Cardiovascular autonomic dysfunction impact on cardiovascular complications across glucose metabolism

PhD dissertation

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Preface

The research presented in this dissertation was conducted during my PhD studies at the Department of Public Health, Aarhus University, and Steno Diabetes Center Aarhus between 2022 and 2025. The project was supported by the Department of Public Health at Aarhus University, Steno Diabetes Center Aarhus, and the European Foundation for the Study of Diabetes/Sanofi European Diabetes Research Programme in Diabetes associated with Cardiovascular Disease. The aim was to better understand the impact of cardiovascular autonomic dysfunction on heart disease in individuals with diabetes or at high risk of diabetes. I am grateful for the opportunity to explore how autonomic dysfunction can be demonstrated by investigating variations in heart rate responses across different durations and conditions to gain insights into cardiovascular risk, which remains an ongoing issue in current research. It is my hope that the findings of this dissertation will contribute to a deeper understanding of the clinical potential of long-term heart rate variability measures and standardized cardiovascular autonomic reflex tests in identifying individuals at risk for cardiovascular disease across all stages of glucose metabolism. In doing so, I hope this work contributes to the broader effort of improving care for individuals at risk of developing diabetes, as well as those living with diabetes.

Other work and collaboration during the PhD

Much of the work I have been involved in during my PhD is not fully reflected in this dissertation. I would like to take this opportunity to provide an overview and acknowledge the many collaborative efforts that emerged during my time as a PhD student. In my work on diabetes epidemiology, I have been deeply curious about future methods in research. This led me to become a peripheral member of the Hulman Lab, a group with an open heart and a strong foundation in critical thinking, focused on machine learning and clinical prediction. Together with Adam Hulman and Anders Isaksen, we investigated how people perceive the use of artificial intelligence in healthcare. On this project, I am grateful to Lasse Bjerg, Annelli Sandbæk, and the rest of the Health in Central Denmark (HICD) steering committee for integrating our questionnaire into their cohort. I also appreciate Kasper Normann's help with prompt data management. This collaboration resulted in one original research paper and two other submitted manuscripts based on wave 2022 and 2024 of data collection from the HICD cohort.

We set out to extend the generalisability of the CANCAN findings to populations without type 2 diabetes. This led us to use data from the Lolland-Falster Health Study (LOFUS). I am deeply grateful to Randi Jepsen for the collaboration on accessing the cohort and for her support in getting the biobank samples analysed. The data is now ready for use in the study. Further appreciation goes to Marie Mathilde Bjerg Christensen, Christian Stevns Hansen, and Jesper Fleischer for updating reference values for CARTs using LOFUS data and for generously sharing their expertise on the measurements.

In my last year of the PhD, I was fortunate to exchange research environments with the Baker Heart and Diabetes Institute. I had the privilege of working alongside a proficient team of epidemiologists consisting of PhD students, postdoctoral researchers, and senior scientists, using data from the PREDICT study involving patients with type 2 diabetes. I was impressed by how closely research and clinical care are integrated. Furthermore, their dedication to utilizing cohorts and organizing multinational data resources was truly admirable. I would like to extend a special thank you to Professor Dianna Magliano, Senior Researcher Julian Sacre, and Professor Jonathan Shaw for their valuable input on using questionnaires to screen for heart failure subtypes and for helping to shape a study that will be submitted soon.

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This PhD journey has been a remarkable adventure. The best part has undoubtedly been all the people I have met and worked with along the way. I would like to express my deepest gratitude to colleagues, collaborators, friends, and family for their support throughout this journey and for making the ride both fun and exciting.

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Thank you to all my friends at Steno Diabetes Center Aarhus (SDCA) and the Department of Public Health at Aarhus University who have supported my project, inspired great discussions, and shared fun times: Adam, Omar, Luke, Daniel I, Anders, Benjamin, Jie, Livie, Helene, Sidsel, Manuel, Christian, and Ole-Emil.

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No adventure is truly exciting without cultural exchange—whether through the lovely visits from Peter and Ieva at SDCA or by putting myself in new research environments. To Professor Dianna Magliano and Professor Jonathan Shaw, thank you for including me in the Epidemiology group at the Baker Heart and Diabetes Institute in Melbourne. It was a truly enriching stay, both scientifically and socially. I would like to extend my sincere thanks to Julian Sacre for his valuable support and insightful contributions in deepening my understanding of the challenges involved in diagnosing heart failure subtypes. To the PhD students and post-docs at 7/11, Della, Forough, Elizabeth, Jedidiah, Lei, Mahtab, Kanika and Joanna, thank you for making me feel so welcomed and giving me a wonderful and fun experience of Melbourne.

To my brothers, Esben and Jalte, thank you for putting up with me and for helping me loosen up and have fun. To my parents, thank you for supporting me throughout my life and for standing by me in every path I have chosen. Much of my curiosity for applying mathematics and statistics to understand the world stems from my grandfather, Ebbe Schaarup, who spent countless hours teaching me. Last but not least, to my lovely girlfriend Freja, thank you for always being kind, supportive, and willing to join the adventure.

Papers in the dissertation

Study I

Cardiovascular autonomic dysfunction is linked with arterial stiffness across glucose metabolism: The Maastricht Study

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). preprint at 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

Cardiovascular autonomic dysfunction precedes cardiovascular disease and all-cause mortality: 11-year follow-up of the ADDITION-PRO study

Jonas R. Schaarup, Lasse Bjerg, Christian S. Hansen, Erik L. Grove, Signe T. Andersen, Dorte Vistisen, Søren Brage, Annelli Sandbæk, Daniel R. Witte (2025). preprint at medRxiv 2024.12.18.24319131; doi: https://doi.org/10.1101/2024.12.18.24319131 (accepted at Diabetes, Obesity and Metabolism)

Study III

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

Jonas R. Schaarup, Lasse Bjerg, Christian S. Hansen, Daniel R. Witte, Henrik H. Thomsen, Jesper Fleischer, Rodica Pop-Busui, Annelli Sandbæk, Signe T. Andersen. (submitted to Diabetes Care)

Additional publications

The 4 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

Schaarup JR, Aggarwal R, Dalsgaard E-M, Norman K, Dollerup OL, Ashrafian H, Witte DR, Sandbæk A, Hulman A.**Perception of artificial intelligence-based solutions in healthcare among people with and without diabetes: A cross-sectional survey from the health in Central Denmark cohort**.Diabetes Epidemiology and Management, 2023. https://doi.org/10.1016/j.deman.2022.100114

Jie Zhang, Christina Andersen, Anja Olsen, Jytte Halkjær, Kristina Elin Petersen, **Jonas Frey Rosborg Schaarup**, Christian S Antoniussen, Daniel R Witte, Christina C Dahm. **Life-long Body Mass Index Trajectories and Cardiometabolic Biomarkers-The Danish Diet, Cancer, and Health-Next Generations Cohort**. 2025. (accepted at International Journal of Obesity)

Becker, E., Emmertsen, K. J., **Schaarup, J. F. R.**, Iversen, L. H., Hovdenak, I., & Laurberg, T. (2025). **The impact of diabetes status on postoperative outcomes after rectal cancer surgery: a population-based cohort study**. Colorectal Cancer, 14(1). https://doi.org/10.1080/1758194X.2025.2489302

Pre-prints

Jonas R. Schaarup, Anders Aasted Isaksen, Kasper Norman, Lasse Bjerg, Adam Hulman. (2025).**Trust in large language model-based solutions in healthcare among people with and without diabetes: a cross-sectional survey from the Health in Central Denmark cohort**. medRxiv 2025.02.24.25322734; doi: https://doi.org/10.1101/2025.02.24.25322734 (under review at BMJ digital health and AI)

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)

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Abbreviations

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
DBP: Diastolic blood pressure
ECG: Electrocardiogram
eGFR: Estimated glomerular filtration rate
FPG: Fasting plasma glucose
GLP1RA: Glucagon-like peptide-1 receptor agonists
GWAS: Genome-Wide Association Studies
HbA1c: Haemoglobin-A1c
HDL: High-density lipoprotein cholesterol
HF: High-frequency range (0.15–0.4 Hz)
HRV: Heart rate variability
IR: Incidence rate
IRR: Incidence rate ratio
LDL-C: Low-density lipoprotein cholesterol
LF: Low-frequency range (0.04–0.15 Hz)
MACE: Three-point major adverse cardiovascular events
MAP: Mean arterial pressure
NGM: Normal glucose metabolism
NT-proBNP: N-terminal pro-B-type natriuretic peptide
OGTT: Oral glucose tolerance test
OR: Odds ratio
PAEE: Physical activity energy expenditure
pNN50: Proportion of NN intervals differing by more than 50 ms
RPAQ: Recent Physical Activity Questionnaire
RMSSD: Root mean square of successive differences between NN intervals
SBP: Systolic blood pressure
SD: Standard deviation
SDANN: Standard deviation of the averages of NN intervals in 5-minute segments
SDNN: Standard deviation of NN intervals
SDNN index: Mean of the SDs of all NN intervals for all 5-minute segments
SGLT2i: Sodium glucose co-transporter type 2 inhibitors
SES: Socioeconomic status
T2D: Type 2 diabetes mellitus
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)

1.Introduction

Diabetes mellitus is a growing global health concern, posing pressing challenges for public health systems.[1] As prevalence increases, more individuals are exposed to a higher risk of premature mortality and cardiovascular disease (CVD).[1] At the same time, individuals are living longer with diabetes and therefore endure extended periods under the burden of diabetes-related complications.[2] Despite advancements in cardiovascular care, coronary artery disease and heart failure are still often detected at more advanced stages, such as during ischemia, major cardiovascular events, or the onset of symptomatic heart failure in patients with diabetes.[3,4]

Over recent 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, the assessment of 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, individuals with prediabetes often remain outside structured treatment pathways.[9,10] Although diabetes contributes to autonomic dysfunction, it remains 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 the relationship between specific diurnal responses and the risk of 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

This background introduces the concept of type 2 diabetes (T2D) and its associated cardiovascular risk. An overview is then provided of various cardiovascular complications, including arteriosclerosis, atherosclerosis, and heart failure. Finally, cardiovascular autonomic function (autonomic function) is described, along with its potential to enhance our understanding of cardiovascular disease (CVD).

2.1. Type 2 diabetes and prediabetes

The progression from normal glucose metabolism (NGM) to T2D is characterized by sustained elevations in blood glucose levels. T2D is defined by a progressive decline in beta-cell function, most often as a consequence of chronic insulin resistance.[14,15] Insulin resistance occurs when tissues such as muscle and liver lose their sensitivity to insulin.[15] As a result, glucose is not effectively taken up by these tissues and remains in circulation.[15] Meanwhile, beta-cell function deteriorates, leading to a diminished insulin response to glucose levels.[15] Years before a T2D diagnosis, these changes contribute to rising fasting and postprandial glucose levels.[14]

The body regulates glucose through various mechanism. During fasting, pancreatic alpha cells secrete glucagon, which stimulates hepatic glucose production via glycogenolysis and gluconeogenesis.[15] 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.[15] Insulin and incretins work together to suppress hepatic glucose production, while insulin promotes glucose uptake in muscle and adipose tissue.[15] 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.[15] 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]

The World Health Organization (WHO) and American Diabetes Association (ADA) diagnostic criteria for T2D 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 percent (48 mmol/mol).[16,17] The OGTT measures glucose levels two hours after the ingestion of a standard 75 gram glucose load in the fasting state.[17] Progression towards diabetes 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.[18] Many complications of diabetes, such as macrovascular disease, neuropathy, cancer, and cognitive impairment, may begin to develop at earlier stages of dysglycemia.[19–21] This stage is referred to as prediabetes or high risk of diabetes and is defined by fasting plasma glucose levels between 6.1 and 6.9 mmol/L, 2 hour plasma glucose levels between 7.8 and 11.0 mmol/L (WHO criteria), and HbA1c levels between 5.7 and 6.4 percent (39 to 47 mmol/mol) (ADA criteria).[17] In parallel with the growing prevalence of T2D, the prevalence of prediabetes is also increasing.[9]

Risk factors for progression to T2D and its complications range from genetic predisposition to lifestyle and socioenvironmental factors. The most common risk factor for diabetes is central obesity.[22] The accumulation of diabetes risk factors is associated with a combination of adverse changes in CVD risk factors, including increases in low density lipoprotein (LDL) cholesterol, triglycerides, and systolic blood pressure, along with decreases in high density lipoprotein (HDL) cholesterol.[23]

Diabetes increases the risk of both microvascular and macrovascular complications, which are major contributors to the morbidity and mortality associated with the disease.[15] Beyond conventional CVD risk factors, chronic hyperglycemia promotes the formation of harmful byproducts such as reactive oxygen species and advanced glycation end products, which drive oxidative stress and inflammation.[24] These processes contribute to endothelial dysfunction and vascular damage.[24] While the general mechanisms underlying macrovascular complications are well described, the identification of preclinical stages of CVD and the differentiation of CVD risk between individuals at high risk of diabetes and those with established T2D require further clarification.[10]

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.[25] At the individual level, these exposures often manifest through more proximal biological risk factors, including hypertension, hypercholesterolemia, diabetes, and obesity.[26] Along the causal pathway, these intermediate conditions tend to cluster, thereby accelerating 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.[27] Risk factors contribute to distinct pathophysiological mechanisms across different types of CVD, involving structural, signaling, inflammatory, and hemodynamic changes within the cardiovascular system.[27–29] 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.[30–33]

2.2.1. Arteriosclerosis

Evidence emphasizes the role of vascular aging in early disease development, extending beyond the traditional focus on cardiovascular endpoints.[29] Arteriosclerosis, commonly referred to as arterial stiffness, has been identified as 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.[34] Arterial stiffness has been found to arise from progressive remodeling of the arterial wall.[29,35] This remodeling has been 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.[34] Remodeling of the arterial wall has been shown to increase systolic blood pressure and reduce coronary perfusion, thereby contributing to the development of hypertension and, eventually, cardiovascular disease.[36] Additionally, arterial stiffness has been associated with elevated pulsatile load on the microcirculation, promoting the progression of chronic kidney disease, vascular dementia, and Alzheimer's disease.[29]

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 often at specific sites such as the coronary and carotid arteries.[37] This chronic process can lead to progressive occlusion of the vessel, contributing to reduced oxygen supply to the heart[38], often leading to symptoms of angina.

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.[39] 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.[39] 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 (MI).[39] 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.[40,41]

Myocardial infarction

MI occurs due to the rupture of an atherosclerotic plaque in the coronary arteries, triggering thrombus formation that blocks blood flow.[42] This leads to oxygen deprivation (ischemia) and subsequent myocardial injury or necrosis.[42] If untreated, this process can cause extensive cardiac damage and fatal arrhythmias.[42] Over the past decades, the incidence of MI has declined in high-income countries with a marked reduction in MI-related mortality.[43] 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.[44,45] Medically, the improved preventive management of hypertension and hyperlipidemia has reduced the burden of atherosclerotic disease.[43] In acute care, the widespread adoption of evidence-based interventions such as thrombolytic therapy, percutaneous coronary interventions (including stenting), and coronary artery bypass grafting has improved survival and outcomes following MI.[46] In T2D, the risk of MI is elevated by 72%, with an approximately threefold risk among patients under 60 years compared to age under 60 without T2D.[47] Similar to the general population, MI incidence and fatality have declined among individuals with diabetes.[48,49]

Stroke

The majority of strokes are ischemic and result from an obstruction in a cerebral artery. The process often begins with the development of atherosclerotic plaques at the carotid artery bifurcation, which can lead to the formation of emboli[50]. These emboli may travel through the bloodstream and become lodged in the cerebral arterial tree, ultimately causing an ischemic stroke[50]. 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.[51,52] Ischemic stroke remains one of the global leading contributors to mortality and disability.[53] The incidence, prevalence, and cause-specific mortality of stroke remain high but have stagnated, although some declines have been observed in high-income countries.[54] Individuals with elevated glucose levels, as measured by fasting plasma glucose, OGTT, or HbA1c, have a 26% higher risk of stroke compared to those with normal glucose levels.[55,56] In T2D, the ischemic stroke risk is elevated almost two-fold compared with individuals without diabetes.[47]

2.2.3. Heart failure

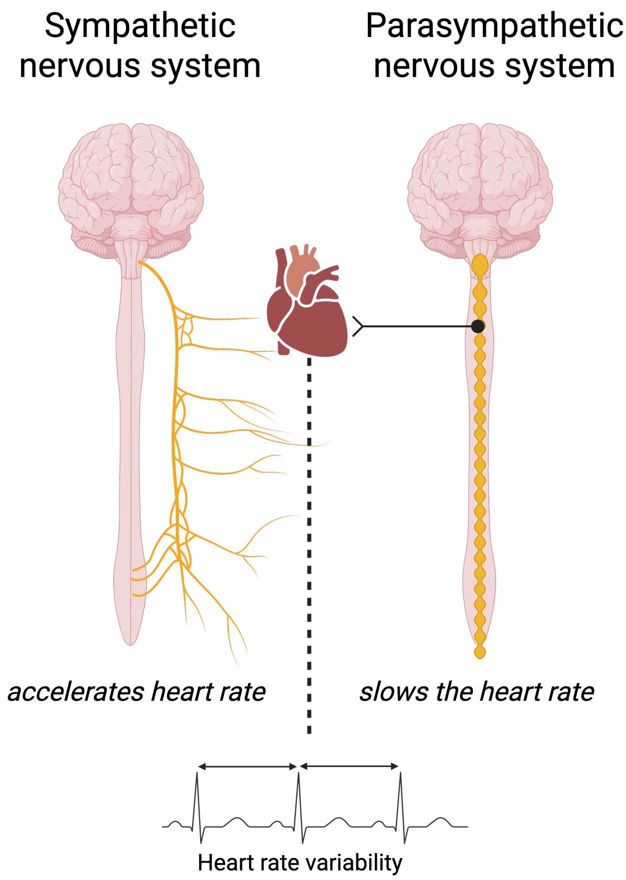
Heart failure develops gradually with age and often accelerates with the progression of T2D. As prevention and treatment of CVD have improved survival in recent years, the prevalence of heart failure has increased, while the incidence remains stable, but may rise with aging populations.[57]

