Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease

Christine D. Johansen†, Rasmus H. Olsen†, Lene R. Pedersen†, Preman Kumarakthurai†, Mette R. Mouridsen†‡, Zeynep Binici‡, Theodoros Intzilakis§, Lars Køber‡, and Ahmad Sajadieh†,§

1Department of Cardiology, Copenhagen University Hospital of Bispebjerg, Bispebjerg Bakke 23, Copenhagen NV 2400, Denmark; 2Department of Cardiology, Copenhagen University Hospital of Herlev, 2730 Herlev, Denmark; 3Department of Cardiology, Copenhagen University Hospital of Amager, 2300 Copenhagen, Denmark; and 4Department of Cardiology, Rigshospitalet, 2100, Copenhagen, Denmark

Aims
Increased heart rate (HR) is a predictor of all-cause and cardiovascular (CV) mortality. We tested which measure of HR had the strongest prognostic value in a population with no apparent heart disease.

Methods and results
Six hundred and fifty-three men and women between the age of 55 and 75 years were included in the Copenhagen Holter Study and underwent 48 h ambulatory electrocardiographic (ECG) monitoring. Resting HR was measured after at least 10 min of rest. Twenty-four-hour HR was derived from the mean time between normal-to-normal RR intervals (MEANNN). Night-time HR was derived from a 15 min sequence between 2:00 and 2:15 a.m. The median follow-up time was 76 months, and an adverse outcome was defined as all-cause mortality and the combined endpoint of CV death, acute myocardial infarction (AMI), and revascularization. All three measures of HR were significantly associated with all-cause mortality, also after adjustment for conventional risk factors. We found an association between all three measures of HR and CV events in analyses adjusted for sex and age. However, when adjusting for CV risk factors, the association with resting HR and 24 h HR disappeared. In a fully adjusted model, only night-time HR remained in the model, hazard ratio $= 1.17 (1.05–1.30), P = 0.005$.

Conclusion
In middle-aged subjects with no apparent heart disease, all measures of increased HR were associated with increased mortality and CV risk. However, night-time HR was the only parameter with prognostic importance after multivariable adjustment.

Keywords
Night-time heart rate • Resting heart rate • 24 h heart rate • Mortality • Prognosis

Introduction
Epidemiological studies have shown that resting heart rate (HR) is a predictor of all-cause mortality and cardiovascular (CV) mortality in subjects with as well as without diagnosed CV disease, and the effect is independent of traditional CV risk factors.1–4 Increased HR is associated with a poor prognosis, and the importance of resting HR as a risk factor in the general population is recognized by the European Guidelines on CV Prevention.5
However, HR is a highly variable parameter that is influenced by numerous factors. The sino-atrial node (SA-node) is affected by physical and mental activities, sleep stages, and environmental factors through the autonomic nervous system, reflex regulation, and circulating hormones. HR has a circadian variation, with a higher HR during daytime compared with night-time. During non-rapid eye movement (REM) sleep, there is a relative vagal dominance in the regulation of HR. This phase is periodically interrupted by REM sleep, where HR increases as vagus nerve tone is withdrawn, and sympathetic nerve activity reaches levels higher than during waking.

It is not clear which measure of HR has the strongest prognostic value. Thus, a better estimate of an individual’s habitual HR might be obtained from 24 h or night-time recordings as compared with resting HR and hence a measure with a stronger prognostic value.

The aim of the present study was to compare the prognostic value for CV morbidity and mortality of resting HR, 24 h average HR, and night-time HR in middle-aged and elderly men and women with no apparent heart disease.

Methods
This study is part of the Copenhagen Holter study, which aimed to address the value of 48 h ambulatory electrocardiographic (ECG) monitoring in risk assessment of middle-aged and elderly men and women with no apparent heart disease.

