**Cardiovascular autonomic dysfunction and cardiovascular disease: the ADDITION-PRO study**

Jonas R. Schaarup1, 2, Lasse Bjerg1,2, Christian S. Hansen4, Erik L. Grove, Signe T. Andersen2, Dorte Vistisen4, 5, Annelli Sandbæk1,2, Daniel R. Witte1,2

1 Department of Public Health, Aarhus University, Bartholins Allé 2, Aarhus, 8000, Denmark

2 Steno Diabetes Centre Aarhus, Palle Juul-Jensens Boulevard 11, Aarhus, 8200, Denmark

3 Aarhus University, Aarhus, Nordre Ringgade 1, Aarhus, 8000, Denmark

4 Steno Diabetes Centre Copenhagen, Ib Juuls Vej 83, Herlev, 2730, Denmark

5 Department of Public Health, University of Copenhagen, Øster Farimagsgade 5, Copenhagen, 1353, Denmark

**Word count**: xxxx (excluding figure legends)

**Number of Figures and Tables**:

**Corresponding author:**  
Jonas R. Schaarup, MSc in Public Health

Department of Public Health, Aarhus University, Bartholins Allé 2, Aarhus, 8000, DK

E-mail: jfrscha@ph.au.dk

Phone number: +45 29 93 68 99

**Abbreviations**

CAN: Cardiovascular autonomic neuropathy

CVD: Cardiovascular disease

ECG: Electrocardiogram

HRV: Heart rate variability

rHR: Resting heart rate

SDNN: The standard deviation of normal-to-normal R-R intervals

mHR: mean heart rate

**Abstract**

**Objective**

To examine the association between components in long-term heart rate variability and cardiovascular disease.

**Methods**

Week-long heart rate variability and mean heart rate (mHR) were measured in 2,088 participants from the ADDITION-PRO between 2009-2011. Cardiovascular disease events and all-cause mortality were followed up in Danish patient registries until 2021.

**Results**

**-**

**Conclusion**

*-*

Introduction

Cardiovascular disease remains the world's leading cause of death. Heart failure remains a diabetes complication that leads to poorer prognosis and lower quality of life. Thus, early identification of high-risk individuals is needed for improved risk detection and prevention.

People with a high risk of diabetes have an increased risk of CVD and mortality [1]. The increased risk consists of a combination of elevated levels of body weight, blood glucose, low-density lipid cholesterol, triglycerides, and blood pressure, as well as lifestyle factors of unhealthy diet, smoking, and physical inactivity [2]. Among individuals with a high risk of diabetes, diabetes-related microvascular and macrovascular complications are present and can accelerate the CVD risk [3] [4]. Autonomic cardiovascular dysfunction and cardiovascular autonomic neuropathy are present throughout the diabetic continuum [5] increasing the risk of CVD [6]. Cardiovascular autonomic dysfunction leads to sympathetic overactivity that likely increases arterial shear stress and causes alteration in cardiac function. Heart rate variability (HRV) is recognized as a valid biomarker for assessing cardiovascular autonomic function, measuring the heartbeats controlled by the sinoatrial node that receives input from the autonomic nervous system. HRV expresses the beat-to-beat variation between normal RR intervals that are calculated into time domain indices. Most studies have demonstrated autonomic dysfunction linked with CVD with changes in short-term heart rate variability which yield resting heart rate patterns in supine rest during inspiration and expiration. Few studies have investigated long-term 24-hour HRV and its association with CVD. However, the long-term measures of a single measurement day can reflect random activities on a particular day i.e., physical activity, sleep and more. Longer heart rate recordings over multiple days capture heart rate activity under regular free-living conditions and thus may yield a more valid assessment of day-to-day autonomic function.