Heart failure may arise as a consequence of atherosclerosis, arteriosclerosis, or both, contributing to myocardial ischemia, pressure overload, and structural cardiac changes.[58] 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.[58] It is a complex cardiovascular disease caused by structural and functional changes in the heart musculature, affecting systolic and/or diastolic pumping function.[58] Heart failure is generally classified into two subtypes: heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF).[58] Both subtypes involve cardiac remodeling but are defined by left ventricular ejection fraction (LVEF).[58] 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.[58] HFrEF is often a consequence of repeated, non-fatal MIs. These events can leave behind scar tissue in the myocardium, impairing the heart's ability to contract effectively and leading to progressive systolic dysfunction.[59]

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.[4,60] Over the past decades, the prevalence of HFpEF has increased with an aging population and more individuals living with conditions such as hypertension, diabetes, and obesity.[59] 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.[59] 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).[4,59] As a result, HFpEF is commonly underdiagnosed and consequently detected at more severe stages, leading to hospitalization.[4,59]

2.3. Cardiovascular autonomic dysfunction

The cardiovascular system is regulated by the autonomic nervous system, which influences heart rate and vasoconstrictuion through the sympathetic and parasympathetic nerves.[32] 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.[32] It also slows AV nodal conduction, predominantly via the left vagus nerve, thereby prolonging atrioventricular conduction time.[32] 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, stress, eating and physical activity.[32]



(Source: Author)

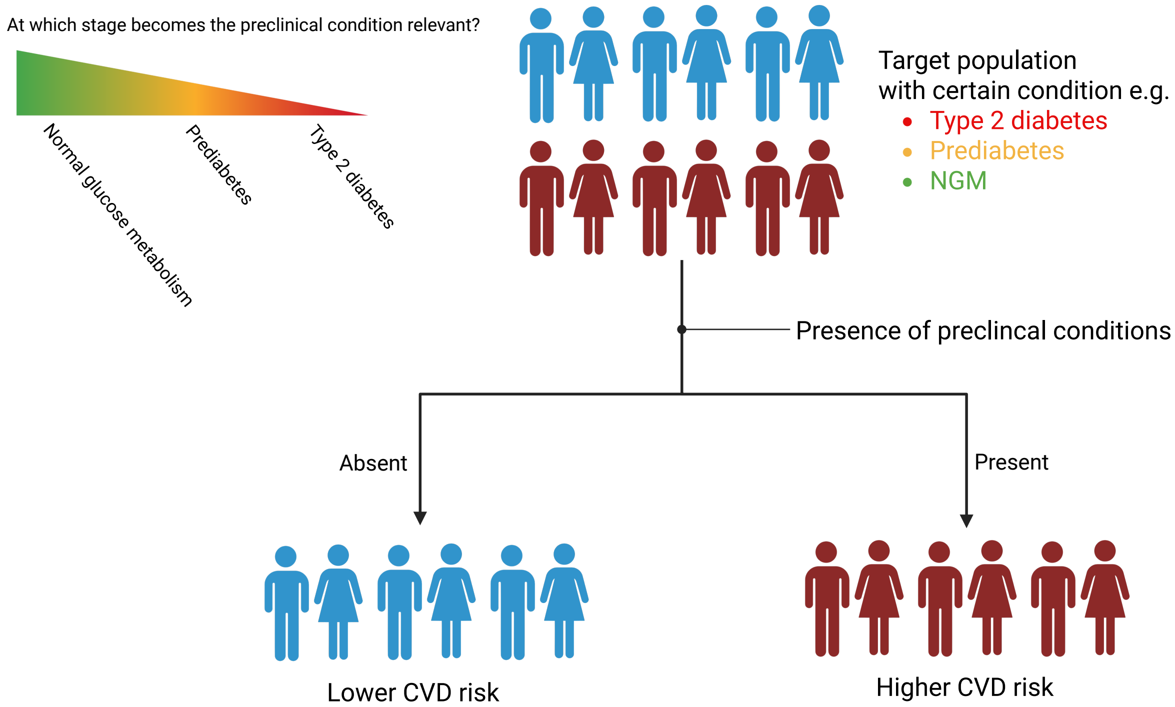
In youth, the autonomic nervous system is highly adaptive and responsive to living conditions, maintaining autonomic balance. However, with aging, there is a gradual decline in parasympathetic function and an increase in sympathetic activity. Additionally, metabolism-related conditions such as obesity and diabetes have been shown to further contribute to cardiovascular autonomic dysfunction (autonomic dysfunction).[61] Autonomic dysfunction reflects a stressed cardiometabolic environment, as both dysfunction in lipid and glucose metabolism are associated with increased sympathetic activity.[61] 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.[61] Therefore, the relationship between autonomic dysfunction and cardiometabolic factors is likely a vicious cycle.[62] The consequences can lead to autonomic dysfunction/neuropathy (CAN), resulting in dysregulation in heart rate and vascular dynamics. CAN prevalent in 12-73% in indiviudals with T2D is liked to CVD diabetic kidney disease, and all-cause mortality.[7,63,64] In this dissertation, 'autonomic dysfunction' will be used as the broader term, while ‘CAN’ will refer specifically to autonomic dysfunction resulting from neuropathy in diabetes.

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.[6] 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 (circadian) pattern.[13] Most studies have examined autonomic function using short-term ECG recordings at rest.[5] 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).[6] 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.[7,65–67]T2D alters the expression of sympathetic bursts, as measured by resting muscle sympathetic nerve activity (MSNA). MSNA is elevated in individuals with both T2D and hypertension, compared to those who are normotensive, regardless of whether they have diabetes or not.[68] Parasympathetic activity is also impaired in individuals with high cardiometabolic risk and T2D, as reflected by reduced baroreflex sensitivity and lower HF and RMSSD short-term HRV.[69] Before onset of diabetes and during progression of diabetes long-term (24-hour) HRV has shown to be lower compare to those with .[8,62] Cardiovascular autonomic reflex tests (CARTs) and orthostatic hypotension are considered the gold standard for assessing CAN.[70] 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.[70] Both HRV and CARTs have shown to be associated with cardiovascular disease, heart failure, and all-cause mortality, primarily in populations with T2D or established cardiovascular disease.[5,63,71] 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 prevention and treatment of T2D. The 2022 ADA/EASD guidelines for the management of hyperglycemia in T2D recommend, cardioprotective medication (glucagon-like peptide-1 receptor agonists [GLP-1RA] and Sodium-Glucose Transport Protein 2 inhibitors [SGLT2i]) as first-line options for individuals at high cardiovascular risk.[72] Due to their benefits in heart failure, SGLT2i 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.[72] However, no additional preclinical markers are recommended to identify individuals at higher CVD or HF risk or for younger individuals. 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.[73,74] During the progression and following the onset of T2D, preclinical indicators of

CVD risk become apparent, providing potential opportunities 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 clinical characteristics.[75] This approach aids in identifying patients at highest risk for further prognostic or diagnostic purposes, identifying subgroups that require further evaluation, specific treatment, or lifestyle modifications.[75]



(Source: Author)

Autonomic dysfunction despite its relationship with cardiovascular complication has not been used in clinical practice in Denmark. Larger epidemiological cohort studies encompassing various stages of diabetes risk, from to prediabetes, onset of T2D, and longer term progression of T2D, serve as valuable resources for identifying risk-stratification opportunities. Epidemiological studies provide a broad representation of the target population, enabling an understanding of the relationship between autonomic dysfunction and cardiovascular complications across different levels of care: public health, primary care, and secondary care. By utilizing observational cohorts, we have the potential to determine when, along the trajectory of diabetes progression, autonomic function becomes a meaningful factor for cardiovascular risk stratification.[75]

3.Aim and hypothesis

The overarching hypothesis of this dissertation are that:

- CAN and autonomic dysfunction are associated with CVD and act as an early risk factor for heart failure and other cardiovascular complications, including stroke, and myocardial infarction in patients with prediabetes and/or T2D.

- Autonomic dysfunction is associated with higher levels of sub-clinical meassures of CVD such as carotid-femoral pulse wave velocity and carotid artery distensibility.

This dissertation investigates the hypothesis by addressing the following three aims:

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 participants with NGM, prediabetes or T2D.

Study II: Quantify the longitudinal association of multiday and hourly HRV with incidence of ischemic-related 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 (NT-proBNP), WATCH-DM risk, and New York Heart Association (NYHA) classification scores among individuals with T2D.

4.Materials and methods

4.1. Overview of the studies

Table 4.1.: 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 T2D: 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 individuals with NGM, prediabetes, or T2D	2082 individuals with high risk of diabetes	176 patients with T2D visiting outpatients clinics
Data sources	Observational phenotyping cohort from The Maastricht Study in the Netherlands	Cohort study of selected individuals 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, NYHA classification, and WATCH-DM risk score
Statistical analysis	Linear regression	Poisson regression	Logistic regression
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

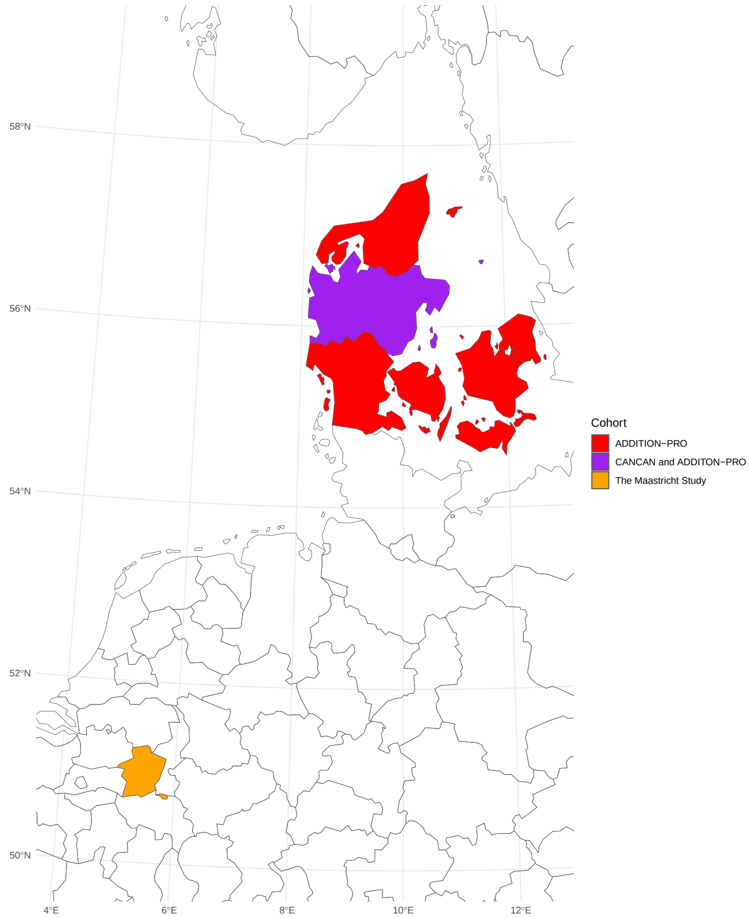


Figure 4.1.: **Map of Study populations**

4.1.1.1. Study I - The Maastricht Study

The Maastricht Study is a prospective, observational phenotyping study of the general population in the province of Limburg, located in the southern part of the Netherlands. The recruitment of individuals with T2D was emphasized through the regional Diabetes Patient Registry, with the aim of extensively phenotyping individuals with T2D and those in intermediate stages of the disease. 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).[76] In the analysis of Study I, the study among 7449 individuals 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. Among those participants with prior CVD were excluded[77]. Approval was granted by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). Written informed consent was given by all participants.[77]

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 T2D in general practice, with the aim of identifying individuals with screen-detected T2D for recruitment into the ADDITION trial. The objective of ADDITION-PRO is 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 T2D, identified through a stepwise screening program that incorporated the Danish diabetes risk score from the Inter99 study[78]. 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 T2D 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 T2D 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 disorders 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, participants with at least a 48-hour recording were included for the primary analysis, and then participants with hourly measures of physical acceleration during the hourly HRV recording were included in the second analysis. Participants with prior CVD ten years before inclusion were also excluded.

Disease history and follow-up data for the population were obtained from Denmark's 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: Viborg Regional Hospital and Regional Hospital Gødstrup. The aim is 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 T2D in Central Denmark. A total of 200 adults (>18 years) with T2D and a disease duration of over one year were included. 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 were informed about the study by their doctor during a telephone call. Those interested were invited to attend a dedicated meeting before their annual diabetes exam, during which study details were discussed. Recruitment was conducted from 2021 to 2024. In Study III, participants without a valid NT-proBNP measurement were excluded.

4.2. Study variables

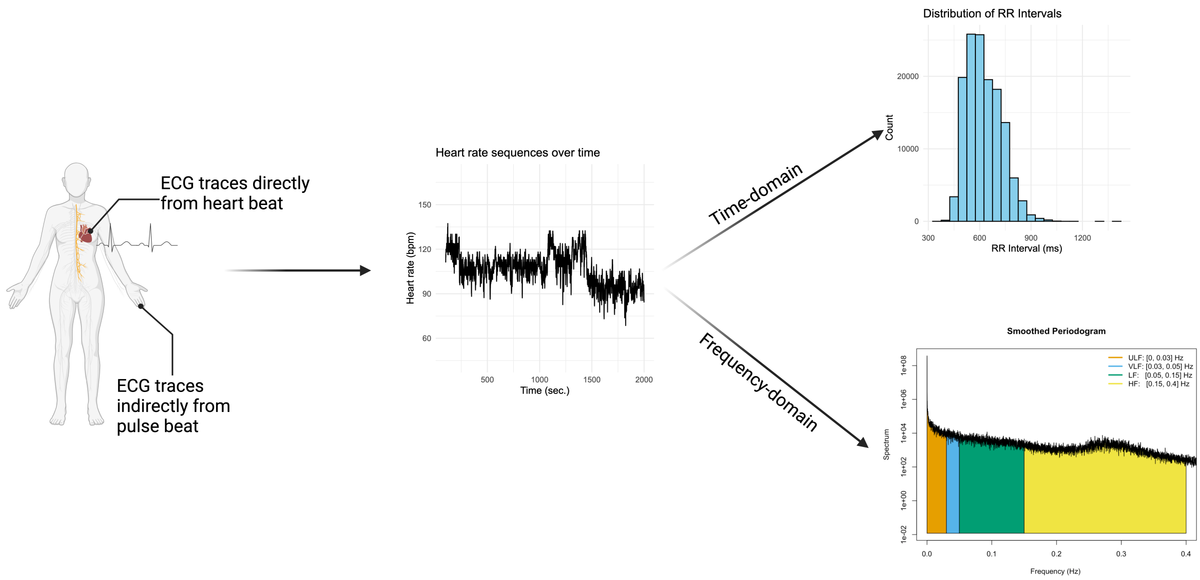
4.2.1. Measures for autonomic dysfunction/ neuropathy

Left: Holter monitor; Middle: Actiheart; Right: Vagus™ device

Figure 4.2.: **Heart rate monitors**

Heart rate variability

In Studies I–III, different devices were used to capture the distance between each heartbeat, defined as RR intervals, from electrocardiogram traces—either directly from heartbeat traces or indirectly from pulse traces. From these, a sequence of successive heartbeat intervals was extracted to calculate time- and frequency-domain HRV.



Time- and Frequency domain HRV indices (Source: Author)

Figure 4.3.: **Heart rate variability calculations**

Time-domain indices

Time-domain measures of HRV are based on the statistical distribution of normal-to-normal (NN) heartbeat intervals. Descriptions of time-domain indices are summarized in Table 4.2.

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[79].
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[79]

Frequency-domain indices

Frequency-domain HRV indices are derived from sequences of NN intervals that have been 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, are reflective of rapid autonomic changes, while longer oscillations are indicative of autonomic responses to posture changes, circadian rhythms, or other physiological processes. Descriptions of frequency-domain indices are summarized in Table 4.3. .

