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FULL RESEARCH PAPER
Diabetes and metabolic disorders

Circadian heart rate fluctuations predict cardiovascular and all-cause mortality in type 2 and type 1 diabetes: a 21-year retrospective longitudinal study

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Aims Circadian heart rate (HR) fluctuations are associated with cardiovascular health. We examined their relationship with microvascular disease and long-term survival in patients with diabetes.

Methods and results In this secondary analysis from the CHAMP1ON cohort of 497 adults with metabolic disease, 349 participants who had type 1 or type 2 diabetes, baseline 24-h ambulatory blood pressure and HR monitoring (ABPM), and survival data over a 21-year observational follow-up were included. Clinical features, microvascular complications, and mortality rates were examined in participants with low circadian HR fluctuations [24-h HR standard deviation (SD) below the median of 30.4] and blunted nocturnal HR dip (<10%). Low 24-h HR SD and blunted nocturnal HR dip were associated with an adverse cardiometabolic risk profile and 12–23% higher prevalence of cardiac autonomic neuropathy and nephropathy. After 6251 person-year follow-up [21.0 (14.0–21.0) years], a total of 136 (39%) deaths occurred, of which 100 (68%) of cardiovascular cause. The low 24-h HR SD group had a higher risk for both cardiovascular [adjusted hazard ratio (aHR) 2.00, 95% confidence interval (CI) 1.30–3.08, $P = 0.002$] and all-cause mortality (aHR 1.61, 95% CI 1.13–2.29, $P = 0.009$), compared with high 24-h HR SD. Similarly, patients with blunted nocturnal HR dip had a higher risk for cardiovascular (aHR 1.63, 95% CI 1.08–2.46, $P = 0.019$) and all-cause mortality (aHR 1.69, 95% CI 1.20–2.38, $P = 0.003$), compared with those with preserved nocturnal HR dip.

Conclusion Impaired circadian HR fluctuations are associated with microvascular disease and long-term cardiovascular and all-cause mortality in diabetes. The ABPM-derived HR measures may provide a widely available and inexpensive risk stratification tool in this high-risk population.

Lay summary Circadian heart rate (HR) fluctuations are associated with cardiovascular health. We examined their relationship with microvascular disease and long-term survival in patients with diabetes. Impaired HR fluctuations measured by 24-h ambulatory blood pressure and HR monitoring (ABPM) were associated with an adverse cardiometabolic risk profile, higher prevalence of cardiac autonomic neuropathy and nephropathy, and higher risk for cardiovascular and all-cause mortality over a 21-year follow-up. The ABPM-derived HR measures may provide a cost-effective risk stratification tool in this high-risk population.

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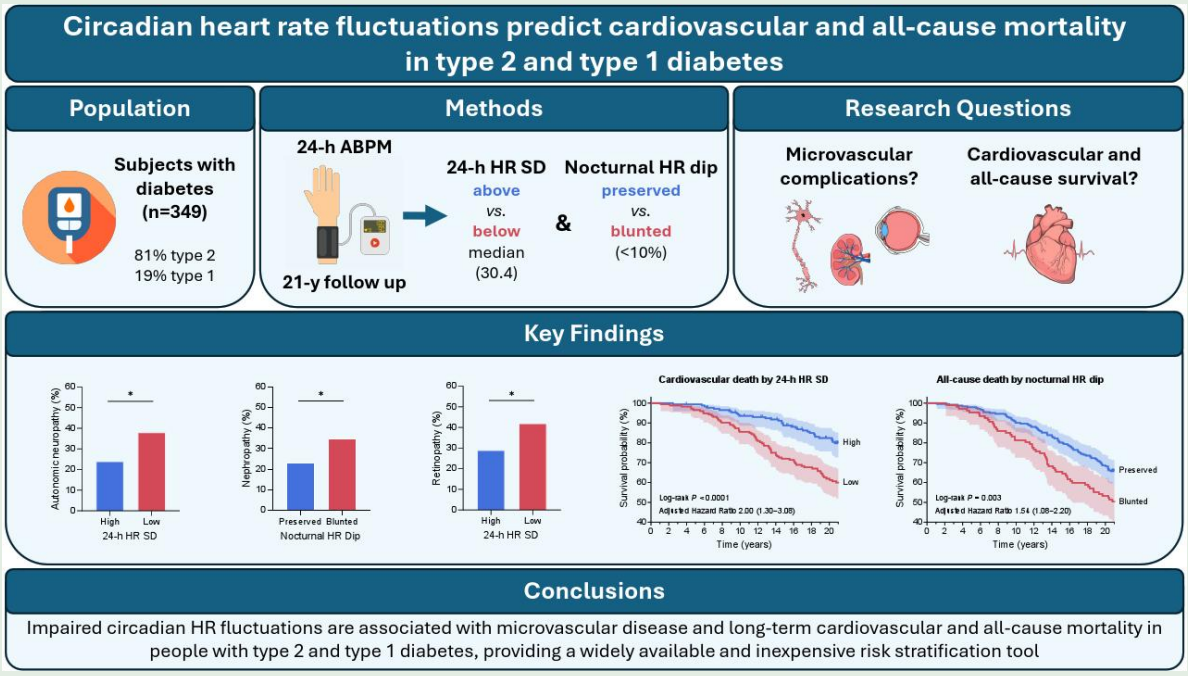
Graphical Abstract



Keywords Heart rate variability • Ambulatory blood pressure monitoring • Nocturnal dip • Mortality • Diabetes

Introduction

Heart rate (HR) and its physiological variability have been increasingly recognized as indicators of cardiovascular and overall health.[1,2] In the late 1990s, both resting HR[3] and day-to-night variations in HR[4] were associated to survival in elderly subjects with hypertension. A blunted nocturnal HR dip, defined as a HR decline lower than 10% from daytime to nighttime, has later been associated with an increased risk of death for all causes over a 7-year follow-up in a cohort of 3957 subjects referred for ambulatory blood pressure and HR monitoring (ABPM), independently from potential confounding factors.[5] Further confirmed by other reports,[6] impaired circadian HR patterns are emerging as novel cardiovascular risk factors with promising applications in high-risk populations.[7]

