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Effects of urinary cortisol levels and resting heart rate on the risk for fatal and nonfatal cardiovascular events

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ABSTRACT

Background and aims: Higher cortisol levels are associated with cardiovascular mortality in the elderly. It is unclear whether this association also exists in a general population of younger adults and for non-fatal cardiovascular events. Likewise, resting heart rate is associated with cardiovascular mortality, but fewer studies have also considered non-fatal events. The goal of this study was to investigate whether twenty-four-hour urinary cortisol (24-h UFC) levels and resting heart rate (RHR) predict major adverse fatal and non-fatal cardiovascular events (MACE) in the general population.
Methods: We used data from a subcohort of the PREVEND study, a prospective general population based cohort study with a follow-up of 6.4 years for 24-h UFC and 10.6 years for RHR. Participants were 3432 adults (mean age 49 years, range 28–75). 24-h UFC was collected and measured by liquid chromatography—tandem mass spectrometry. RHR was measured at baseline in a supine position for 10 min with the Dinamap XL Model 9300. Information about cardiovascular events and mortality was obtained from the Dutch national registry of hospital discharge diagnoses and the municipal register respectively.
Results: 24-h UFC did not significantly increase the hazard of MACE (hazard ratio = 0.999, 95% confidence interval = 0.993–1.006, p = 0.814). RHR increased the risk for MACE with 17% per 10 extra heart beats per minute (hazard ratio = 1.016, 95% confidence interval = 1.001–1.031, p = 0.036) after adjustment for conventional risk factors.
Conclusions: In contrast to 24-h UFC, RHR is a risk marker for MACE in the general population.

1. Introduction

Psychosocial stress is a well-known risk factor for cardiovascular disease (CVD) [1]. Hyperactivation of the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic nervous system (SNS), leading to increased cortisol levels and resting heart rate are postulated to be amongst the mechanisms behind this association [2].

This is plausible as cortisol has a direct effect on various risk factors for CVD. Increased levels of cortisol can affect blood pressure [3], BMI [4], waist circumference [4], fasting glucose levels [4], and HDL levels [4]. Moreover, glucocorticoids may also adversely influence remodeling after myocardial infarction via inhibition of angiogenesis [5], and induction of fibrosis via activation of the mineralocorticoid receptor [6]. An elevated resting heart rate (RHR) in turn might influence cardiovascular outcome by increasing cardiac oxygen demand [7], decreasing coronary blood flow by decreasing the duration of the diastole [8], lowering endothelial shear stress [9], and increasing the risk of plaque rupture [10].

Two recent prospective studies showed that elevated levels of cortisol predict cardiovascular death amongst elderly people with [11] and without preexisting CVD [11,12]. To our knowledge, studies in a younger population investigating whether physiological levels of endogenous cortisol are associated with increased incidence in cardiovascular events are lacking. Moreover, the studies in elderly people did not investigate the relationship of cortisol levels with non-fatal cardiovascular events. Regarding the effects of RHR, in populations without known CVD, RHR was found to be a risk factor

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for both cardiovascular death [13—16] and morbidity [14,15,17], although some studies did not find any relationship with non-fatal cardiovascular events [18—20].

In the current study we assessed for the first time in a general population cohort whether higher levels of cortisol are an independent risk factor for major adverse fatal and non-fatal cardiovascular events (MACE). Moreover, we intended to replicate the results of previous studies with regard to higher RHR and the risk for MACE.

2. Materials and methods

2.1. Study population

We used data from a subcohort of the Prevention of REnal and Vascular ENd stage Disease (PREVEND) study. PREVEND is population cohort study originally designed to investigate microalbuminuria as a risk factor for renal disease and CVD. The recruitment of participants for PREVEND has been extensively described elsewhere [21]. In brief, 8592 subjects completed the baseline screening survey in 1997—1998 (T1), rendering the PREVEND study cohort. Subjects with insulin dependent diabetes mellitus and pregnant women were excluded from the study population. The PREVEND study is enriched for albuminuria which is a risk factor for developing renal disease. To obtain a representative random sample of the Groningen general population for the current study, we included all subjects with a urinary albumin concentration (UAC) < 10 mg/L that completed the first screening (N = 2592) and added a random subset (n = 840) from the "overrepresented" subjects with an UAC > 10 mg/L proportional to the degree of overrepresentation. This resulted in a group of 3432 subjects with a population representative ratio of albuminuria negative and positive subjects forming the basis for the current study. The average age was 49 years, minimum and maximum were 28—75 years. Follow-up measurements took place between January 2002 and November 2003 (T2). Average time between T1 and T2 was 4.1 years. The study was approved by the local Medical Ethical Committee for human research of the University Medical Center Groningen (UMCG). All participants were aged 18 or older and provided written informed consent for participation in this study.

