has been approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written i

4.1.1.2. Study II - ADDITION-PRO

The ADDITION-PRO study is a prospective, population-based cohort nested within the Danish arm of the ADDITION-Europe study. ADDITION was originally designed as a stepwise screening program for type 2 diabetes in general practice, aiming to identify individuals with screen-detected type 2 diabetes for recruitment into the ADDITION trial. ADDITION-PRO aims to investigate early markers of CVD and metabolic dysfunction in individuals in different tiers of diabetes risk.

The ADDITION-Europe screening program identified a large number of individuals with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and normoglycemia despite having risk factors for diabetes and CVD. Participants for ADDITION-PRO were recruited from the original ADDITION-DK screening cohort, which included individuals from 190 general practices across Denmark. The recruitment strategy focused on individuals at high risk of diabetes without type 2 diabetes, identified through a stepwise screening program that incorporated the Danish diabetes risk score from the Inter99. This assessment, conducted between 2001 and 2006, considered factors such as age, sex, history of gestational diabetes, family history of diabetes, known hypertension, BMI, and physical activity. High-risk individuals were further screened for type 2 diabetes using blood measurements, including HbA1c, random blood glucose, FPG, and OGTT. Those with screen-detected diabetes, confirmed by a second OGTT, were invited to participate in the ADDITION trial. High risk individuals without type 2 diabetes were further considered in as the sampling frame for ADDITION-PRO.

Between 2009 and 2011, a follow-up health examination was conducted at four ADDITION-DK study centers to establish a cohort baseline. Eligible participants were those still alive, residing near the research centers (Steno Diabetes Center Copenhagen, Aarhus University Hospital, Holstebro Hospital, and the Hospital of South West Jutland, Esbjerg), and who had not withdrawn consent. Eligibility criteria included individuals aged 40–70 years who had previously undergone diabetes screening in ADDITION-DK. Exclusion criteria included pregnancy, psychological or psychiatric disorder preventing informed consent, and life-limiting conditions. One key feature of the data collection was the precise measurement of physical activity and energy expenditure using a combined chest worn accelerometer/heart rate monitor (ActiHeart), which recorded acceleration and heart rate over a week. In study II, we included participants with a least 48-hour recording for our first analysis, and then include those participants with hourly measures of physical acceleration during the hourly HRV recording for th second analysis. We also excluded participant with prior CVD ten years before inclusion.

Disease history and follow-up data for the population were obtained from Denmark's unique national registry system, which allows linkage of health records using the personal Civil Registration Number assigned to all citizens. The following national registries were accessed to collect information on incident CVD and mortality, medication use, and healthcare utilization: the National Patient Registry (hospital admissions and outpatient contacts), the National Health Service Registry (general practice visits), the Medical Prescription Registry, the Diabetes Registry, and the Cause of Death Registry.

4.1.1.3. Study III - CANCAN

The CANCAN Study is an observational study conducted at two hospital outpatient clinics in Viborg Regional Hospital and Regional Hospital Gødstrup. It aims to implement a screening protocol for identifying high-risk individuals using CAN assessments, continuous glucose monitoring, and heart failure indicators. All measures were part of routine clinical care for type 2 diabetes in Central Denmark. We included 200 adults (>18 years) with type 2 diabetes with duration of over one year. Exclusion criteria were recent laser-treated eye disease (3 months), pregnancy, lactation, life-threatening illness, or cognitive impairment preventing consent. Participants were identified via electronic records and informed about the study by their doctor during a telephone call. Those interested attended a dedicated meeting before their annual diabetes exam, where study details were discussed. Recruitment took place from 2021 to 2024. In study III, participants without a valid NT-proBNP measurement were excluded.

4.2. Study variables

4.2.1. Measures for cardiovascular autonomic dysfunction/ neuropathy

Figure 4.2.: Left: Holter monitor Middle: Actiheart Right: Handheld Vagus™ device

Heart rate variability

In study I-III a device was used to capture the distance between each heartbeat defined as RR intervals from electrocardiogram traces either directly from heart-beat traces or indirectly from pulse traces. From this a sequence of successive heart beat intervals is extracted to calculate HRV. The pool of hearbeat data, we extrapolated time-domain and frequency-domain HRV indices.

Time-domain indices

Time-domain measures of HRV are based on the statistical distribution of normal-to-normal (NN) heartbeat intervals. Description of time-domain indices are summarized in **?@tbl-td**.

Frequency-domain indices

Frequency-domain HRV indices are derived from sequences of NN intervals transformed into the spectral domain using Fourier transformation. These indices quantify heart rate oscillations over different timescales. Short-term variations, such as respiratory sinus arrhythmia, reflect rapid autonomic changes, while longer oscillations capture autonomic responses to posture changes, circadian rhythms, or other physiological processes. Description of frequency-domain indices are summarized in **?@tbl-fq**.

Holter recordings in study I

All ECG recordings were obtained using a 12-lead Holter system (Fysiologic ECG Services, Amsterdam, the Netherlands) over 24 hours, as previously described. Participants were instructed to follow their regular daily activities but avoid showering during the

Table 4.2.: **Box 1** Time-domain indices reflections of autonomic function

Time-domain HRV	Description
Standard deviation of NN heart beat intervals (SDNN, in ms)	Measures the total variation in interbeat intervals and reflects both sympathetic and parasympathetic activity[6].
SD of the averages of NN intervals in 5-minute segments throughout the recording (SDANN, in ms)	Measures variations in 5-minute mean interbeat intervals, primarily reflecting autonomic fluctuations associated with the circadian rhythm[6]
Mean of the SDs of all NN intervals for all 5-minute segments (SDNN index, in ms)	Measures the average short-term variability in interbeat intervals across successive 5-minute periods, reflecting both sympathetic and parasympathetic modulation of heart rate[6]
NN50 count divided by the total number of all NN intervals (pNN50, percentage)	Measures the proportion of successive interbeat intervals differing by more than 50 ms, primarily reflecting parasympathetic (vagal) activity[51].
Square root of the mean of the sum of squares of differences between adjacent NN intervals (RMSSD, in ms)	Measures variation in successive interbeat intervals during inhalation and exhalation, primarily reflecting parasympathetic (vagal) activity[51]

Table 4.4.: **Box 2** Frequency-domain indices reflections of autonomic function

Frequency domain HRV	Description
Variance of all NN intervals 0.4 Hz, total power (TP, in ms^2)	Measures the total variation in interbeat intervals, reflecting both short- and long-term autonomic regulation by the sympathetic and parasympathetic nervous system[6].
Ultra low-frequency range (ULF, in ms^2 0.003 Hz)	Measures very long-term oscillations in interbeat intervals, influenced by autonomic responses to circadian rhythms, physical activity, metabolic processes, and thermoregulation [52][53].
Very-low-frequency range (VLF, in ms^2; 0.003–0.04 Hz)	Measures oscillations in interbeat intervals over 5-minute periods, reflecting the activity of the renin–angiotensin system and peaks in sympathetic nervous system activity, while also depending on parasympathetic modulation[54][50].
Low-frequency range (LF, in ms^2; 0.04–0.15 Hz)	Measures intermediate oscillations in interbeat intervals, reflecting a combination of sympathetic and parasympathetic nervous system activity, particularly associated with baroreflex function and blood pressure regulation[55].
High-frequency range (HF, in ms^2; 0.15–0.4 Hz)	Measures short-term oscillations during inspiration and expiration, reflecting parasympathetic modulation of heart rate via the vagus nerve, and closely associated with respiratory sinus arrhythmia[56].

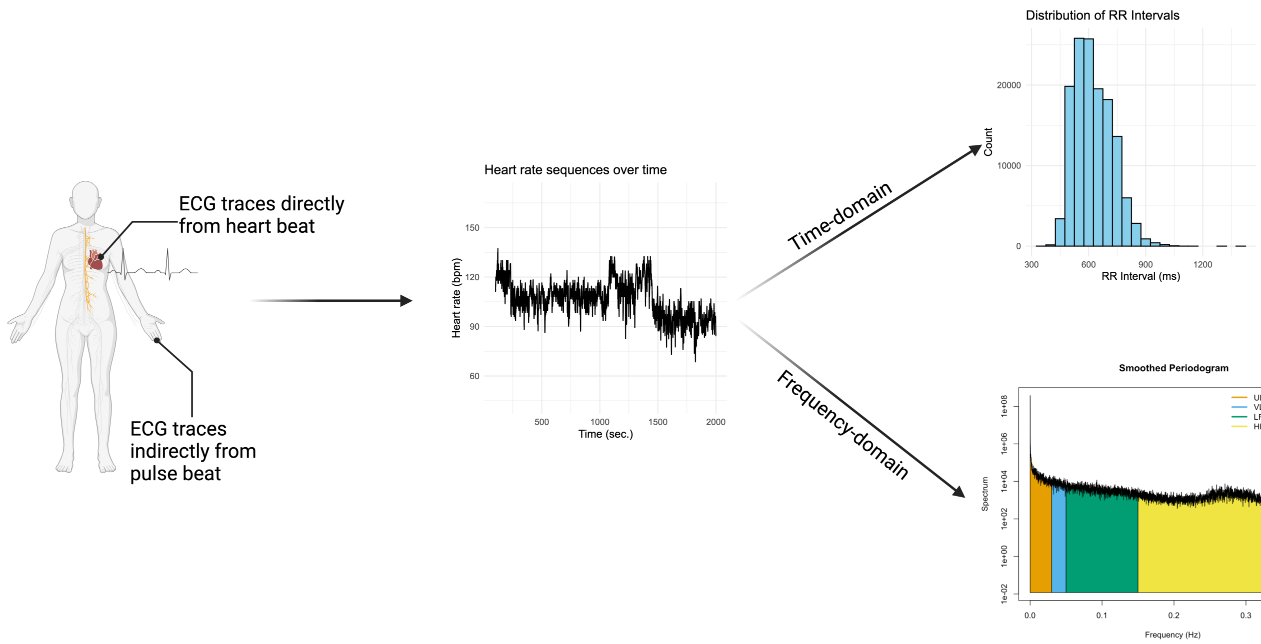

Figure 4.3.: Heart rate variability. (Source: Author)

recording. The ECG data were processed using proprietary Holter Analysis Software (Fysiologic ECG Services), where artefacts and ectopic beats were excluded through automated processing and manual validation. A minimum recording duration of 18 hours was required for further analysis. Inter-beat intervals between consecutive sinus beats were provided in milliseconds (ms). Time-domain HRV indices were calculated, including SDNN, SDANN, RMSSD, SDNN index, and pNN50. Frequency-domain measures were derived using Fast Fourier Transform, including TP, ULF, VLF, LF, and HF. Outliers were removed. HRV indices were standardised by their mean and SD, and composite Z-scores were computed for time and frequency-domain measures, respectively. This selection of indices covers the main sources of HRV variance.

ActiHeart heart rate and physical activity in study II

Heart rate was measured using a combined accelerometer and heart rate monitor (ActiHeart, CamNTech, Cambridge, UK), recording uniaxial acceleration and heart rate. The data collection and processing methods have been described previously. Mean heart rates were recorded in 30-second epochs, and HRV was derived as the variation between consecutive normal heartbeats on the ECG. HRV calculations were performed using the RHRV package (version 4.2.7) in R, including SDNN, SDANN, SDNN index, TINN, and mean HR (mHR). We tested our approach on a dataset with full access to all interbeat intervals to validate our algorithm[57]. These indices have shown high validity for HRV indices based on global distribution (e.g. SDNN, SDANN, SDNNi) in 24-hour recordings. HRV indices were calculated by week, 24-hour cycle, and hour of the day, with hourly values averaged across recording days.

Vagus device for cardiovascular autonomic reflex test in study III

CAN was diagnosed using cardiovascular autonomic reflex tests (CARTs), the gold standard for CAN assessment. R-R intervals were derived from an ECG signal using the Vagus™ device (Medicus Engineering, Aarhus, Denmark). We used pulse rate ratios measured under different conditions. Three standardized cardiovascular autonomic reflex tests (CARTs) were performed—lying-to-standing, deep breathing, and the Valsalva manoeuvre, following a standardized protocol conducted between 8:00 a.m. and 2:00 p.m., after 10 minutes of supine rest. Smoking and caffeine intake were prohibited two hours before testing. Each test was conducted once by trained examiners.

Manifest CAN was defined as two or more abnormal CARTs using age-specific cut-off values (ref.). The Vagus™ device's accuracy has been validated against FDA standards and stationary devices, showing moderate to high reproducibility (ref.).

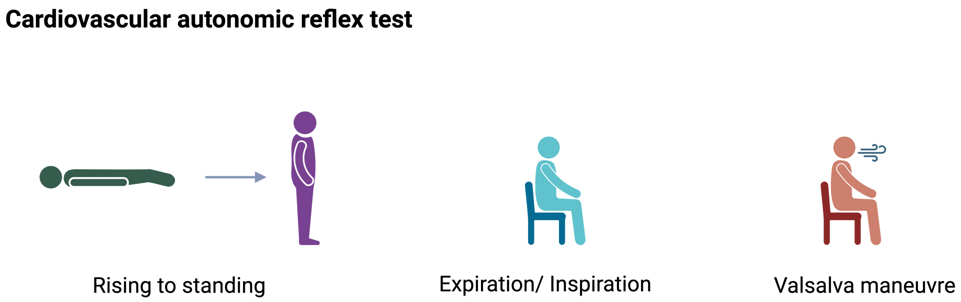

Figure 4.4.: CART

HRV was derived from all CARTs using autoregressive spectral analysis. Time domain measures included SDNN and RMSSD, while frequency domain measures included LF, HF, and total power. Orthostatic hypertension was defined as a sustained drop in systolic blood pressure of 20 mmHg or diastolic blood pressure of 10 mmHg within three minutes of standing (ref.).

4.2.2. Confounders and variables for instrumental bias

Across Studies I, II, and III, a comprehensive set of covariates and potential confounders were assessed, including lifestyle factors, clinical measurements, biochemical markers, and socioeconomic indicators.

Smoking status was self-reported in all studies, categorized as never, former, or current (Study I), current/ex/never (Study II), and smoker/non-smoker (Study III). Alcohol consumption was recorded as average weekly units in all three studies. Physical activity was assessed via self-report in Studies I, II, III, with Study I capturing total and moderate-to-vigorous activity (hours/week), Study II used the Recent Physical Activity Questionnaire (RPAQ) to calculate physical activity energy expenditure (PAEE), and Study III classifying activity as sedentary or non-sedentary. In Study II also used combined accelerometry and heart rate monitoring (ActiHeart) to estimate PAEE. Study II included register-based data on socioeconomic status at baseline, including education length, income, and employment status. All studies included measurements of body mass index (BMI), waist circumference, and systolic and diastolic blood pressure, obtained during clinical examinations.