Within two well-defined postal regions in Copenhagen city, all men aged 55, as well as all women and aged 60, 65, 70, and 75 years (n = 2969) were contacted. The study subjects filled out a questionnaire on CV risk factors, use of medication, and medical history. The subjects were ranked according to the number of the following self-reported risk factors: hypertension, diabetes mellitus, smoking habits, familial predisposition to cardiac disease (sudden death or acute myocardial infarction (AMI) in a parent or sibling before the age of 60), obesity (body mass index > 30), or known hypercholesterolaemia.

The exclusion criteria were manifest ischaemic heart disease, angina pectoris, congestive heart failure, valvular heart disease, congenital heart disease, arrhythmic heart disease, including permanent atrial fibrillation, medical treatment for any heart disease, a history of stroke, cancer, or other significant or life-threatening diseases (cirrhosis hepatitis, renal insufficiency needing dialysis, and chronic lung disease needing home-oxygen therapy), Usage of medication affecting the SA-node for non-cardiac reasons was not an exclusion criterion, being home-oxygen therapy). Technical reasons for exclusion were unacceptable or incomplete Holter recordings, period/periods of atrial fibrillation during the recordings and electrocardiographic findings suggestive of cardiac disease (Minnesota code: I 1-2, II-1, IV-1).

Figure 1 shows the study population at different exclusion steps. All together, 653 subjects participated in this Holter study. All participants were subject to a physician-based interview, physical examination, including anthropometric measurements, fasting laboratory testing, and 48 h Holter monitoring.

The study was performed from 1998 to 2000. Current results are based on data from the second follow-up, which was performed 7 years after study start.

Laboratory testing and C-reactive protein assay
Laboratory testing was conducted between 7 and 10 a.m. after overnight fasting. Standard analyses, including blood glucose and cholesterol measurements, were immediately performed on a Hitachi 7170 automated analyser. For future analyses, both plasma and serum were stored at −70 °C. Analyses of high-sensitive C-reactive protein were performed on the original sample 4 years after it was obtained and measured by immunofluorescence technique using ‘Kryptor’ manufactured by BRAHMS (Saint-Ouen, France). N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was measured by electrochemiluminescence technique using Elecsys 2010 provided by Roche (Basel, Switzerland). Detection range: 0.6–4130 pmol/L, CV within-run: 1.9–2.7%.

Definition of variables
Blood pressure and resting HR were measured with the patient in a sitting position after at least 10 min of rest. Diabetes mellitus was defined as known diabetes or fasting plasma glucose of ≥7 mmol/L.

A continuous 48 h Holter recording was made using two-channel SpaceLabs tape recorders (9025, SpaceLabs, Inc., Redwood, WA, USA), and analysed by FT3000 Medical Analysis and Review Station. From the 48 h Holter recording, the first 24 h was selected for analyses (2nd to 25th hour). Trained personnel at the Holter laboratory of the Copenhagen University Hospital of Hvidovre performed the analysis.

Mean HR was derived from ‘mean normal to normal’ (MEANNN), which is the mean time between normal R to R-waves of ECG in milliseconds. In these terms, MEANNN is representing the average 24 h HR, i.e. 60 000/MEANNN = average 24 h HR in beats per minute (b.p.m). The night-time HR was derived from a 15 min sequence between 2:00 and 2:15 a.m. The difference between max HR registered during the night, and the mean night-time HR was calculated to evaluate the fluctuations and their amplitude.

The reproducibility of ambulatory HR was examined by re-evaluation of 26 Holter recordings. Coefficients of variation for paired evaluations were 1.4%, P > 0.1.

Sensitivity analyses were performed to assess the stability of night-time HR. Retrospectively, we measured and analysed night-time HR between 1 and 1.15 a.m., and between 3 and 3.15 a.m.

To assess the stability of the data in the population of patients who did not use β-blockers, we reanalysed the data after exclusion of these subjects from analyses.