Diabetes has profound consequences on the cardiovascular system leading to higher incidence and mortality from cardiovascular events even after accounting for traditional risk factors, whose mechanisms remain to be elucidated.[8] Therefore, novel means to better stratify cardiovascular risk in this population are intensely warranted. Impaired cardiovascular rhythmicity is typically observed in both type 2 (T2D)[9,10] and type 1 diabetes (T1D),[11] prompting growing interest in its relationship with cardiovascular disease. Specifically, alterations in resting HR[12] or day–night BP and HR patterns have been related to autonomic dysfunction,[13,14] diabetic complications,[13,15,16] and higher risk of 4- to 5-year cardiovascular events.[14,17,18] Nevertheless, available studies are either small, focused on resting HR or nocturnal HR dip alone, combining HR and BP patterns, or examining the relationship of HR patterns to specific complications and/or short-term cardiovascular outcomes. To the best of our knowledge, a comprehensive evaluation of the clinical features and complication burden of individuals with diabetes and altered HR variations is currently unavailable. Moreover, there is a lack of long-term survival data in relation to circadian HR fluctuations in the diabetic population.

To fill these gaps in knowledge, our co-primary objectives are (i) to describe the clinical, metabolic, and cardiovascular features of reduced daily HR fluctuations in patients with diabetes; (ii) to quantify the associated microvascular complication burden; and (iii) to explore the long-term prognostic value for cardiovascular and all-cause mortality. To these aims, we retrospectively analysed cross-sectional and 21-year longitudinal data of participants with diabetes from the '*CHronic diabetes complications and All-cause Mortality in Pisa from 1999 ONwards*' (CHAMP1ON) study cohort, whose extensive metabolic and cardiovascular characterization, comprehensive assessment of microvascular complications, and long-term follow-up grant a thorough depiction of the features associated to impaired HR fluctuations while allowing adjustment for multiple potential confounders. We hypothesized that impaired 24-h HR fluctuations are associated with a higher diabetic complication burden and long-term mortality. Thus, early identification of impaired HR patterns could provide a simple and unexpensive tool for cardiovascular risk stratification in this high-risk population, in which residual risk estimation remains a significant clinical issue.

Methods

Study population

We retrospectively analysed clinical data of 497 consecutive outpatients attending the University Hospital of Pisa between 1999 and 2000, who were recruited into the CHAMP1ON observational study cohort, as previously described.[19–21] Inclusion criteria of the CHAMP1ON study were age between 18 and 75 years, both women and men, history of diabetes or prediabetes (either impaired fasting glucose or impaired glucose tolerance). Most patients presented at baseline with comorbidities commonly associated with diabetes, including dyslipidaemia, hypertension, and/or obesity.

Exclusion criteria were concomitant acute or chronic diseases associated with a significant reduction in life expectancy, including lung, hepatic, neoplastic or inflammatory diseases, end-stage chronic kidney disease (CKD), and cardiovascular events in the previous 12 months.

In the present study, participants of the CHAMP1ON study cohort without diabetes ($n = 44$), with incomplete or unavailable ABPM ($n = 63$), or with unavailable survival data ($n = 41$) were excluded (*Figure 1*). Thus, final analyses were performed on a subset of 349 participants with diabetes, whose characteristics are representative of the original CHAMP1ON diabetic cohort (see Supplementary material online, *Table S1*).

Patients' characterization and prognosis assessment

At baseline, a detailed clinical history was obtained using standardized questionnaires. All participants underwent a physical examination by a trained physician and a comprehensive clinical and biochemical characterization, including blood and urine sampling for routine chemistry, metabolic profiling, and inflammatory markers assessed at the hospital central laboratory. The diagnosis of diabetes was made in accordance with the 1997 American Diabetes Association (ADA) guidelines (i.e. fasting plasma glucose > 126 mg/dL, plasma glucose > 200 mg/dL and classic symptoms of diabetes, 2-h plasma glucose > 200 mg/dL during an oral glucose tolerance test).[22] Patients with diabetes were screened for the presence of abnormal BP patterns and microvascular complications, namely neuropathy, nephropathy, and retinopathy, by an expert and trained physician (G.F.), with the same instruments and under standardized conditions, as previously detailed.[19–21] After enrolment, participants periodically attended the clinic in relation to their clinical needs and were treated according to the best clinical practice in effect at the time of the visits. The vital status of study participants was verified in April 2021 by interrogating the Italian Health Card database, which provides updated information on all current Italian residents, as well as regional and national registries for identifying the cause of death.

The study protocol, including long-term data collection and longitudinal analyses on main health outcomes, was approved by the local Ethics Committee at the University Hospital of Pisa in 1999. Written informed consent was obtained from all participants before enrolment.

Blood pressure and heart rate variability measurements

Twenty-four-hour ABPM recordings were performed at the time of enrolment while patients continued their anti-hypertensive treatment, if any. Patients were instructed to engage in normal activities but to avoid strenuous exercise and to keep their arm relaxed and motionless during each measurement. An oscillometric ABP Monitor (Takeda TM3420, Tokyo, Japan) with adequate cuff size was mounted on the non-dominant arm between 8:00 a.m. and 10 a.m. and removed 24 h later. Blood pressure and HR readings were taken every 15 min from 7:00 a.m. to 10:00 p.m. (daytime) and every 30 min from 10:00 p.m. to 7:00 a.m. (nighttime). Initial measurements were concomitantly measured by a mercury sphygmomanometer to verify the agreement between the two modes of measurement (deemed acceptable within a range of 5 mmHg). The ABPM reading was considered acceptable if at least 70% of the BP recordings were valid, according to current guidelines.[23] Daily HR fluctuations were measured using the standard deviation (SD) of HR values registered during ABPM, differentiating daytime from nighttime readings, as well as 24-h readings, as previously published.[5,24] Nocturnal dip was measured as follows: (mean daytime value − mean nighttime value)/mean daytime value. A ≥10% decline in average nighttime HR compared with average daytime HR was considered normal, as previously published.[5] In accordance with the current European Society of Cardiology/European Society of Hypertension (ESC/ESH) guidelines for the management of arterial hypertension,[23] patients were classified as hypertensive if they showed either one of the following criteria: systolic BP (SBP) ≥130 mmHg and/or diastolic BP (DBP) ≥80 mmHg during 24-h ABPM recording, SBP ≥ 135 mmHg and/or DBP ≥ 85 mmHg during daytime ABPM recording, SBP ≥ 120 mmHg and/or DBP ≥ 70 mmHg during nighttime ABPM recording, or treatment with at least one anti-hypertensive drug.