Table 4.3.: **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.[80,81]
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.[82,83]
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.[84]
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.[85]

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.[76] Participants were instructed to follow their regular daily activities but avoid showering during the 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.[8,77] 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.[77]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.[77]

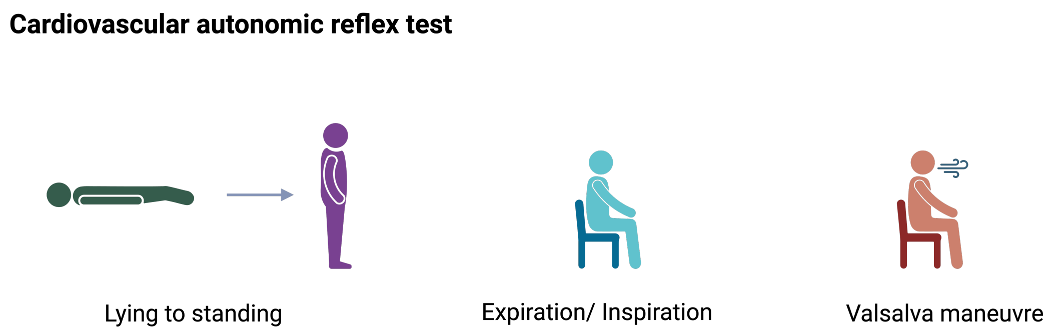
ActiHeart heart rate and physical activity in study II

Heart rate was measured using a combined accelerometer and heart rate monitor (ActiHeart, CamNTech, Cambridge, UK), which recorded uniaxial acceleration and heart rate. The data collection and processing methods have been described previously. Mean heart rates were recorded in 30-second epochs. Based on an algorithm, distributions of inter-beat intervals were calculated in each 30-second epoch.[86] HRV calculations were performed using the RHRV package (version 4.2.7) in R.[86,87] The algorithm was tested on a dataset with full access to all inter-beat intervals.[86] HRV indices based on global distribution in 24-hour recordings showed high validity.[87] HRV indices including SDNN, SDANN, SDNN index, TINN, and mean heart rate (HR) were calculated by week, 24-hour cycle, and hour of the day, with hourly values averaged across recording days.[87]

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).[70,88] Pulse rate ratios were measured under different conditions.[88] Three standardized cardiovascular autonomic reflex tests (CARTs) were performed: (1) lying-to-standing, (2) deep breathing, and (3) the Valsalva manoeuvre, following a standardized protocol conducted between 8:00 a.m. and 2:00 p.m., after 10 minutes of supine rest.[88] Smoking and caffeine intake were prohibited two hours before testing. Each test was conducted once by trained examiners.[88]

Manifest CAN was defined as two or more abnormal CARTs using age-specific formula.[70] The Vagus™ device's accuracy has been validated against FDA standards and stationary devices, showing moderate to high reproducibility.[89] Orthostatic hypotension 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.[88]



(source: Author)

Figure 4.4.: **Cardiovascular autonomic reflex test**

4.2.2. Confounders and variables for instrumental bias

Across Studies I, II, and III, a comprehensive set of covariates and potential confounders was assessed, including lifestyle factors, clinical measurements, biochemical markers, and socioeconomic indicators.[77,87,88]

Smoking status was self-reported in all studies and 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.[77,87,88] Physical activity was assessed via self-report in Studies I, II, and III. In Study I, total and moderate-to-vigorous activity (hours/week) was recorded. Study II used the Recent Physical Activity Questionnaire (RPAQ) to calculate physical activity energy expenditure (PAEE), while Study III classified activity as sedentary or non-sedentary.[77,87,88] In addition, Study II used combined accelerometry and heart rate monitoring (ActiHeart) to estimate PAEE.[87] Register-based data on socioeconomic status at baseline, including education length, income, and employment status, were included in Study II.[87] All studies included measurements of body mass index (BMI), waist circumference, and systolic and diastolic blood pressure, obtained during clinical examinations.[77,87,88]

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.[77,87,88] Study I also included a 2-hour oral glucose tolerance test (OGTT) to classify glucose metabolism status based on FPG and OGTT (normal, prediabetes, T2D) using WHO 2006 criteria, excluding HbA1c as a diagnostic criterion.[77] Study III additionally measured creatinine, estimated glomerular filtration rate (eGFR), and urine albumin-to-creatinine ratio.[88]

Self-reported history of CVD and use of anti-hypertensive, glucose-lowering, and lipid-lowering medications were collected in all studies.[77,87,88] In Study II, history of CVD events in the 10 years prior to baseline was retrieved from national registers.[87] In Study III, history of CVD was collected from electronic patient records.[88]

4.3. Outcomes

4.3.1. Arterial stiffness

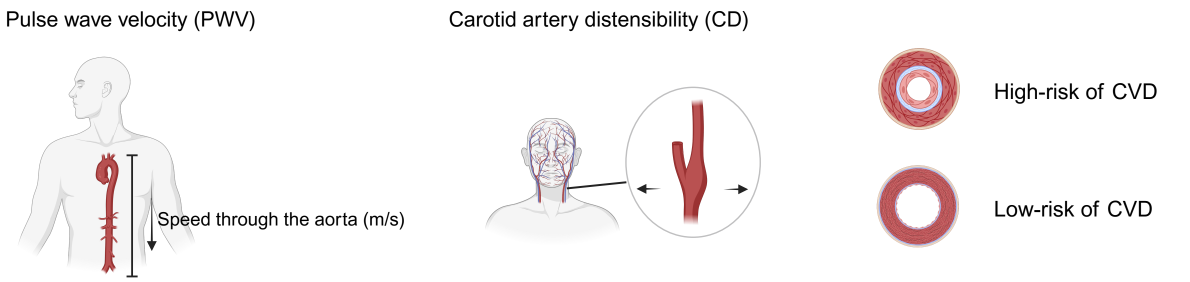
Arterial stiffness is characterized by arteriosclerosis and atherosclerosis properties of the arteries. The stiffness of different segments of the vascular musculature can be assessed both locally and dynamically. Aortic and carotid stiffness were assessed as markers of arterial stiffness, following previously described procedures.[90]

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 was calculated based on the time between the ECG systole and the arrival of the pressure wave at the femoral and carotid measurement sites, along with the distance between these two measurement sites. cf-PWV was 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.[90]

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 oscillometric device (Accutorr Plus, Datascope, Montvale, NJ, USA).[90]



Measures of arterial stiffness, measured dynamically through the decending aorta and locally ay carotid sites. (Source: Author)

Figure 4.5.: **Aortic and carotid stiffness**

4.3.2. Indicators of heart failure

N-terminal prohormone of brain natriuretic peptide (NT-proBNP) is a natriuretic peptide that can be used to detect patients with heart failure and monitor its progression. It is derived from B-type natriuretic peptide (BNP), a cardiac neurohormone synthesized and secreted in response to stretched cardiomyocytes 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, a blood sample was taken at the study site. A description of the NT-proBNP analysis of plasma samples is provided in the supplementary material in Appendix Study III.[88]

A modified version of the validated WATCH-DM heart failure risk score was used. The risk score is based on nine variables: two binary (history of myocardial infarction and coronary artery bypass grafting) and seven continuous (age, BMI, systolic/diastolic BP, serum creatinine, HDL cholesterol, and HbA1c). Scores range from 0–39 and are categorized as very low (11), low (12–13), moderate (14–15), high (16–18), and very high (19) risk.[88,91,92]

NYHA class stage I–IV was included. Heart failure symptoms were defined as NYHA class II–IV, assessed by a physician.[88]

Table 4.4.: **?(caption)**

(a) Definition of CVD and Heart failure

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, KPA

4.3.3. Cardiovascular events

Information on CVD events and mortality was obtained from the Danish National Patient Registers until 2021 by utilizing the ICD-10 codes for stroke, myocardial infarction, cardiovascular death, cardiovascular revascularization, and heart failure. Three-point major adverse cardiovascular events (MACE) was defined as myocardial infarction, stroke, cardiovascular revascularization, and cardiovascular death.[87]

4.4. Statistical Methods

4.4.1. Cross-sectional analysis

Study I

In Study I, multiple linear regression was used to investigate associations between 24-hour HRV and arterial stiffness. Model 1 was adjusted for age, sex, education, glucose metabolism status, and mean arterial pressure (MAP) to account for the oversampling of individuals with T2D and potential instrumental bias of arterial pressure flow. Model 2 included additional adjustments for smoking behavior, alcohol consumption, physical activity, BMI, HbA1c, triglycerides, total-to-HDL cholesterol ratio, and medication use. Arterial stiffness measures were log-transformed to ensure normally distributed residuals and were back-transformed into percentage change estimates. A sex interaction term was added to assess whether the association differed by sex. Sensitivity analyses were performed excluding individuals on antihypertensive treatment or glucose-lowering medication.[77]

Study III

In Study III, logistic regression models were applied to investigate the association between CAN and heart failure, using elevated NT-proBNP (concentration >125 pg/ml) as the primary outcome. Adjustments were made for age, sex, diabetes duration, smoking behavior, alcohol consumption, BMI, HbA1c, triglycerides, total cholesterol, antihypertensive medication, eGFR, and prior CVD. Sensitivity analyses were performed excluding participants with beta-blocker treatment or prior CVD. Logistic regression was also applied to assess the odds of CAN being associated with heart failure symptoms, defined as NYHA class II or higher, adjusting for covariates in the primary analysis. Linear regression was employed to evaluate differences in the WATCH-DM risk score between individuals with and without CAN.[88]

4.4.2. Time-to-event analysis

In Study II, Poisson regression models were used to quantify the associations between multiday HRV and cardiovascular events, as follow-up data were undisturbed over time and to avoid assumptions of proportional hazards.[93] 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 whether the association of HRV exhibited 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 (DAGs), two models were fitted: Model 1 adjusted for age and sex, while Model 2 further adjusted for education, smoking, alcohol consumption, physical activity (PAEE calculated from the 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 for the influence of these factors. Missing covariates were handled using multiple imputation. Each individual's follow-up period began at the time of their inclusion in the baseline examination.[87]

To calculate age-specific incidence rates (IR), follow-up ended at the earliest occurrence of CVD, heart failure, all-cause mortality, or the end of the study period. The follow-up time was divided into one-year intervals based on the individual's age. Using this age-split data, incidence rates of CVD, heart failure, and all-cause mortality were analysed in relation to HRV, with age treated as a time-varying covariate in a Poisson regression model.

4.4.3. Effect modification

Effect modification was assessed to determine whether the association between an exposure and an outcome varied depending on the level of a third variable, known as the effect modifier.[94]

In Study I, it was hypothesized that the association between 24-hour HRV and arterial stiffness was stronger in strata of diabetes progression (normal glucose metabolism, prediabetes, T2D). Therefore, an interaction term between HRV and diabetes status was included to observe the size of the association across strata.[77] A subsidiary analysis was conducted in a subpopulation without T2D to assess whether the effect was modified by HbA1c. In Study II, the variation in the association between multiday HRV and CVD endpoints by sex was quantified to explore potential biological dimorphism.[87] In Study III, the presence of an association between CAN and elevated NT-proBNP in the subgroup without symptoms (NYHA class < II) was examined. It was hypothesized that no significant effect modification would be observed between groups with and without symptoms. Similarly, the persistence of the association in the group classified as low to moderate risk of heart failure was explored, based on the WATCH-DM risk score.[88]

A significant effect modification 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) was used to handle missing data. This procedure imputes missing values through an iterative series of predictive models, generating plausible estimates while preserving the relationships within the data. To avoid assigning the same confidence to imputed values as to observed values, Rubin's rules were followed. Rubin's rules combine results from multiple imputed datasets by pooling estimates of interest (e.g., means or regression coefficients) using their within- and between-imputation variances.[95] This approach ensures valid statistical inferences by accounting for the uncertainty introduced by missing data.

In Study II, confounders were imputed to include as many participants as possible and to avoid excluding individuals with or without cardiovascular or mortality events. The dataset was imputed 10 times. In Study III, missing CARTs were imputed, as a proportion of participants had non-valid tests due to insufficient air in the Valsalva manoeuvre, unstable heartbeats, or data errors. These variables were used as auxiliary variables in the imputation to reduce bias.[96] All available variables on biochemical measures, diagnoses, medications, and causes of non-valid CARTs were used to impute each missing CART using predictive mean matching.[88]

4.4.5. Instrumental bias

In Studies I–III, physiological properties were investigated using dynamic measures and biomarkers to quantify autonomic function, arterial stiffness, and cardiac function. Other conditions may influence the properties being measured, thereby introducing instrumental bias.

Vascular Stiffness

In Study I, arterial stiffness was measured using cf-PWV and carotid distensibility. 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 distensibility).[97] To account for this, adjustments were made for MAP in the models.[77]

Cardiovascular autonomic function

In Study II, autonomic function was assessed using multiday HRV recordings and hourly HRV measurements. Previous studies have shown 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, HRV was pre-adjusted 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, a similar pre-adjustment approach was applied by regressing concurrent heart rate and physical acceleration to account for physical activity.[87]

Biomarker of Heart Failure

In Study III, kidney function and overweight are known to influence NT-proBNP levels independently of heart failure.[58] The model was adjusted to account for the confounding effect of eGFR on NT-proBNP levels in the analysis.[88]

5. Results

In this section, study population characteristics and findings from analysis will be presented.

5.1. Study I

5.1.1. Descriptive

In The Maastricht Study, 7,449 participants were included between November 2010 and December 2020, of whom 1,316 reported a history of CVD.[77] A total of 4,379 participants had valid 24-hour HRV measurements, and among these, 3,673 had a valid measurement of either CD or cf-PWV. Study population included 3673 participants. The study population represented diabetes risk of NGM (65%), prediabetes (15%), and T2D (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 T2D: 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 T2D: 12.5 (9.9, 16.0) $\times$ 10 ^{3}/kPa. SDNN (ms) was highest in NGM and lowered with worsening glucose metabolism: NGM: 138ms (117, 164), prediabetes: 127ms (106, 152), and T2D: 116ms (96, 139). Further description of characteristics by diabetes are described in Table 5.1.

Table 5.1.: Study charteristics in The Maastricht Study (Study I) by diabetes status

Characteristic	Normal glucose metabolism N = 2,389	Prediabetes N = 538	Type 2 Diabetes N = 746
Women	1,361 (57%)	258 (48%)	265 (36%)
Age (years)	58 (51, 64)	62 (57, 68)	63 (57, 68)
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/m^2)	25.0 (22.9, 27.4)	27.2 (24.9, 30.1)	28.8 (26.0, 31.7)
HbA1c (%)	5.35 (5.17, 5.63)	5.63 (5.35, 5.90)	6.54 (6.08, 7.09)
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)			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)
TP (ms^2)	12,596 (8,880, 17,498)	10,615 (7,134, 15,374)	8,880 (6,064, 12,722)
ULF (ms^2)	10,771 (7,392, 15,142)	8,948 (5,852, 13,374)	7,524 (5,036, 11,001)
VLF (ms^2)	1,198 (833, 1,692)	1,015 (685, 1,478)	816 (541, 1,267)
HF (ms^2)	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)
Glucose-lowering medication	0 (0%)	0 (0%)	519 (70%)
Antihypertensive medication	431 (18%)	199 (37%)	478 (64%)
Beta blockers	149 (6.2%)	77 (14%)	195 (26%)
Lipid-lowering medication	280 (12%)	141 (26%)	484 (65%)

Note:

makecell[l]Categorical variables: N (%); Continous variables: Median (IQT:25th-75th) Table are adapted from supplementary material in Appendix Study I

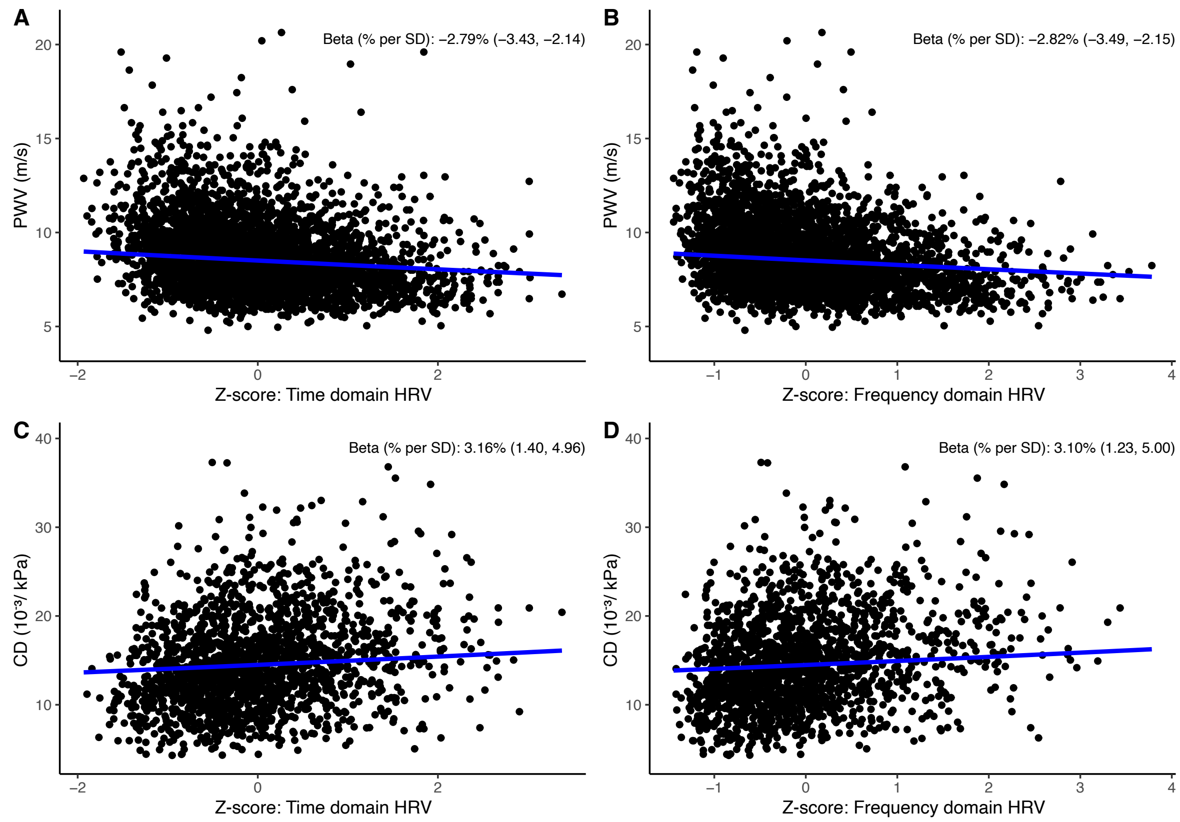
5.1.2. 24-hour HRV and arterial stiffness