Endpoints
Data on death and CV events were obtained through the National Central Patient Registry. The National Hospital Registry keeps records on all hospital admissions in Denmark since 1978, and each hospitalization is classified according to the 10th revision of the International Classification of Diseases (ICD-10). The diagnosis of AMI (ICD-10: I21–I22) in the National Hospital Registry has been validated and has a sensitivity of 91% and predictive value of 93%. All deaths, hospital admissions, and discharges in Denmark are reported to this registry within 2 weeks. Hospital admissions were additionally studied from hospital discharge letters. The diagnosis of AMI was based on history, typical ECG changes, and elevation of cardiac enzymes. A CV endpoint was defined as CV death, AMI, or coronary revascularization, whichever occurred first.

Ethics
Before inclusion, all participants gave their written informed consent. The study was approved by the regional Ethics Committee for the city of Copenhagen and Frederiksberg. The study protocol was designed to comply with the Declaration of Helsinki.
Statistical analysis
Statistical analyses were performed using SAS statistical software program (SAS Institute Inc., Cary, NC, USA, version 9.1). For normally distributed variables, mean and SD are presented; otherwise median values and inter-quartile range are presented. The associations between HR and other baseline variables were assessed by Pearson or Spearman correlation coefficients, Student’s t-test, or Kruskal–Wallis test. To evaluate the independent associations, multivariate linear regression analyses, followed by forward selection, were performed. In the forward selection models, a P-value of ≤0.10 was used as a criterion to enter the model and P ≤ 0.05 to stay in the model. Cox proportional hazard models were used to evaluate risk factor adjusted associations of variables of interest with death or CV events. The assumption of proportional hazards was assessed by visual judgment of the log-minus-log survival plots. High-sensitive C-reactive protein was dichotomized at 3.0 mg/mL in agreement with current guidelines.11 Adjustment for relevant covariates was performed stepwise in four models. Model 1: adjustment for sex and age, Model 2: adjustment for conventional risk factors: sex, age, total cholesterol, systolic blood pressure (SBP), diabetes mellitus, and smoking habits, Model 3: adjustment for conventional risk factors and biomarkers, Model 4: additional adjustment for use of β-blockers, and Model 5: further adjustment for other relevant medications [angiotensin-converting enzyme (ACE)-inhibitors/angiotensin II receptor blockers, diuretics and calcium channel blockers]. Stepwise adjustments were performed to evaluate the mathematical stability of the models when adding more covariates to the models. Event-free survival in different groups was illustrated by Kaplan–Meier curves.

Results
Population
The baseline characteristics of the study population are listed in Table 1. Table 2 shows the significant univariate associations of different measures of HR and baseline variables of interest. As shown all measures of HR are higher in women, smokers, diabetics, and subjects with a lower level of physical activity. In multiple regression analyses, resting HR was related to smoking and high-sensitive C-reactive protein, while 24 h HR was associated with sex, smoking, high-sensitive C-reactive protein, NT-proBNP, and SBP, and night-time HR was associated with sex, smoking, high-sensitive C-reactive protein, and low physical activity level.

Follow-up and endpoints
The median follow-up was 76 months (inter-quartile range: 74–78). During the follow-up period, 80 participants died (22 from CV death,
selection model, only 24 h HR remained in the model. Further adjustment for biomarkers and \( \beta \)-blocker usage, the association with 24 h HR and resting HR reappeared (Table 3, Model 4). After additional adjustment for other relevant medication (ACE-inhibitors/angiotensin II receptor blockers, diuretics, and calcium channel blockers), all three measures of HR remained significantly associated with CV events (Table 3, Model 5). In a forward selection model with inclusion of all three measures of HR, conventional risk factors, biomarkers, and \( \beta \)-blocking agents, only night-time HR, among the three measures of HR, remained in the model, hazard ratio = 1.17 (1.05–1.30), \( P = 0.005 \). When only resting HR and 24 h HR were tested in a forward selection model, none remained in the model. The median value of fluctuation from the mean night-time HR to maximum night-time HR was 32 (range: 11–86). These fluctuations were not associated with all-cause mortality but were inversely associated with CV events in a multivariate Cox-model; hazard ratio = 0.84, 95% confidence interval (CI): 0.72–0.98, \( P = 0.029 \) after adjustment for all conventional risk factors, high-sensitive C-reactive protein, NT-proBNP, and use of \( \beta \)-blockers.