2.2. 24-h urinary free cortisol

24-h urinary free cortisol (24-h UFC) was measured at T2. Participants were asked to collect urine samples in a polypropylene container on two consecutive days prior to the visit to the outpatient clinic. They were carefully instructed to urinate into the container during the 24-h collection period and refrigerate the sample until delivery to the laboratory. 24-h urine collection was available on at least one day for 2761 people and at both days for 2710 people. Urinary creatinine was measured to assess completeness of the 24-h urine collection. We used the following formula to assess completeness: incomplete urine ≤ 0.7 of [mmol urinary creatinine $\times$ 113]/[21 $\times$ kilograms of body weight] [22], as this has been proven to be the most sensitive method to detect incompleteness [23]. Only samples which were complete according to the above formula were used for the current study. Urinary free cortisol (UFC) was measured by liquid chromatography—tandem mass spectrometry (LC—MS/MS) analysis. The lower detection limit of the assay was 0.3 nmol/l. At low, middle, and high concentrations, intra-assay variation ranged from 1.3 to 2.4% while inter-assay variation ranged from 3.8 to 7.8%. 24-h UFC was calculated by multiplying urinary volume with cortisol concentration and is expressed in nmol per 24-h. We used the mean of the two samples on two consecutive days to reflect HPA axis function. In the

case when values of only one day were available we used this value instead of the mean.

2.3. Resting heart rate

RHR and blood pressure Blood pressure was measured, in supine position, every minute for 10 min, with an automatic device (Dinamap XL Model 9300, Johnson—Johnson Medical, Tampa, FL, USA).

2.4. Follow-up and outcomes

Information about cardiovascular events was obtained from the Dutch national registry of hospital discharge diagnoses (PRISMANT). Information about mortality was obtained from the municipal register. Information on the cause of death was acquired by linking the number of death certificates to the primary cause of death as coded by the Dutch Central Bureau of Statistics. The outcome of interest was a combined end-point of fatal and non-fatal major adverse cardiovascular events (MACE). MACE was defined as acute myocardial infarction (ICD-code 410), acute and subacute ischemic heart disease (411), occlusion or stenosis of the precerebral (433) or cerebral arteries (434) and the following procedures: coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, and other vascular interventions namely percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels. Mortality from any other cause was censored.

2.5. Medication use

Medication (antihypertensive, lipid-lowering, antidiabetic) was self-reported and substantiated with information of drug-use from the IADB.nl, which contains dispensing information from 55 community pharmacies in the Northern part of the Netherlands, covering on average 500 000 persons annually (www.IADB.nl) and almost the entire population of PREVEND study participants [24]. The database's pharmacy information includes, among others, name of the drug, anatomic—therapeutic—chemical (ATC) classification and date of prescription. Medication records of patients are virtually complete because of high patient pharmacy commitment in the Netherlands [25] We extracted information on drug prescriptions from 100 days prior until 100 days after the date of the visit to our research facilities.

2.6. Covariates

Each survey in the PREVEND study consisted of one to two visits to an outpatient unit. Participants completed a questionnaire on demographics, CVD history, lifestyle and medication use before the visit. Height and weight were measured and a fasting blood sample was drawn. Body mass index was calculated as the ratio between weight and height squared. Smoking status was assessed by self-report. Participants were considered smokers if they had smoked in the previous year and previous smokers if they had quit smoking more than one year ago. Hypertension was defined as systolic blood pressure $\geq$ 140 mm Hg or diastolic blood pressure $\geq$ 90 mm Hg following the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure criteria, or use of antihypertensive medication. Hyperlipidemia was defined as cholesterol level >6.5 mmol/L when a history of hyperlipidemia was absent, or use of lipid-lowering drugs. Diabetes was defined as fasting glucose level 7.0 mmol/L, nonfasting glucose level 11.1 mmol/L, or use of antidiabetic medication. Prior history of CVD at inclusion of the study was defined as self-report of

cerebrovascular accident, coronary heart disease, or peripheral vascular disease requiring surgery.