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 (see see Figure 5.1 A and B). 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.[77] 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.[77]

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 (see see Figure 5.1 C and D). 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; 2.6), respectively.[77] 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.[77]



A: Percentage PWV per SD in time-domain composite z-score B: Percentage PWV per SD in frequency-domain composite z-score C: Percentage higher CD per SD in time-domain composite z-score D: Percentage CD per SD in frequency-domain composite z-score. All regression lines were adjusted for being a male, 60 years old, low educational level, without prediabetes or type-2 diabetes, and with 96mmHg MAP. From Figure 1 in Appendix Study I[77]

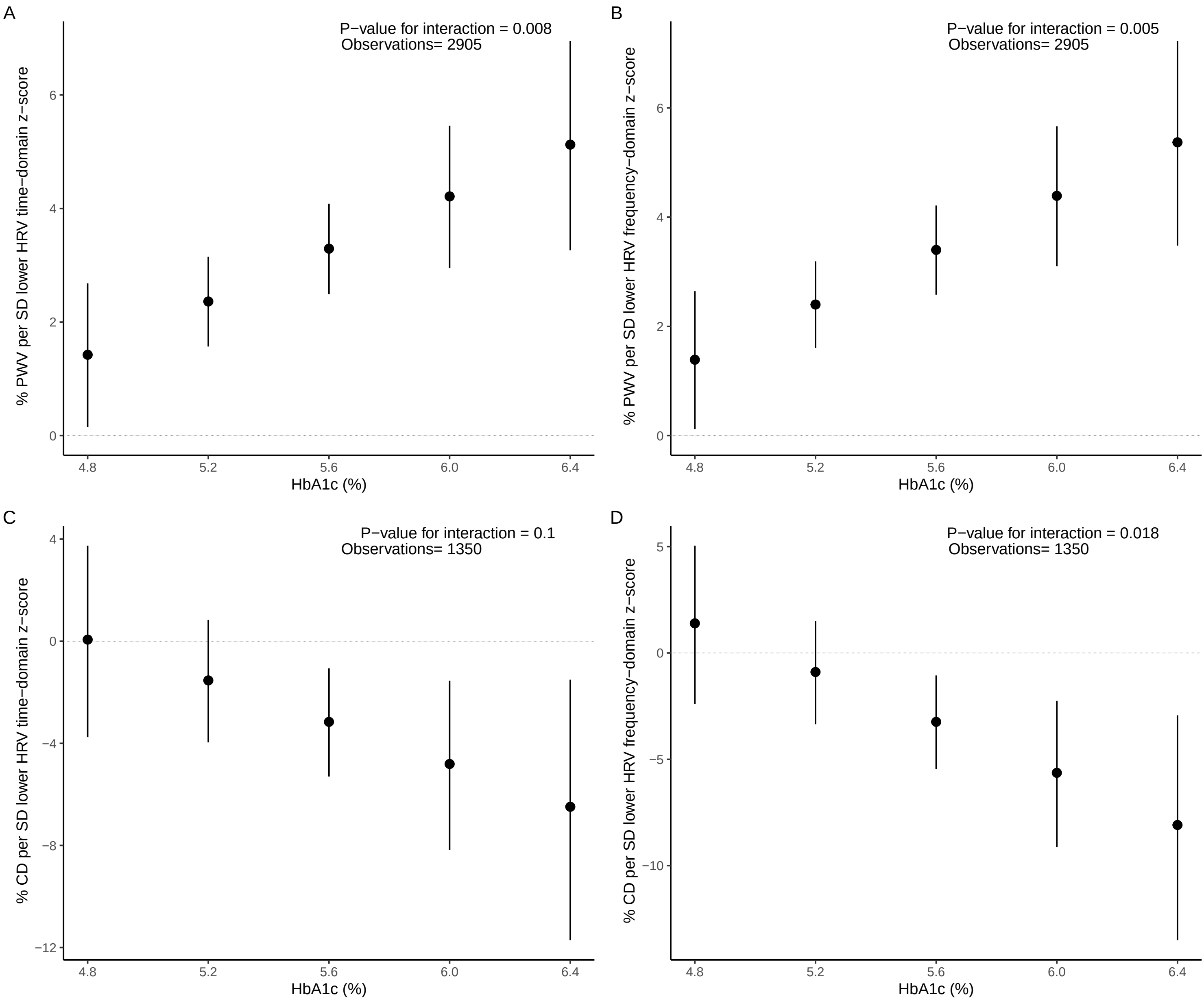
Figure 5.1.: **Association between HRV and arterial stiffness**

5.1.3. Effect modification of diabetes status

The association between HRV time-domain Z-scores and cf-PWV and CD was significantly modified by prediabetes (cf-PWV: -4.9 [CI: -6.5; -3.2] $^{interaction(*)^{p-value<0.01}}$ CD: 8.0 [CI:3.8; 12.5]$^{*^{p-value<0.01}}$) but not by T2D (cf-PWV: -3.5 % [CI: -4.8; -2.1)] $^{*^{p-value<0.1}}$ CD: 4.8 % [CI:1.3; 8.4]$^{*^{p-value<0.1}}$)[77]. For the indices SDNN and SDANN, the association with both cf-PWV and CD was significantly modified by both prediabetes and T2D[77].

The association between HRV frequency-domain Z-score and cf-PWV was statistically significant modified from NGM by prediabetes (-5.7 %[CI:-7.4; -3.9]$^{*^{p-value<0.01}}$) and T2D (-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 T2D (5.3 %[CI:1.4; 9.4]$^{*^{p-value<0.1}}$)[77]. For the indices total power and ULF, the association with both cf-PWV and CD was statistically significant modified by both prediabetes and T2D. Mean inter beat interval association with cf-PWV or CD was not significantly modified by diabetes status[77].

As no stepwise increase was observed in the modification of glucose metabolism status from prediabetes to T2D,the subgroup with T2D was excluded 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 6.4% was associated with an 8.1% lower (CI: -13.5; -2.9) CD, which was 4.8% to 9.5% lower compared to the magnitude of association at HbA1c levels of 5.6% and 4.8% (see Figure 5.1 D). No association between HRV frequency domain Z-score and CD was observed at HbA1c levels between 4.8% and 5.2%.



A: Percentage PWV per SD in time-domain composite z-score B: Percentage PWV per SD in frequency-domain composite z-score C: Percentage higher CD per SD in time-domain composite z-score D: Percentage CD per SD in frequency-domain composite z-score. Model adjusted for sex, age, educational status, diabetes status, and MAP, physical activity, smoking behaviour, alcohol use, body mass index, hba1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication. Figure was based on data in Study I[77]

5.2. Study II

5.2.1. Descriptive

The ADDITION-PRO population consisted of 1,627 participants with at least 48-hour HRV measures, while 1,432 had all hours 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%). SDNN was categorized into five groups: 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).

Characteristics are described 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 rHR (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.

Table 5.2.: Study characteristics in The ADDITION-PRO Study (Study II) by SDNN categories

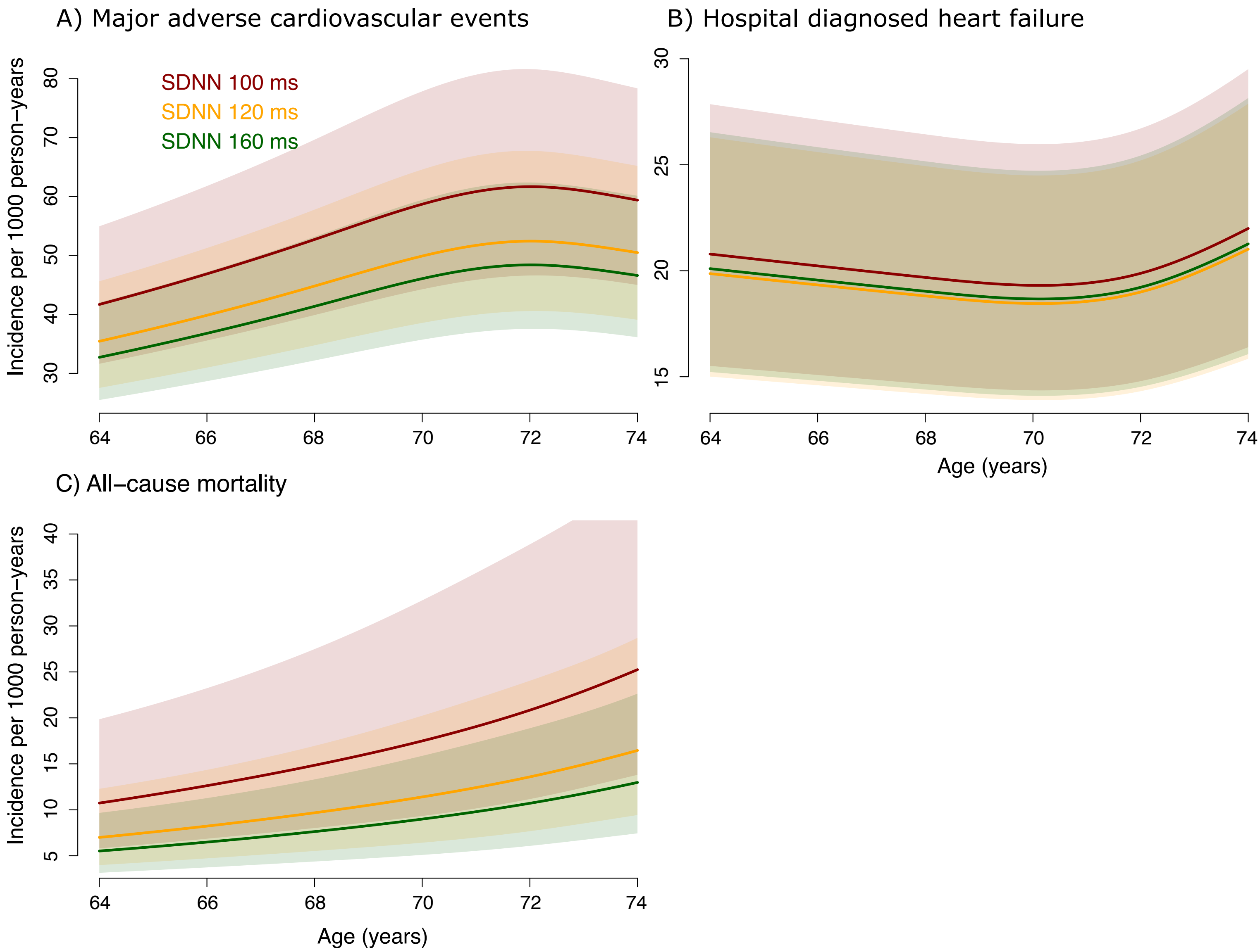
SDNN Categories	Overall, N = 1,625	<100 ms, N = 148	100-120 ms, N = 312	120-140 ms, N = 457	140-160 ms, N = 346	>160 ms, N = 362
Women	759 (47%)	80 (54%)	164 (53%)	251 (55%)	143 (41%)	121 (33%)
Age (years)	65.9 (6.8)	67.4 (6.9)	65.7 (6.9)	66.0 (6.7)	65.5 (6.6)	66.0 (7.0)
Physical activity energy expenditure (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						
Current	263 (16%)	40 (28%)	70 (23%)	65 (14%)	41 (12%)	47 (13%)
Prior	750 (47%)	58 (40%)	145 (47%)	214 (47%)	162 (47%)	171 (48%)
Never	598 (37%)	47 (32%)	95 (31%)	174 (38%)	140 (41%)	142 (39%)
BMI (kg/m²)	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)
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)
VO2 max (mL/kg/min)	26.6 (7.8)	24.8 (7.5)	24.8 (7.5)	26.1 (6.8)	27.0 (8.0)	28.7 (8.7)
Resting heart rate (bpm)	57.3 (7.3)	67.8 (5.7)	63.3 (5.0)	58.4 (4.5)	55.0 (4.2)	49.8 (4.9)
Anti-hypertensive medication (yes)	753 (47%)	88 (61%)	149 (48%)	216 (47%)	147 (43%)	153 (43%)

Note:

makecell[l]Categorical variables: N (%); Continous variables: Median (IQT:25th-75th Table are based on data from Study II

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.[87] 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).[87] In model with pre-adjustment for rHR, the proportion of the association explained between HRV and MACE, HF, and all-cause mortality was 14%, 25%, and 19%, respectively.[87] When knots were included in the model, the risk was found to be higher as SDNN dropped below approximately 120–110 ms (around the 20th percentile), suggesting a potential threshold for elevated risk.[87] Therefore, age-specific IR were calculated at SDNN levels of 100 ms, 120 ms, and 160 ms, respectively.



Multiday SDNN levels (100 ms, 120 ms, 160 ms) by age-specific incidence rates for A) major adverse cardiovascular events, B) heart failure hospitalisation, and C) all-cause mortality. Model adjusted for age, sex, education, alcohol consumption, smoking behavior, physical activity, body mass index, total cholesterol, and Hba1c. Figure was based on data in Study II[87]

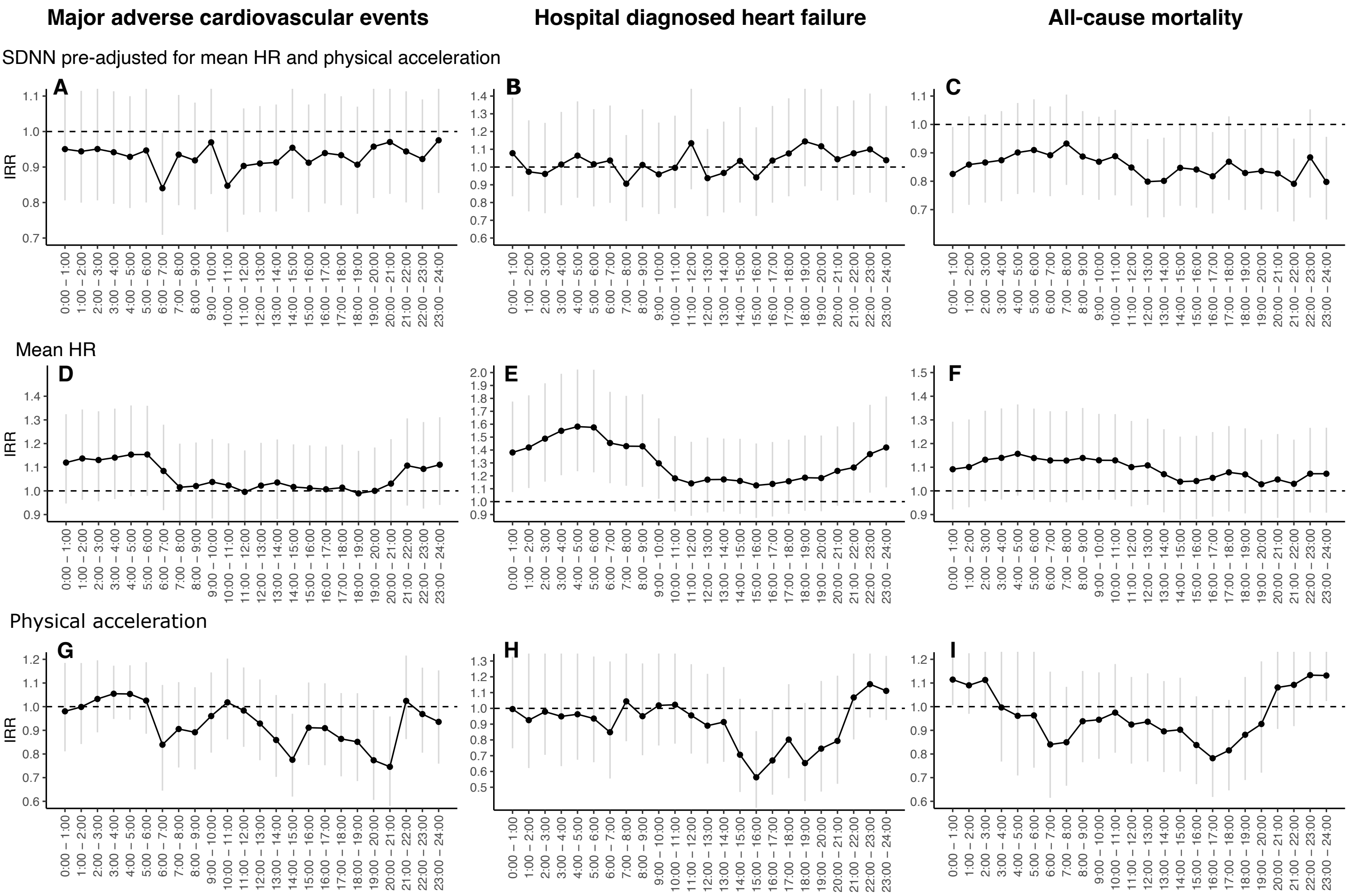
Figure 5.2.: **Multiday SDNN and mean HR association with major adverse cardiovascular events, heart failure, and all-cause mortality**

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 was observed to become 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 was observed to become higher with age.