Event rates for quintiles of the three different measures of HR are illustrated in Figure 2. As shown there seems to be a threshold of risk at about 70 beats/min for resting HR and 24 h HR, but for night-time HR, it seems to be at about 60 beats/min.

### All-cause mortality

All three measures of HR were significantly associated with all-cause mortality in sex- and age-adjusted Cox proportional hazard models (Table 3). In multivariable analyses, adjusted for sex, age, and conventional risk factors (total cholesterol, diabetes, current smoking, andhypertension), the associations remained significant. Further adjustment for prognostic significant biomarkers (high-sensitive C-reactive protein and NT-proBNP) and use of \( \beta \)-blockers did not change the associations. Nor did additional adjustment for other relevant medication (ACE-inhibitors/angiotensin II receptor blockers, diuretics, and calcium channel blockers) (Table 3). Using forward selection including all three HR measures, only night-time HR stayed in the model suggesting that this has the strongest correlation to all-cause mortality, hazard ratio = 1.07 (1.02–1.13), \( P = 0.02 \). When only resting HR and 24 h HR were tested in a forward selection model, only 24 h HR remained in the model. Figure 3 illustrates the event-free survival estimates of the subjects in quintiles of different measures of HR.

### Cardiovascular events

All three measures of HR were associated with CV events both in univariate and in age and gender adjusted analysis (Table 3). However, when adjusting for CV risk factors, the association with resting HR and 24 h HR was no longer present. Yet, when further adjusting for biomarkers and \( \beta \)-blocker usage, the association with 24 h HR and resting HR reappeared (Table 3, Model 4). After additional adjustment for other relevant medication (ACE-inhibitors/angiotensin II receptor blockers, diuretics, and calcium channel blockers), all three measures of HR remained significantly associated with CV events (Table 3, Model 5).

### Sensitivity analysis

The night-time HR measured from 2:00 to 2:15 a.m. (65.9 ± 10.4) was closely correlated with night-time HR measured from 1 to 1:15 a.m. (67.6 ± 10.5), and from 3 to 3:15 a.m. (66.3 ± 10.5), respectively, with correlation coefficients of 0.90 and 0.92; \( P < 0.0001 \) for both associations. Multivariable adjusted hazard ratio and 95% CI for the association with CV events for night-time HR at 1 a.m. was 1.16 (1.01–1.35), \( P = 0.036 \), and at 3 a.m. 1.25 (0.98–1.30), \( P = 0.09 \), respectively. Very similar results were found for all-cause mortality.

Reanalysis of the data after exclusion of \( \beta \)-blocker users did not change the results (data not shown).

### Collinearity between HR variables

Collinearity was found with Pearson correlation coefficients of 0.83 (between 24 h HR and night-time HR), 0.62 (between 24 h HR and resting HR), and 0.50 (between night-time HR and resting HR), all \( P < 0.0001 \).

### Stratified by risk profile

To investigate whether the key findings were consistent in subjects with low-risk profile (0–1 risk factor) as well as in subjects with high-risk profile (≥2 risk factors), age and gender-adjusted Cox proportional hazard analyses were performed stratified by risk groups with all-cause mortality as endpoint. We found night-time HR to be predictive in both subjects with 0–1 risk factor (hazard ratio = 1.22, 95% CI: 1.04–1.43, \( P = 0.02 \)) and in subjects with ≥2 risk factors (hazard ratio = 1.29, 95% CI: 1.14–1.46, \( P < 0.0001 \)). Twenty-four h HR was borderline significant in subjects with 0–1 risk factor (hazard ratio = 1.24, 95% CI: 1.01–1.53, 0.09, respectively. Very similar results were
and significant in subjects with ≥2 risk factors (hazard ratio = 1.26, 95% CI: 1.08–1.48, P = 0.004). Resting HR was still a significant predictor in the subjects with 0–1 risk factor (hazard ratio = 1.03, 95% CI: 1.01–1.06, P = 0.01) but not in subjects with ≥2 risk factors (hazard ratio = 1.02, 95% CI: 0.99–1.04, P = 0.118).
### Table 3  Cox proportional hazard models showing associations between different measures of heart rate with different all cause mortality and cardiovascular (CV) events