Blood samples were analyzed in all studies for HbA1c, fasting plasma glucose (FPG), triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol. Study I also included a 2-hour oral glucose tolerance test (OGTT) to classify glucose metabolism status based on FPG and OGTT (normal, prediabetes, type 2 diabetes) using WHO 2006 criteria, excluding HbA1c as a diagnostic criterion. Study III additionally measured creatinine, estimated glomerular filtration rate (eGFR), and urine albumin-to-creatinine ratio.

Self-reported history of cardiovascular disease (CVD) and use of anti-hypertensive, glucose-lowering, and lipid-lowering medications were collected in all studies. In Study II, history of CVD events in the 10 years prior to baseline were retrieved from national registers. In Study III, history of CVD was collected electronic patient records.

4.3. Outcomes

4.3.1. Arterial stiffness

Arterial stiffness characterized arteriosclerosis and atherosclerosis properties of the arteries. The stiffness of different trees of the vascular musculature can assessed both locally and dynamically. Aortic and carotid stiffness were assessed as markers of arterial stiffness, following previously described procedures[58].

Pulse wave velocity

Aortic stiffness was measured by carotid-femoral pulse wave velocity (cf-PWV) using applanation tonometry (SphygmoCor, Atcor Medical, Sydney, Australia), with the median of at least three consecutive recordings included in the analysis. cf-PWV is calculated from the time between the ECG systole and the arrival of the pressure wave at the femoral and carotid measurement sites and the distance between these two measurement sites. It is measured with participants in a supine position following a 10-minute rest period. The aortic path length was determined using a tape measure by subtracting the carotid-to-sternal notch distance from the femoral-to-sternal notch distance[58].

Carotid artery distensibility

Carotid stiffness was assessed by the carotid artery distensibility coefficient (CD), based on ultrasound imaging of the left common carotid artery using a 7.5 MHz linear probe (MyLab 70, Esaote Europe, Maastricht, the Netherlands). CD was calculated as ΔD/braPP, where ΔD represents carotid distension and braPP is brachial pulse pressure. Mean heart rate and mean arterial pressure (MAP) were recorded every five minutes using an oscillometer device (Accutorr Plus, Datascope, Montvale, NJ, USA)[58]..

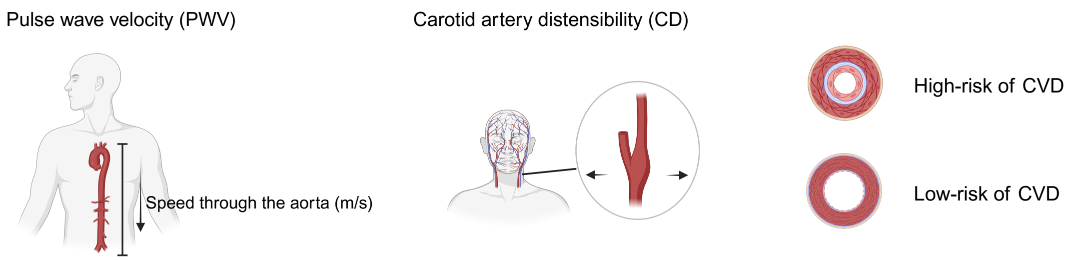

Figure 4.5.: Measures of arterial stiffness, measured dynamically at the aortic and local carotid sites. (Source: Author)

4.3.2. Biomarker of heart failure

N-terminal prohormone of brain natriuretic peptide (NT-proBNP) is a neuretic peptide that can be used to detect patients with heart failure and the progression. It derives from B-type natriuretic peptid (BNP) which is a cardial neurohormon, that is syntezied and secreted as response to streched cariomycytes and cardiac volume overload. After secretion, proBNP is cleaved, releasing the active hormone BNP along with the remaining N-terminal fragment, known as NT-proBNP. In Study III, blood sample were taken at Study cite. Description of the NT-proBNP analysis of plasma samples is described in supplementary material [ref.].

Table 4.6.: **?(caption)**

Outcome	**Diagnosis codes**
Heart failure	ICD: I50
Three-point MACE	
• Stroke	ICD: I61 - I64
• Myocardial infarction	ICD: I21-I24
• Cardiovascular death	ICD: I20-I28, I42, I46
• Cardiovascular revascularization	SKA: KPAE10, KPAE25, KPAF10, KPAF20, KPAF21, KPAF22, KPAH10, KPAH20, KPAH21, KPEE, KPEF, KPEH, KPEP, KPEQ, KPFE,, KPFH, KPFP, KPFQ

4.3.3. Cardiovascular events

Information on CVD events and mortality was obtained from the Danish National Patient Registers until 2021. ICD-10 codes for stroke, myocardial infarction, cardiovascular death, cardiovascular revascularization, and heart failure. We defined three-point major adverse cardiovascular events (MACE) as myocardial infarction, stroke, cardiovascular revascularization, and cardiovascular death.

4.4. Statistical Methods

4.4.1. Cross-sectional analysis

In Study I, we used multiple linear regression to investigate associations between multiday HRV and arterial stiffness. Model 1 adjusted for age, sex, education, glucose metabolism status, and mean arterial pressure (MAP) to account for the oversampling of individuals with type 2 diabetes and potential instrumental bias of arterial pressure flow. Model 2 included additional adjustments for smoking behavior, alcohol consumption, physical activity, body mass index, HbA1c, triglycerides, total-to-HDL cholesterol ratio, and medication use. Arterial stiffness measures were log-transformed to ensure normally distributed residuals and back-transformed into percentage change estimates. We add interaction sex to oberve if the association differed between sex. We performed sensitivity analyses excluding individuals on antihypertensive treatment or glucose-lowering medication. In Study III, we applied logistic regression models to investigate the association between CAN and heart failure, using NT-proBNP as the primary outcome. We adjusted for age, sex, and diabetes duration, smoking behavior, alcohol consumption, body mass index, HbA1c, triglycerides, total cholesterol, and antihypertensive medication, eGFR and prior CVD. We performed sensitivity analyses excluded participants with beta-blocker treatment or prior CVD.

4.4.2. Time-to-event analysis

In Study II, we used Poisson regression models to quantify the associations between HRV and cardiovascular events, as follow-up data were undisturbed over time and to avoid assumptions of proportional hazards[59]. Multiday HRV was modelled using splines with knots at predefined percentiles to assess non-linear associations. Hourly HRV was analysed separately for each hour to observe if the association of HRV had diurnal variation. Both HRV and mHR were standardized by their mean and standard deviation to ensure comparability. Based on assumptions about potential confounding pathways summarized in directed acyclic graphs (DAG), we fitted two models: Model 1 adjusted for age and sex, while Model 2 further adjusted for education, smoking, alcohol consumption, physical activity (physical activity energy expenditure (PAEE) calculated from Recent Physical Activity Questionnaire RPAQ), body mass index, total cholesterol, and HbA1c. Additional analyses were performed with HRV pre-adjusted for concurrent heart rate and physical acceleration to account the influence of these factors. Missing covariates were handled using multiple imputation. [Add age specific incidence rates]

4.4.3. Effect modification [det kan evt. kortes ned]

Effect modification is used to assess whether the association between an exposure and an outcome varies depending on the level of a third variable, known as the effect modifier. This means that the observed relationship between the exposure and the outcome is not uniform across all subgroups. Instead, it differs across strata defined by the effect modifier[60].

In Study I, we hypothesize that the association between 24-hour and arterial stiffness was stronger in strata of progression of diabetes (normal glucose metabolism, prediabetes, type 2 diabetes). We therefore first stratified by diabetes status to observe the size of the association across strata. We then combine all groups and include an interaction term between HRV and diabetes status. We did subsidiary analysis to check if the effect was modified by dysglycemia by stratifying HbA1c and fasting plasma glucose into deciles. In Study II, we quatified whether the association between multiday HRV and CVD endpoints varied by sex to explore potential biological dimorphism.

In Study III, we aimed to determine whether the association between CAN and elevated NT-proBNP is present in the subgroup without symptoms, defined as NYHA class < II. Hence, we hypothesized no significant effect modification between groups with and without symptoms. Similarly, we explored whether the association remains present in the group classified as low to moderate risk of heart failure, based on the WATCH-DM risk score.

A significant effect modification between the exposure and the effect modifier in all analyses was defined as an interaction term with a p-value < 0.05.

4.4.4. Multiple imputed by chained equations

Multiple Imputation by Chained Equations (MICE) is a method for handling missing data in datasets. This procedure imputes missing values through an iterative series of predictive models, generating plausible estimates while preserving the relationships within the data. To avoid one imputation for missing value could give the value the same confidence as the a non-missing value, we followed Rubins Rule. Rubin's rules in MICE combine results from multiple imputed datasets by pooling estimates of interest (e.g., means or regression coefficients) using their within- and between-imputation variances. Thus, we ensure valid statistical inferences by accounting for the uncertainty introduced by missing data.

In Study II, we imputed confounders to include as many participants and avoid excluding population with our without cardiovascular or mortality events. We imputed dataset 10 times. In Study III, we imputed missing CART, as a proportion of participants had non-valid test due to insufficient air in the valsalva manuevre, unstable heart beats or data error. These variables was used as auxiliary variables in imputation to reduce bias[61]. All available variables of biochemical measures, diagnosis, medication and cause of non-valid CART was used to impute each missing CART using predictive mean matching.

4.4.5. Instrumental bias

In Study I-III we are investigating the body properties by dynamic measures and biomarkers to quantify autonomic function, arterial stiffness, and cardiac function. Other conditions may affect the properties we are attempting to measure, and thus are causing instrumental bias.

Vascular Stiffness

In Study I, we used measurements of arterial stiffness using cf-PWV and carotid distensibilty. Both measures are influenced by arterial pressure at the time of examination.

Arterial pressure affects the propagation of the pressure wave through the aorta (cf-PWV) and the expansion and contraction of the carotid artery (carotid distensibilty) [ref.]. To account for this, we adjusted for mean arterial pressure in our models.

Cardiovascular autonomic function

In Study II, we assessed cardiovascular autonomic function using multiday HRV recordings and hourly HRV measurements. Studies have highlighted that HRV is dependent on heart rate, and low HRV may simply reflect a higher resting heart rate (rHR). To adjust for this without overcorrecting for a collinear variable, we pre-adjusted HRV by regressing rHR on HRV, extracting the residuals, and using these as the pre-adjusted determinant. For hourly HRV, variability in heart rate may be influenced by changes in physical activity, creating a risk that HRV serves as a proxy for movement rather than autonomic function. To address this, we applied a similar pre-adjustment approach by regressing concurrent heart rate and physical acceleration to account for physical activity.

Biomarker of Heart Failure

In Study III, kidney function and overweight are know to influence NT-proBNP levels independently of heart failure. We adjusted the model to account for the blurred effect of eGFR on NT-proBNP levels in the analysis.

5. Results

In this section, I will summarize study population characteristics and findings from analysis.

5.1. Study I

5.1.1. Descriptive

In The Maastricht Study, [10,000 participated by Date], of those 1316 reported prior CVD. Participants who had valid 24-hour HRV measured was 4379 and of those 3673 had a valid measurement of either CD or cf-PWV. Study population included 3673 participants. Further characteristic are described in the study I manuscript [Table 1] [refeernce to study I].

5.1.2. 24-hour HRV and arterial stiffness

Time-domain HRV

In the fully adjusted model 2, cf-PWV was 2.8% (CI: 2.1; 3.4) lower, while CD was 3.3% (CI: 1.5; 5.1) higher per SD higher in HRV time-domain Z-score. Among the time-domain indices, SDNN, SDNNi, and SDANN showed the strongest associations, with cf-PWV being lower by 2.5% (CI: 2.0; 3.1), 2.5% (CI: 1.9; 3.4), and 2.2% (CI: 1.7; 2.7), respectively. Conversely, CD was higher by 3.2% (CI: 1.7; 4.7), 3.0 % (CI: 1.4; 4.6), and 2.8% (CI: 1.3; 4.3), respectively. RMSSD and pNN50 showed a weaker association with cf-PWV (-1.1% [CI: -1.4; -0.4], and -1.1 [-1.7; -0.6]), while no evidence for an association was found with CD.