Echocardiography

A comprehensive transthoracic echocardiography examination was performed at rest by trained cardiologists at the University Hospital of Pisa according to clinical guidelines.[25] Data collected included left ventricle (LV) thickness, volumes, geometry, and ejection fraction (EF); LV volumes and EF were calculated from the apical two- and four-chamber views using the modified Simpson's rule according to international recommendations.[26] Left ventricular hypertrophy was defined as an LV mass/body surface area > 115 g/m^2 in men and >95 g/m^2 in women; concentric remodelling was defined as a relative wall thickness (RWT) ≥ 0.43 with normal LV mass index as per guidelines.

Microvascular complications

A trained physician (G.F.) performed the screening of diabetic microvascular complications, with standardized procedures at fixed times of the day. The presence of cardiac autonomic neuropathy (CAN) was determined in 205 patients through a battery of cardiovascular tests using a portable computerized system (Cardionomic, Medimatica, Martinsicuro, Italy). Cardiac autonomic neuropathy was diagnosed if patients had at least two tests among lying-to-standing, standing-to-lying, and deep breathing showing reduced HR variability (HRV) and/or orthostatic hypotension, defined as a reduction in SBP of ≥20 mmHg within 3 min of standing, as per standard protocols.[23] Peripheral neuropathy was screened through a standardized questionnaire, physical exam, and monofilament testing in 230 patients. If the screening test was positive, the diagnosis was confirmed by

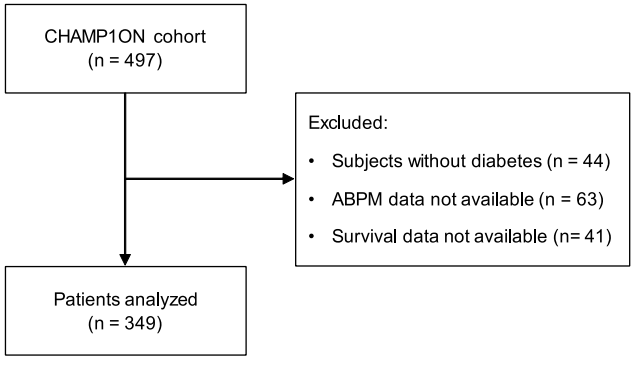


Figure 1 Study flowchart of patients' selection.

electroneurography performed by trained neurologists at the University Hospital of Pisa. Chronic kidney disease was assessed by determining estimated glomerular filtration rate (eGFR) with the CKD-EPI creatinine equation (n =320) and albumin excretion rate above 20 µg/min with an overnight urine collection, excluding urine samples that were indicative of significant urinary tract infection or haematuria (n = 299). Diabetic nephropathy was defined as CKD in the absence of other clear primary causes of kidney damage.[27] A total of 248 patients underwent dilated funduscopic examination by an expert ophthalmologist using an ophthalmoscope for the detection of retinopathy, in accordance with ADA's criteria.[28] Pre-proliferative or proliferative diabetic retinopathy was defined by the presence of any characteristic lesions, including intra-retinal microvascular abnormalities, microaneurysms, haemorrhages, cotton wool spots, venous beading or dilation, hard exudates, and new vessels.[28]

Statistical analyses

Variables were tested for normality using the Shapiro–Wilk test. Continuous normally distributed variables are presented as mean ± standard deviation (SD), and non-normally distributed variables are presented as median (interquartile range). The study population was divided based on the median value of 24-h HR SD of 30.4 (low vs. high) and on the presence or absence of a normal nocturnal HR dip. Differences between groups were tested using the χ^2 test for nominal variables and analysis of variance (ANOVA) or Kruskal–Wallis test for normally or non-normally distributed continuous variables, respectively. Kaplan–Meier curves were compared with the log-rank test. The Cox proportional hazard model was used to determine the hazard ratios for cardiovascular and all-cause mortality. Multivariate models were systematically adjusted for age (above vs. below the median, which was 60 years), sex, body mass index (BMI) (above vs. below 30 kg/m^2), good vs. poor glycaemic control (HbA_{1c} above vs. below 7.5%), type of diabetes (T1D vs. T2D), and uncontrolled hypertension at ABPM according to ESC/ESH guidelines.[23] These variables were selected based on clinical relevance, statistical significance in survival analyses ($P < 0.10$), and number of events in each category to ensure model power and reliability. To explore possible non-linear relationships and validate the cut-off point for 24-h HR SD of 30.4, a restricted cubic spline analysis was performed (see Supplementary material online, *Figure S1*). This approach determined non-linear trends in the association between 24-h HR SD and the relative hazards for cardiovascular death (both crude and adjusted models) and all-cause death (only crude model), identifying three optimal spline points (11.13, 30.23, and 170.71). The second spline corresponded to a relative hazard of ~1 and was almost identical to the median 24-h HR SD. Using the two proposed cut-off points for 24-h HR SD (30.4 vs. 30.23) leads to the same participants' dichotomous classification and does not affect study findings. To examine the effect of 24-h HR SD separately from nocturnal HR dip, we repeated the analyses after the exclusion of patients with blunted nocturnal HR dip (n = 107), all belonging to the low 24-h HR SD group. The proportional hazards assumption was respected in all models. Given an estimated 20% cardiovascular mortality rate in the low-risk group (i.e. high 24-h HR SD),[21] a total sample size of at least 340 subjects (170 per group) was calculated to provide 85% statistical power to detect a 15% higher cardiovascular mortality rate in the high-risk group (i.e. low 24-h HR SD), deemed clinically relevant, in a two-tailed group comparison ($\alpha = 0.05$). Statistical analyses were performed using JMP Pro software version 17.0.0 (SAS Institute Inc., Cary, NC, USA) at a two-sided α level of 0.05.