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

From the hourly recordings, a clear periodicity in SDNN, heart rate, sleep patterns, and physical acceleration was observed (see Figure S4 in supplemental material Appendix Study II). Mean (SD) SDNN was found to increase 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).[87] A similar circadian pattern was observed in heart rate, although its peak was reached two hours later, starting at 9 AM (76.7 [10.9] bpm).[87] After peaking, heart rate was observed to remain stable throughout the afternoon before gradually decreasing.[87]

In Figure 5.3, hourly SDNN (preadjusted for heart rate and physical acceleration), heart rate, and physical acceleration associations were examined. Models were adjusted for age, sex, education, alcohol consumption, smoking behavior, BMI, total cholesterol, and HbA1c. The morning response of SDNN was found to be most indicative of MACE, with the strongest association observed from 6–7 AM (IRR: 0.84; 95% CI: 0.71–1.00 per SD higher SDNN) (see Figure 5.3 A).[87] 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).[87] Across all hours, a plausible association between SDNN and heart failure was observed. However, this association disappeared after adjustment for physical acceleration and heart rate (see Figure 5.3 B).[87] In contrast, heart rate between 10 PM and 9 AM was associated with heart failure (IRR 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).[87] No clear trends of association were observed between heart rate and all-cause mortality.[87]



SDNN preadjusted for concurrent physical acceleration and heart rate, as well as mean heart rate (HR) and physical acceleration, were measured hourly from 00:00 to 24:00. The IRR for MACE, heart failure hospitalization, and all-cause mortality are shown by hourly associations of: (A–C) preadjusted SDNN, (D–F) mean HR, and (G–I) physical acceleration. Models were adjusted for age, sex, education, alcohol consumption, smoking behavior, body mass index, total cholesterol, and HbA1c. Figure adapted from Appendix Study II[87]

Figure 5.3.: **Diurnal heart rate variability and heart rate association with major adverse cardiovascular events, heart failure, and all-cause mortality risk**

5.3. Study III

5.3.1. Descriptive

In study III, 176 participants had measures of NT-proBNP. CAN was present in 31% (n = 54) of participants (36% among those with valid CAN measurements (Figure 5.4 A)).[88] Meanwhile, 23% (n = 40) were unable to complete the CART assessment adequately, primarily due to insufficient air pressure during the Valsalva manoeuvre (n = 21).[88] 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 23%) and declined eGFR (< 60ml/min/1.73 m^2) (35% 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 T2D (median 19 years vs 15 years), and higher use SGLT2i (65% vs 60%) but lower use of GLP-1RA (63% vs 70%). No other difference in clinical characteristic was observed (see Table 5.3).[88]

Table 5.3.: Study characteristics in The CANCAN Study (Study IIY) by CAN status

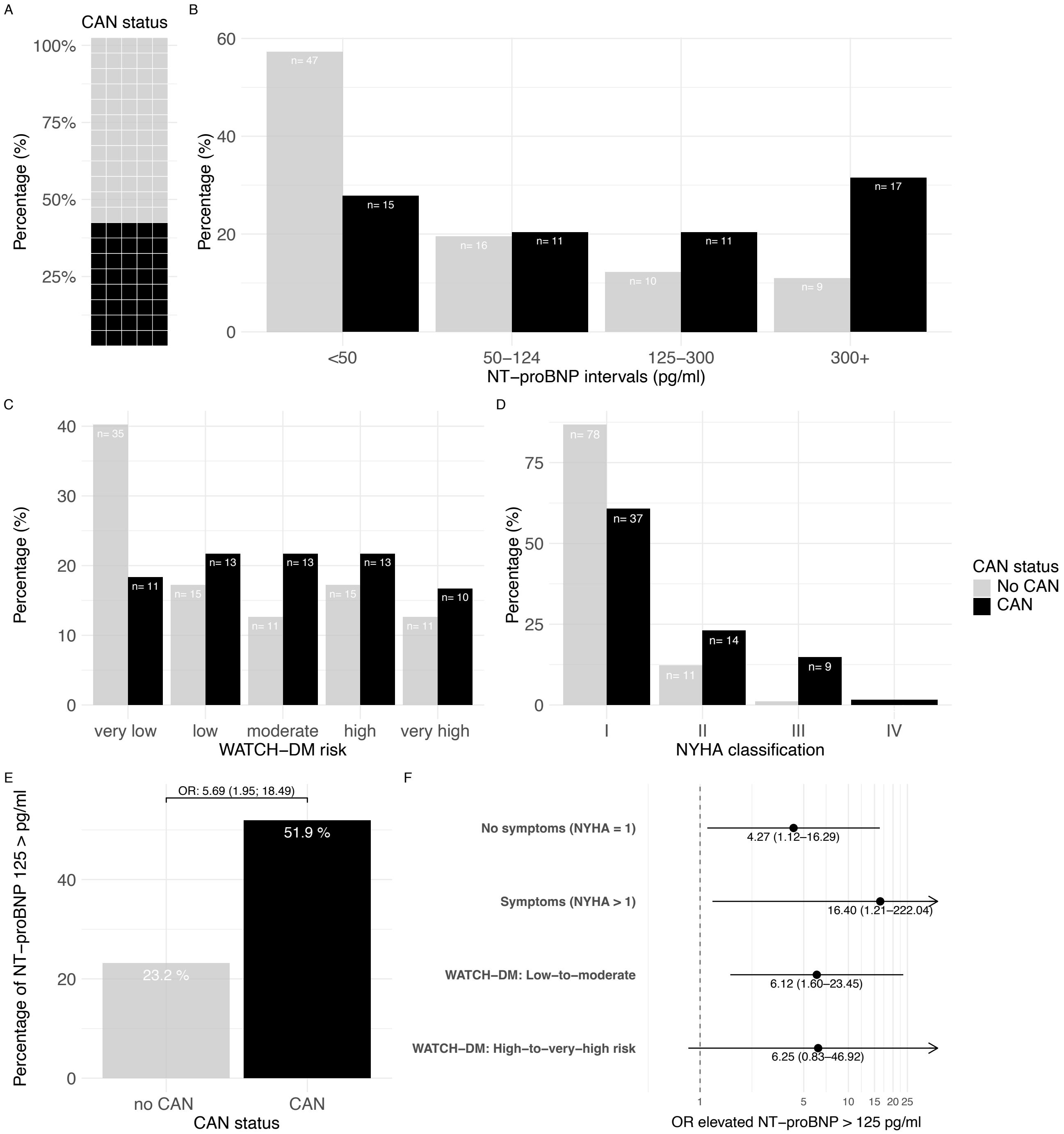
CAN status	Missing	Overall, N = 176	CAN missing, N = 40	No CAN, N = 82	CAN, N = 54
Sex (Women)	0	68 (39%)	19 (48%)	27 (33%)	22 (41%)
Age (years)	0	63 (55; 70)	68 (61; 75)	61 (52; 69)	62 (56; 68)
BMI (kg/m²)	2	32 (28; 37)	30 (26; 34)	33 (28; 38)	33 (30; 36)
Duration of diabetes (years)	0	17 (11; 24)	20 (13; 30)	15 (9; 21)	19 (13; 24)
HbA1c (mmol/mol)	0	64 (56; 80)	65 (56; 85)	64 (55; 78)	64 (57; 76)
Total cholesterol (mmol/L)	2	3.90 (3.23; 4.78)	3.70 (3.33; 4.18)	4.10 (3.33; 4.88)	3.75 (3.03; 4.98)
HDL (mmol/L)	2	1.00 (0.88; 1.20)	1.00 (0.90; 1.30)	1.00 (0.90; 1.20)	0.97 (0.80; 1.18)
Triglycerides (mmol/L)	3	2.00 (1.30; 2.90)	2.10 (1.10; 2.80)	1.95 (1.30; 2.90)	2.05 (1.40; 2.98)
Systolic blood pressure (mmHg)	1	133 (123; 143)	135 (127; 147)	131 (123; 142)	133 (120; 143)
Diastolic blood pressure (mmHg)	1	76 (68; 82)	73 (66; 79)	78 (72; 83)	74 (66; 82)
Resting heart rate (bpm)	6	77 (66; 84)	68 (62; 80)	78 (69; 84)	80 (67; 89)
Lying to standing (RR ratio)	8	1.02 (1.01; 1.06)	1.03 (1.02; 1.05)	1.05 (1.01; 1.08)	1.01 (1.00; 1.02)
Deep breathing (RR ratio)	4	1.13 (1.07; 1.26)	1.15 (1.10; 1.28)	1.18 (1.11; 1.30)	1.07 (1.03; 1.08)
Valsalva maneuver (RR ratio)	45	1.24 (1.13; 1.36)	1.20 (1.14; 1.25)	1.32 (1.25; 1.45)	1.11 (1.08; 1.16)
NT-proBNP (pg/ml) categories	0				
< 50		72 (41%)	10 (25%)	47 (57%)	15 (28%)
50-124		38 (22%)	11 (28%)	16 (20%)	11 (20%)
125-300		28 (16%)	7 (18%)	10 (12%)	11 (20%)
> 300		38 (22%)	12 (30%)	9 (11%)	17 (31%)
Any antihypertensive medication (yes)	0	140 (80%)	33 (83%)	61 (74%)	46 (85%)
Beta-blockers (yes)	0	52 (30%)	11 (28%)	19 (23%)	22 (41%)
Glucose-lowering medication					
Metformin (yes)	0	123 (70%)	26 (65%)	56 (68%)	41 (76%)
SGLT2-inhibitors (yes)	0	81 (46%)	12 (30%)	40 (49%)	29 (54%)
GLP1 RAs (yes)	0	91 (52%)	15 (38%)	47 (57%)	29 (54%)
Insulin (yes)	0	140 (80%)	33 (83%)	66 (80%)	41 (76%)

Note:

makecell[l]Categorical variables: N (%); Continous variables: Median (IQT:25th-75th Table are adapted from Table 1 in Appendix Study III

5.3.2. CAN and indicators of heart failure

A greater proportion of individuals with CAN were observed to exhibit 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).[88] 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.[88] Among the cardiovascular autonomic reflex tests (CART), the Valsalva manoeuvre was found to demonstrate 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).[88] No significant association was identified for the lying-to-standing test (OR 0.80, 95% CI: 0.32; 1.97; n = 54/108).[88] After imputation of missing CART data, the OR for CAN in relation to elevated NT-proBNP declined to 2.94 (95% CI: 1.37; 6.56).[88] 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.[88] 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 statistically significant (p = 0.4).[88] Similar associations were observed 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).[88] Participants with CAN were found to have 1.7 (95% CI: 0.3 to 3.0) point higher WATCH-DM risk scores compared to those without CAN.[88] The OR of presenting with NYHA class II or higher was 5.51 (95% CI: 1.9 to 15.97) in the group with CAN.[88]



A: Percentage distribution by CAN status (no CAN, CAN). B: Percentage distribution of NT-proBNP level categories stratified by individuals with and without CAN. C: Percentage distribution of WATCH-DM risk score stratified by individuals with and without CAN. C: Percentage distribution of NYHA classification stratified by individuals with and without CAN. E: Percentage of individuals with NT-proBNP > 125 pg/ml among those with and without CAN and adjusted odds ratio from Model 4. F: Effect modification of the association between CAN and NT-proBNP by symptoms defined by NYHA classification (symptoms: NYHA II vs no symptoms: NYHA = I) and risk score defined by WATCH-DM risk (very-low-to-moderate vs high-to-very-high risk). Figure from Appendix Study III[88]

Figure 5.4.: **Distribution of NT-proBNP, NYHA Class, and WATCH-DM Score by CAN Status, and association of CAN with Elevated NT-proBNP**

6. Discussion

The aim of this dissertation is to understand how cardiovascular autonomic dysfunction and CAN affect the risk of CVD across stages of glucose metabolism. Given the rising prevalence of prediabetes and T2D, and their association with increased risks of CVD and heart failure, there is a pressing need for earlier indicators to help healthcare providers intervene in a timely manner and prevent progression to more advanced stages of cardiovascular complications. One promising approach involves leveraging data from wearable devices and standardized screening tools. Heart rate dynamics and variability across different circumstances may hold promise as accessible indicators for timely cardiovascular risk stratification.

This chapter presents a summary of the main findings from this dissertation, interpreted in the context of existing evidence in the field, and discusses their clinical relevance across different levels of healthcare. Moreover, the strengths and limitations of the methods and results will be discussed.

6.1. Summary of findings

In this dissertation, autonomic dysfunction, defined by long-term HRV and standardized CARTs, and its relationship with cardiovascular complications were studied across three different cohorts representing populations at varying levels of prevention and care, including public health, primary care, and secondary care. In The Maastricht Study (Study I), I investigated autonomic dysfunction, measured by 24-hour HRV, and arterial stiffness, measured dynamically along the descending aorta and locally at the carotid site among individuals with NGM, prediabetes, and T2D. Lower HRV was associated with higher aortic and carotid stiffness. This association was evident regardless of glucose metabolism status and was more pronounced in individuals with prediabetes or T2D. The modifying effect of dysglycemia was confirmed by a statistically significant stronger association across higher HbA1c levels. Z-scores of time- and frequency-domain measures showed the strongest associations, primarily driven by HRV indices reflecting total variation in interbeat intervals (SDNN, SDANN, SDNN index, ULF, VLF, TP).

Study II focused on individuals at higher risk of developing diabetes, using data from the ADDITION-PRO cohort. In Study II, lower SDNN, measured over multiple days, was associated with 18, 24, and 21 percent higher risk per SD for ischemic-related CVD, hospitalization for heart failure, and all-cause mortality, respectively. The risk became higher at SDNN levels below 120 ms, supported by a greater difference in incidence rates between individuals with 100 ms and 120 ms than the difference observed between individuals with 120 ms and 160 ms. Hourly measures suggested a specific time point related to ischemic-related CVD, as lower SDNN recorded between 6:00 and 7:00 AM was associated with MACE. Adjustment using the residuals method for concurrent heart rate and physical movement did not explain the observed association. Hourly SDNN was associated with all-cause MRR, although no specific time point showed an exceptionally strong association. While no association between hourly SDNN and heart failure was observed, higher heart rate during the night hours from 02:00 to 06:00 AM was linked to a higher risk of heart failure hospitalization.

These findings suggest that both long-term HRV measures and hourly HRV responses may serve as indicators of CVD risk. However, a key observation is that long-term HRV was assessed under free-living conditions, which restricts the comparability of results to standardized tests of autonomic function, although it provides insights that are more comparable to what may be found with long-term wearable devices. In the CANCAN Study (Study III), standardized CARTs were used to define CAN and to describe indicators of heart failure, including elevated NT-proBNP, WATCH-DM risk, and NYHA classification, among individuals with and without CAN in a population with T2D. In CANCAN, two out of five had CAN. Compared to individuals without CAN, these individuals more often showed signs of heart failure, including elevated NT-proBNP levels, higher WATCH-DM risk scores, and higher classifications on the NYHA. CAN was associated with elevated NT-proBNP levels and these persisted even among individuals without heart failure symptoms based on NYHA classification, as well as among those categorized as having low to moderate heart failure risk according to the WATCH-DM score.

In summary, various aspects of autonomic dysfunction and cardiovascular complications were investigated in populations with NGM, prediabetes, or T2D. The overall findings showed that autonomic function, assessed through heart rate dynamics of long-term HRV and diurnal HRV or heart rate responses to autonomic reflex tests, is associated with a higher risk of CVD and heart failure. This relationship appears to be stronger in more severe stages of dysglycemia.

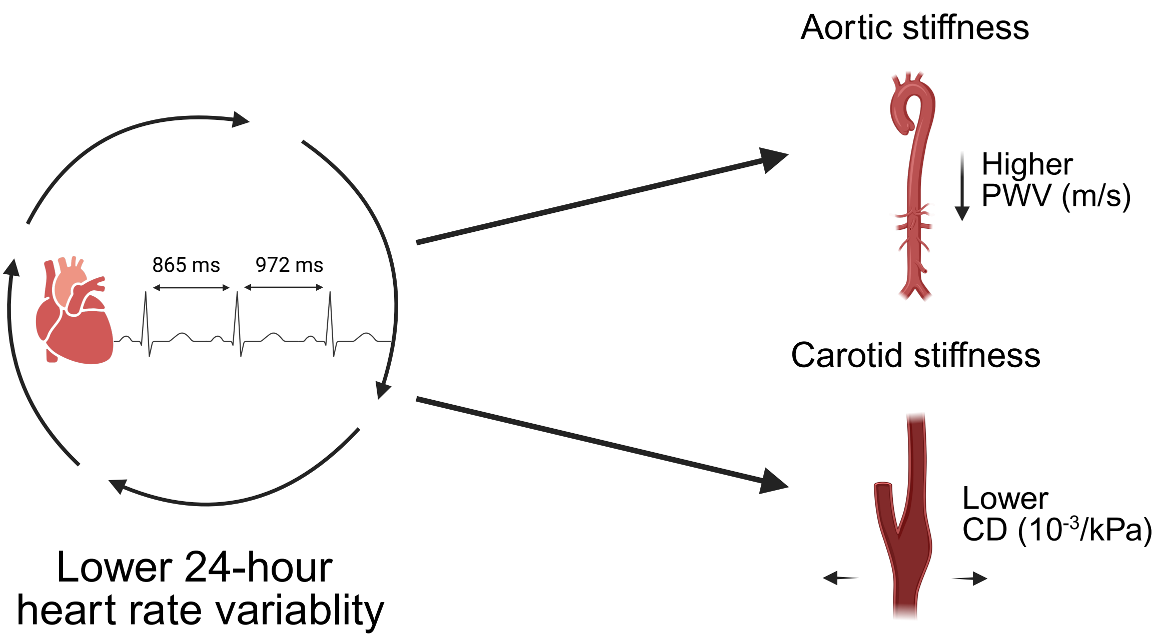
Moreover, among individuals with T2D, the presence of CAN may help identify those at higher risk of heart failure, even in the absence of heart failure symptoms.