Frequency-domain HRV

In the fully adjusted model 2, cf-PWV was 2.8% (CI: 2.1; 3.5) lower, while CD was 3.2% (CI: 1.3; 5.1) higher per SD higher in HRV frequency-domain Z-score. Among the frequency-domain indices, total power, VLF, and ULF showed the strongest associations, with cf-PWV being lower by 2.2% (CI: 1.7; 2.8), 2.4% (CI: 1.9; 4.0), and 2.1% (CI: 1.5;

Table 5.1.: Study charteristics by diabetes status

Characteristic	**Normal glucose metabolism** N = 2,389	**Prediabetes** N = 538	**Type 2 Diabetes** N = 746
Sex			
Men	1,028 (43%)	280 (52%)	481 (64%)
Women	1,361 (57%)	258 (48%)	265 (36%)
Age (years)	58 (51, 64)	62 (57, 68)	63 (57, 68)
Total physical activity (hours/week)	13 (9, 19)	13 (9, 19)	12 (7, 17)
Moderate to vigorous physical activity (hours/week)	5.3 (3.0, 8.3)	4.5 (2.3, 7.5)	3.8 (1.5, 6.8)
BMI (kg/m²)	25.0 (22.9, 27.4)	27.2 (24.9, 30.1)	28.8 (26.0, 31.7)
Waist (cm)	89 (81, 97)	98 (90, 105)	103 (96, 112)
HbA1c (%)	5.35 (5.17, 5.63)	5.63 (5.35, 5.90)	6.54 (6.08, 7.09)
Fasting plasma glucose (mmol/L)	5.10 (4.80, 5.40)	5.90 (5.40, 6.30)	7.40 (6.60, 8.50)
LDL (mmol/L)	3.20 (2.70, 3.90)	3.30 (2.60, 4.00)	2.40 (1.80, 3.10)
HDL (mmol/L)	1.60 (1.30, 2.00)	1.40 (1.20, 1.80)	1.30 (1.00, 1.50)
Total cholesterol (mmol/L)	5.50 (4.80, 6.20)	5.50 (4.80, 6.30)	4.50 (3.90, 5.20)
Triglycerides (mmol/L)	1.05 (0.80, 1.45)	1.39 (1.03, 1.90)	1.51 (1.08, 2.14)
Duration of type-2 diabetes (only for diagnosed participants)	NA (NA, NA)	NA (NA, NA)	3 (0, 9)
Mean IBI (ms)	838 (775, 907)	815 (760, 897)	806 (744, 889)
SDNN (ms)	138 (117, 164)	127 (106, 152)	116 (96, 139)
RMSSD (ms)	26 (21, 34)	24 (19, 33)	22 (17, 31)
SDANN (ms)	125 (103, 149)	113 (92, 139)	103 (84, 127)
SDNNi (ms)	55 (46, 65)	50 (41, 60)	44 (36, 54)
pNN50 (%)	7 (3, 13)	5 (2, 10)	4 (2, 9)
TP (ms²)	12,596 (8,880, 17,498)	10,615 (7,134, 15,374)	8,880 (6,064, 12,722)
ULF (ms²)	10,771 (7,392, 15,142)	8,948 (5,852, 13,374)	7,524 (5,036, 11,001)
VLF (ms²)	1,198 (833, 1,692)	1,015 (685, 1,478)	816 (541, 1,267)
LF (ms²)	421 (257, 651)	328 (200, 540)	261 (154, 422)
HF (ms²)	94 (57, 158)	78 (47, 138)	63 (36, 117)
Systolic blood pressure (mmHg)	123 (114, 133)	129 (122, 140)	130 (122, 139)
Diastolic blood pressure (mmHg)	75 (71, 80)	78 (73, 83)	76 (72, 81)
Mean arterial pressure (mmHg)	95 (88, 102)	99 (93, 107)	98 (92, 105)
Carotid artery distensibility (10-3/kPa)	15.0 (11.8, 18.8)	13.5 (10.4, 16.9)	12.5 (9.9, 16.0)
Carotid-femoral pulse wave velocity (m/s)	8.08 (7.28, 9.16)	8.96 (7.84, 10.32)	9.36 (8.16, 10.80)
N_HT	833 (35%)	317 (59%)	590 (79%)
Antihypertensive medication	431 (18%)	199 (37%)	478 (64%)
med_HT_beta	149 (6.2%)	77 (14%)	195 (26%)
Lipid-lowering medication	280 (12%)	141 (26%)	484 (65%)

2.6), respectively. Conversely, CD was higher by 2.7% (CI: 1.2; 4.2), 2.4% (CI: 0.9; 4.1), and 2.6% (CI: 1.1; 4.1), respectively. HF showed a weaker association with cf-PWV (-0.9% [CI: -1.4; -0.4]), while no evidence for an association was found with CD. Mean interbeat interval was associated with 2.4 % (CI: 1.8; 2.9) lower cf-PWV and 4.5% (3.1; 6.1) higher CD.

5.1.3. Effect modification of diabetes status

The study population represented diabetes risk of normal glucose metabolism (65%), prediabetes (15%), and type 2 diabetes (20%). The median (IQR) cf-PWV (aortic stiffness) became higher with diabetes status: NGM: 8.08 m/s (7.28, 9.16), prediabetes: 8.96 m/s (7.84, 10.32), and type 2 diabetes: 9.36 m/s (8.16, 10.80). CD (carotid stiffness) decreased: NGM: 15.0 (11.8, 18.8), prediabetes: 13.5 (10.4, 16.9), and type 2 diabetes: 12.5 (9.9, 16.0) $\times$ 10 ^{3}/kPa. SDNN (ms) was highest in NGM and decreased with worsening glucose metabolism: NGM: 138ms (117, 164), prediabetes: 127ms (106, 152), and type 2 diabetes: 116ms (96, 139). Further description of charaterisitcs by diabetes are described in Table 5.1.

The association between HRV time-domain Z-scores and cf-PWV and CD was significantly modified by prediabetes (cf-PWV: -4.9 [CI: -6.523; -3.243] $^{interaction(*)^{p-value<0.01}}$ CD: 8.0 [CI:3.8; 12.5]$^{*^{p-value<0.01}}$) but not by type 2 diabetes (cf-PWV: -3.5 % [CI: -4.8; -2.1)] $^{*^{p-value<0.1}}$ CD: 4.8 % [CI:1.3; 8.4]$^{*^{p-value<0.1}}$). For the indices SDNN and SDANN, the association with both cf-PWV and CD was significantly modified by both prediabetes and type 2 diabetes.

The association between HRV frequency-domain Z-score and cf-PWV was significantly modified from normal glucose metabolism by prediabetes (-5.7 %[CI:-7.4; -3.9]$^{*^{p-value<0.01}}$) and type 2 diabetes (-3.9 %[CI:-5.4; -2.3]$^{*^{p-value<0.05}}$) while CD was only modified by prediabetes (8.3 %[CI:3.6; 13.2]$^{*^{p-value<0.01}}$) but not by type 2 diabetes (5.3 %[CI:1.4; 9.4]$^{*^{p-value<0.1}}$). For the indices total power and ULF, the association with both cf-PWV and CD was significantly modified by both prediabetes and type 2 diabetes. Mean inter beat interval association with cf-PWV or CD was not significantly modified by diabetes status.

As I did not observe a stepwise increase in the modification of glucose metabolism status from prediabetes to type 2 diabetes, I excluded the subgroup with type 2 diabetes to test whether the association was gradually modified by dysglycemia. In this subgroup, the association between HRV time and frequency domain Z-scores and measures of arterial stiffness was modified by HbA1c (range of interaction p-values: 0.1 to 0.005) (see Figure Figure 5.1). For example, per SD lower HRV frequency domain Z-score at HbA1c 6.4% was associated with a 5.4% higher (CI: 3.5; 7.2) cf-PWV, which was 2.0% to 4.0%

higher compared to the magnitude of association at HbA1c levels of 5.6% and 4.8% (see see Figure 5.1 B). In CD, per SD lower HRV frequency domain Z-score at HbA1c

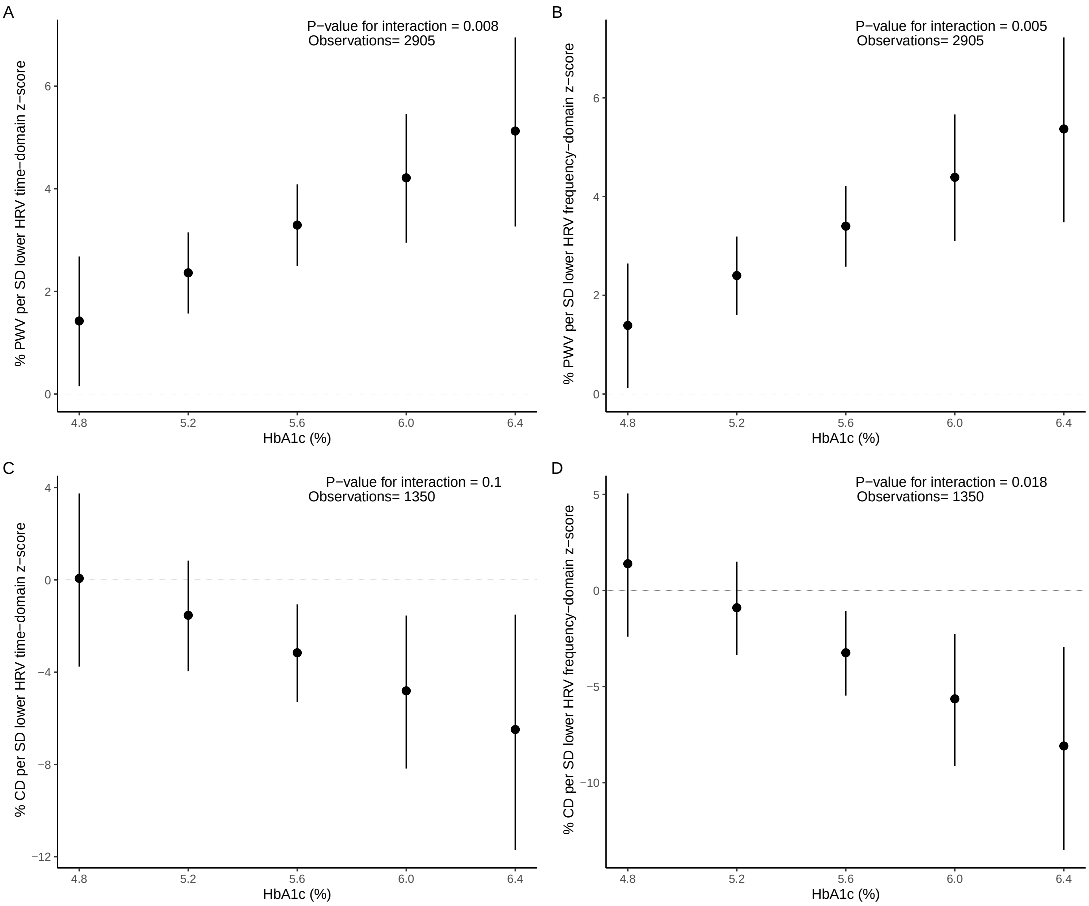

Figure 5.1.: **?(caption)**

Table 5.2.: Study participants charteristics

Characteristic	Overall, N = 1,625	<100, N = 148	100-120, N = 312	120-140, N = 457	140-160, N = 346	>160, N = 362
sex						
Men	866 (53%)	68 (46%)	148 (47%)	206 (45%)	203 (59%)	241 (67%)
Women	759 (47%)	80 (54%)	164 (53%)	251 (55%)	143 (41%)	121 (33%)
Age (years)	65.9 (6.8)	67.4 (6.9)	65.7 (6.9)	66.0 (6.7)	65.5 (6.6)	66.0 (7.0)
Physical activity energy expenditure (KJ / day)	53.1 (25.1)	46.8 (24.0)	49.4 (21.0)	50.7 (21.5)	57.6 (27.2)	57.5 (29.2)
Alcohol consumption (units per week)	9.2 (9.5)	11.3 (10.8)	10.2 (11.3)	8.9 (8.5)	8.5 (9.2)	8.7 (8.2)
Smoking status						
1	263 (16%)	40 (28%)	70 (23%)	65 (14%)	41 (12%)	47 (13%)
2	750 (47%)	58 (40%)	145 (47%)	214 (47%)	162 (47%)	171 (48%)
3	598 (37%)	47 (32%)	95 (31%)	174 (38%)	140 (41%)	142 (39%)
BMI (kg/m^2	27.7 (4.7)	28.1 (5.4)	28.2 (4.6)	28.0 (4.7)	27.7 (4.9)	26.9 (4.2)
Waist circumference (cm)	96.7 (13.4)	98.0 (14.9)	98.2 (13.2)	96.7 (13.6)	96.7 (13.1)	94.8 (12.5)
Systolic blood pressure (mmHg)	133.7 (17.3)	134.2 (16.3)	133.7 (17.6)	133.5 (17.8)	133.4 (16.9)	133.8 (17.5)
Diastolic blood pressure (mmHg)	81.9 (10.4)	83.8 (10.1)	82.7 (10.2)	81.7 (10.6)	82.1 (10.2)	80.6 (10.3)
Pulse rate (bpm)	67.4 (10.9)	77.7 (11.2)	72.6 (9.3)	67.9 (9.3)	65.3 (9.3)	60.0 (9.8)
HbA1c (%)	5.8 (0.5)	5.9 (0.9)	5.9 (0.6)	5.8 (0.5)	5.7 (0.4)	5.7 (0.4)
Triglycerides (mmol/L)	1.3 (0.7)	1.5 (0.9)	1.4 (0.7)	1.3 (0.6)	1.2 (0.7)	1.1 (0.6)
Total cholesterol (mmol/L)	5.4 (1.1)	5.2 (1.0)	5.4 (1.2)	5.4 (1.1)	5.4 (1.0)	5.4 (1.0)
HDL cholesterol (mmol/L)	1.6 (0.4)	1.6 (0.4)	1.5 (0.5)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)
LDL cholesterol (mmol/L)	3.2 (1.0)	3.0 (1.0)	3.2 (1.1)	3.2 (1.0)	3.3 (0.9)	3.3 (0.9)
Urine albumin-creatine ratio (mg/g)	25.9 (132.8)	36.4 (105.9)	47.9 (275.1)	19.6 (48.2)	19.4 (67.7)	16.4 (36.3)
vo2max	26.6 (7.8)	24.8 (7.5)	24.8 (7.5)	26.1 (6.8)	27.0 (8.0)	28.7 (8.7)
rest_hr	57.3 (7.3)	67.8 (5.7)	63.3 (5.0)	58.4 (4.5)	55.0 (4.2)	49.8 (4.9)
med_any_anti_hypertensive	753 (47%)	88 (61%)	149 (48%)	216 (47%)	147 (43%)	153 (43%)

5.2. Study II

5.2.1. Descriptive

In ADDITION-PRO population consisted of 1,627 participant with a least 48-hour HRV measures, while 1,432 had all hour represented with hourly HRV and physical acceleration. The study population included different tiers of diabetes risk: 154 individuals at low risk (9%), 889 at high risk (51%), 314 with impaired fasting glucose (IFG) (18%), 226 with impaired glucose tolerance (IGT) (13%), and 161 with both IFG and IGT (9%). I splitted SDNN into categories by very-low (SDNN< 100 ms), low (SDNN 100-120 ms), middle (SDNN 121-140 ms), high (SDNN 141-160 ms) and very-high (SDNN >160 ms).

Charteristics are desribed in Table 5.2. Participants in the lowest SDNN group (<100 ms) were older (67.4 ± 6.9 years), had higher BMI (28.1 ± 5.4), HbA1c (5.9 ± 0.9), triglycerides (1.5 ± 0.9 mmol/L), and resting heart rate (67.8 ± 5.7 bpm), were more likely to use anti-hypertensive medication (61%), and had lower physical activity energy expenditure (46.8 ± 24.0 kJ/day) compared to those with higher SDNN levels.

5.2.2. Multiday HRV and MACE, heart failure, and all-cause mortality.

The mean multiday SDNN was 139.0 (32.3) ms, and the mean heart rate was 73.5 (9.1) bpm. In the fully adjusted model, SDNN per SD was associated with a lower incidence rate ratio (IRR) for MACE 0.82 (CI: 0.69; 0.97), heart failure 0.76 (CI: 0.58; 0.99), and mortality rate ratio of 0.79 (CI: 0.66; 0.94). When I pre-adjusted for resting heart rate, the proportion of the association explained between HRV and MACE, HF, and all-cause mortality was 14%, 25%, and 19%, respectively. I included knots in the model, which showed that the risk became higher when SDNN fell below 120 to 110 ms (approximately below the 20th percentile), suggesting a potential cut-off point for higher risk. I therefore calculated the incidence rate (IR) at SDNN levels of 100 ms, 120 ms, and 160 ms, respectively, and plotted these as a function of age.