<table>
<thead>
<tr>
<th></th>
<th>Resting heart rate</th>
<th>Night-time heart rate</th>
<th>24 h heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>$\chi^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>All cause mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.14 (1.04–1.24)</td>
<td>8.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.11 (1.01–1.22)</td>
<td>5.1</td>
<td>0.024</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.10 (1.01–1.21)</td>
<td>4.4</td>
<td>0.037</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.11 (1.01–1.22)</td>
<td>4.5</td>
<td>0.034</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.11 (1.01–1.22)</td>
<td>4.5</td>
<td>0.033</td>
</tr>
<tr>
<td>CV events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.14 (1.03–1.26)</td>
<td>6.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.10 (1.00–1.23)</td>
<td>3.4</td>
<td>0.066</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.10 (0.99–1.23)</td>
<td>3.0</td>
<td>0.081</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.14 (1.02–1.27)</td>
<td>5.0</td>
<td>0.025</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.12 (1.00–1.26)</td>
<td>4.0</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Model 1: sex and age adjusted.
Model 2: adjusted for systolic blood pressure, age, sex, smoking, diabetes, and total cholesterol.
Model 3: adjusted for variables as in Model 2 and high-sensitive C-reactive protein and NT-proBNP.
Model 4: adjusted for variables as in Model 3 and use of $\beta$-blockers.
Model 5: adjusted for variables as in Model 4 and use of ACE-inhibitors/angiotensin II receptor blockers, diuretics, and calcium channel blockers.

Hazard ratio for each increment of 5 b.p.m.

---

**Figure 3** Event-free survival estimates for the quintiles of the three different measures of heart rate.
Discussion

In this study, we found that all three measures of HR were significantly associated with all-cause mortality. The major finding was that night-time HR seems to be better than resting HR and 24 h HR in predicting the risk of all-cause mortality as well as cardiac events. This result persisted after adjusting for all traditional CV risk factors, high-sensitive C-reactive protein, NT-proBNP, and use of β-blockers.

A number of prospective studies have reported an association between HR and all-cause mortality as well as cardiac events.\(^1\)–\(^3\),\(^6\),\(^12\) In addition, HR is also associated with conventional CV risk factors.\(^6\) HR is positively associated with sub-clinical inflammation and increased HR may also be a sign of sub-clinical heart disease. The poor prognosis associated with high HR may be partially due to these effects. However, this study shows that all measures of HR are associated with mortality even after further adjustment for these biomarkers. To our knowledge, this is the first study to address the prognostic value of HR after adjustment for high-sensitive C-reactive protein and NT-proBNP. Less attention has been given to which measure of HR that holds the strongest prognostic power. The predictive value of 24 h HR for mortality was documented by Aronow et al.\(^13\) Unfortunately, the predictive power was not compared with that of resting HR. Palatini et al.\(^14\) measured HR three times from 60 s assessments to overcome the assumption that HR is a highly variable measurement with low reproducibility. They analysed all measures individually and all showed a close correlation to mortality. In another study, 24 h HR was compared with resting HR in predicting death from non-CV causes. They were both associated to mortality with similar predictive power.\(^15\)

In the present study, night-time HR was measured between 2:00 and 2:15 a.m., where participants were assumed to be sleeping. In this situation, interference of sensory input as well as physical and mental activities was minimized, and the mean HR significantly lower compared with resting HR and 24 h HR, which was expected according to the well-known circadian rhythm of HR.\(^6\) By removing these external influences, we find it biologically plausible to assume that night-time HR is a better measure of HR, and according to our findings a stronger predictor of all-cause mortality.