2.7. Statistical analysis

For statistical analysis, 24-h UFC and RHR were analyzed as a continuous measure, and divided into quintiles to assess if the association was linear. We used Cox proportional hazards regression to investigate the associations between 24-h UFC or RHR (in separate models), and the outcome variable MACE. Model 1 was adjusted only for sex and age. Model 2 was additionally adjusted for smoking status, hypertension, hyperlipidemia, diabetes mellitus, and history of CVD. Model 3 was the same as model 2, but with additional adjustment for creatinine clearance and BMI. The lowest category (e.g. the 1st quintile) was always used as a reference category. For sex, males were used as the reference category. The use of corticosteroids leads to negative feedback on the HPA axis and thus to lower endogenous levels of 24-h UFC. Thus, for the analysis of 24-h UFC, we excluded participants using inhalation, local, gastrointestinal, or systemic glucocorticosteroids (15%). For the analyses on the effects of RHR, we excluded people on antihypertensive medication (14%) as this might either directly (beta-blocker, calcium channel blocker) or indirectly influence the values of RHR. The proportional hazards assumption was checked by graphically assessing the log-log minus function of all predictor variables. The assumption was met for all predictor variables in all analyses. Unlike RHR, 24-h UFC was not measured at baseline, but only at the second assessment wave (T2). As any longitudinal study, PREVEND suffered from some loss to follow-up. We therefore used multiple imputation techniques to correct for attrition bias (supplement 1). The conclusions drawn from the Cox regression models on the original data and the imputed data sets were in accordance with each other. The estimates below are pooled estimates of the imputed data. Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 20.0. and Stata Statistical Software: Release 12. StataCorp. A two-sided P value of ≤0.05 was considered statistically significant.

3. Results

3.1. Population characteristics at T1 and T2

Descriptive statistics of the characteristics of our study population at T1 and T2 are provided in Table 1. There were slightly more women than men at T1 and T2 (56%). The average age at T1 was 49 years with a minimum of 28 and a maximum of 75 years. The median follow-up time from T1 was 10.6 years and the median follow-up time from T2 was 6.4 years. From T1 until follow-up in January 2009, a total of 263 MACE took place. From T2 until follow-up in January 2009 a total of 161 MACE took place. During the PREVEND study, 588 T1 participants (17%) did not show up at the follow-up visit (T2).

3.2. The prediction of MACE by 24-h urinary free cortisol

After excluding people on corticosteroid medication, a sample of 2792 people remained, in which 121 MACE took place. Table 2 shows the results of the three Cox proportional hazard models assessing the association between quintiles of 24-h UFC and MACE. 24-h UFC did not significantly influence the hazard of MACE. The traditional risk factors for MACE: sex, age, smoking status, hypertension, hyperlipidemia, and previous history of CVD were all highly significant. To exclude the possibility that our negative findings could be explained by the use of artificial cut-off scores, we also ran two models adjusted only for sex and age with the

continuous measure of 24-h UFC as a predictor. MACE was not significantly predicted by the continuous measure of 24-h UFC (p = 0.814). To investigate whether 24-h UFC levels might be only a significant predictor for people of a certain age, we also investigated a model with a multiplicative interaction term between 24-h UFC and age. The interaction term between 24-h UFC and age (p = 0.791) was not statistically significant. Likewise, an interaction term between sex and 24-h UFC (p = 0.090) was not significant. We excluded the possibility that our null findings could be explained by mixing a population with and without a history of CVD by rerunning the analyses excluding persons with a history of CVD. After excluding those with a history of CVD, a population of 2687 people remained in which 97 MACE took place. 24-h UFC was again not a significant predictor of MACE (p = 0.923).

3.3. The prediction of MACE by resting heart rate

After exclusion of persons using antihypertensive medication a population of 2823 people remained, in which 156 MACE occurred. Table 3 shows the results of the three Cox proportional hazard models assessing the association between quintiles of RHR and MACE. Compared to the lowest quintile, the risk for MACE was twice as high in the fourth and the fifth quintile while adjusting for sex and age. Only the fifth quintile of heart rate remained a significant predictor of MACE in the model adjusted for sex, age, hypertension, smoking status, hyperlipidemia, diabetes mellitus, and CVD history. In the final model, with additional adjustment for BMI and creatinine clearance, the fifth quintile remained a significant predictor of MACE. We repeated the analyses for the continuous variable of RHR (data not reported in table). Like the quintiles, the continuous variable of RHR was a highly significant predictor of MACE while adjusting for sex and age (HR = 1.074, 95% CI = 1.059–1.089, p < 0.001). After addition of hypertension, smoking status, hyperlipidemia, diabetes mellitus, and CVD history to the model, the continuous variable of RHR remained a significant predictor of MACE, but its effects were attenuated (HR = 1.016, 95% CI = 1.001–1.031, p = 0.036). In the final model, where also BMI and creatinine clearance were added the continuous variable of heart rate remained a significant predictor, increasing the risk for MACE with 16% per 10 extra heart beats per minute (HR = 1.016, 95% CI = 1.001–1.031, p = 0.036). We reran the same models after exclusion of people with a history of CVD. In fully adjusted models, the continuous variable of RHR (HR = 1.016, 95% CI = 1.001–1.031, p = 0.040) and the highest quintile (HR = 2.073, 95% CI = 1.184–3.629, p = 0.011) remained significant predictors of MACE in people without prior CVD.