*Theme: Circadian variation in HRV and CVD*

Earlier studies have shown that the circadian variation in heart rate and HRV are present, presumably affected by a complex interplay between endogenous rhythmicity and response to external cardiovascular stressors. Hence, different time points could reflect autonomic responses in the body’s different physiological states. With the highest CVD incidence observed between early morning and noon, the variation timing of CVD events through the circadian rhythm exists [7]. No single mechanism can be pointed out to explain the diurnal variation, but the cardiovascular response to the increase in sympathetic activity in the morning could be a potential indicator. Hence, the diurnal variation in cardiovascular autonomic response may capture important information about future CVD risk.

*Theme: The usefulness of detecting autonomic dysfunction and CAN by HRV. Technologies facilitate opportunities for continuous monitoring in free-living conditions.*

In diabetes risk, we are observing more wearable technologies taken into use for treatment and prevention i.e., continuous glucose monitors. Additionally, the increase in the use of smartwatches and other wearable devices allows the monitoring of people's physiological data i.e. pulse, heart rate, heart rate variability, blood oxygen, and lifestyle measurement of physical activity. Wearable devices have future potential for improved monitoring and target intervention based on physiological data. However, how to use large data from wearables remains to be investigated.

*Theme: What to look for, at which time point and how well the predictor is*

Studies have investigated 10-second to 24-hour recordings of HRV and its association with CVD [6]. However, studies comparing short-term recordings with long-term recordings and comparing hourly time points in the diurnal clock are scarce. Such comparisons could inform what specific types of data from free-living heart rate patterns have clinical implications for cardiovascular disease.

*Theme: Aim and hypotheses*

This study aims to zoom from week-long to hourly recordings of HRV and investigate the CVD risk information of long-term and diurnal variation in hourly HRV. Our hypothesis is 1) week-long HRV measures are more robust in capturing detailed HRV patterns and giving information about CVD risk. 2) The assessed risk of CVD varies between hourly HRV throughout the diurnal clock.

Methods

*Study population*

Data were obtained from 1,800 participants with a high risk of diabetes in the Danish ADDITION-PRO study 2009-2011.

CHECK HANAN paper!!!!

*Exposure*

* Describe ActiHeart data collection and preprocessing

*Then, the normal-to-normal (NN) sinus rhythm was determined from the recordings with an automated algorithm to identify R-R intervals without the presence of arrhythmias, ectopic beats and/or branch blocks. (CHECK ACTHIHEART GUIDE)*

Based on ActiHeart, we had measures of heart rate traces for up to 7 days. Mean heart rates with prediction intervals were obtained every 30-second epoch. We did not have access to the series of successive IBI, in the period of measurement. Therefore, we generated IBIs in the period of every 30-second interval based on the 30-second epoch of mean heart rate and prediction intervals. As earlier data from studies have shown that IBIs are normally distributed per 30-second epoch, we generated the IBI 30-second distribution by using mean heart rate and standard deviation. To calculate SD from prediction intervals, we ensured that the prediction intervals differed symmetrical from the mean by calculating the difference between the upper and lower prediction intervals from the mean heart rate and visually observing their symmetry over time. Using the RHRV (version xx) package in R, we calculated HRV indices. As we did not have successive time-series measurements, we only used HRV indices that are based on the distribution of RR intervals which is only available in time-domain and geometrical HRV indices. We included the standard deviation between normal-to-normal heartbeat intervals (SDNN), the standard deviation of the 5-minute average NN intervals (SDANN), the SDNN index (SDNNi), and the triangular interpolation of NN interval histogram (TINN). All HRV indices were calculated by week, 24-hour cycle, and hour per day. As there were multiple cycles from both 24 hours and hours, we took the mean of all combined cycles.

To account for cardiac automatism from concurrent rHR, interbeat interval (IBI) was regressed on the logarithm of each HRV index. From each model, we added individual intercepts and residuals together and transformed the sum to the original HRV scale. (see function in supplementary)

*Outcome*

We defined CVD events by including ICD-10 diagnostic codes for stroke, myocardial infarction, heart failure, and cardiovascular death, and surgical codes for cardiovascular revascularization. Information on CVD events and mortality, as well as all-cause mortality was followed up in the Danish National Patient Registries until 2021.