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

This dissertation shows that autonomic dysfunction, measured by HRV and CARTs, is associated with CVD risk across the spectrum of glucose metabolism dysregulation. This association is evident in measures of arteriosclerosis, atherosclerotic events, all-cause mortality, and heart failure in individuals at high risk of diabetes, as well as in indicators of heart failure in patients with T2D.

6.2.0.1. Arteriosclerosis

In Study I, autonomic dysfunction, measured by 24-hour HRV, was shown to be cross-sectionally associated with arterial stiffness, measured both dynamically (cf PWV) and locally (CD). Adjustments for demographic and lifestyle factors, as well as a range of cardiovascular risk factors, did not explain the association. This suggests that autonomic responses under free-living conditions may contribute to the development of arterial stiffness. The majority of studies in the field have shown an association between autonomic dysfunction, as measured by short-term HRV during rest, and arterial stiffness in populations with either type 1 or T2D.[98,99] Study I further extended this perspective by examining long-term HRV in a population without diabetes or prediabetes.



(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 higher arterial stiffness.[100,101] Two possible mechanisms may explain how autonomic dysfunction is related to arterial stiffness.

First, autonomic dysfunction may raise vascular tone in large arteries, reducing elasticity. Animal studies support this, showing that proper autonomic regulation is essential for maintaining aortic elasticity.[31] This effect may, in part, be transient and reversible if autonomic function is restored. However, chronic sympathetic overstimulation can lead to structural remodeling and sustained stiffness.[102] While such findings cannot be directly extrapolated to humans, they suggest plausible biological pathways.[103]

Second, the autonomic nervous system controls heart rate and cardiac contractility.[101]] Autonomic dysfunction, characterized by increased sympathetic activity and reduced parasympathetic tone, elevates rHR and arterial shear stress, potentially contributing to structural arterial stiffening. Data from the Whitehall II study found that a steeper 10-year decline in short-term resting HRV was associated with greater aortic stiffness five years later.[103]

The association between 24-hour HRV and arterial stiffness (Study II) was modified by dysglycemia, suggesting dysglycemia may induce CAN that can affect arterial stiffness, even before the onset of T2D.[8,104,105] Data from the Whitehall II study showed that aortic stiffness increased more steeply with higher HbA1c values among non-diabetic individuals[106], supporting this notion. In the subpopulation in Study I without diabetes, a modification by HbA1c in both aortic and carotid stiffness was observed. The modifying effect of HbA1c suggests that hyperglycaemia amplifies the consequences of autonomic dysfunction.

6.2.0.2. Atherosclerosic events

Study II showed that individuals with a preclinical stage of autonomic dysfunction, measured by multiday HRV, face a higher risk of incident ischemic CVD, heart failure, and all-cause mortality.

Multiday HRV was assessed to capture autonomic activity in real-life settings across several days. These results are consistent with earlier research linking lower HRV (including measures from 10 seconds to 24 hours) to CVD and mortality.[5,107] Findings from Study II build on existing evidence by: (1) focusing on a population at elevated risk for diabetes, (2) employing multiday HRV recordings, and (3) identifying specific times of day when HRV patterns were indicative of ischemic-related cardiovascular risk. By using both week-long and hourly data, specific periods that better indicate long-term risk were identified. A strength of using multiday HRV recordings is that they provide more robust insights into individual autonomic patterns by averaging autonomic responses across typical daily conditions. This reduces the influence of random fluctuations caused by factors such as physical activity, emotional states, or sleep on any single day.[108]

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 contribute to arterial stiffness, a dynamic and potentially modifiable process that impairs vasodilation and increases hemodynamic stress[109–111], thereby elevating the risk of ischemic events.

Second, the autonomic nervous system innervates the adventitia layer of blood vessels, modulating vascular tone via sympathetic fibres.[32]Although plaques form in the intima, higher plaque burden has been linked to increased local sympathetic nerve density, possibly through neuroinflammatory pathways.[112] Autonomic dysfunction likely exerts systemic effects on the vasculature[32], making a direct role in plaque formation uncertain, but it may compromise vasodilatory capacity during ischemic or thrombotic events. Notably, reduced sympathetic innervation has attenuated plaque formation in animal models, supporting indirect effects on atherogenesis.[112]

Third, autonomic dysfunction has been shown to interfere with signalling pathways controlling heart rhythm, potentially leading to arrhythmias. Earlier studies have shown lower short-term HRV was associated with incident atrial fibrillation (AF), with a higher risk among participants with T2D.[107,113] This supports the role of autonomic dysfunction in arrhythmogenesis, which increases the risk of myocardial infarction and stroke.[114] Study II did not include atrial fibrillation (AF) as an outcome due to limitations in Danish registries prior to 2016, which often failed to distinguish between short- and long-term AF, thereby compromising diagnostic validity.[115]

Study II 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 Genome-Wide Association Study (GWAS) in the UK Biobank of short-term HRV supports this by identifying mechanisms involving G-protein signaling, pacemaker activity, and mitochondrial function as likely mediators of the genetic contribution to

HRV. These pathways influence vagal control, cardiac excitability, and energy metabolism.[116] Although derived from short-term recordings, these genetic associations may reflect autonomic traits that persist across different time scales and reinforce the notion of a biological basis for inter-individual differences in HRV, preceding and independent of the onset of dysglycaemia. A Mendelian randomization study using data from the Rotterdam Study found that genetically predicted HRV was associated with a higher risk of AF.[117] 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.[118] 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.[118] No GWAS has yet been conducted for long-term HRV. Therefore, it is unclear whether the genetic influences identified for short-term HRV are applicable to long-term HRV. Future GWAS efforts targeting long-term HRV could help establish causal relationships to CVD by leveraging methods such as Mendelian randomization and advancing understanding of the genetic architecture underlying autonomic regulation under a full day.

6.2.0.3. Heart failure

The relationship between cardiovascular autonomic dysfunction and heart failure is complex.[119] On one hand, autonomic dysfunction contributes to cardiac remodelling and eventual heart failure.[30,120] On the other hand, it may reflect compensatory mechanisms of the progression of cardiac remodelling and declining cardiac output.[30] Findings in Study I and II demonstrated a relationship between autonomic dysfunction and heart failure both cross-sectionally in a population with T2D and prospectively in individuals representing different tiers of diabetes risk. However, the extent to which this relationship supports one explanation over the other is difficult to determine due to limitations in the data, as both baseline and follow-up measures of heart failure and HRV are lacking.

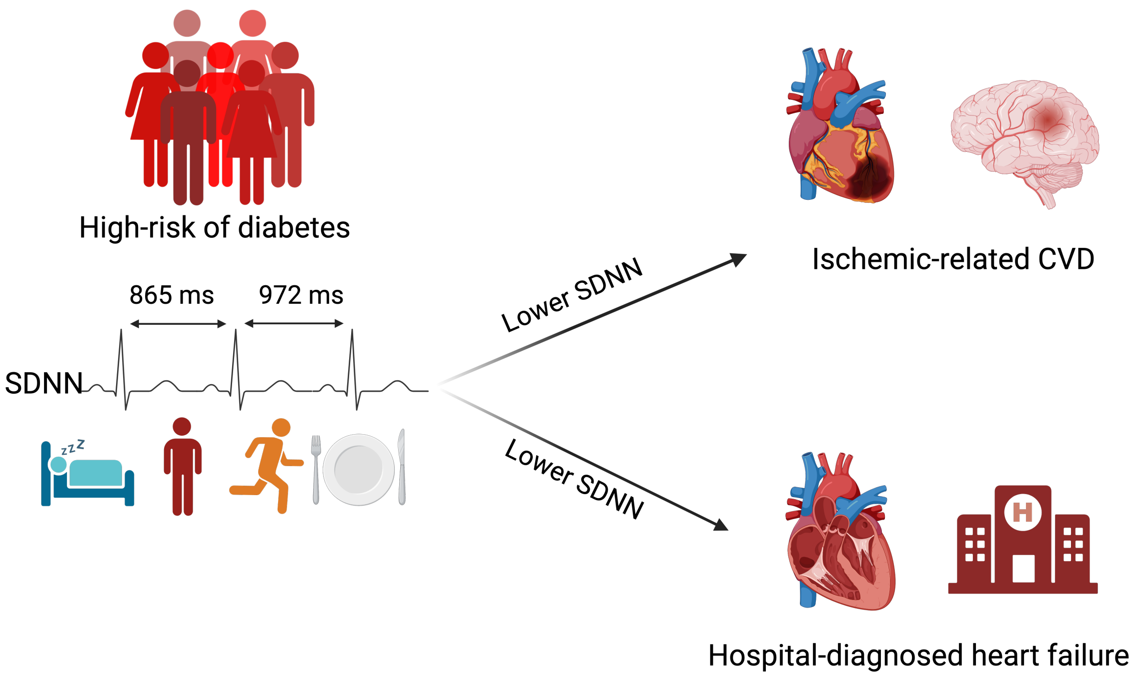
Previous studies have shown that both short- and long-term HRV are associated with incident heart failure in populations with and without T2D.[121–124] Findings from Study II extended prior work by applying multiday HRV recordings to a population at high risk for diabetes and by: (1) assessing the role of resting heart rate in the HRV–heart failure relationship, and (2) identifying time-of-day heart rate patterns associated with heart failure risk.

Several mechanisms may underlie this relationship. Arterial stiffness is known to contribute to cardiac remodelling by increasing cardiac afterload and reducing coronary perfusion through earlier return of the reflected pulse wave.[125] This suggests that autonomic dysfunction may indirectly influence heart failure through arterial stiffness. However, structured methods such as mediation analysis with repeated measures are needed to clarify these pathways.

Study II showed that multiday 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 resting short-term HRV was longitudinally associated with echocardiographic measures reflecting systolic function, suggesting that autonomic dysfunction contributes to cardiac remodelling.[120] 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 multiday HRV that was linked to heart failure risk. This suggests that the association is not driven by isolated shifts in autonomic activity in response to circadian stressors, but rather by a consistently impaired autonomic balance under free-living conditions.

In Study III, individuals with CAN were found to have higher risk of elevated NT-proBNP, a biomarker of myocardial stress and early heart failure. This supports the interpretation that CAN contributes to both structural and functional cardiac changes, reflected in elevated NT-proBNP levels.

Reverse causation cannot be ruled out, as autonomic dysfunction may also reflect compensatory responses to progressing heart failure.[30] CAN and cardiac remodelling may interact in a reinforcing cycle: autonomic dysfunction increases sympathetic tone and reduces parasympathetic modulation, promoting cardiac stress and remodelling, which in turn further impairs autonomic regulation.[24] This feedback loop may accelerate heart failure progression, although this remains beyond the scope of the current data and analysis.



(Source: Author)

6.3. Clinical implications

The dissertation investigates autonomic dysfunction in populations ranging from NGM to T2D and yields insights relevant for individuals who engage with the healthcare system at different levels. No specific role has yet been defined for autonomic dysfunction in clinical decision-making within healthcare, as current treatment and intervention options specifically targeting autonomic function remain limited. Although the results do not point directly to where and how implementation of autonomic dysfunction in clinical practice may make sense, the included studies broadly represent situations relevant to public health, primary care, and secondary care settings. In the following section, the clinical implications of using autonomic dysfunction in the prevention of CVD will be discussed. 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.

6.3.1. Public health

A central strategy in preventing CVD is the early identification and multifactorial treatment of individuals at high risk.[126] Public health initiatives support this by promoting healthy lifestyles, facilitating early screening for risk factors, and improving access to essential care and medications.[127,128] Long-term HRV may enhance these efforts by identifying individuals with elevated cardiovascular risk and by tracking their physiological response to lifestyle changes.

Evidence from Study I showed that lower long-term HRV was associated with higher arterial stiffness, as measured by cf-PWV and CD, even in individuals without T2D. One standard deviation lower HRV corresponded to the effect of 2.7 additional years of ageing on aortic stiffness (cf-PWV) and 1.6 years on carotid stiffness (CD).[77] These cross-sectional findings suggest that HRV may serve as a marker of early vascular ageing and cardiovascular risk. Supporting this, the Whitehall II study demonstrated a longitudinal relationship between short-term HRV and aortic stiffness.[103] Together, these findings highlight the potential of HRV as an indicator of vascular health.

Within the public health setting, individuals with prediabetes represent a particularly vulnerable group at risk for comorbidities.[129] They often fall between structured care pathways, sometimes encouraged to reassess their cardiovascular risk at more frequent intervals, other times not offered any additional measures or attention beyond general lifestyle advice. Notably, Studies I and II demonstrated that the associations between long-term HRV and CVD risk were especially pronounced in this population. In those at high risk of diabetes, a one standard deviation (33 ms) lower multiday SDNN was equivalent to 4.5 additional years of ageing for ischemic-related CVD and 2.2 to 2.4 years for heart failure.[87] On a population level, lower HRV (SDNN: 100 ms) in individuals with prediabetes was associated with a higher incidence rate of CVD, heart failure, and all-cause mortality compared to individuals with normal-to-higher HRV (SDNN: 120 to 160 ms). These findings reinforce the role of HRV as an early and sensitive marker of cardiovascular health in populations at cardiometabolic risk.

While these findings highlight HRV's potential, practical implementation faces several challenges. Historically, long-term HRV monitoring has required specialized equipment such as Holter ECG recorders.[6] However, the growing popularity of wearable devices offers a promising alternative.[130] These devices provide a non-invasive, user-friendly way to collect heart rate and HRV data over time, under free-living conditions like those examined in Study II.[11]

If HRV monitoring proves effective in helping individuals maintain a healthy, age-adjusted HRV range through lifestyle changes and prompts healthcare engagement when HRV deteriorates, it could become a meaningful tool for long-term health tracking. A cross-sectional study of 8 million individuals found that those who took more steps per day had higher HRV, suggesting that HRV may also reflect a healthy lifestyle.[13] This notion has been longitudinally supported in the Whitehall II study.[131] In addition to the ADDITION-PRO study, the inclusion of data from future observational cohorts that incorporate wearable devices, along with precise lifestyle measures such as physical activity, diet, and sleep, could enhance the understanding of patterns influencing long-term heart rate variability and diurnal autonomic responses.[132,133]

A major public health challenge lies in ensuring equitable access to wearable technology.[11]Individuals from lower socioeconomic backgrounds are less likely to own such devices, raising concerns about health disparities.[11] Despite this, there is encouraging evidence that the general population is open to share health data with public institutions and support the use of AI in disease monitoring.[11,134]

In summary, integrating wearable HRV monitoring into public health strategies could represent a transformative step in proactive cardiovascular care. It holds potential for early detection, personalized prevention, and timely referral to primary care when risk levels increase.

6.3.2. Primary care

Cardiovascular risk in primary care is assessed using clinical evaluations and standardized risk prediction tools to identify individuals at elevated risk.[135] Management focuses on lifestyle modification, pharmacological therapy, and regular monitoring to reduce cardiovascular events.[135] In this health care setting, long-term HRV may offer added value by improving the precision of cardiovascular risk stratification and by serving as a marker to monitor the effectiveness of preventive strategies.

Long-term HRV may improve ranking of individual risk when added to established clinical risk scores. Tools such as SCORE2 and the Framingham Risk Score are widely used in primary care to guide cardiovascular risk assessment.[136,137] In Study I, models adjusted for conventional CVD risk factors supported the potential added value of 24-hour HRV in relation to arterial stiffness, a surrogate marker of CVD risk. Study II extended this perspective by demonstrating associations between multiday HRV and incident CVD and heart failure. However, these findings are based on associations and do not include formal prediction modeling[138], and therefore cannot determine whether incorporating long-term HRV or CARTs into existing risk scores improves predictive performance beyond current guidelines. This study design was not feasible in ADDITION-PRO, as the cohort did not represent high-risk diabetes populations typically identified in primary care (e.g., elevated HbA1c). Likewise, CANCAN is limited by its small sample size and recruitment from secondary care. To assess predictive value, cohorts should reflect individuals with T2D or those at high risk, as defined by current clinical practice. Few studies suggest that 24-hour HRV may improve risk discrimination for CVD and all-cause mortality in individuals with T2D[139], and for stroke and heart failure in older adults.[121,140] However, these studies often lack calibration or validation in large-scale cohorts and have not been integrated with widely used risk scores such as SCORE2 or the Framingham Risk Score.