4. Discussion

In the current study we investigated whether 24-h UFC levels and RHR were independent predictors of major fatal and non-fatal adverse cardiovascular events. Higher RHR did indeed significantly increase the risk of MACE. Unlike RHR and traditional cardiovascular risk factors, 24-h UFC did not significantly contribute to the risk of MACE.

4.1. Limitations

There are several limitations that need to be considered when interpreting our results. Heart rate is susceptible to influences from the environment, and although it was measured in a very standardized way, with a 10-min recording, 24-h holter registration could have increased accuracy. Moreover, as PREVEND is an observational study, it can never establish a causal link, as residual confounding might still exist. We did for instance not adjust for

Table 1
Population characteristics at T1 and T2.

Variable	T1 N = 3432	T2 N = 2773[a]
MACE (N)	263	161
Myocardial infarction	72	40
(Sub)acute ischeamic heart disease	63	39
Occlusion or stenosis of:		
- Precerebral arteries	14	8
- Cerebral arteries	27	19
CABG	22	14
PTCA	26	14
PTA of peripheral vessels	2	1
Bypass grafting of aorta	10	5
Carotid endarterectomy or carotid stenting	7	6
Death by MACE	20	15
Mean survival time MACE (SE)	3925 (12)	2719 (7)
Age	49 (12)	53 (12)
Sex (female)	56%	56%
Cortisol in nmol/24-h	n.a.	69 (47—96)
Quintiles:		
- 1st	n.a.	$1 \leq UFC \leq 43$
- 2nd	n.a.	$43 < UFC \leq 59$
- 3rd	n.a.	$59 < UFC \leq 79$
- 4th	n.a.	$79 < UFC \leq 106$
- 5th	n.a.	$106 < UFC \leq 666$
Resting heart rate	69 (10)	n.a.
Quintiles:		
- 1st	$42 \leq RHR \leq 61$	n.a.
- 2nd	$61 < RHR \leq 66$	n.a.
- 3rd	$66 < RHR \leq 71$	n.a.
- 4th	$71 < RHR \leq 77$	n.a.
- 5th	$77 < RHR \leq 115$	n.a.
Smoking		
− Never smoked	32%	32%
− Previous smoker	33%	40%
− Current smoker	35%	28%
BMI	26 (4)	27 (4)
Creatinine clearance	101 (26)	102 (25)
Hypertension	27%	28%
Diabetes (yes)	3%	5%
Hyperlipidemia (yes)	23%	22%
History of CVD (yes)	5%	4%
Antihypertensive medication (yes)	14%	18%
Glucocorticoid medication (yes)	n.a.	15%

[a] Unimputed data after the exclusion of people with events before T2. CVD = cardiovascular disease, BMI = body mass index, MACE = a combined end-point of fatal and non-fatal Major Adverse Cardiovascular Event, N = number of events, SE = standard error of the mean, CABG = coronary artery bypass grafting, PTCA = percutaneous transluminal coronary angioplasty, PTA = percutaneous transluminal angioplasty, UFC = urinary free cortisol, RHR = resting heart rate. Descriptives are given of non-missing values as either percentages or mean with standard deviation between brackets unless indicated otherwise.

Table 2
Association of MACE with 24-h UFC in multivariable Cox regression models.[a]

Variable	Model 1 HR	95% CI	p	Model 2 HR	95% CI	p	Model 3 HR	95% CI	p
24-h UFC 2nd	0.741	0.253—2.170	0.583	0.820	0.273—2.469	0.724	0.823	0.273—2.479	0.728
24-h UFC 3rd	0.710	0.272—1.859	0.485	0.740	0.275—1.991	0.550	0.745	0.276—2.010	0.560
24-h UFC 4th	0.844	0.313—2.278	0.737	0.916	0.328—2.555	0.866	0.924	0.329—2.594	0.880
24-h UFC 5th	0.743	0.261—2.120	0.577	0.737	0.255—2.129	0.572	0.744	0.254—2.180	0.588
Age	1.086	1.068—1.104	<0.001	1.064	1.043—1.087	<0.001	1.063	1.039—1.087	<0.001
Sex (female)	0.382	0.258—0.564	<0.001	0.457	0.302—0.692	<0.001	0.444	0.285—0.690	<0.001
Previous smoker				1.306	0.709—2.405	0.391	1.309	0.711—2.411	0.387
Current smoker				2.768	1.532—5.001	0.001	2.757	1.523—4.933	0.001
Hypertension				2.515	1.467—4.310	0.001	2.534	1.459—4.401	0.001
Diabetes				1.307	0.692—2.470	0.408	1.308	0.691—2.477	0.409
Hyperlipidemia				1.564	1.020—2.398	0.040	1.566	1.012—2.393	0.044
CVD history				1.896	1.124—3.199	0.017	1.871	1.101—3.180	0.021
BMI							0.999	0.947—1.054	0.979
Creatinine clearance							0.998	0.987—1.009	0.726