Results

Clinical, metabolic, and cardiovascular features of reduced daily heart rate fluctuations

A total of 349 patients with diabetes were included in this analysis, of whom 284 (81%) patients were diagnosed with T2D and 65 (19%) with T1D. Baseline characteristics of these subjects are presented in *Table 1*.

When divided based on 24-h HR SD (*Table 1*), subjects with low 24-h HR SD displayed older age, higher prevalence of hypertension, higher values of systo-diastolic BP, triglycerides, inflammation markers, lower eGFR, and more frequent therapy with statins and beta-blockers compared with the group with high 24-h HR SD. Echocardiographic measures showed no difference in either LV remodelling or systolic function between groups. No other significant group differences were observed.

Blunted nocturnal HR dip was diagnosed in almost a third (n = 107, 31%) of the whole cohort (*Table 1*) and was associated with older age, higher prevalence of hypertension, higher BMI, SBP, triglycerides, and inflammation markers, lower eGFR, and lower LVEF (although within normal reference range). No differences in pharmacological therapies were evident between the groups with preserved or blunted nocturnal HR dip.

Mean values and variability (SD) of 24-h, diurnal, and nocturnal HR in patients stratified based on 24-h HR SD and nocturnal HR dip are reported in *Table 2*. Patients with low 24-h HR SD had lower mean HR values during both daytime and 24-h, while displaying higher nocturnal mean HR, compared with those with high 24-h HR SD. By design, HR SD was lower during daytime and 24-h among the group with low 24-h HR SD, while there were no group differences during night rest hours. A total of 107 of 175 patients (61%) in the low 24-h HR SD group had blunted nocturnal HR dip, while all the patients in the high 24-h HR SD group had preserved nocturnal HR dip. Patients with blunted nocturnal HR dip had higher nocturnal mean HR values, but similar 24-h and diurnal mean HR values, compared with the group with preserved nocturnal HR dip. Conversely, they showed lower 24-h and diurnal HR SD, but similar nocturnal HR SD. The prevalence of low 24-h HR SD was significantly higher in the group with blunted nocturnal HR dip (n = 107, 100%), compared with those with preserved HR dip (n = 68, 28%; $P < 0.0001$).

Microvascular complications

The prevalence of diabetic microvascular complications in relation to circadian HR patterns at enrolment is reported in *Figure 2*. Patients with low 24-h HR SD were more frequently diagnosed with CAN (38% vs. 24%, $P = 0.028$), nephropathy (33% vs. 21%, $P = 0.019$), and retinopathy (42% vs. 29%, $P = 0.043$) compared with those with high 24-h HR SD. Similarly, a blunted nocturnal HR dip was associated with higher prevalence of CAN (47% vs. 24%, $P = 0.001$) and nephropathy (35% vs. 23%, $P = 0.049$).

Survival analysis

After 6251 person-years of follow-up [median follow-up 21.0 (14.0–21.0) years], a total of 136 (39%) deaths occurred, of which 100 (74%) for cardiovascular causes. Kaplan–Meier curves for 24-h HR SD groups (low vs. high) and nocturnal HR dip patterns (blunted vs. preserved) are shown in *Figure 3*.

Low 24-h HR SD was associated with higher mortality rate for both cardiovascular (n = 67, 38.3%, 22.5 events per 1000 person-years) and all causes (n = 83, 47.4%, 27.9 events per 1000 person-years) compared with high 24-h HR SD (cardiovascular death: n = 33, 19.0%, 10.1 events per 1000 person-years, $P < 0.0001$; all-cause death: n = 53, 30.5%, 16.2 events per 1000 person-years, $P = 0.001$), as well as reduced mean overall survival (16.8 ± 0.4 years vs. 18.5 ± 0.3 years, log-rank $P = 0.0006$). Consistently, the low 24-h HR SD group had a higher risk for both

Table 1 Baseline characteristics of the whole study cohort and of subgroups stratified by 24-h heart rate standard deviation (24-h HR SD) or nocturnal HR dip

	Whole cohort ($n = 349$)	24-h HR SD		Nocturnal HR dip	
		High ($n = 174$)	Low ($n = 175$)	Preserved ($n = 242$)	Blunted ($n = 107$)
Clinical characteristics					
Age, years	57 ± 12	55 ± 13	60 ± 10*	56 ± 12	59 ± 11*
Women/men, %	52/48	52/48	52/48	52/48	54/46
BMI, kg/m^2	29.4 ± 5.9	28.9 ± 5.9	29.8 ± 5.9	28.9 ± 5.5	30.6 ± 6.6*
T2D/T1D, %	81/19	81/19	82/18	80/20	84/16
Duration of diabetes, years	10 (4–20)	8 (4–19)	11 (4–22)	10 (4–20)	10 (3–22)
24-h systolic BP, mmHg	131 ± 16	128 ± 15	134 ± 16*	129 ± 15	135 ± 17*
24-h diastolic BP, mmHg	75 ± 8	74 ± 8	76 ± 8*	74 ± 8	77 ± 9
Hypertension, %	79	71	85*	73	89*
Controlled hypertension, %	12	11	13	12	14
Uncontrolled hypertension, %	59	52	67*	55	72*
Active smokers, %	32	35	29	35	24
Metabolic variables					
Fasting glucose, mg/dL	174 ± 66	174 ± 69	173 ± 67	173 ± 67	173 ± 70
HbA_{1c}, %	8.6 ± 2.1	8.5 ± 2.1	8.7 ± 2.1	8.5 ± 2.0	8.9 ± 2.3
Total cholesterol, mg/dL	215 ± 46	213 ± 46	218 ± 46	215 ± 45	216 ± 48
HDL-c, mg/dL	48 ± 14	49 ± 12	47 ± 16	49 ± 12	47 ± 16
LDL-c, mg/dL	182 ± 43	183 ± 45	182 ± 41	184 ± 43	179 ± 42
Triglycerides, mg/dL	162 ± 98	150 ± 99	175 ± 96*	157 ± 103	174 ± 88*
eGFR, mL/min/1.73 m^2	81 ± 21	85 ± 20	78 ± 22*	84 ± 20	76 ± 23*
Albuminuria, %	21	16	25	18	27
Urate, mg/dL	5.4 (4.3–6.6)	5.1 (4.2–6.3)	5.8 (4.6–7.1)*	5.2 (4.2–6.4)	5.8 (4.9–7.1)*
C-reactive protein, mg/dL	0.4 (0.3–0.9)	0.4 (0.3–0.8)	0.5 (0.3–1.0)	0.4 (0.3–0.8)	0.5 (0.4–1.2)*
ESR, mm/2 h	32 (20–44)	27 (17–38)	35 (24–55)*	29 (19–42)	35 (25–54)*
Homocysteine, µmol/L	10 (8–13)	10 (8–13)	10 (8–13)	10 (8–12)	11 (8–13)
Fibrinogen, mg/dL	377 (320–435)	373 (313–430)	383 (320–448)	368 (318–430)	392 (323–486)
Echocardiography					
LVMi, g/m^2	122 ± 27	118 ± 26	125 ± 28	120 ± 27	125 ± 28
RWT, ratio	0.45 ± 0.05	0.44 ± 0.05	0.45 ± 0.06	0.44 ± 0.05	0.45 ± 0.06
LVEF, %	59 ± 6	60 ± 5	58 ± 7	60 ± 5	58 ± 8*
Therapy					
ACEi/ARB, %	46	41	52	44	52
Beta-blockers, %	6	3	9*	5	9
Calcium channel blockers, %	28	25	31	25	36
Alpha1 antagonists, %	14	13	16	12	19
Alpha2 agonists, %	4	2	5	3	6
Diuretics, %	15	13	17	13	18
Oral glucose-lowering drugs, %	46	47	45	47	45
Insulin, %	34	29	38	32	37
Daily insulin dose, IU	39 (30–48)	40 (32–48)	38 (30–48)	40 (30–48)	39 (30–48)
Statin, %	9	5	14*	9	9