*Covariates*

Lifestyle factors of smoking and alcohol consumption (average units per week), as well as CVD disease history, and anti-hypertensive, glucose-lowering, and lipid-lowering medication use, were reported through a self-reported questionnaire. Physical activity was hourly estimated based on combined accelerometry and heart rate data from ActiHeart recordings. The hourly estimates were summarized into the proportion of time in the metabolic equivalent of task (MET) categories and physical activity energy expenditure kilojoules per day (PAEE) . Furthermore, sleep where defined by the period of MET below 1.04, within the . Blood measurement haemoglobin A1c (HbA1c), oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), triglycerides, total-, high density- (HDL) and low density- (LDL) cholesterol were derived from blood samples. Body mass index (BMI), waist circumference, and systolic and diastolic blood pressure were measured at the participant's clinical examination. From the Danish Registries, we collected information on 10-year prior CVD events, medication use, and socioeconomic status at baseline (length of education, income, work status).

*Statistical analysis*

Baseline characteristics were described to characterize the distribution of continuous variables (median, 25th and 75th percentile) and frequencies (numbers, percentage) for categorical variables.

Individual risk time was determined at baseline data collection in ADDITION-PRO (2009-2011) and ended at the time of CVD events or the end of follow-up (31 December 2021). First, we used Poisson regression models to investigate the association between heart rate variability indices and four-point MACE (including myocardial infarction, stroke, cardiovascular revascularization, and heart failure) as well as all-cause mortality. We fitted three models. Model 1 included adjustments of age and sex. Model 2 was further adjusted for education, alcohol consumption, smoking behavior, physical activity, systolic blood pressure, body mass index, total cholesterol, and Hba1c, and Model 3 with the addition of anti-hypertensive, and glucose-lowering medication, and prior CVD event before baseline. In the analysis of CVD, we accounted for competing risks of death. To investigate for non-linearity of HRV (splines), we included defined knots based on percentiles in HRV distribution. We then split risk time along with individual follow-up time in one-year intervals to estimate the incidence rate per 1000 years at different levels of HRV.

Because of biological sex differences in the development of CVD and women genuinely having a lower heart rate variability than men, we stratified the analysis by sex.

Second, we performed a similar analysis for 24-hour HRV and each hour of HRV in a 24-hour cycle. In the analysis of hourly HRV, we did not include knots and spline.

Both multiple imputation and complete case analyses will be conducted in the R statistical computing environment (version X).

Zoom study:

* Weekly HRV
* Mean daily HRV.
  + Stratified by weekday.
* Mean hour HRV
  + Stratified by hour.

Results

From the entire cohort, 1748 (84%) participants had HRV measured and calculated (**Fig. 1**). The mean interval of follow-up time for CVD and mortality was 12.5 years. The study population included 54% of men with a mean (SD) age of 66.1 (6.8) years and a mean BMI of 27.8 kg/m2 (4.7). The mean week-long SDNN was 139.1 ms (32.7) and the heart rate was 73.2 bpm (9.3). Ninety-seven (5.5%) had prior CVD events while 46% had hypertension. Further characteristics of the participants are described in **Table 1**.

The study population were in total followed up for 19,251 person-years until the end of follow-up time or mortality event (Individual mean follow-up: 9.21 years). There were 184 incident cases of CVD (10 per 1000 person-years) and 181 all-cause mortality events (9.4 per 1000 person-years).

**Long-term HRV**

In model 1, per SD higher week-long SDNN was inversely associated with a 0.80 (CI: 0.68; 0.93) lower incidence rate ratio of CVD and 0.750 (CI: 0.63; 0.88) lower incidence rate ratio. Full adjustment of confounders and correction heart mean IBI did not materially change the estimates. TINN showed similar association with CVD event, but was not observed in the SDANN, SDNNi, mHR. For mortality

IRR from the week-long HRV was comparable with mean 24-hour SDNN.