At age 65, the IR per 1000 person-years for MACE was 44.2 (CI: 33.5; 58.3) at SDNN = 100 ms, which was higher than the rates observed at SDNN = 120 ms (IR: 37.6 [CI: 29.2; 48.3]) and SDNN = 160 ms (IR: 34.7 [CI: 27.0; 44.5]) (Figure 5.2 A). The IR became higher with age, reaching its peak at age 72. For heart failure at age 65, the IR was 20.5 (CI: 15.3; 27.5) at SDNN = 100 ms, slightly higher than at SDNN = 120 ms (IR: 19.6 [CI: 14.8; 25.9]) and SDNN = 160 ms (IR: 19.8 [CI: 15.0; 26.2]) (Figure 5.2 B). The IR remained stable until age 70, after which it became higher. For all-cause mortality at age 65, the IR was 11.6 (CI: 6.3; 21.4) at SDNN = 100 ms, higher than at SDNN = 120 ms (IR: 7.6 [CI: 4.3; 13.3]) and SDNN = 160 ms (IR: 6.0 [CI: 3.4; 10.4]) (Figure 5.2 C). The IR for all-cause mortality became higher with age.

5.2.3. Hourly HRV and MACE, heart failure, and all-cause mortality.

From the hourly recordings, I observed a clear periodicity in SDNN, heart rate, sleep patterns, and physical acceleration. Mean (SD) SDNN increased from 5–6 AM (70.2 [28.8] ms), peaking at 8–9 AM (92.1 [29.0] ms), followed by a gradual decline, reaching its lowest point around 2 AM the next day (64.1 [28.1] ms). A similar circadian pattern was observed in heart rate, although its peak occurred two hours later, starting at 9 AM (76.7 [10.9] bpm). After peaking, heart rate remained stable throughout the afternoon before gradually decreasing.

In Figure 5.3, I observe hourly SDNN (preadjusted for heart rate and physical acceleration), heart rate, and physical acceleration association. Models was adjusted for age, sex, education, alcohol consumption, smoking behavior, BMI, total cholesterol, and Hba1c. The morning response of SDNN was most indicative of MACE, with the strongest association observed from 6–7 AM (IRR: 0.84; 95% CI: 0.71 to 1.00 per SD higher SDNN) (see Figure 5.3 A).Heart rate between 12 AM and 6 AM showed a small trend toward higher risk of MACE (IRR range: 1.11 to 1.15 per SD higher heart rate), although none of the confidence intervals exceeded one (see Figure 5.3 D). Across all hours, there was a plausible association between SDNN and heart failure. However, this association disappeared after adjusting for physical acceleration and heart rate (see Figure 5.3 B). In contrast, heart rate between 10 PM and 9 AM was associated with heart failure (IRR

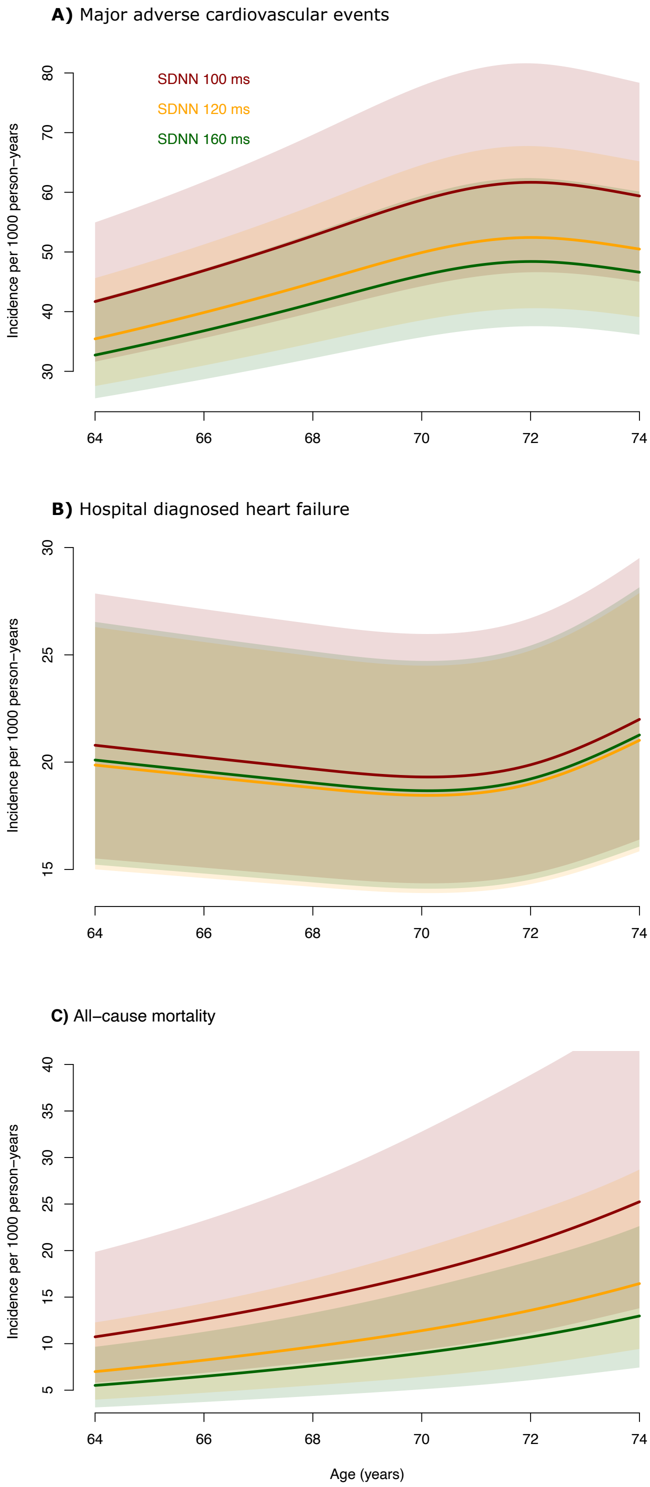

Figure 5.2.: Multiday SDNN levels (100 ms, 120 ms, 160 ms) by age-specific incidence rates for A) major adverse cardiovascular events, B) heart failure, and C) all-cause mortality

range: 1.37 to 1.58 per SD higher heart rate) (see Figure 5.3 E). SDNN was consistently associated with all-cause mortality across all hours, with a stronger inverse association observed between 12 PM and 1 AM (IRR range: 0.79 to 0.88 per SD higher SDNN) (see Figure 5.3 C). No clear trends of association were observed between heart rate and all-cause mortality.

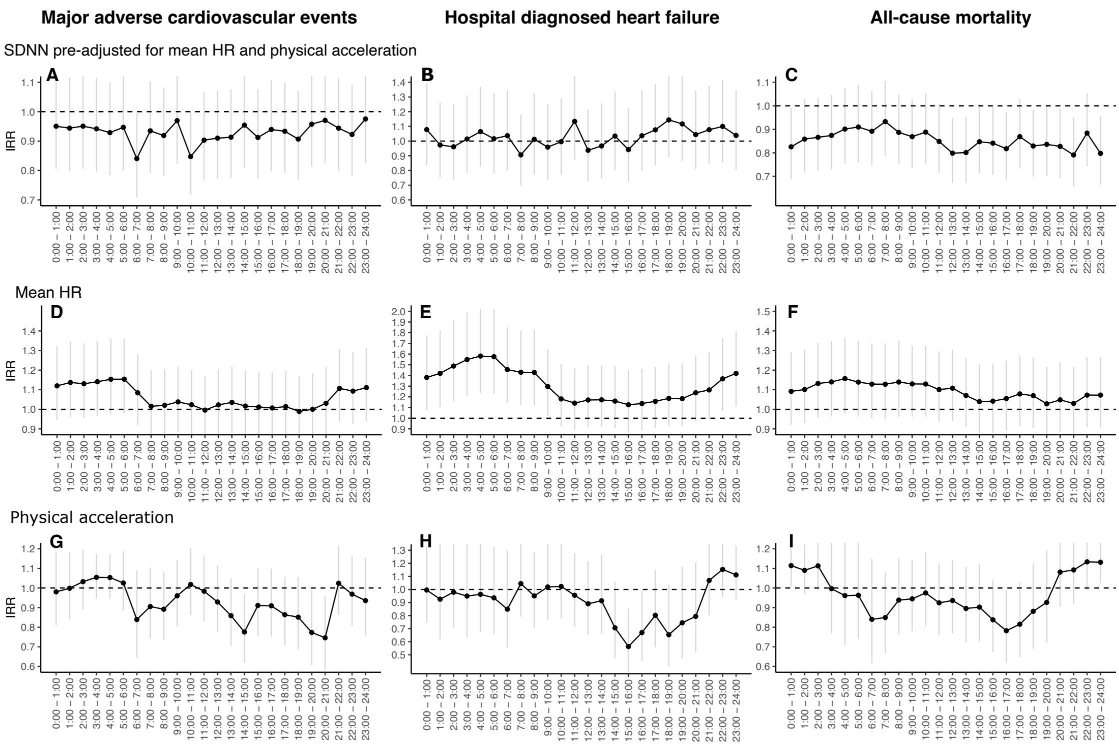

Figure 5.3.: Hourly SDNN levels (100 ms, 120 ms, 160 ms) by age-specific incidence rates for A) major adverse cardiovascular events, B) heart failure, and C) all-cause mortality. Figure adapted from [authors]. —. (Paper II, appendix)

5.3. Study III

5.3.1. Patients characteristics

In study III, 179 participants with type 2 had measures of NT-proBNP and performed the CART test. CAN was present in 30% (n = 54) of participants (36% among those with valid CAN measurements (Figure 5.4 A)). Meanwhile, 24% (n = 43) were unable to complete the CART assessment adequately, primarily due to irregular heart rhythms (n = 8) or insufficient air pressure during the Valsalva manoeuvre (n = 21). Compare to those without CAN, the participants with CAN were more women (41 % vs 33 %), were more sedentary (45% vs 36%), had a higher proportion with prior major CVD (41% vs 20%) and declined eGFR (< 60) (36% vs 22%), higher levels of triglyceride (median 2.05 mmol/L vs 1.95 mmol/ L), were slightly older (median 62 years vs 61 years), had longer duration of type 2 diabetes (median 19 years vs 15 years), and higher use SGTL2-inhibitors (65% vs 60%) but lower use of GLP-1 RA (63% vs 70%). No other difference in clinical characteristic was observed.

5.3.2. Relationship between CAN and elevated NT-proBNP

A greater proportion of individuals with CAN exhibited elevated NT-proBNP levels (>125 pg/ml) (51.9%, n=52/78) compared to those without CAN (23.2%, n=26/112)(Figure 5.4 E). The fully adjusted odds ratio (OR) for elevated NT-proBNP in individuals with CAN was 5.69 (95% CI: 1.95; 18.49) relative to those without CAN. Among the cardiovascular autonomic reflex tests (CART), the Valsalva maneuver demonstrated the strongest association with NT-proBNP (OR 9.00, 95% CI: 2.88; 33.09; n=51/75), followed by deep breathing (OR 3.30, 95% CI: 1.17; 9.77; n=33/133) and orthostatic hypertension (OR 4.04, 95% CI: 1.27; 13.77; n=24/146). No significant association was identified for the lying-to-standing test (OR 0.80, 95% CI: 0.32; 1.97; n=54/108). After imputing missing CART data, the OR for CAN in relation to elevated NT-proBNP declined to 2.94 (95% CI: 1.37; 6.56). Sensitivity analyses, which excluded participants using beta-blockers or those with a history of CVD, resulted in a smaller sample size and wider confidence intervals, though the overall association remained unchanged. CAN was associated with elevated NT-proBNP in individuals both without (NYHA I; OR = 4.3, 95% CI: 1.1; 16.3) and with heart failure symptoms (NYHA II; OR = 16.4, 95% CI: 1.2; 222.0), though the interaction was not significant (p = 0.4). Similar associations were seen across WATCH-DM risk groups: very-low-to-moderate (OR = 6.1, 95% CI: 1.6; 23.5) and high-to-very-high (OR = 6.3, 95% CI: 0.83; 46.9).