Continuous variables are presented as mean ± SD or median (interquartile range). Asterisks (*) mark a *P*-value of <0.05 for group differences between high and low 24-h HR SD or blunted and preserved nocturnal HR dip. Group differences were tested using the χ^2 test for nominal variables and ANOVA or Kruskal–Wallis test for normally or non-normally distributed continuous variables, respectively.

cardiovascular [crude hazard ratio (cHR) 2.34, 95% CI 1.54–3.55, $P < 0.0001$] and all-cause mortality (cHR 1.82, 95% CI 1.29–2.56, $P = 0.001$), which remained statistically significant after adjustment for age, sex, BMI, glycaemic control, type of diabetes, and hypertension [adjusted hazard ratio (aHR) 2.00, 95% CI 1.30–3.08, $P = 0.002$ and 1.61, 95% CI 1.13–2.29, $P = 0.009$, respectively].

Similarly, patients with blunted nocturnal HR dip had higher mortality rate for cardiovascular ($n = 41$, 38.3%, 23.0 events per 1000

Table 2 Ambulatory heart rate (HR) analysis of participants stratified by 24-h HR standard deviation (24-h HR SD) or nocturnal HR dip

	24-h HR SD			Nocturnal HR dip		
	High ($n = 174$)	Low ($n = 175$)	*P*	Preserved ($n = 242$)	Blunted ($n = 107$)	*P*
Mean 24-h HR, b.p.m.	75 ± 10	73 ± 10	0.003	73 ± 10	75 ± 9	ns
Mean diurnal HR, b.p.m.	82 ± 11	76 ± 10	<0.0001	79 ± 11	77 ± 9	ns
Mean nocturnal HR, b.p.m.	66 ± 10	70 ± 11	<0.0001	65 ± 10	74 ± 10	<0.0001
Blunted nocturnal HR dip, %	0	61	<0.0001	—	—	—
24-h HR SD	55.1 ± 26.6	17.5 ± 7.0	<0.0001	46.3 ± 26.8	13.9 ± 5.8	<0.0001
Diurnal HR SD	12.6 ± 3.6	9.8 ± 3.3	<0.0001	11.9 ± 3.7	9.8 ± 3.4	<0.0001
Nocturnal HR SD	6.2 ± 3.1	6.0 ± 2.8	ns	6.0 ± 2.9	6.2 ± 3.0	ns
Low 24-h HR SD, %	—	—	—	28	100	<0.0001

Continuous variables are presented as mean ± SD. Group differences were tested using the χ^2 test for nominal variables and ANOVA or Kruskal–Wallis test for normally or non-normally distributed continuous variables, respectively.

person-years) and all causes ($n = 53$, 49.5%, 29.8 events per 1000 person-years) compared with the group with preserved HR dipping (cardiovascular death: $n = 59$, 24.4%, 13.2 events per 1000 person-years, $P = 0.010$; all-cause death: $n = 83$, 34.3%, 18.6 events per 1000 person-years, $P = 0.009$), as well as reduced mean overall survival (16.5 ± 0.5 years vs. 18.2 ± 0.3 years, log-rank $P = 0.003$). The blunted nocturnal HR dip group also had higher crude and adjusted risk for both cardiovascular (cHR 1.83, 95% CI 1.23–2.72, $P = 0.003$; aHR 1.63, 95% CI 1.08–2.46, $P = 0.019$) and all-cause mortality (cHR 1.69, 95% CI 1.20–2.38, $P = 0.003$; aHR 1.54, 95% CI 1.08–2.20, $P = 0.017$).

After exclusion of 107 patients with blunted nocturnal HR dip, the remaining 68 patients in the low 24-h HR SD group showed a higher crude and adjusted risk for cardiovascular mortality (cHR 2.24, 95% CI 1.34–3.74, $P = 0.002$; aHR 1.90, 95% CI 1.12–3.24, $P = 0.018$) and a higher crude mortality risk for all causes (cHR 1.62, 95% CI 1.04–2.54, $P = 0.034$; aHR 1.43, 95% CI 0.90–2.26, $P = 0.129$), compared with the high 24-h HR SD group.

