**Autonomic dysfunction and cardiovascular disease: the ADDITION-PRO study**

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**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

**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 (rHR) was measure 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**

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Introduction

*CVD epidemiology in prediabetes – heart failure*

Cardiovascular disease remains the world leading cause of death. While

Early detection 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. The increased risk consists 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. Among individuals with a high risk of diabetes, diabetes-related complications may accelerate the CVD risk. Autonomic cardiovascular dysfunction and cardiovascular autonomic neuropathy have been shown to be present throughout the diabetic continuum increasing the risk of CVD. Autonomic cardiovascular 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. Hence, HRV expresses the beat-to-beat variation between normal RR intervals, which can be calculated into time- and frequency 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 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.

*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 timepoint 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 is present [1]. No single mechanism can be pointed out to explain the diurnal variation, but it has been suggested to be linked to cardiovascular response to the increase in sympathetic activity in the morning. Hence, the diurnal variation in cardiovascular autonomic response may capture important information about future CVD risk.

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

In diabetes, 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 treatment based on physiological data. However, how to use large data from wearables remains to be investigated.

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

Studies have investigated from 10 seconds to 24-hour recordings of HRV and its association with CVD. 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.

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) weeklong HRV measures are more robust in capturing detailed HRV patterns and giving information about CVD risk. 2) 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.

*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)*

*To account for cardiac automatism from concurrent rHR, we also included inter-beat interval (IBI) corrected HRV indices (cHRV): cSDNN, following the approach previously described in Van Roon et al (see formula in supplemental material). (DISCUSS THIS MATTER WITH SUP- CSH MIGHT HAVE SOMETHING TO SAY)*

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 time-series of successive IBI, in the period of measurement. Therefore, we generated IBIs in the time span 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 assured that the prediction intervals were symmetric and differed from the mean by calculating the difference between the upper and lower prediction interval 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 can only use HRV indices that are based on the distribution of RR intervals which is only available in time-domain and geometrical HRV indices. We included include standard deviation between normal-to-normal heartbeat intervals (SDNN), the standard deviation of the 5-minute average NN intervals (SDANN), SDNN index (SDNNi), HRV triangular index, and triangular interpolation of NN interval histogram (TINN). All HRV indices were calculated by week, day, and hour per day.

*Outcome*

We defined CVD by 5-point MACE including, stroke, myocardial infarction, heart failure, cardiovascular revascularization, and cardiovascular death. 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 based on combined accelerometry and heart rate data from ActiHeart recordings (measures PAEE and MET). 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 prior CVD events, medication use, and socioeconomic status (length of education, income, work status).

*Statistical analysis*

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

Zoom study:

* Weekly HRV
* Mean daily HRV
* Mean hour HRV
* CVD timepoint of the day???

Predictive value of most risk hour, day, and weekly.

* Split time explanation

First, we used Poisson regression models to investigate the association between week-long heart rate variability indices and five-point MACE (including myocardial infarction, stroke, and heart failure) as well as all-cause mortality. We fitted two models. Model 1 will include 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. We accounted for competing risks of death. To investigate for non-linearity (splines), we included defined knots based on percentiles in HRV distribution. 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 each hour of HRV in a day. In the analysis of hourly HRV, we did not include knots and spline.

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

Results

From the entire cohort, 1500 (xx%) participants had HRV measured and calculated (**Fig. 1**). The mean interval of follow-up time for CVD and mortality was 10.4 years. Further characteristics of the participants are described in **Table 1**. Xx had prior CVD events.

Discussion

* Main findings
* 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*

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**Authors' contributions**

Study concept and design: JRS, DRW, LB, DV, 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, had 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.

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**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

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**Tables and figures**

**Table 1:** Baseline characteristics

**Figure 1:** Study flowchart

**Figure 2*:* NOT REAL RESULT (ONLY VISUALISATION)**

A red line graph with numbers

Description automatically generated

**Figure 3: NOT REAL RESULT (ONLY VISUALISATION)**

A graph of a graph showing the number of hrv hours

Description automatically generated

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 |  |  |