**24-hour HRV**

**Hourly HRV**

Discussion

* Main findings

Week-long SDNN and linked with a 20% risk reduction per SD for both CVD and mortality. SDANN and SDNNi try to account for non-stationarity activity. These indices were not linked with any outcome after further adjustments. Total variation from SDNN and TINN were therefore strongest associated. Taking the mean of multiple 24-hour HRV did not change the association compared with week-long. (should we examine a random 24 hour cycle?). When the HRV periods were divided into hourly cycles, the SDNN response in the morning from 6:00-7:00 showed an association with increased CVD risk. Whereas the hours during the early morning, afternoon, evening and night were more consistently associated with all-cause mortality risk.

Week-long mHR is not associated with CVD but with heart failure and all-cause. mortality. However, night-time mHRs are linked with a higher risk of CVD (maybe because the individual is non-dipping their heart rate during sleep).

* Confounders
* Medication
* If the morning HRV indicates high-risk CVD – the sympathetic system increases arterial pressure and triggers fatal arrhythmia for sudden cardiac death.

*Strengths and limitations*

* *Selection bias and collider bias*
* *Long follow-up*
* *Long term HRV*
* *No successive IBI data*

*Conclusion*

.

**Acknowledgements**

We would like to acknowledge all participating women and men in the ADDITION-PRO, as well as research scientists, study and data managers and clinical and administrative staff who made the study possible. We would like to thank Luke W. Johnston for his expertise in data wrangling, as well as Else-Marie Dalsgaard and Kasper Norman for their help with using Danish registries. We will also express our gratitude to Marianne Pedersen for her contribution as data manager on ADDITION-PRO.

**Authors' contributions**

Study concept and design: JRS, DRW, LB, DV, ELG, CSH. Contributed to the data: DRW, DV. Planning the statistical analysis: JRS, DRW, LB. Conducted the statistical analysis: JRS. All authors contributed to, critically revised, and approved the final version of the manuscript. JRS is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Funding**

JFRS, DRW, AS, and LB are employed at Steno Diabetes Center Aarhus, and CSH and DV are employed at Steno Diabetes Center Copenhagen. Both institutions are partly funded by a donation from the Novo Nordisk Foundation. The funders had no role in the design of the study. DRW and JRS are supported by EFSD/Sanofi European Diabetes Research Programme in diabetes associated with cardiovascular disease.

**Ethics**

The UK NHS Health Research Authority London-Harrow ethics committee approved the study, which was conducted in accordance with the Helsinki Declaration with written informed consent from all participants.

**Conflicts of interests**

All the authors declare that there is no duality of interest associated with their contribution to this manuscript.

**Availability of data and materials**

ADDITION-PRO data

References

1. Barr ELM, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, et al. Risk of Cardiovascular and All-Cause Mortality in Individuals With Diabetes Mellitus, Impaired Fasting Glucose, and Impaired Glucose Tolerance. Circulation. 2007;116(2):151-7. doi: doi:10.1161/CIRCULATIONAHA.106.685628.

2. Beulens J, Rutters F, Rydén L, Schnell O, Mellbin L, Hart H, et al. Risk and management of pre-diabetes. European Journal of Preventive Cardiology. 2020;26(2\_suppl):47-54. doi: 10.1177/2047487319880041.

3. Houben AJHM, Stehouwer CDA. Microvascular dysfunction: Determinants and treatment, with a focus on hyperglycemia. Endocrine and Metabolic Science. 2021;2:100073. doi: <https://doi.org/10.1016/j.endmts.2020.100073>.

4. Sörensen BM, Houben AJHM, Berendschot TTJM, Schouten JSAG, Kroon AA, van der Kallen CJH, et al. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction. Circulation. 2016;134(18):1339-52. doi: 10.1161/CIRCULATIONAHA.116.023446.

5. Coopmans C, Zhou TL, Henry RMA, Heijman J, Schaper NC, Koster A, et al. Both Prediabetes and Type 2 Diabetes Are Associated With Lower Heart Rate Variability: The Maastricht Study. Diabetes Care. 2020;43(5):1126-33. doi: 10.2337/dc19-2367.

6. Hillebrand S, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, et al. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose–response meta-regression. EP Europace. 2013;15(5):742-9. doi: 10.1093/europace/eus341.

7. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the framingham heart study population. The American Journal of Cardiology. 1987;60(10):801-6. doi: <https://doi.org/10.1016/0002-9149(87)91027-7>.

**Tables and figures**

**Table 1:** Baseline characteristics

**Figure 1:** Study flowchart

**Figure 2*:* Diurnal HRV and HR**

**Figure 3: Splines for weekly and daily**

Variable list

| **Variables from ADDITION-PRO dataset** | Available at DST | variable name |
| --- | --- | --- |
| Age (years) | x | age\_fup |
| Sex (male) | x | p\_gv\_sex |
| Socioeconomic status | x |  |
| Smoking status | x | smoke\_q\_4\_a  smoke\_cigarettes\_day\_q4\_b  p\_gq\_smoke |
| Physical activity (PAEE kj\_kg\_day) |  | PAEE\_kj\_kg\_day |
| *Step test results* |  |  |
| 1. VO2 max (Cardiorespiratory function) |  | Pred.VO2max |
|  |  |  |
| Alcohol comsuption (units per week) | x | alkohol\_week\_unit\_total |
| BMI (kg/m2) | x | bmi |
| Height (cm) |  | height |
| Weight (kg) |  | ????? |
| Waist circumference (cm) | x | waist\_av |
| Hip circumference (cm) |  | hip\_av |
| Fat percentage ( % ) | x | fat\_pc |
| Visceral fat |  | vat |
| Subcutaneous fat |  | sat |
| Systolic blood pressure (mm hg) | x | sbp\_av |
| Diastolic blood pressure (mm hg) | x | dbp\_av |
| Hours fasting |  | hours\_fast~g |
| HbA1c (%) |  | p\_lab\_hba1c |
| LDL cholesterol (mmol/L) |  | p\_lab\_ldl |
| HDL cholesterol (mmol/L) |  | p\_lab\_hdlc |
| Total cholesterol (mmol/L) |  | p\_lab\_chol |
| Triglycerides (mmol/L) |  | p\_lab\_trig |
| Insulin resistance |  | homa\_ir |
| Beta cell function |  | homa\_b |
| Insulin sensitivity |  | homa\_s |
| Insulin sensitivity (Gutt’s index) | Doubblecheck with DW | isi |
| 2 hour glucose tolerance test (0 min plasma glucose) |  | p\_lab\_pglu\_0 |
| 2 hour glucose tolerance test (30 min plasma glucose) |  | p\_lab\_pglu\_30 |
| 2 hour glucose tolerance test (120 min plasma glucose) |  | p\_lab\_pglu\_120 |
| 2 hour glucose tolerance test (0 min GIP) |  | p\_lab\_gip\_0 |
| 2 hour glucose tolerance test (30 min GIP) |  | p\_lab\_gip\_30 |
| 2 hour glucose tolerance test (120 min GIP) |  | p\_lab\_gi~120 |
| 2 hour glucose tolerance test (0 min GLP-1) |  | p\_lab\_glp1\_0 |
| 2 hour glucose tolerance test (30 min GLP-1) |  | p\_lab\_glp~30 |
| 2 hour glucose tolerance test (120 min GLP-1) |  | p\_lab\_gpl~120 |
| 2 hour glucose tolerance test (0 min insulin response) |  | p\_lab\_insu\_0 |
| 2 hour glucose tolerance test (30 min insulin response) |  | p\_lab\_insu\_30 |
| 2 hour glucose tolerance test (120 min insulin response) |  | p\_lab\_in~120 |
| Kidney function markers | ask marianne |  |
| Mean heart rate (bpm) |  |  |
| Median heart rate (bpm) |  |  |
| *Heart rate variability indices*   * Standard deviation of Normal to Normal intervals (ms) * SDANN * SDNNi * HRVi * TINN |  |  |
| Medication use?   * Glucose lowering medication * Cardioprotective medication   + antihypentensive     - types of antihypertensive * lipid lowering medication |  |  |