Discussion

This retrospective longitudinal study demonstrates that alterations in the physiological circadian fluctuations of HR, identified as low 24-h HR SD or blunted nocturnal HR dip, are associated with an adverse cardiometabolic risk profile, increased prevalence of microvascular complications, and higher cardiovascular and all-cause mortality in a well-characterized cohort of patients with long-standing T1D and T2D followed up for more than 20 years. Our data provide support to the prognostic value of ABPM-derived HR variation analysis in diabetes, showing that circadian HR control derangements are highly prevalent in this population and are predictors of increased cardiovascular and all-cause mortality, independent of potential confounders. To our knowledge, this is the first report to associate circadian HR fluctuations with a comprehensive clinical, metabolic, and cardiovascular characterization including a complete screening for microvascular complications. Furthermore, our study provides the longest follow-up to date and the first evidence of a reduced survival in patients with diabetes and impaired daily HR fluctuations.

Few previous studies have considered the impact of disrupted HR variations on prognosis in people with diabetes without overt cardiovascular disease, while the description of the associated clinical characteristics has been often overlooked. The thorough baseline characterization of our cohort adds valuable information to the existing literature describing the relationship between deranged HR patterns and cardio–renal–metabolic parameters and microvascular complications, while allowing adjustments for multiple confounding factors in survival analyses. Despite similar glycaemic control, patients with deranged HR fluctuations have slightly older age, higher BMI, higher indices of systemic inflammation, worse metabolic and BP control, and slightly lower LVEF in those with blunted nocturnal HR dip (*Table 1*). A higher prevalence of complications was noticeable in the groups with abnormal HR patterns, particularly CAN and nephropathy (*Figure 2*). These observations suggest that HRV either takes part in or is a proxy of a progressive damage to the cardiovascular system. In fact, the higher prevalence of elderly and patients with CKD supports the role of microvascular dysfunction in worsening HR fluctuations, either direct or mediated by subclinical autonomic damage. The association between lower 24-h HR SD and higher BP values confirms earlier reports suggesting a synergistic effect of hypertension and diabetes on HR control and myocardial damage.[29,30] Moreover, obesity and systemic inflammation may be involved in reducing HR variations in diabetes,[31] as supported by our finding of higher BMI and inflammatory markers in patients with blunted nocturnal HR dip.

Previous studies described an increased risk of cardiovascular events in patients with diabetes and altered resting HR[12,14,17] and HR fluctuations.[18] More reports focused on electrocardiogram (ECG)-derived HRV in patients with diabetes and overt CAN,[32] established cardiovascular disease,[9,33–35] or CKD.[36] Nevertheless, it was demonstrated that ECG-based HRV adds little to standard autonomic tests in terms of prognosis.[37] Despite the wide accessibility to data generated during 24-h ABPM, only a few studies have addressed ambulatory HR variations in diabetes,[6,11,13,15] linking it to glycaemic control,[11] microalbuminuria,[15] LV dysfunction,[13] and cardiovascular risk factors,[6] but never specifically exploring the prognostic value of abnormal HR patterns on mortality. Furthermore, compared with our cohort, the clinical characterization of previous studies was limited, and statistical analysis was often performed on a combination of HR and BP indices. In the present investigation, the analysis of HR patterns showed a significantly lower survival in patients with reduced 24-h HR SD (*Figure 3A* and *B*), expanding the current knowledge by adding information about its relation to all-cause and cardiovascular mortality in this high-risk population. Similarly, the incapacity of reducing HR during nighttime sleep

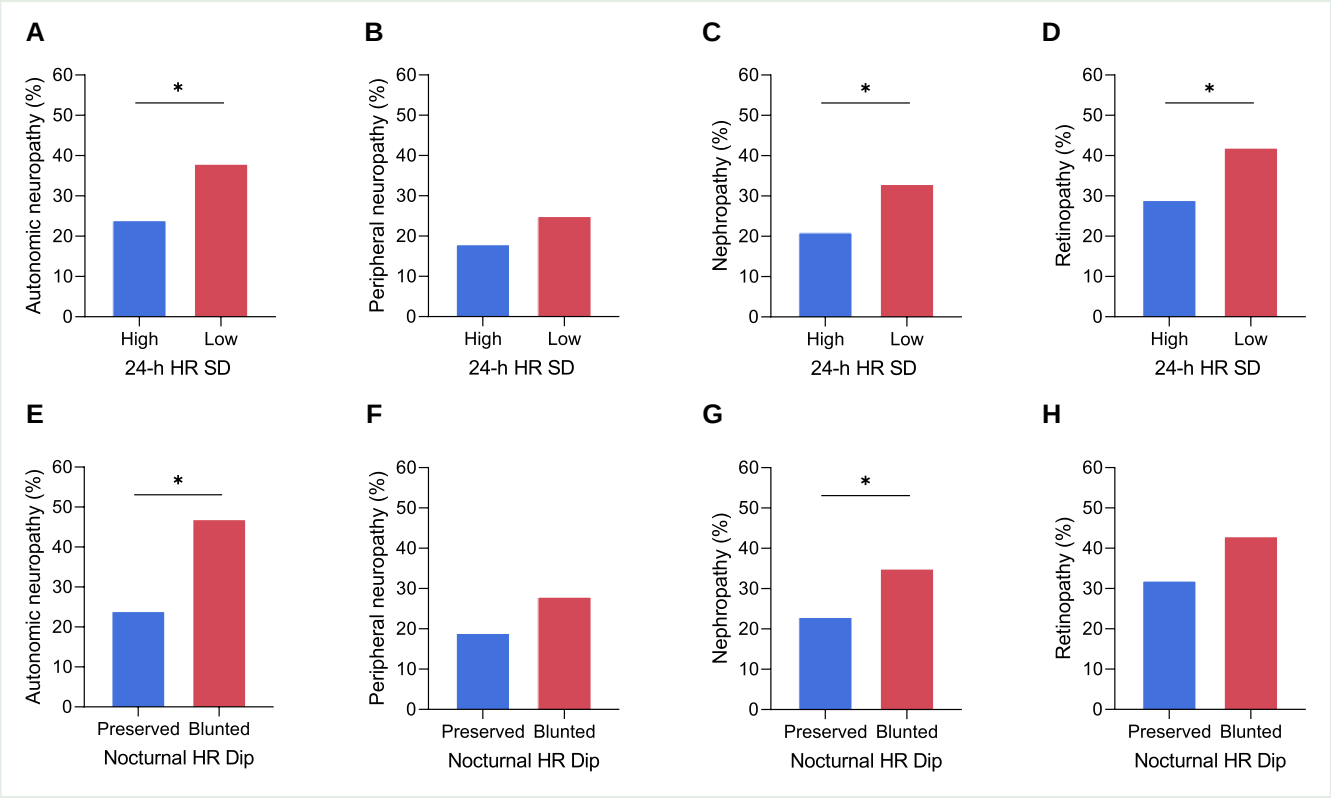


Figure 2 Diabetic microvascular complications. Prevalence of autonomic (*A*) or peripheral neuropathy (*B*), nephropahty (*C*), and retinopathy (*D*) in participants with high or low 24-h heart rate standard deviation (24-h HR SD). Prevalence of autonomic (*E*) or peripheral neuropathy (*F*), nephropahty (*G*), and retinopathy (*H*) in participants with blunted or preserved nocturnal HR dip.