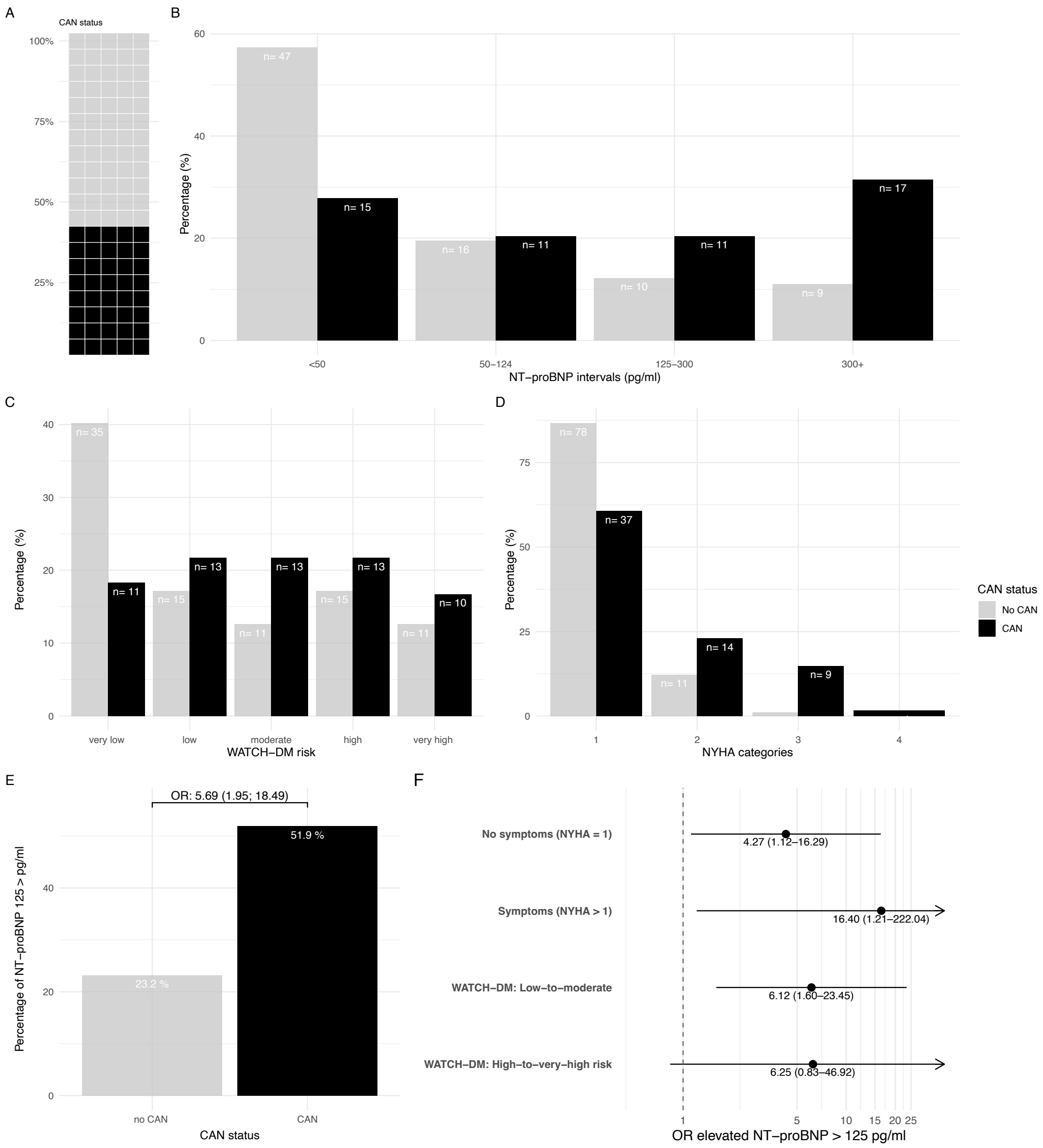

Figure 5.4.: Relationship betweeen CAN and indicators for heart failure. Figure from [authors]. Cardiovascular autonomic neuropathy and indices of heart failure in type 2 diabetes: The CANCAN Study. (Paper III, appendix)

6. Discussion

6.1. Discussion of results

The aim of this thesis is to understand how cardiovascular autonomic dysfunction and CAN affect the risk of cardiovascular disease across stages of glucose metabolism. I included conditions such as heart failure, stroke, and myocardial infarction, as well as subclinical markers like cf-PWV and CD. The thesis includes populations ranging from normal glucose metabolism to type 2 diabetes and considers individuals engaged at different levels of the healthcare system. This chapter discusses the results and conclusions in relation to existing evidence and addresses their clinical implications across the levels of the healthcare system.

The challenges related to the growing population with type 2 diabetes and the risk of developing diabetes are addressed at multiple levels within the healthcare system.

- Public health focuses on preventing diabetes and its complications across all age groups, from childhood to older adulthood.
- Primary care, especially general practitioners, plays a central role in identifying individuals at risk of diabetes and cardiovascular disease. General practitioners also manage patients with uncomplicated type 2 diabetes.
- Outpatient clinics, led by endocrinologists, are responsible for treating patients with more advanced stages of diabetes and for managing complex cases.

To address the aim of the dissertation, I used three different cohorts that reflect various levels of prevention and care. In Study I, I approached the question from a public health perspective by using data from The Maastricht Study, including individuals aged 40 and above, representing all stages from normal glucose metabolism to type 2 diabetes. In this broader population, I demonstrated a link between lower 24-hour HRV and cardiovascular risk, measured by arterial stiffness. This association was modified by glucose metabolism, showing a stronger relationship in individuals with prediabetes and type 2 diabetes.

This led to a focus on individuals at higher risk of developing diabetes, using data from the ADDITION-PRO cohort. Individuals with prediabetes may benefit from structured guidance in primary care to prevent progression to type 2 diabetes and related complications such as cardiovascular disease. In Study II, I showed that in a population with prediabetes, lower multiday HRV was associated with a higher risk of cardiovascular disease, heart failure, and all-cause mortality.

A key challenge in managing type 2 diabetes lies in the complexity of clinical decision-making, which is often applied uniformly across a heterogeneous patient population. As the duration of diabetes increases, the disease typically progresses, leading to a higher prevalence of both microvascular and macrovascular complications. This raises the need to identify early subgroups of individuals who may benefit from more structured and personalized treatment strategies based on their risk profile. However, to support such an approach, reliable and standardized tools are required to accurately detect and classify high-risk phenotypes. To uncover this perspective, we collected data for the CANCAN study, which included individuals with type 2 diabetes who were referred to secondary care at an outpatient clinic by general practitioners. In Study III, I showed that among individuals with type 2 diabetes, those with CAN had a higher risk of heart failure, measured by elevated NT-proBNP levels, and this association remained significant in subgroups without heart failure symptoms or with a low-to-moderate heart failure risk score.

Cardiovascular autonomic dysfunction, indicated by lower HRV or abnormal values in CARTs, is associated with increased cardiovascular risk across all stages of glucose metabolism. In the next section, I will discuss potential mechanisms and explore the clinical utility of HRV and CARTs at different stages of diabetes risk.

6.2. Cardiovascular autonomic dysfunction and its impact on heart disease across glucose metabolism

Based on our studies, I have shown that cardiovascular autonomic dysfunction, measured by HRV and CARTs, is associated with cardiovascular disease risk across the spectrum of glucose metabolism. This association is evident through measures of arteriosclerosis, atherosclerotic events, all-cause mortality, and heart failure in individuals at high risk of diabetes, as well as through indications of heart failure in patients with type 2 diabetes.

6.2.0.1. Arteriosclerosis

In Study I, I demonstrated that autonomic dysfunction, as measured by 24-hour HRV, is associated with arterial stiffness, assessed both dynamically (cf-PWV) and locally (CD).

This suggests that autonomic responses under free-living conditions contribute to the development of arterial stiffness.

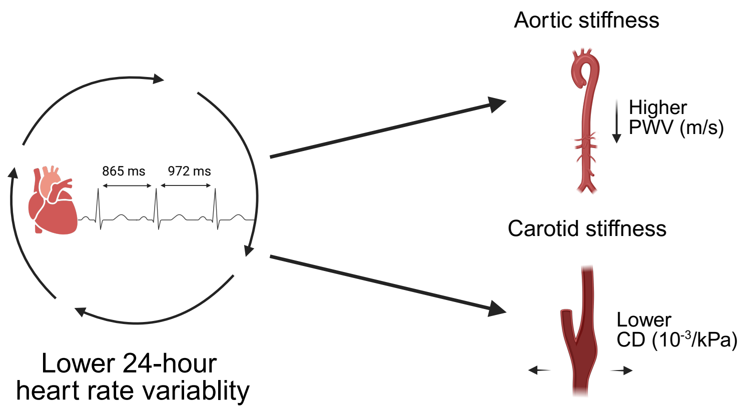

Figure 6.1.: Autonomic dysfunction and arterial stiffness. (Source: Author). —.

Arterial stiffness is not only a structural marker of vascular ageing but is also dynamically modulated by local endothelial signals and autonomic nervous system activity. Several studies have demonstrated a link between elevated sympathetic tone and increased arterial stiffness. Two possible mechanisms may explain how autonomic nervous function is related to arterial stiffness. First, autonomic dysfunction may increase the vascular tone of large arteries, thereby impairing arterial elasticity. Animal studies support this notion. In rats, proper autonomic regulation has been shown to be essential for maintaining aortic elasticity, and heightened sympathetic activity has been shown to damage elastin fibres, resulting in stiffer arteries. While such findings cannot be directly extrapolated to humans, they suggest plausible biological pathways. Second, the autonomic nervous system regulates heart rate and cardiac contractility. Autonomic dysfunction typically manifests as both reduced HRV and elevated resting heart rate. Arterial shear stress increases as a result of heightened sympathetic activity and parasympathetic withdrawal. A higher resting heart rate may contribute to arterial stiffness by altering blood flow dynamics and increasing shear stress. Our earlier study using data from the Whitehall II cohort showed that a steeper decrease in short-term (5-minute) HRV over a ten-year period was linked with higher levels of aortic stiffness in the subsequent five years[62].

Previous studies have shown an association between autonomic dysfunction, as measured by short-term HRV during rest, and arterial stiffness[63]. Our data from Study I extended this perspective by using long-term HRV. Its association with arterial stiffness was modified by dysglycemia, suggesting that autonomic dysfunction may lie on the pathway from dysglycemia to the development of arterial stiffness, even before the onset of type 2 diabetes. Data from the Whitehall II study showed that aortic stiffness increased more steeply with higher HbA1c values among non-diabetic individuals[64]. In the subpopulation in Study I without diabetes, I observed modification by HbA1c in both aortic and carotid stiffness. The modifying effect of HbA1c suggests an amplified impact of hyperglycemia on the consequences of autonomic dysfunction. Chronic hyperglycemia, reflected in elevated HbA1c levels, promotes the formation of advanced glycation end products and the overproduction of reactive oxygen species[23]. These byproducts contribute to oxidative stress, which in turn exacerbates autonomic dysfunction, often evidenced by reduced HRV[[65]][66]. In our analysis, several time-domain and frequency-domain HRV measures based on the total variability were modified by diabetes status, whereas the association with mean interbeat interval remained unchanged. I suspect that the deterioration of HRV indicators may partly reflect the pathogenesis of autonomic neuropathy, as a consequence of harmful byproducts resulting from hyperglycemia. This suggests that lower HRV may indicate a different risk profile for arterial stiffness, compared to heart rate alone.

6.2.0.2. Atherosclerosis

In Study II, I showed that individuals with a preclinical stage of autonomic dysfunction, measured by week-long HRV, face a higher risk of incident ischemic cardiovascular disease, heart failure, and all-cause mortality.

Multiple mechanisms may explain how autonomic dysfunction contributes to the initiation and progression of ischemic events and stroke. First, as discussed in Study I, autonomic dysfunction may promote arteriosclerosis, leading to arterial stiffness. This stiffness impairs vasodilation and may enhance vasoconstriction, increasing hemodynamic stress and the risk of plaque rupture and thrombus formation[67]. In this context, findings from Study I may not entirely distinguish between arterial stiffness and atherosclerosis, as shown by data from the Rotterdam Study[68]. As plaques develop, the associated increase in sympathetic nerve density around the arteries could reduce arterial elasticity. In a smaller study of people with type 2 diabetes, lower HRV was linked with increased carotid atherosclerosis[69].

Second, the autonomic nervous system innervates the adventitia layer of blood vessels, where it modulates vascular tone via sympathetic fibres. Although atherosclerotic plaques form in the intima layer, recent in vivo studies have demonstrated that increased plaque burden is associated with higher local sympathetic nerve density, likely mediated by neuroinflammatory processes. Notably, reducing sympathetic innervation has been shown to attenuate plaque formation in animal models[70]. These findings suggest that autonomic dysfunction may not only reflect but also actively contribute to atherogenesis.

Third, autonomic nervous dysfunction has been shown to interfere with signalling pathways controlling heart rhythm, potentially leading to arrhythmias that disturb cardiac contraction. Data from the Atherosclerosis Risk in Communities study illustrated that lower short-term HRV was associated with incident atrial fibrillation over 20 years, with a higher risk among participants with type 2 diabetes[71]. This supports the role of autonomic dysfunction in the development of arrhythmogenesis, which increases the risk of myocardial infarction and stroke. However, in Study II, I did not include incident atrial fibrillation as an outcome. Future research is needed to explore whether it could explain the higher risk of major adverse cardiovascular events. A study of individuals with coronary artery disease showed that stress-induced HRV was associated with myocardial infarction, even more than resting HRV, suggesting that reduced parasympathetic modulation of heart rate under stress may play a role in ischemia[72]. In our week-long recordings, our data likely captured episodes of stress-induced HRV under free-living conditions, such as during the awakening stages in the morning. Capturing autonomic responses to daily living and their alignment with circadian rhythms may provide valuable insights into cardiovascular risk. Therefore, understanding autonomic responses to tasks is relevant for comprehending their role in cardiovascular risk, beyond short-term measures taken at rest. Including data to monitor real-time activity, such as physical activity, would add value by capturing physiological responses to bodily demands. This could enable the inclusion of heart rate responses (e.g., from rest to standing) and other relevant measures of autonomic function, such as heart rate recovery after physical movement, which is a known risk factor for cardiovascular disease and all-cause mortality [**colechristopherr?**][73].

Our study focused on long-term HRV under free-living conditions, capturing stress-responsive periods such as morning awakening. These recordings likely reflect underlying autonomic dynamics relevant to cardiovascular risk. A genetic study in the UK biobank of short-term HRV support this by identifying mechanisms involving G-protein signaling, pacemaker activity, and mitochondrial function. These pathways influence vagal control, cardiac excitability, and energy metabolism[74]. Although derived from short-term recordings, the genetic associations may reflect autonomic traits that persist across different time scales, thereby reinforcing the biological basis of HRV. A Mendelian randomization study using data from the Rotterdam Study found that genetically predicted HRV was associated with an increased risk of atrial fibrillation[75]. However, this association did not extend to all-cause mortality or cardiovascular death in the UK Biobank cohort, where only phenotypically measured HRV showed a significant relationship with these outcomes[76]. Interestingly, the genetic determinants of HRV exhibited pleiotropic relationships with several autonomic traits, including resting heart rate, heart rate response during exercise, and post-exercise recovery dynamics[76]. No genome-wide association study (GWAS) has been conducted for long-term HRV.

6.2.0.3. Heart failure

The relationship between cardiovascular autonomic dysfunction and heart failure is complex[77]. On one hand, autonomic dysfunction may represent a complication that contributes to cardiac stress, sympathetic overactivation, and eventual heart failure. On the other hand, it may reflect the progression of cardiac remodelling and declining cardiac output. Our findings demonstrated a relationship between autonomic dysfunction and heart failure both cross-sectionally in a population with type 2 diabetes and prospectively in individuals representing different tiers of diabetes risk. However, our data are limited in determining the extent to which this relationship supports one explanation over the other, as we lack baseline and follow-up measures of both heart failure and HRV.