Long-term HRV may also help classify preclinical autonomic dysfunction, enabling targeted interventions in a subgroup of patients to prevent CVD. The increasing availability of wearable devices capable of capturing long-term HRV data presents a practical opportunity for continuous monitoring in primary care. These devices may facilitate earlier detection of autonomic dysfunction and support more personalized approaches to cardiovascular risk management. However, the clinical utility of stratifying patients based on preclinical autonomic dysfunction remains uncertain. These considerations are only actionable if interventions in this subgroup can be shown to lower cardiovascular risk. Emerging evidence suggests that both pharmacological and lifestyle interventions can improve HRV in the short term.[141–143] For example, high-intensity interval training has been shown to improve autonomic function in obese individuals with and without T2D.[144] Similarly, lifestyle changes in individuals with prediabetes have been associated with improvements in short-term HRV, which may partly explain a reduction in diabetes risk independently of weight loss.[145] Nevertheless, it remains unclear whether these effects on HRV are sustainable over time and whether they translate into long-term cardiovascular protection. In many cases, improvements in autonomic function may be mediated indirectly through changes in cardiometabolic markers such as glucose levels, lipid profiles, body weight, maximal oxygen uptake, and blood pressure.[145–147]

Despite these uncertainties, monitoring autonomic function through long-term HRV may offer a valuable tool for assessing cardiovascular risk and tracking the impact of preventive strategies. In Denmark, prediabetes, defined by HbA1c, is present in 7.1% of adults.[148] One in five of these individuals develops T2D within five years[148], while others either remain in the prediabetic stage or return to normoglycemia. Despite their higher risk of CVD and heart failure[149], individuals with prediabetes are not captured by existing preventive strategies. This underscores the need for early and precise risk assessment.[10] Given that the cardiovascular consequences of autonomic dysfunction appear to be more pronounced in individuals with prediabetes compared to those with normoglycemia, HRV has the potential to help identify those at elevated CVD risk within this group. However, evidence demonstrating improved risk prediction and sustained effects leading to better cardiovascular outcomes is needed to establish its relevance for integration into primary care.

6.3.3. Secondary care

In secondary care, endocrinologists assess cardiovascular and heart failure risk by integrating advanced diagnostics, biomarker analysis, and imaging to detect early heart failure, guided by symptoms and risk profiles. The treatment of patients with T2D is guided by evidence-based therapies and multidisciplinary collaboration.[4,58,72] The ADA/EASD 2022 consensus on Management of Hyperglycemia in T2D emphasizes that early detection of heart failure in individuals with T2D is crucial. This enables timely initiation of therapies such as SGLT2i, which have demonstrated significant benefits in lowering heart failure-related outcomes.[72] 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.[58] Still, there is a need to identify optimal approaches for recognizing and diagnosing heart failure in clinical care, as broad echocardiographic screening in T2D is time-consuming and costly.[4]

Study III demonstrated that CAN may help identify individuals at higher risk of heart failure, beyond what is captured by symptoms or existing risk scores. These findings support considering CAN as a relevant risk factor for heart failure and suggest it may have value in future risk stratification strategies in T2D. A clinical advantage of using CARTs is that they are standardized tests performed under controlled conditions.[70,89] CARTs have proven to be reliable and reproducible, with reference values established in large population studies.[70,89] Beyond these findings and the established evidence of higher heart failure risk, CAN also identifies individuals at high risk for overall CVD, kidney disease, and early mortality in the T2D population.[63,64]

In Study III, I observed that two out of five participants had CAN, highlighting it as a complication with considerable prevalence. Theore, detecting CAN may uncover an often-overlooked condition that is common in individuals with T2D.

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.[58] However, its specificity varies across heart failure phenotypes, being less specific for detecting HFpEF compared to HFrEF.[58] Therefore, additional evaluation using echocardiography are warranted. Beyond classifying heart failure phenotypes, echocardiography identifies preclinical stages of heart failure through the detection of functional or structural cardiac abnormalities. Including CAN in structured assessments of heart failure could help clarify to which extent CAN overlaps with cardiac abnormalities. 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. SGLT2i are recommended as second-line treatment in T2D and have demonstrated benefits in lowering the risk of heart failure, CVD, and kidney function decline, complications commonly associated with CAN.[63,64] Current guidelines recommend initiating these therapies based on a history of CVD, heart failure, or the presence of conventional high-risk cardiovascular factors.[72] However, the specific impact of SGLT2i 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 direct clinical implications of the findings in Study III are limited. The generalizability of the results is restricted, as the study population consisted of patients with T2D receiving secondary care. Two out of five patients with CAN showed to have a history of CVD, a group already at higher risk of heart failure due to their prior diagnosis. This overlap may influence the interpretation of CAN as an independent risk factor. Therefore, these findings need to be validated in a broader population with T2D, including individuals without a history of CVD. Doing so would allow for greater generalizability of the results to the broader T2D population, particularly those receiving care in primary settings.

6.4. Strengths and limitations

6.4.1. 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, temporality cannot be established, nor can it be confirmed whether changes in the outcome were caused by the exposure. Based on prior evidence, the direction of the associations in Study I was inferred using physiological knowledge and findings from epidemiological and in vivo studies.[103]

Study III focused 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 T2D who may be progressing early toward heart failure. Whether cardiac function progressively worsens due to the underlying mechanisms of CAN remains to be fully established.[88]

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, lowering the risk of reverse causation. The prospective design 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.

Causality cannot be ascertained from the findings in Study I and Study II, and more causally focused methods are needed. These will be discussed in detail in the *Perspectives* section.

6.4.2. Internal validity

Cardiovascular autonomic function was assessed in this project both under free-living conditions and in response to standardized test procedures conducted during clinical visits. Additionally, dynamic measurements were used to evaluate arterial stiffness both locally and by velocity, and biomarker assessments were performed to determine the presence of heart failure. In this section, the validity of 24-hour, multiday, and hourly HRV measurements is discussed, along with the standardized tests of CAN. The validity of the included outcomes is also addressed, and the strengths and limitations of using MACE as a time-to-event outcome are examined.

6.4.2.1. Long-term HRV (24 hours) and autonomic function

A main consideration in HRV analysis is the reliability of raw inter-beat interval data from ECG recordings. Accurate HRV measures depend on continuous and correctly sequenced inter-beat intervals. Frequency-domain analyses depend on the inter-beat interval sequence, as well as time-domain measures, such as RMSSD and pNN50.[6] In Study I, data from a 12-lead Holter system was used, which is considered the gold standard for long-term ECG recordings. In Study II, data from the Actiheart device was used for HRV. The device was configured to record continuously over a seven-day period. It captured 30-second epochs of mean heart rate intervals. HRV was estimated from the inter-beat interval distributions using a validated algorithm.[86] 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.[86]

Autonomic nervous function, as measured by long-term HRV in free-living conditions, may also be influenced by behavioral factors such as physical activity, sleep, meal timing, smoking, caffeine intake, alcohol consumption, and medication use.[70,108,150,151] These factors can potentially mask or mimic underlying physiological dysfunction during recordings, but they may also elicit the HRV responses of interest.[108] HRV is shaped by both daily behaviors and long-term lifestyle patterns[152] In Studies I and II, habitual physical activity was accounted for, and in Study

II, hourly HRV was adjusted for physical movement during recordings to test the influence of concurrent activity.

Anti-hypertensive medications, especially beta-blockers, are known to increase HRV in randomized controlled trials.[150] In sensitivity analyses in Studies I and III, excluding participants on anti-hypertensive treatment did not materially change the estimates. Therefore, these participants where kept and adjusted for medication use in the full models.

Beyond the behavioral and pharmacological contributions to HRV, a physiological distinction cannot be made as to whether autonomic dysfunction is primarily driven by higher sympathetic activity or lower parasympathetic tone, as HRV is a proxy of these modulations.[6,79–85] It remains unclear whether cardiovascular complications stem mainly from sympathetic overactivity or parasympathetic withdrawal.

HRV levels are influenced by heart rate, as lower resting heart rate allows for greater variability[153,154]. In Study I, adjustment for heart rate was deliberately omitted from the models, as its inclusion could introduce multicollinearity. Additionally, elevated heart rate is driven by higher sympathetic activity and may act as a mediator in the pathway leading to arterial stiffness.[77] 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.[70] In Study II, the residuals method was used to pre-adjust HRV measures for resting heart rate. This adjustment 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 for hourly associations, where the outcome of heart failure was similarly affected by heart rate pre-adjustment.

The three studies demonstrate approaches to identifying CVD risk: (1) selecting appropriate HRV indices, (2) segmenting time intervals, and (3) assessing HRV under defined conditions. Findings Study I indicated that associations between long-term HRV indices and arterial stiffness vary, with RMSSD and HF showing weaker associations. Similar patterns have been observed in long-term HRV measures among individuals with type 2 diabetes.[99] However, previous research has shown that these indices can be informative when analyzed in 5-minute segments.[103,141,155] Additionally, SDNN exhibited varying associations with CVD risk depending on the time of day. It was also observed that, in CARTs, the Valsalva maneuver and deep breathing test were more indicative of heart failure. These insights highlight the relevance of aligning HRV methods with study objectives.

6.4.2.2. Cardiovascular autonomic reflex test

CART provides a practical approach for screening for autonomic dysfunction and has been shown to be a reliable method.[89] 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.[70] A limitation of the CARTs in this study was the high prevalence of participants who were unable to complete the all the tests, primarily due to missing data from the Valsalva maneuver.

6.4.2.3. Measures of cardiovascular risk

In Study I, arterial stiffness measures, including cf-PWV and CD, are influenced by MAP, which may confound the assessment of vascular stiffness. In Study I, the observed associations were attenuated by adjustment for MAP. However, the associations remained statistically significant.

In Study II, outcomes were based on CVD events, heart failure, and causes of death from Danish national registries. Potential misclassification and underreporting, especially of heart failure, may have led to underestimation of associations.[156]

In Study III, NT-proBNP was used as a primary indicator of heart failure. While NT-proBNP is a validated biomarker for early-stage heart failure and useful for ruling out the condition, its specificity varies by HF phenotype.[58] Thus, a determination of HFpEF or HFrEF cannot be made. NT-proBNP diagnostic accuracy is influenced by factors such as AF, obesity, and kidney function.[58] Individuals with AF were excluded by design. Analysis was adjusted for BMI, which did not affect the association between CAN and elevated NT-proBNP. After adjusting for eGFR, the association became stronger, suggesting that lower kidney function may have masked the true link between CAN and heart failure risk.

6.4.3. External validity

6.4.3.1. Selection bias

The Maastricht Study

The target population in Study I was intended to represent individuals at different stages of glucose metabolism. However, the analysis may have been affected by selection bias in the representation of individuals with T2D. Participants in the Maastricht Study were recruited based on their ability and willingness to attend multiple research visits and receive personal health feedback, which likely attracted health-conscious individuals with higher education levels.[157] As a result, individuals with T2D were relatively healthy, with a median disease duration of three years and a low prevalence of complications. Those who completed both long-term ECG and arterial stiffness assessments may have represented an even healthier subgroup. This selection bias may have limited the generalizability of the findings to the broader T2D population and may explain why the effect modification did not differ step-wise from that observed in individuals with prediabetes.

ADDITION-PRO

The target population in Study II was intended to represent individuals at high risk of developing T2D. 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. Those with high scores were invited for further testing using HbA1c or random glucose measurements.[158]

This recruitment strategy involved selection by design, as the source population was defined based on specific risk criteria. The questionnaire prioritized risk factors such as older age and hypertension, leading to overrepresentation of these groups.[159] Prediabetes was identified only after biochemical testing, while the risk score was primarily designed to detect undiagnosed T2D.[158] Although this selection process was intentional and aligned with the ADDITION-PRO objectives, the generalizability of the findings to the broader population at risk for T2D may have been limited.

In addition, selection bias may have occurred due to differential participation in the ADDITION screening program. Healthier individuals were more likely to participate, both by completing the risk questionnaire and by attending follow-up testing.[160] As a result, the baseline risk for CVD in ADDITION-PRO participants may have been lower compared to the target population.

CANCAN

The target population in Study III was intended to represent individuals with T2D treated in outpatient clinics. In Denmark, patients with T2D are referred to diabetes specialists at outpatient clinics when their general practitioner is unable to stabilize their condition. A strength of the CANCAN sampling strategy was that patients were already attending endocrinology consultations, and the study examination required only additional time during their visit, without the need for extra transportation or appointments. Assessing selection bias in this study is challenging, as inclusion depended on referral practices by general practitioners.[161] These practices may have varied individually, with differing thresholds for referring patients to specialized care based on clinical judgment and patient characteristics.

6.4.3.2. Generalisability

The generalisability of the findings has been considered in the context of the targeted recruitment strategies used in each study, which were aimed at including individuals across a spectrum of diabetes risk, from NGM to established T2D. As a result, the findings are most applicable to populations with similar clinical profiles and healthcare settings.

Studies I–III included individuals at high risk of diabetes and those with T2D. Therefore, the associations between cardiovascular autonomic dysfunction and cardiovascular outcomes or surrogate biomarkers are relevant to individuals with some degree of diabetes risk and progressed T2D. Study I suggested that the link between autonomic dysfunction and cardiovascular risk, as measured by arterial stiffness, was also present in individuals with NGM, though to a lesser extent. This finding, supported by replication in the Whitehall II cohort, indicated that the observed relationship may extend beyond high-risk groups and into the general population.[103] In Study III, participants represented a higher-risk diabetes group among Danish diabetes patients, while more stable patients remained under general practitioner care. Consequently, the prevalence of heart failure indicators and CAN was likely higher in this selected group than in patients managed in primary care, and thus the extension of the findings to broader T2D populations is limited.

By design, younger individuals with prediabetes or young-adult-onset T2D were underrepresented in the studies. This group may have been overlooked in current research and warrants further attention in future studies.[148,162] The applicability of the findings to other countries may be influenced by differences in demographic composition, risk factor distributions, healthcare systems, and stages of economic development. These factors can affect both the prevalence of diabetes and cardiovascular disease and the nature of their associations. While the study populations were primarily of Nordic and Western European descent, differences in ethnic composition are only one of several factors that may influence external validity. These regions also share relatively well-organized, publicly funded healthcare systems, which may differ substantially from those in other parts of the world and further affect the applicability of the findings.

7.Perspective

This dissertation has investigated the impact of autonomic function on cardiovascular complications across different stages of glucose metabolism. Understanding when and how physiological signals reflect elevated CVD risk is essential for the development of early and effective prevention strategies. The incorporation of HRV into digital health solutions could be used to support personalized feedback mechanisms, enabling timely lifestyle or therapeutic interventions and contributing to more adaptive and preventive healthcare strategies. Based on the findings and conclusions, further perspectives are proposed 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

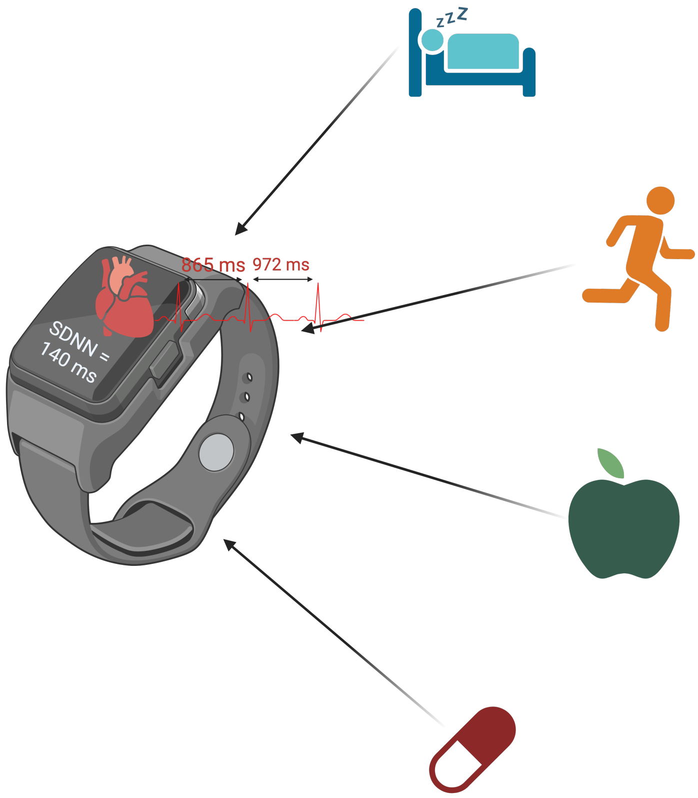
Wearable devices enable comprehensive data collection on behavioral (e.g., sleep and physical activity) and physiological (e.g., heart rate, ECG, temperature) parameters.[130] These devices offer a broader and more feasible approach to long-term heart rate monitoring. Despite growing interest in wearable-based 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

Lower long-term HRV has been identified as a risk factor for CVD, associated with arterial stiffness and clinical endpoints. Furthermore, findings indicate that specific HRV and heart rate patterns under free-living conditions may enhance early risk detection, independent of concurrent physical activity. For improved risk assessment, future predictive models should move beyond adjusting for physical activity as a confounder and instead integrate multiple physiological signals, such as HRV responses to sleep and activity patterns, to better capture dynamic health states. Machine learning offers powerful tools to analyze complex raw time-series data, including interbeat intervals and accelerometer signals, potentially improving risk prediction beyond traditional HRV summary metrics[163]. However, the limited interpretability of these models remains a key barrier to clinical adoption. Nevertheless, HRV may help identify individuals at elevated cardiovascular risk using wearable devices, potentially without relying on blood pressure or blood-based measures, though this remains to be validated.

Assessment of response to intervention

HRV represents a potential target for intervention, as low HRV may reflect adverse lifestyle patterns. Behaviors such as disrupted sleep, physical inactivity, diet, and irregular meal timing have been shown to influence circadian fluctuations in HRV.[141,152,164,165] HRV has also been shown to respond to pharmacological interventions. For example, beta-blockers have been shown to increase HRV, while GLP-1RA may reduce it.[150,166]



(Source: Author)

Figure 7.1.: **Hypothetical scenario of lifestyle and treatment adaptation using HRV in wearable devices**

Future research may leverage wearable devices to monitor the effectiveness of behavioral and pharmacological interventions on HRV at the individual level. This approach may support precision real-time monitoring to identify lifestyle patterns or treatments that promote cardiovascular health through HRV modulation or uncover potential side effects.[75]

However, standardization and transparency across wearable device brands remain a challenge for both research and clinical use. While smartwatches offer convenient heart rate monitoring, their accuracy varies due to reliance on photoplethysmography, which can be affected by motion and other external factors, especially during physical activity.[167,168] Despite these limitations, ongoing improvements in sensor technology and algorithm calibration are likely to enhance the reliability of wearable-derived HRV and heart rate data.[169] Open data formats are important to ensure that detailed data (e.g., interbeat intervals)[169,170] from various devices can be used consistently in health prediction algorithms, rather than relying only on summarized outputs.