appears to be a powerful indicator of worse outcomes in diabetes (*Figure 3C and D*), in line with previous observations in large non-diabetic cohorts.[3–5,38] Of note, we observed higher cardiovascular mortality in the groups with abnormal circadian HR patterns despite more frequent use of beta-blockers and statins (*Table 1*), further supporting the hypothesis that impaired HR patterns are independently associated with outcome in this population.[39]

The biological plausibility of our results derives from the known crucial role of HR control: a dysfunction in the delicate interplay of organs and systems regulating cardiovascular responses to everyday stimuli is a clear sign of pathology.[40] Thus, it is not surprising that alterations in HR fluctuations become especially manifest during daytime, when external stimuli are more abundant, compared with nighttime and rest (*Table 2*). The inability to increase HR to match daily activities (low 24-h HR SD) and/or to decrease it during rest (blunted nocturnal HR dip) denotes an advanced dysfunction of the neuro-cardiovascular system. An incapacity of regulating HR to sustain effort intensity can be the first sign of cardiovascular dysfunction and is associated with multiple traits of subclinical heart failure in diabetes.[41] Furthermore, recent evidence demonstrated that impaired 24-h HR variations in diabetes are associated with early markers of cardiovascular disease such as vascular and valvular stiffening, as well as subclinical systolic dysfunction.[16,42] Heart rate fluctuation analysis is not only used as a neuro-cardiological index but also has an increasingly important role in estimating global health as evidence-based medical practice.[7] A better understanding of pathophysiological mechanisms of HR variation impairments in diabetes may have substantial clinical relevance, as there are currently no effective therapeutic means. In fact, while HRV in diabetes can be improved by exercise training,[43] there are no effective drugs specifically targeting it. Intriguingly, the thiazolidinedione pioglitazone, which reduces atherosclerosis-related events and mortality, can effectively restore the cardiovascular circadian clock in humans and may be useful in this setting.[44]

Our findings have relevant applications, as the early recognition of abnormal circadian HR patterns in clinical practice can help refine risk stratification, allowing the identification of the patients who will benefit the most from intensive preventive strategies. The detection of a blunted nocturnal HR dip can prompt the search for microvascular complications and subtle myocardial dysfunction and support early treatment with drugs with proven prognostic benefits.[45] In this scenario, ABPM is a simple, widely available, non-invasive, and inexpensive tool that can provide HR data under conditions of wakefulness and sleep over a 24-h cycle. As HR is less dependent than BP on movement and daily activities, this is an advantage for estimating prognosis.[5] In the CHAMP1ON study, patients were instructed to spend a 'normal day' while wearing the ABPM to reflect the usual HR fluctuations in free-living conditions. Despite our study relies on a single ABPM measurement at baseline, the demonstrated accuracy and reproducibility of the method provide consistent information regarding circadian HR variations at a specific time point.[5,46] Indeed, the same methodology, employing a single baseline ABPM measurement, has been used before in larger cohorts, providing similar outcomes.[3–5,38] Our data therefore support the role of ABPM as a screening and monitoring tool to allow the diagnosis of abnormal BP and HR circadian variations to