Findings from Study I confirmed the relationship between autonomic dysfunction and arterial stiffness. It is well established that arterial stiffness is linked to cardiac remodelling, as increased cf-PWV leads to an earlier return of the reflected pulse wave to the aorta, which increases cardiac afterload and reduces coronary perfusion pressure[78]. Therefore, autonomic dysfunction may have an indirect effect on heart failure, potentially mediated by arterial stiffness. However, structured analyses are needed to confirm these pathways. For example, through mediation analysis to assess the direct and indirect effects of arterial stiffness, with autonomic dysfunction as the main determinant In Study II, I observed that week-long HRV was associated with incident heart failure, and approximately one-fourth of the risk was explained by resting heart rate. Data from the Rotterdam Study showed that short-term HRV was longitudinally associated with echocardiographic measures reflecting systolic function, suggesting that autonomic dysfunction contributes to cardiac remodelling[79]. In contrast to MACE outcomes, findings from Study II showed no specific time point in hourly HRV that was associated with heart failure. Instead, it was the overall daily pattern captured by week-long HRV that was linked to heart failure risk. This suggests that the association is not driven by isolated shifts in autonomic activity, but rather by a consistently impaired autonomic balance under free-living conditions. The effect appears to be driven in part by a failure to show appropriate decreases in heart rate during rest, as individuals with higher hourly heart rates at night had an increased risk of heart failure. In Study III, I observed that individuals with CAN had higher risk of elevated levels of NT-proBNP, a biomarker of myocardial stress and early heart failure. Therefore, CAN is associated with hemodynamic consequences that contribute to both structural and functional cardiac remodeling, which in turn leads to elevated NT-proBNP levels.

We cannot exclude the possibility that autonomic dysfunction represents an elevated demand for compensatory mechanisms as heart failure progresses. Studies have shown that patients with heart failure and lower HRV tend to have a worse prognosis in terms of mortality. If low HRV or the presence of CAN were primarily driven by existing cardiac complications, it would suggest that individuals with these conditions exhibit more pronounced sympathetic overactivity as a consequence of heart failure progression, indicating potential reverse causation. Hence, elevated sympathetic activity during rest may reflect a greater reliance on compensatory mechanisms to maintain cardiac output. More precise measures are needed to assess whether sympathetic activity acts as a primary driver of heart failure or as a secondary compensatory response to cardiac dysfunction. In addition, it remains unclear to what extent the parasympathetic nervous system can act as a protective mechanism to counterbalance sympathetic dominance, and whether a decline in HRV reflects a breakdown of this balance. The two pathways (autonomic neuropathy and cardiac remodelling) are not mutually exclusive and may interact in a reinforcing cycle. Autonomic dysfunction can lead to increased sympathetic tone and reduced parasympathetic modulation, placing the heart under chronic stress and promoting structural and functional changes[23]. In turn, cardiac remodelling may impair autonomic regulation, further exacerbating the imbalance. This interplay may create a self-perpetuating loop that accelerates the progression of heart failure. However, this remains beyond the scope of our current data and analysis.

6.2.1. Clinical implications

I have discussed the utility of different cohorts representing public health, primary care, and secondary care in addressing the impact of autonomic dysfunction on CVD, as well as the possible mechanisms involved. I will now turn to the clinical implications of using autonomic dysfunction in the prevention of CVD. If long-term HRV or CARTs are to be considered for improving risk stratification, it is important to determine at what stage in the progression of diabetes risk, and at which level of care, autonomic dysfunction becomes meaningful for early detection and intervention. [I further assess the role[Or the potential.. You haven't really examined it's role in a healthcare setting] of cardiovascular autonomic function in healthcare, particularly in risk stratification and enabling the early detection of individuals at high risk for cardiovascular complications]

6.2.1.1. Public health

A key preventive strategy for cardiovascular disease is the identification and treatment of high-risk individuals[80]. Public health approaches complement this by promoting healthy lifestyles, ensuring early screening for risk factors, and improving access to essential care and medications.

Studies I and II demonstrated a strong association between long-term HRV and cardiovascular disease risk, with particularly pronounced associations in individuals with prediabetes and type 2 diabetes. These findings suggest that HRV metrics could serve as early indicators for stratifying individuals who may benefit from preventive interventions. hTe longitudinal relationship, based on repeated measures of HRV and cf-PWV, demonstrates that changes in HRV are associated with alterations in aortic stiffness[62]. Thus, a declining HRV trend detected by smartwatches may help identify individuals who require more intensive interventions.

I examined a general population across the full spectrum of glucose metabolism. Our findings revealed that low long-term HRV, is consistently associated with arterial stiffness, regardless of glycemic status. In Study I, one standard deviation lower HRV was equivalent to the effect of 2.7 additional years on cf-PWV and 1.6 years on CD[81]. In Study II, long-term measures of HRV were strongly associated with cardiovascular risk, with an effect size equivalent to 4.5 additional years of aging for major adverse cardiovascular events and 2.2 to 2.4 years for heart failure per one standard deviation (33 ms) lower in multiday SDNN intervals[82]. Autonomic dysfunction is known to precede the development of hypertension[[83]][84], which is an early and major risk factor for subsequent cardiovascular disease. Our results support the role of HRV as an early marker of cardiovascular health, both in the general population and among those with elevated cardiometabolic risk. As such, our studies suggest that monitoring cardiovascular risk progression or remission through long-term HRV may be valuable.

A limitation of implementing HRV monitoring, especially for long-term recordings, has been the demanding equipment requirements, such as the need for ECG recorders like Holter monitors. In recent years, wearable devices have become increasingly popular among the general public[11]. These devices offer an easy, non-invasive way to collect heart rate and HRV data and to monitor their progression over time. Further challenges include ensuring accurate measurement of interbeat intervals and determining which HRV indices are most suitable as markers of cardiovascular health. In diabetes and cardiovascular research, 24-hour HRV indices that reflect heart rate responses to inspiration and expiration (such as RMSSD, HF, and pNN50) are more sensitive to behavioral influences and therefore have not consistently shown strong associations with cardiovascular or metabolic outcomes, compared to measures based on total variability (SDNN, SDANN, TP, ULF, VLF, LF) [[44]][881]. However, the utility of these respiration-related indices appears to improve when measured during rest in shorter segments, such as 5-minute recordings(ref.).

If HRV monitoring proves effective by helping individuals maintain a healthy, age-adjusted HRV range through lifestyle changes, and by prompting healthcare engagement in response to sustained deterioration of HRV, then HRV could become a meaningful marker for long-term health monitoring. A cross-sectional study involving 8 million individuals with HRV data from wearable devices found that those who took more steps per day exhibited higher HRV[13]. A key public health challenge in integrating wearable devices into healthcare is ensuring equitable access, as individuals from lower socioeconomic groups are significantly less likely to own or use such devices. This raises the risk of health disparities by overlooking high-risk populations with lower income or education levels. Despite these concerns, there is encouraging evidence that the general population is open to digital health innovations. Studies have shown that most individuals are willing to share their health data with public health institutions and to allow algorithm-based systems, such as artificial intelligence, to collaborate with healthcare professionals in monitoring disease [[11]][85]. Therefore, monitoring HRV through wearable devices may serve as a first step in tracking health status and facilitating timely referrals to primary care when risk levels increase.

6.2.1.2. Primary care

Cardiovascular risk in primary care is assessed using clinical evaluations and standardized risk prediction tools to identify individuals at elevated risk (ref.). Management focuses on lifestyle modification, pharmacological therapy, and regular monitoring to reduce cardiovascular events (ref.). As the clinical paradigm shifts from a focus on ischemia toward more preventive strategies targeting atherogenesis, greater attention is being paid to individual risk factors that predispose patients to plaque formation[86]. Individuals with prediabetes live with elevated cardiometabolic risk, particularly for ischemic CVD and heart failure[87]. Early detection are important as CVD and heart failure leads to lower life-expectancy and quality of life.

However, many individuals with prediabetes remain in this state or return to normoglycemia. Despite their increased risk, they often fall outside structured assessment pathways in primary care, highlighting a critical gap in preventive strategies. This underscores the need for early and precise risk assessment[10]. In this section, I evaluate the integration of autonomic function assessment into routine cardiovascular risk stratification, with a particular emphasis on individuals with prediabetes or type 2 diabetes.

A central question is whether autonomic dysfunction can serve not only as a marker of underlying pathophysiology but also as a clinically useful risk indicator for cardiovascular disease and heart failure. If so, it could help identify patients at higher risk and thereby improve risk stratification. This concept can be explored from two complementary perspectives. First, long-term measurements of HRV may enhance the precision of individual cardiovascular risk prediction when added to established clinical risk scores.

Second, these measurements may help identify preclinical manifestations of cardiovascular autonomic dysfunction, enabling targeted interventions in a subgroup of patients to prevent future cardiovascular disease.

This raises the question of whether incorporating long-term HRV into traditional cardiovascular risk models, such as the Systematic COronary Risk Evaluation 2 (SCORE2) and the Framingham Risk Score, adds predictive value[88,89]. Our findings in Study I support a potential added value of 24-hour HRV to arterial stiffness, which is considered a surrogate marker of cardiovascular disease risk. In Study II, I extended this perspective by demonstrating a link between multiday HRV and hard endpoints of cardiovascular disease and heart failure. However, our findings are based on associations and do not include outcome prediction[90]. Therefore, a limitation of our study is that I did not evaluate whether incorporating long-term HRV or CARTs into established risk scores improves detection compared to existing guidelines for cardiovascular disease and heart failure. While most biomarkers have shown limited incremental value beyond established predictors such as age, sex, lipid levels, diabetes status, and blood pressure, some studies suggest that 24-hour HRV may improve risk discrimination for CVD and all-cause mortality in individuals with type 2 diabetes[91], and for stroke in older adults[92]. However, a key limitation of these studies is that their reference models have not been calibrated or validated in large-scale cohorts or in combination with established risk scores such as SCORE2 and the Framingham Risk Score.

The increasing availability of wearable devices capable of capturing long-term HRV data presents a practical opportunity for continuous monitoring in primary care settings. These devices may facilitate earlier detection of autonomic dysfunction and support more personalized approaches to the prevention and management of cardiovascular risk. However, the clinical utility of stratifying patients based on preclinical autonomic dysfunction remains uncertain. These considerations are only actionable if interventions targeting autonomic function can be shown to reduce cardiovascular risk. Emerging evidence suggests that both pharmacological and lifestyle interventions can improve HRV in the short term[93,94]. For example, high-intensity interval training has been shown to improve autonomic function in obese individuals with and without type 2 diabetes, although benefits in HRV were observed only in those without diabetes[95]. Similarly, lifestyle changes in individuals with prediabetes have been associated with improvements in short-term HRV, which may partly explain the reduction in diabetes risk independently of weight loss[96].

Nevertheless, it remains unclear whether these effects are sustainable over time and whether they translate into long-term cardiovascular protection. Particularly in the context of lifestyle improvements and intensified diabetes management, much of the observed enhancement in autonomic function may be mediated indirectly through improvements in cardiometabolic markers such as glucose levels, lipid profiles, body weight, maximal oxygen uptake, and blood pressure. Despite current uncertainties, monitoring autonomic function through long-term HRV may offer a valuable tool for assessing cardiovascular risk and tracking the impact of preventive strategies. However, its clinical utility must be confirmed through robust evidence demonstrating sustained effects and improved cardiovascular outcomes.

6.2.1.3. Secondary care

In secondary care, endocrinologists assess cardiovascular and heart failure risk by integrating advanced diagnostics, biomarker analysis, and imaging to detect early dysfunction. The treatment of patients with type 2 diabetes is guided by evidence-based therapies and multidisciplinary collaboration. The ADA/EASD 2022 consensus on Management of Hyperglycemia in Type 2 Diabetes emphasizes that early detection of heart failure in individuals with type 2 diabetes is crucial, as it enables timely initiation of therapies such as SGLT2 inhibitors, which have demonstrated significant benefits in reducing heart failure-related outcomes[97]. A major challenge in diabetes care is detecting heart failure before symptoms appear, as patients with symptomatic heart failure face a higher risk of mortality and more frequent hospitalizations[4]]. The AHA, ACC, and HFSA 2022 guidelines recommend identifying individuals at risk of heart failure based on factors such as diabetes, poor glycaemic control, uncontrolled hypertension, hyperlipidaemia, elevated BMI, albuminuria, renal dysfunction, and a history of CVD[98]. Still, there is a need to identify optimal approaches for recognizing and diagnosing heart failure in clinical care, as broad echocardiographic screening in type 2 diabetes is time-consuming and costly[4].

I demonstrated that CAN may help identify individuals at increased risk of heart failure, beyond what is captured by symptoms or existing risk scores. Our findings support considering CAN as a relevant risk factor for heart failure and suggest it may have value in future risk stratification strategies in type 2 diabetes. A clinical advantage of using CARTs is that they are standardized tests performed under controlled conditions. As discussed, these tests are reliable and reproducible, with reference values established in large population studies[47]. Beyond our findings and the established evidence of increased heart failure risk, CAN in the type 2 diabetes population also identifies individuals at high risk for cardiovascular disease, kidney disease, and early mortality. In Study III, I observed that two out of five participants had CAN, highlighting it as a prevalent complication. Therefore, detecting CAN may uncover an often-overlooked condition that is common in individuals with type 2 diabetes.

Clinical stratification of care includes two key considerations: (1) CAN should be further evaluated for associated cardiovascular complications, such as heart failure; and (2) cardiopreventive strategies should be initiated earlier in this subgroup.

First, patients with CAN may benefit from further cardiovascular assessment, including the use of sensitive biomarkers or echocardiography. NT-proBNP is a strong predictor of heart failure and a validated biomarker for ruling out the condition (ref.). However, its specificity varies across heart failure phenotypes, being less specific for detecting HFpEF compared to HFrEF. Therefore, additional evaluation using echocardiography is warranted. Echocardiography not only helps classify heart failure phenotypes but also identifies preclinical stages of heart failure through the detection of functional or structural cardiac abnormalities. An important question remains: to what extent does CAN overlap with cardiac abnormalities identified via echocardiography? Including CAN in structured assessments of heart failure could help clarify this relationship. Determining the diagnostic and prognostic value of CAN, particularly its sensitivity and specificity in detecting HFrEF and HFpEF, requires further investigation.

Second, the presence of CAN may justify earlier initiation of protective therapies. SGLT2 inhibitors are recommended as second-line treatment in type 2 diabetes and have demonstrated benefits in reducing the risk of heart failure, cardiovascular disease, and kidney function decline, complications commonly associated with CAN. Current guidelines recommend initiating these therapies based on a history of cardiovascular disease, heart failure, or the presence of conventional high-risk cardiovascular factors. However, the specific impact of SGLT2 inhibitors on the progression of cardiorenal outcomes in patients with CAN remains to be fully understood. Furthermore, while antihypertensive treatment is a cornerstone of cardiovascular risk management, whether specific classes of antihypertensive agents offer protective effects in patients with CAN remains to be explored.