7.2. Risk-stratification

The distinct roles of long-term HRV and CART in cardiovascular risk stratification remain to be fully established. Building on the concept of continuous monitoring through wearable technology, long-term HRV presents two promising avenues that warrant further investigation:

- **Enhancement of existing risk scores:** HRV may improve the predictive accuracy of established cardiovascular risk models, such as SCORE2 or the Framingham Risk Score.
- **Support for treatment decisions:** Long-term HRV may also help optimize the timing of treatment initiation and guide intermediate clinical decisions.

Cardiovascular risk assessment

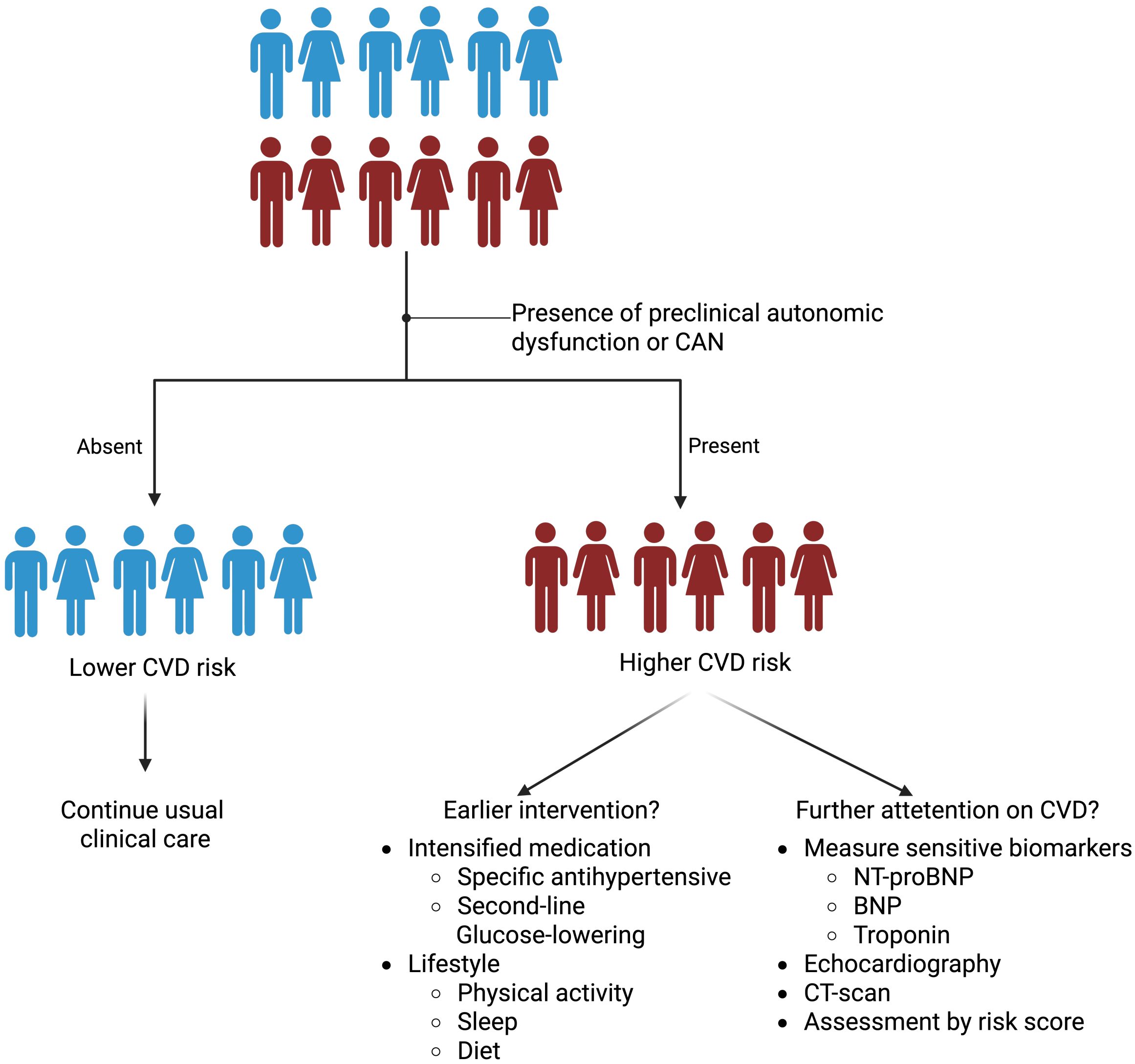
Digital CVD risk calculators can be used to optimize the timing of follow-up assessments and treatment initiation. Analyses from Steno Diabetes Center Copenhagen have suggested that annual retinopathy screening may not be necessary for all patients. Instead, prediction models using clinical variables can be used to determine optimal re-screening intervals.[171] In prediabetes, a key concern is overmedicalization, as many individuals do not progress to T2D or develop complications.[14] Therefore, efforts to identify subgroups in prediabetes are needed to enable timely prevention of cardiovascular complications.[10] In T2D, data-driven methods using clinical characteristics have been used to identify who will benefit most from intensive treatment.[172] Whether wearable technologies, such as those measuring HRV, can improve individualized screening intervals and identify individuals who require closer clinical attention remains to be investigated.

Timing and treatment decisions

In addition to optimizing the timing of follow-up assessments, cardiovascular risk stratification can also guide when to initiate treatment. In type 1 diabetes, for example, elevated CVD risk scores are used to inform decisions about starting lipid-lowering therapy.[173] Wearable-derived data may also support intermediate treatment decisions.[75] In a UK population with T2D in clinical practice, patient characteristics have been used to predict whether SGLT2i or GLP1RA will better improve HbA1c levels.[174,175] A further step would be to include long-term HRV as a clinical characteristic to enhance the prediction of treatment response. This could enable more precise stratification of therapy or lifestyle interventions based on the most effective option for each individual. Whether incorporating long-term HRV into predictive models can improve the personalization of treatment, particularly for therapies with cardiovascular effects, remains to be demonstrated. This conceptual framework may also have potential for guiding the selection of first-line antihypertensive medications.

As discussed in the clinical implications of CAN in T2D, it remains unclear how well a CAN diagnosis predicts heart failure risk in the broader T2D population seen in primary care. Intermediate clinical decisions are needed for patients diagnosed with CAN to determine whether to proceed with further evaluation for heart failure using echocardiography or to initiate specific cardioprotective therapy.

In summary, future research should uncover whether identifying individuals with high-risk of CVD based on autonomic dysfunction, using HRV or CAN assessed through CART, can support personalized and timely cardiovascular screening or interventions.[75]



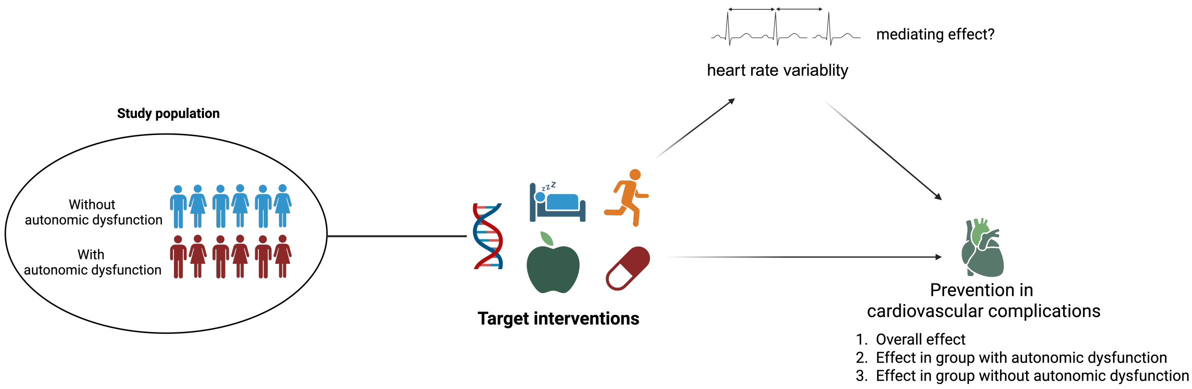
(Source: Author)

Figure 7.2.: **Conceptual model for risk stratification by autonomic dysfunction**

7.3. Effective causal modifiable marker

The findings support a potential etiological link between long-term HRV and CVD risk, providing preliminary evidence consistent with a causal relationship. However, the observed association does not confirm causality, and further research is needed to determine whether HRV directly influences CVD outcomes. While randomized controlled trials are the gold standard for establishing causality, isolating the direct effect of HRV is particularly challenging. Interventions that affect HRV often do so indirectly through changes in weight, inflammation, or insulin sensitivity. Similarly, pharmacological treatments may improve HRV as a secondary effect, such as through blood pressure reduction from antihypertensive medications. This makes it difficult to determine whether modifying HRV itself leads to improved cardiovascular outcomes.

To address these limitations, modern epidemiological methods such as Mendelian randomization and structured causal mediation analysis offer promising alternatives. These approaches can be used to infer causality from observational data and estimate indirect effects using trial data. Notably, no GWAS has yet investigated the genetic determinants of long-term HRV. Establishing such associations is essential for understanding its genetic architecture and for using genetic variants as unconfounded proxies to assess HRV's causal role in CVD. However, a challenge arises from findings in short-term HRV, which show considerable pleiotropy. This may complicate the use of Mendelian randomization, as the method relies on the assumption of no horizontal pleiotropy.[176]



(Source: Author)

Figure 7.3.: **Suggested mediation of HRV by intervention/observation in the prevention of CVD**

Future cardiometabolic intervention trials and longitudinal cohorts, whether focused on lifestyle or pharmacological strategies, should, where feasible, include repeated HRV measurements.[77] In trials, structured mediation analyses are enabled and used to determine whether modifying autonomic function is associated with sustained improvements in cardiovascular outcomes. Such evidence could clarify whether interventions like antihypertensive medications or lifestyle changes in physical activity, diet, and sleep can causally and sustainably improve CVD risk through HRV modulation. Using observational data with repeated measurements, similiar interventions could be emulated by targeted trails. A further option is to use a CVD polygenic risk score as the exposure, CVD as the outcome, and HRV as a mediator to test whether genetic variation in CVD-related traits is mediated through HRV.[177]

8.Conclusion

This dissertation has investigated how autonomic dysfunction, assessed by HRV and CARTs, is associated with cardiovascular complications across different stages of glucose metabolism. The findings support the hypothesis that autonomic dysfunction is an early and independent marker of cardiovascular risk.

Autonomic dysfunction was associated with higher arterial stiffness not only in individuals with T2D, but also in those with prediabetes and normal glucose metabolism. A particularly pronounced association was observed in individuals with prediabetes, where lower multiday HRV was linked to a higher risk of cardiovascular disease, heart failure, and mortality. These findings suggest that autonomic dysfunction may contribute to cardiovascular complications even before the onset of T2D, potentially through a modifying effect during the early stages of dysglycemia. Among individuals with T2D, standardized CARTs identified those with CAN who had a higher risk of heart failure, even when asymptomatic and not classified as high risk by risk scores.

Early detection is important, as CVD and heart failure are associated with reduced life expectancy and quality of life. This dissertation has demonstrated the potential of autonomic dysfunction as a clinically relevant marker of cardiovascular risk across the full spectrum of glucose metabolism, including stages prior to the onset of T2D.

Modern epidemiological methods, e.g. mediation analysis and Mendelian randomization, are needed to ascertain causality. Moreover, further research is needed to determine the clinical utility of long-term HRV and CARTs in risk stratification, including their potential to timely initiate individually adapted health assessment or intervention.

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Summary

Background
Cardiovascular autonomic dysfunction is a diabetes complication that has been increasingly recognized as a contributor to cardiovascular disease (CVD). Yet, its clinical utility has remained unclear across stages in the glucose metabolism spectrum.

Aim
This dissertation was aimed at investigating how autonomic dysfunction, assessed by heart rate variability (HRV) and cardiovascular autonomic reflex tests (CARTs), is associated with cardiovascular complications in individuals with normal glucose metabolism (NGM), prediabetes, and type 2 diabetes (T2D).

Methods
In The Maastricht Study, long-term (24 hours) HRV, together with markers of aortic (carotid-femoral pulse wave velocity) and carotid stiffness (carotid artery distensibility), was quantified in 3,673 individuals with either NGM, prediabetes, or T2D. A cross-sectional analysis was conducted to quantify the association between long-term HRV and measures of arterial stiffness across NGM, prediabetes, and T2D. In the ADDITION-PRO cohort, HRV over multiple days was quantified in 1,627 individuals at high risk of diabetes and was linked to Danish National Health Registries for outcomes of CVD, heart failure, and all-cause mortality. Time-to-event analysis was applied to quantify the association between multiday and hourly HRV and CVD, heart failure, and all-cause mortality. In The CANCAN Study of 176 individuals with T2D visiting outpatient clinics, CARTs were performed, with two or more abnormal values defined as cardiovascular autonomic neuropathy (CAN). A cross-sectional analysis was conducted to evaluate the association between CAN and heart failure indices, with N-terminal pro-B-type natriuretic peptide (NT-proBNP) as the primary index.

Results
Lower long-term HRV was associated with higher aortic and carotid stiffness. These associations were observed across all stages of glucose metabolism and were particularly pronounced in individuals with prediabetes or T2D. In a population at high risk of diabetes, lower multiday HRV was linked to a higher incidence of CVD, heart failure, and mortality. Hourly HRV was particularly indicative for CVD in response to the morning period from 6AM to 7AM. In the population with T2D in secondary care, CAN was associated with higher heart failure risk by elevated NT-proBNP, even in asymptomatic individuals and those classified as low-to-moderate risk by conventional risk scores.

Conclusion
Autonomic dysfunction was found to be associated with cardiovascular complications across the glycemic continuum. These findings suggest that HRV and CARTs may serve as early, independent markers of cardiovascular risk and become more relevant to individuals with dysglycemia, even before the onset of T2D.

Resume

Baggrund
Kardiovaskulær autonom dysfunktion er en diabeteskomplickation, som i stigende grad risiko faktor for hjertekarsygdom. Alligevel er dens kliniske anvendelighed fortsat uklar på tværs af stadier af glukosemetabolisme.

Formål
Denne afhandling havde til formål at undersøge, hvordan autonom dysfunktion, målt igennem hjertefrekvensvariabilitet (HRV) og kardiovaskulære autonome reflekstests (KARTs), er associeret med kardiovaskulære komplikationer hos personer med normal glukosemetabolisme (NGM), prædiabetes og type 2-diabetes (T2D).

Metoder
I Maastricht studiet blev langvarig (24 timer) HRV, sammen med markører for aortastivhed (karotis-femoral pulsbølgehastighed) og karotis-stivhed (karotisarteriens distensibilitet), kvantificeret hos 3.673 personer med enten NGM, prædiabetes eller T2D. En tværsnitsanalyse blev udført for at kvantificere sammenhængen mellem langvarig HRV og mål for arteriel stivhed på tværs af NGM, prædiabetes og T2D. I ADDITION-PRO-kohorten blev HRV over flere dage kvantificeret hos 1.627 personer med høj risiko for diabetes og blev koblet til de danske nationale sundhedsregistre for udfald af hjertekarsygdomme, hjertesvigt og dødelighed af alle årsager. En time-to-event analyse blev anvendt til at kvantificere sammenhængen mellem flerdages og timebaseret HRV og CVD, hjertesvigt og dødelighed. I CANCAN-studiet af 176 personer med T2D, der besøgte ambulatorier, blev KARTs udført, hvor to eller flere unormale værdier blev defineret som kardiovaskulær autonom neuropati (KAN). En tværsnitsanalyse blev udført for at evaluere sammenhængen mellem KAN og hjertesvigtindikatorer, med N-terminal pro-B-type natriuretisk peptide (NT-proBNP) som primær indikator.

Resultater
Lavere langvarig HRV var associeret med højere aorta- og karotis-stivhed. Disse sammenhænge blev observeret på tværs af alle stadier af glukosemetabolisme og var særligt udtalte hos personer med prædiabetes eller T2D. I en population med høj risiko for diabetes var lavere flerdages HRV forbundet med højere forekomst af CVD, hjertesvigt og dødelighed. Timebaseret HRV var særligt indikativ for CVD i morgenperioden fra kl. 6 til 7. I populationen med T2D i sekundærsektoren var KAN associeret med højere risiko for hjertesvigt ved forhøjet NT-proBNP, selv hos asymptomatiske personer og dem klassificeret som lav-til-moderat risiko i konventionelle risikoscorer.

Konklusion
Autonom dysfunktion blev fundet at være associeret med kardiovaskulære komplikationer på tværs af det glykæmiske kontinuum. Disse fund tyder på, at HRV og CARTs kan fungere som tidlige, uafhængige markører for kardiovaskulær risiko. Autonom dysfunktion er mere relevant for personer med dysglykæmi, selv før udviklingen af T2D.

A.