Increased HR is a sign of sympathetic hyperactivity and/or reduced parasympathetic tone.\(^6\),\(^16\) A disturbed autonomic balance can influence many vital functions and contribute to a poorer prognosis. It is shown in animal studies that rapid HR accelerates coronary atherosclerosis and is an independent predictor of coronary heart disease.\(^17\) Turbulence and pulsatile stress in the arterial systems occur primarily in the downstream of stroke and are therefore dependent on HR.\(^18\) In cynomolgus monkeys HR-reducing therapy reduced the progression of atherosclerosis.\(^19\) Increased HR by pacing markedly impairs arterial compliance and distensibility.\(^20\) High HR can also increase the risk of coronary thrombosis through increased blood viscosity, platelet activation, and procoagulant state.\(^21\) Furthermore, sympathetic activation promotes the occurrence of life-threatening ventricular arrhythmias.\(^22\) Past studies have shown favourable results by lowering the HR with β-blockers.\(^23\),\(^24\) The recent results of the BEAUTIFUL and the SHIFT study where the I\(_1\) inhibitor ivabradine was examined in patients with coronary artery disease and left ventricular systolic dysfunction and patients with systolic heart failure, respectively, have brought considerable interest in elevated HR as a treatable risk factor in CV disease.\(^25\),\(^26\) Subgroup analysis from the BEAUTIFUL study showed that patients with coronary disease, left ventricular dysfunction, and resting HR of 70 or higher could benefit from ivabradine treatment, as this treatment was associated with a reduction in risk of AMI and need of revascularization.\(^27\) In patients with moderate-to-severe heart failure, the SHIFT trial showed that ivabradine treatment reduced hospitalization and mortality from heart failure. This effect was independently linked to the degree of HR reduction.\(^28\) Treatment with medications with effect on the renin–angiotensin–aldosterone system may theoretically affect the prognostic burden of HR. However, adjustment for these medications in Cox models did not remove the prognostic significance of HR-variables in this study (Table 3, Model 5).

In patients with obstructive sleep apnoea (OSA), cardiac autonomic activity is altered. Several studies have showed an association between OSA and CV disease.\(^29\),\(^30\) Zhu et al.\(^31\) found MEANNN to be the only Holter variable to be associated with OSA. In the present study, we found a reverse association between CV events and amplitude of night-time HR from mean to maximum. This shows that it is a mean level of high rate, and not short periods with rapid increases in HR, that account for the prognostic importance. This is in agreement with the study of Zhu et al. and could reflect that OSA may be a contributor to higher night-time HR in this study.

Recent investigations have shown that the number of heartbeats in a lifetime is quite constant, and this seems to be true in the whole animal kingdom.\(^32\) Thus, a large body of evidence supports high resting HR as a risk factor in various populations including patients with hypertension, coronary artery disease, and heart failure. This enables us to consider HR as a modifiable risk factor with a targeted specific treatment available. This study presents an improved way of measuring this modifiable risk factor.

Limitations

This study is performed in middle-aged and elderly Caucasian subjects with no apparent heart disease or heart-related symptoms. Application to other population should be done with caution. Over-representation of subjects with multiple risk factors in our study population should be taken into consideration when interpreting the results. The relatively small number of subjects and events in this study compared with larger epidemiological studies is to be taken into account; however, the numbers were enough to detect statistically significant differences. The close associations and collinearity between different measures of HR make it very difficult to show an absolute superiority of one of the parameters. However, a better prognostic performance probably due to higher degree of signal-to-noise can be inferred from this study.

We did not use time-dependent analysis of covariates. As a consequence, there is a possibility that hypertensive subject at baseline without any anti-hypertensive treatment could have been started medications affecting HR during the 7 years of follow-up. This would, on the other hand, most likely tend to decrease the association of baseline HR and future events.
Conclusions
In middle-aged and elderly subjects with no apparent heart disease, all measures of increased HR are associated with increased mortality and CV risk; however, night-time HR has the strongest prognostic value. The clinical implications based on this study could be a greater attention to night-time HR and the possibility of intervention based on its value.

Funding
No funding was received in completing this paper, but the original Copenhagen Holter Study was supported by grants from The Danish Heart Foundation.

Conflict of interest: none declared.

References