The clinical implications of our findings in Study III are limited. Tthe generalizability of our results is restricted, as our study population consisted of patients with type 2 diabetes receiving secondary care. I observed that two out of five patients with CAN also had a history of cardiovascular disease—a group already at increased risk of heart failure due to their prior diagnosis. This overlap may influence the interpretation of CAN as an independent risk factor. Therefore, our findings need to be validated in a broader population with type 2 diabetes, including individuals without a history of cardiovascular disease. Doing so would allow for greater generalizability of our results to the broader type 2 diabetes population, particularly those in primary care.

6.3. Strength and limitation

6.3.1. Study design

Cross-sectional design

Studies I and III are based on cross-sectional data, with exposure and outcome measured within a three-month period. The main limitation of this design is that it does not allow us to determine whether the exposure led to the outcome or vice versa. As a result, we cannot establish temporality or confirm whether changes in the outcome were caused by the exposure. However, based on prior evidence, I assume the direction of the associations using physiological knowledge and in vivo studies. In Study I, I based my direction of the association based on longitudinal association based on data from Whitehall II study, showing steeper decrease in short-term HRV are associated with subsequent higher levels of cf-PWV[62]. Moreover, [insert in vivo studies]. In Study II, I attempted to mimic temporality by stratifying individuals with and without known type 2 diabetes, showing that deterioration in glucose metabolism increased the strength of the association. A strength of Study I is its large sample size, which enhances the generalisability of the findings across different glucose metabolism statuses.

In Study III, the design focused more on the clinical diagnosis of CAN and the presence of heart failure. The research question was oriented toward the clinical utility of CAN in identifying patients with type 2 diabetes who may be progressing early toward heart failure. Thus, the aetiological question remains better suited for other study designs. Whether cardiac function progressively worsens due to the underlying mechanisms of CAN remains to be fully established. If confirmed, this would highlight the relevance of CAN as a preclinical marker for early progression to heart failure, potentially serving as a target for preventive strategies.

Longitudinal design

A major strength of Study II is its longitudinal design, where HRV was measured at baseline and outcomes were captured prospectively through national registries. This temporal structure ensures that the exposure (HRV) preceded the outcome, reducing the risk of reverse causation. Although repeated HRV measurements over time would provide deeper insights into autonomic function dynamics, the prospective design still allows for stronger inference of directionality than cross-sectional studies. Furthermore, the use of high-quality registry data ensures complete outcome ascertainment and minimizes loss to follow-up bias.

Based on our studies, I demonstrate initial steps in understanding the relationship between cardiovascular autonomic dysfunction, measured by HRV or CART, and cardiovascular complications. However, I can only establish associations and cannot conclude with certainty that improvements in HRV lead to reduced cardiovascular risk. Therefore, causal inference cannot be drawn from our findings, and more causally focused methods are needed. Mendelian randomization, which uses genetic instruments for exposure, could help address this question. Additionally, structured mediation analysis involving modifications such as medication or lifestyle changes could clarify whether improving HRV or CART reduces cardiovascular risk, using data from intervention studies.

6.3.2. Intern validity

In this project, I aimed to assess cardiovascular autonomic function both in free-living conditions and in response to standardized test procedures during clinical visits. Additionally, I used dynamic measurements to evaluate arterial stiffness both locally and by velocity, and biomarker assessments to determine the presence of heart failure. In this section, I discuss the validity of 24-hour, week-long, and hourly HRV measurements, as well as the standardized tests of CAN. I also address the validity of the included outcomes and discuss the strengths and limitations of using MACE as a time-to-event outcome.

6.3.2.1. Long-term HRV (>24 hours) as measurement for autonomic function

A main consideration in HRV analysis is the reliability of raw inter-beat interval data from ECG recordings. To ensure accurate various HRV measures, the intervals must be captured in a continuous and correctly sequenced manner. Both frequency-domain analyses depend on the integrity of the inter-beat interval sequence, while some time-domain measures, such as RMSSD and pNN50, specifically quantify the variability in the differences between successive intervals.

In Study I, I used data from a 12-lead Holter system, which is considered the gold standard for long-term ECG recordings. This provided a valid reference for evaluating wearable devices. With detailed and sequential inter-beat intervals, I were able to access full range of HRV metrics including, time-domain measures of variability between successive intervals, while frequency-domain measures were derived across power bands from high to ultra-low frequencies.

In Study II, I used the Actiheart device to assess HRV. The device was configured to record continuously over an 8-day period. It captured 30-second epochs of mean heart rate intervals, along with upper and lower prediction intervals. From each epoch, I generated a distribution of inter-beat intervals. An algorithm was applied to estimate HRV from these distributions, and its validation showed strong agreement with established metrics, including SDNN, SDANN, and the SDNN index [6][57]. However, a limitation of this dataset is that it did not allow for the calculation of frequency-domain measures or specific time-domain metrics such as RMSSD or pNN50. High-frequency power, RMSSD, and pNN50 typically show earlier diurnal peaks compared to low-frequency power and SDNN[13]. These measures are more reflective of parasympathetic activity during in- and expiration. It remains to be determined how these hourly measures relate to cardiovascular disease, heart failure, and all-cause mortality.

In the context of this project, which focuses on long-term HRV in diabetes and cardiovascular research, it is important to acknowledge that the autonomic nervous function I aim to assess may also be influenced by behavioral factors such as physical activity, sleep, meal timing, emotions, smoking, caffeine intake, alcohol consumption, medication use, and prior cardiovascular complications. These factors can potentially mask or mimic underlying physiological dysfunction during recordings. Therefore, reduced long-term HRV cannot be interpreted solely as a marker of autonomic function. Moreover, HRV is influenced by lifestyle patterns over time, making it sensitive not only to day-to-day behaviors but also to long-term habits that affect autonomic balance. In Study II, I observed that individuals with lower HRV also had lower habitual physical activity and lower VO_2 max, suggesting that reduced HRV reflects a more sedentary lifestyle and lower cardiorespiratory fitness.

In Studies I and II, I accounted for habitual physical activity, and in Study II, I also adjusted hourly HRV for physical movement during recordings to test the influence of concurrent activity. However, further studies are needed to understand how lifestyle patterns affect long-term HRV recordings on subsequent days, in order to better isolate the behavioral component. In both studies, I excluded participants with prior CVD to preserve the etiological order between autonomic dysfunction and cardiovascular outcomes.

Anti-hypertensive medications, especially beta-blockers, are known to increase HRV in randomized controlled trials[99]. However, in cohort studies, participants using anti-hypertensives generally show lower HRV, likely reflecting a higher burden of cardiovascular complications [ref]. Because beta-blockers target the autonomic nervous system, they may introduce bias in HRV measurements by interfering with the function I aim to assess. In sensitivity analyses in Studies I and III, excluding participants on anti-hypertensive treatment did not materially change the estimates. Therefore, I included these participants and adjusted for medication use in the full models.

HRV levels are influenced by heart rate, as lower resting heart rate allows for greater variability. In Study I, I chose not to adjust for heart rate in our models, as this could introduce multicollinearity. Additionally, elevated heart rate is driven by increased sympathetic activity and may act as a mediator in the pathway leading to arterial stiffness. Our use of full-day recordings captures HRV during both rest and activity, providing a robust representation of autonomic function over a typical day. In contrast, heart rate correction may be more relevant for short-term HRV recordings, where standardized conditions can be affected by random influences such as time of day, smoking, or caffeine intake. These factors would have been relevant in Study III had I included HRV measures. In Study II, I included HRV measures pre-adjusted for heart rate, which accounted for part of the observed associations, particularly with heart failure and all-cause mortality, and to a lesser extent with ischemic-related CVD events. Similar trends were observed in hourly associations, where heart rate pre-adjustment had comparable effects on the outcomes.

[When HRV is analyzed in shorter segments (e.g., hourly or in 5-minute intervals), measures like RMSSD, pNN50, and HF appear to offer new insights into autonomic function and its relevance in diabetes and CVD, e.g during night-time [[100]][93]. SDANN and SDNNi aim to reduce the impact of short-term variability, such as that caused by physical activity, by calculating either the standard deviation of 5-minute segment mean IBI (SDANN) or the mean of standard deviations across 5-minute segments (SDNNi). [include lower-frequency points]. This helps smooth out transient fluctuations and better capture long-term autonomic modulation. Thus, behavioral patterns pose a limitation in physiological research aiming to disentangle the causal pathways between autonomic dysfunction and CVD when using long-term HRV measures. These patterns likely introduce high variability between observations that is not attributable to autonomic function itself.]

6.3.2.2. Cardiovascular autonomic reflex test

CART provides a practical approach for screening autonomic dysfunction and has been shown to be a reliable method[101]. Although certain indices from CARTs may be influenced by factors such as time of day or recent physical activity, these effects are generally minimal. Furthermore, no impact of caffeine intake has been observed on the reference age-based formula[47]. A limitation of the CARTs in this study was the high prevalence of participants who were unable to complete the full battery of tests, primarily due to missing data from the Valsalva manoeuvre.

6.3.2.3. Measures of cardiovascular risk

[Arterial stiffness - NT-proBNP - MACE limitation in aetiological research - Non-specific heart failure and MI and stroke and death - HRV stronger link with MI or stroke - Perspective: decreasing number of events with prolonging time-to-event - Clinical trail moved to high risk population in lower-income countries (South America) - Challenge for coming observational cohorts (need to increase sample size) [The Problem With Composite End Points in Cardiovascular Studies The Story of Major Adverse Cardiac Events and Percutaneous Coronary Intervention]]

6.3.3. External validity

6.3.3.1. Selection bias

The Maastricht Study

In Study I, participants were recruited using multiple strategies, with a focus on enrolling individuals with type 2 diabetes to ensure sufficient statistical power. Recruitment was conducted through mass media campaigns, municipal registries, and the regional Diabetes Patient Registry via mailings. Participation therefore depended on individuals' awareness of the campaigns and their willingness to attend. Patients with type 2 diabetes were actively targeted with additional mail invitations to encourage participation.

ADDITION-PRO

In Study II, participants were recruited through a stepwise screening procedure. Initially, individuals were selected based on a risk score derived from a self-administered questionnaire sent by mail, followed by HbA1c or random glucose measurements. This process introduced several opportunities for selection bias, as individuals had to receive and return the questionnaire and attend a follow-up visit for blood testing if their risk score was high[102].

First, the population consisted of those who responded to the screening questionnaire and those at higher risk who underwent further testing. Second, the questionnaire selected participants based on a risk score designed to identify individuals with type 2 diabetes, while prediabetes was identified through subsequent HbA1c measurements. Risk factors such as age and hypertension contributed to higher scores, potentially leading to overrepresentation of these groups.

Thus, selection bias may arise from both participation in the risk assessment and follow-up attendance in ADDITION, as well as from the instruments used to identify risk, namely, the Inter99 risk score, HbA1c, and random glucose measurements. Healthier individuals are generally more likely to participate in screening and cohort studies [ref.].

CANCAN

In Denmark, patients with type 2 diabetes are referred to diabetes specialists at outpatient clinics when their general practitioner is unable to stabilize their care. As a result, CANCAN participants represent a higher-risk group among Danish diabetes patients, while more stable patients remain under general practitioner care. Consequently, the prevalence of heart failure indicators and CAN is likely higher in this selected group. A strength of the CANCAN sampling strategy is that patients were already attending endocrinology consultations, and the study examination required only additional time during their visit, without extra transport or appointments.

cross this project, selection bias spans different aspects. In Studies I and II, healthier and more health-conscious individuals were more likely to participate, potentially introducing bias. In contrast, participation in Study III was optimized by aligning study assessments with routine consultations. In epidemiology, I aim to match the source population with the target population, but self-selection in participation can limit this. As a result, participants may be healthier and more engaged with healthcare services, reducing contrast between determinants and outcomes in etiological analyses. One possible explanation for the lack of a stepwise increase in the association between HRV and arterial stiffness across prediabetes and type 2 diabetes in Study I is that participants with type 2 diabetes represented a well-treated population. Although the sample may be sufficient to demonstrate a relationship, the magnitude of the association in this group may be limited.

6.3.3.2. Generalisability

The generalisability of our findings can be considered on two levels: how well the results apply to the general population within the country, and how they translate to populations with different ancestries in other countries.

Studies II and III include individuals at high risk of diabetes and those with type 2 diabetes. Therefore, the associations between cardiovascular autonomic dysfunction and cardiovascular outcomes or surrogate biomarkers are relevant to individuals with some degree of diabetes risk. However, whether these associations hold in the general population remains uncertain. Study I suggests that the link between autonomic dysfunction and cardiovascular risk, as measured by arterial stiffness, is also present in individuals with normal glucose metabolism, though to a lesser extent. This finding was supported by replication in the Whitehall II cohort, strengthening the generalisability of the observed relationship[62].

The study populations in Studies II and III consist of individuals of Nordic descent, while Study I includes individuals of Western European descent. Since the constellation of diabetes risk factors may differ in populations of Asian, South American, African, and other ancestries, our findings may not be fully generalisable to these groups. This limitation affects the applicability and magnitude of the observed associations to an unknown degree. Further cohort studies including underrepresented populations are warranted. As our studies focus on diabetes risk, all participants were adults aged 40 years and older. Therefore, our findings are limited to this age group, and whether the results extend to younger adults or children remains to be confirmed. Overall, while our study benefits from including individuals across different levels of diabetes risk, limitations in generalisability remain, particularly for more diverse and younger populations.

7. Perspective

We have discussed the mechanisms and clinical implications of cardiovascular autonomic function across different stages of glucose metabolism. Based on our findings and discussion, we propose further perspectives to define its role in research and healthcare from three aspects: (1) continuous non-invasive health monitoring, (2) risk stratification, and (3) identification as a causal and modifiable marker.

7.1. Continuous monitoring of cardiovascular health

Understanding when and how physiological signals reflect elevated CVD risk is essential for developing early and effective prevention strategies. Incorporating HRV into digital health solutions could support personalized feedback mechanisms, enabling timely lifestyle or therapeutic interventions and contributing to more adaptive and preventive healthcare strategies. Wearable devices enable comprehensive data collection on behavioral (e.g., sleep and physical activity) and physiological (e.g., heart rate, ECG, temperature) parameters[103]. These devices offer a broader and more feasible approach to long-term heart rate monitoring. Despite growing interest in wearable-based monitoring, the integration of HRV into routine cardiometabolic risk assessment remains limited.

Two key aspects highlight the potential applications of monitoring: (1) identification of risk and (2) assessment of response to intervention.