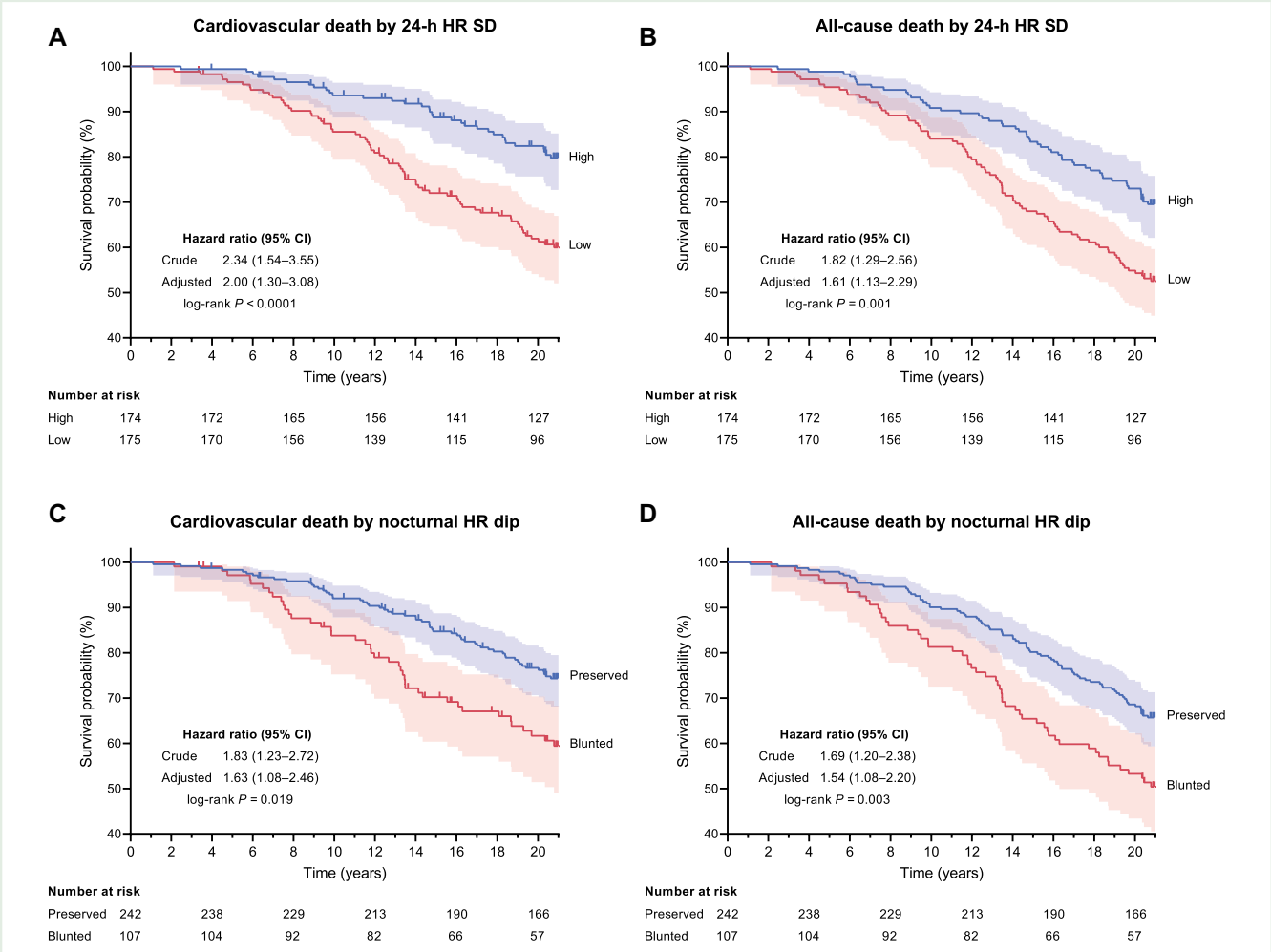


Figure 3 Kaplan–Meier curves and 95% confidence intervals (CI) for subjects stratified by 24-h heart rate standard deviation (24-h HR SD) (*A* and *B*) and nocturnal HR dip (*C* and *D*).

stratify cardiovascular risk and optimize treatment and management of patients with diabetes, promoting evidence-based precision medicine. Diabetes management has changed significantly since the time of recruitment of our cohort, and data should be interpreted in the light of the limited potential of previous therapeutic means in changing the natural history of diabetes—especially for cardiovascular complications. Yet, cardiovascular disease remains the leading cause of death in diabetes,[47] and recent guidelines stress the need to stratify cardiovascular risk for early action and tailored treatments in the prevention of cardiovascular disease in diabetes.

One of the key advantages of our study is the extended follow-up period, spanning more than two decades. This has enabled us to conduct a long-term evaluation of the temporal prognostic impact of altered HR circadian fluctuations. Expanding previous reports, the thorough baseline characterization of our cohort enabled us to give a comprehensive description of the clinical, metabolic, and cardiovascular features associated with impaired daily HR fluctuations in diabetes. Furthermore, we describe for the first time the association of impaired circadian HR patterns with the full spectrum of microvascular complications. Importantly, the utilization of a cost-effective and readily accessible instrument like 24-h ABPM facilitates the straightforward application of our findings in clinical practice. We recognize some limitations to our study. First, we acknowledge that the retrospective design is a potential source of confounding. Second, HR patterns were assessed only at enrolment; nevertheless, previous studies have found a high reproducibility of ABPM-based classification of BP and HRV patterns in diabetes.[5,46] Also, we quantified HR fluctuations using the SD of HR recorded by ABPM instead of the more precise index of beat-to-beat HRV, which is usually calculated based on time or frequency domain measures requiring ECG monitoring; circadian variations and patterns, however, are effectively recognized by ABPM, and our method is in line with previously published protocols.[5] Third, although multiple adjustments were implemented to account for plausible confounders, we could not entirely discount the impact of additional, unmeasured potential mediating factors that could be marked by altered HR patterns, including sleep disorders or extremely sedentary lifestyle, due to the retrospective study design. We acknowledge the potential loss of information due to the categorization of continuous variables before inclusion in multivariable models. However, the categorization was based on either clinically meaningful or data-driven cut points and ensured that each category had sufficient events, improving estimate reliability and statistical power. The study cohort included only outpatients from a single tertiary referral hospital, from which we selected only individuals with diabetes; therefore, a selection

bias cannot be excluded, and the validity of our findings remains to be confirmed in different patient populations. Ultimately, patients were recruited between 1999 and 2000, when best medical practice could not benefit from the currently available anti-diabetic drugs with proven cardiovascular benefits[48] and glucose-, BP-, and lipid-lowering goals were not as strict as today. This is reflected by the loose glycaemic and metabolic control observed in our cohort, which may have contributed to the high death rate and prevalence of microvascular complications. A comparable overall death rate, however, has been reported in a similar, large cohort of patients with diabetes followed up for 20 years.[49]

In conclusion, low 24-h HR variations and blunted nocturnal HR dip are frequent in patients with long-standing diabetes and are associated with an adverse cardiometabolic risk profile, microvascular disease, and increased cardiovascular and all-cause mortality. Identifying impaired circadian HR fluctuations using 24-h ABPM may provide a widely available and inexpensive risk stratification tool in this high-risk population.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Author contributions

L.N.: study design, literature review, database, statistics, data analysis, interpretation of results, tables and graphs ideation and creation, and manuscript writing. M.C.: study design, longitudinal data collection, data analysis, interpretation of results, and drafting of manuscript; L.S. and D.M.: longitudinal data collection, data analysis, and interpretation of results; L.S., N.R.P., S.G., N.C., and G.C.: substantial contribution to the interpretation of data and manuscript drafting; G.F.: baseline data collection, data analysis, substantial contribution to the interpretation of data, and manuscript drafting; S.L.: data analysis, substantial contribution to the interpretation of data and manuscript revision; A.N.: study design, interpretation of results, manuscript editing, and revision; A.S.: study design, longitudinal data collection, interpretation of results, manuscript editing, and revision; and D.T.: study design and supervision, interpretation of results, manuscript editing, and revision.

Conflict of interest: The authors have declared that no conflict of interest exists pertinent to this study.

Data availability

The dataset analysed during the current study is available from the corresponding author on reasonable request.

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