Identification of risk

We ascertained that lower long-term HRV is a risk factor for CVD, as demonstrated by its association with arterial stiffness and hard CVD endpoints. In addition, our findings that specific time points of HRV and heart rate are associated with increased risk of CVD and heart failure suggest that distinct physiological responses captured under free-living conditions may enhance early risk detection. The majority of the observed associations were not explained by movement at the corresponding time points. For risk assessment, instead of merely adjusting for physical activity as a confounding factor, future predictive models could benefit from integrating multiple physiological signals such as HRV, sleep, and activity patterns to better reflect dynamic states of health. Machine learning techniques offer the ability to process complex raw time-series data, such as interbeat intervals and accelerometer signals, and to uncover patterns that may improve risk prediction beyond conventional summary measures of HRV. However, a key limitation of these models is their reduced explainability, which may limit their clinical applicability.

Assessment of response to intervention

HRV highlights a potential target for intervention, given that low HRV may be indicative of adverse lifestyle patterns. For instance, behavioral patterns such as disrupted sleep, inactivity, or irregular meal timing may influence circadian fluctuations in HRV. Evidence from studies on night-shift workers suggests that meal timing affects HRV, with daytime meals leading to higher HRV during night hours[93]. Medications such as beta-blockers and GLP-1 receptor agonists have been shown to influence autonomic function, with effects observed in both 24-hour and hourly HRV measures [[99]][104].

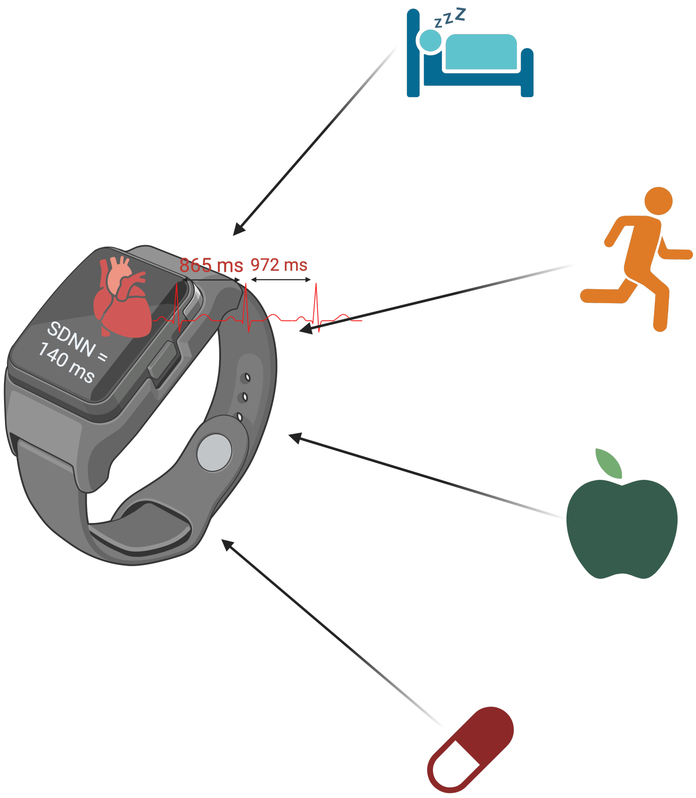

Figure 7.1.: Biofeedback HRV response to lifestyle and treatment solutions. (Source: Author)

Future studies can leverage wearable devices to better understand the behavioral factors and treatment options that contribute to its improvement or deterioration of HRV. This approach may help identify effective lifestyle patterns or medications that improve success of cardiovascular health through modulation of HRV.

Standardization and transparency across different brands of wearable devices remain a challenge for both research and clinical implementation of heart rate and HRV monitoring. While smartwatches offer a convenient method for heart rate measurement, their accuracy can vary, as they rely on photoplethysmography to detect pulse rate at the wrist. This method can be imprecise under certain conditions, particularly during physical activity, due to motion artifacts and other external factors[[105]][106]. Despite these limitations, ongoing improvements in sensor technology and algorithm calibration are likely to enhance the reliability of wearable-derived heart rate and HRV data.

7.2. Risk-stratification

Based on the clinical implications, the distinct roles of long-term HRV and CART in risk stratification remain to be demonstrated and require further investigation.

From the perspective of wearable devices, it remains to be determined whether HRV can serve as an early indicator of CVD risk when used in a risk score alongside simple non-invasive markers such as age, sex, and BMI, and how it compares to current CVD risk scores that include blood-based biomarkers and blood pressure.

A limitation of long-term HRV measurement is the lack of standardization, as data are collected under free-living conditions and may be influenced by daily behaviors, potentially affecting risk classification. Therefore, standardized procedures may be necessary. CART has been shown to be a reliable, non-invasive method that typically takes approximately 10 minutes to complete. As such, a standardized and validated diagnosis of CAN can help identify patients with type 2 diabetes who are at higher risk of CVD, nephropathy, and heart failure. However, the extent to which the diagnosis of CAN identifies heart failure risk and is generalizable to broader populations with type 2 diabetes or even prediabetes, in a clinically relevant context, remains to be assessed.

Our findings suggest that long-term HRV and CAN may serve as potential markers for CVD and heart failure risk among individuals at elevated metabolic risk, helping to identify those who could benefit from preventive strategies. Future research should evaluate whether individuals classified as high-risk based on autonomic dysfunction or CAN should initiate cardiovascular-focused prevention or screening earlier, or receive specific interventions tailored to their condition.

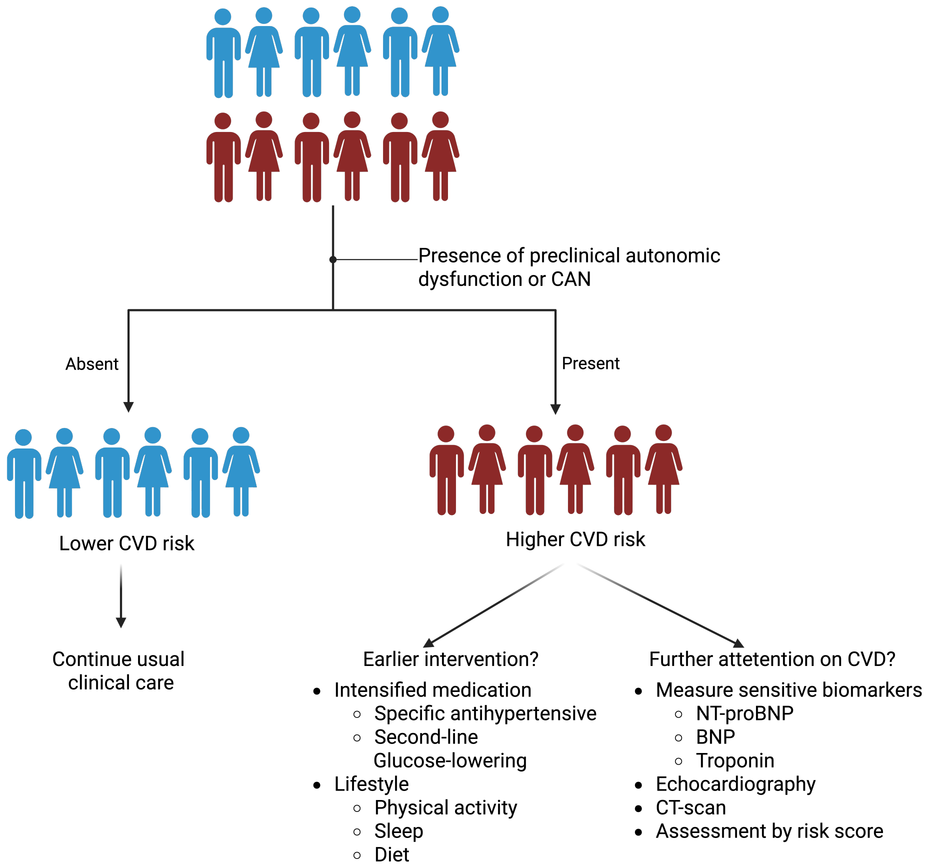

Figure 7.2.: Risk-stratification by autonomic dysfunction. (Source: Author)

7.3. Effective causal modifiable marker

Our findings support an etiological link between long-term HRV and the risk of CVD, providing initial evidence of a potential causal relationship. However, the observed association does not confirm causation, and further research is necessary to determine whether the relationship between HRV and CVD risk is truly causal. Traditionally, epidemiological research has relied on randomized controlled trials to establish causality. However, conducting such trials to isolate the direct effect of HRV is particularly challenging. Interventions that influence HRV often do so indirectly, through changes in lifestyle factors such as weight loss, inflammation, and insulin sensitivity. Similarly, pharmacological treatments may improve HRV as a secondary effect, for example through blood pressure reduction from antihypertensive medications. As a result, isolating the direct modification of HRV is difficult. To address the limitations of clinical trials, modern epidemiological approaches such as Mendelian randomization[107] and structured causal mediation analysis offer alternatives for inferring causality from observational genetic studies and for estimating indirect effects using trial data[108]. To date, no GWAS has been conducted to investigate the genetic determinants of long-term HRV. Establishing such genetic associations is essential for understanding its genetic architecture and for providing unconfounded estimates by using genetic variants as proxies to assess the causal role of HRV in CVD.

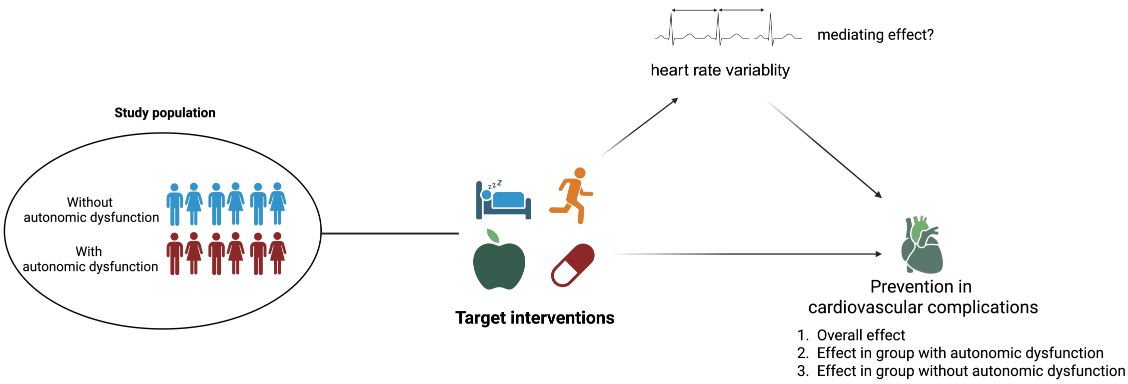

Figure 7.3.: Mediation of HRV by intervention in prevention of CVD. (Source: Author)

A study has demonstrated that reduced HRV mediates the association between glomerular hyperfiltration and mortality[109], indicating an initial potential for HRV as a modifying factor. While this has been shown in observational data, no evidence of such mediation has yet been established in trial data. The Diabetes Prevention Program (DPP) showed that HRV may modify the effect of lifestyle intervention in preventing type 2 diabetes[96]. However, it remains unclear to what extent this modification applies to cardiovascular outcomes, and whether the intervention was more effective among individuals with lower HRV. Future cardiometabolic intervention trials, whether focused on lifestyle modification or pharmacological treatment, should, where feasible, include HRV measurements to enable structured mediation analyses and to better understand whether modifying autonomic function improves cardiovascular outcomes. This could help demonstrate whether modification of HRV through potential strategies such as medications like beta-blockers or lifestyle interventions including physical activity, diet, and sleep has a sustainable effect on CVD outcomes.

Even if HRV proves to be causally linked with cardiovascular complications, its measurement is limited by the inability to distinguish between sympathetic and parasympathetic contributions.

8. Conclusion

Autonomic dysfunction, assessed through 24-hour HRV, is associated with increased arterial stiffness. This relationship is already evident in individuals with normal glucose metabolism and becomes more pronounced in those with prediabetes and type 2 diabetes. In individuals at high risk of type 2 diabetes, lower long-term HRV measured over a week has been linked to ischemic events, heart failure, and all-cause mortality, highlighting HRV's potential as a marker of cardiovascular health. Both HRV and heart rate follow circadian patterns in relation to cardiovascular disease. Higher nighttime heart rate is associated with increased risk of heart failure, and specific morning patterns of HRV have been linked to ischemic events. These findings suggest that both long-term and hourly heart rate and HRV measures provide valuable prognostic information. Structured testing of cardiovascular autonomic function in individuals with type 2 diabetes can detect those with CAN and may help identify individuals at higher risk of heart failure.

We have established an association between HRV and cardiovascular complications. However, the underlying mechanisms remain unclear. It is not yet known whether autonomic dysfunction, as indicated by low HRV, is a marker of developing arteriosclerosis, atheroma, or cardiac remodeling, or whether it plays a causal role in their development. While the pathogenic pathways leading to cardiovascular risk appear similar across the spectrum of glucose metabolism, dysglycemia may amplify the impact of autonomic dysfunction. Whether lower long-term HRV in individuals with prediabetes or type 2 diabetes reflects a distinct physiological mechanism involving neuropathy, compared to those with normal glucose metabolism, remains an open question.

Structured studies assessing screening strategies may help determine the optimal timing, population, and methods for incorporating HRV and CART into clinical practice. Furthermore, trial designs that focus on either lifestyle interventions or targeted pharmacological modulation of HRV are needed to clarify the clinical role of HRV and CART in cardiovascular prevention. When using HRV and standardized CART, it is important to carefully consider how the data are applied in relation to specific research objectives, ranging from physiological mechanisms to clinical diagnosis. Long-term HRV and its hourly fluctuations provide insight into autonomic responses under free-living conditions. Further research is needed to determine whether modifying these measures can yield sustained preventive effects on cardiovascular disease and mortality.

CARTs offer a standardized approach for diagnosing CAN. Clarifying the clinical utility of CARTs in assessing cardiovascular and heart failure risk through the identification of CAN is essential for advancing precision care in individuals with type 2 diabetes. Echocardiographic studies can help establish the link between CAN and the risk of specific heart failure subtypes. Future research should carefully select HRV measures aligned with specific clinical or research objectives. Long-term HRV and CART have demonstrated potential in cardiovascular risk assessment and should be integrated to evaluate whether autonomic function assessments can monitor treatment or lifestyle effectiveness or guide stratified cardiovascular risk decisions in individuals with prediabetes or type 2 diabetes.

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A. More results

Some results that wouldn't fit into the main thesis

B. Another appendix

Something else