[a] After exclusion of people on corticosteroid. medication. BMI = body mass index, 24-h UFC = twenty-four-hour urinary free cortisol, CVD = cardiovascular disease, HR = hazard ratio, 95% CI = 95% confidence interval of the hazard ratio, p = p-value.

Table 3
Association of MACE with resting heart rate in Multivariable Cox Regression Models.[a]

Variable	Model 1			Model 2			Model 3		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
RHR 2nd	1.707	0.990—2.943	0.054	1.643	0.953—2.833	0.074	1.654	0.948—2.885	0.076
RHR 3rd	1.026	0.541—1.948	0.936	0.879	0.462—1.675	0.695	0.895	0.467—1.716	0.738
RHR 4th	2.026	1.180—3.476	0.010	1.592	0.920—2.754	0.096	1.635	0.938—2.850	0.083
RHR 5th	2.626	1.569—4.397	<0.001	1.942	1.151—3.276	0.013	1.934	0.137—3.290	0.015
Age	1.074	1.059—1.089	<0.001	1.073	1.056—1.090	<0.001	1.067	1.049—1.084	<0.001
Sex (female)	0.275	0.193—0.391	<0.001	0.295	0.205—0.424	<0.001	0.260	0.147—0.386	<0.001
Previous smoker				1.246	0.751—2.066	0.395	1.277	0.763—2.138	0.351
Current smoker				3.104	1.957—4.925	<0.001	3.252	2.034—5.202	0.001
Hypertension				1.337	0.940—1.900	0.106	1.286	0.887—1.845	0.172
Diabetes				0.943	0.384—2.312	0.897	0.942	0.383—2.138	0.897
Hyperlipidemia				2.010	1.454—2.788	<0.001	1.987	1.434—2.753	<0.001
CVD history				1.733	0.948—3.168	0.074	1.704	0.930—3.121	0.085
BMI							1.049	1.002—1.098	0.040
Creatinine clearance							0.993	0.986—1.001	0.074

[a] After exclusion of people who use antihypertensive medication. BMI = body mass index, RHR = resting heart rate, CVD = cardiovascular disease, HR = hazard ratio, 95% CI = 95% confidence interval of the hazard ratio, p = p-value.

physical activity levels while physical fitness is associated with both heart rate [26] and cardiovascular outcomes. A recent study demonstrated, however, that heart rate is an independent risk factor for mortality after adjustment for cardiorespiratory fitness as indexed by VO_2-max [13]. Another limitation of the current study is that the PREVEND study suffered from attrition and no-show at the scheduled follow-up visit at T2 at which 24-h UFC was measured. Individuals with missing data had in general more cardiovascular risk factors and experienced more events. As participants needed to be event free until the second survey to be included in the current study, our negative results might also in part be due to a survivor selection bias. Furthermore, the occurrence of death from other than cardiovascular causes might have precluded participants from experiencing MACE (competing risk phenomena). These limitations, however, also apply to previous cohort studies. In the INCHIANTI and the WHITEHALL II study missingness was also related to lower socioeconomic status and having more cardiovascular risk factors. Complete case analysis can yield biased estimates [27]. Unlike the two other studies, we used multiple imputation to mitigate the effects of missingness and to preserve power as is advised also for survival analysis [27,28]. The outcome MACE was known for every participant including those that had dropped out. Yet, our conclusion remained the same with both complete case analysis and analyses of the imputed sets. We can, however, never exclude the possibility that selection bias negatively influenced our results.

4.2. Strengths

The PREVEND study also has several strengths. It is a large population representative cohort in which both predictors and outcomes were well measured. MACE were assessed by using the Dutch national registry of hospital discharge diagnoses and the Dutch national bureau of statistics. Therefore, detailed information was available on both fatal and non-fatal events. Furthermore, medication use was substantiated with information from the database of pharmacy-dispensing data. Compared to the INCHIANTI study, the only other study which measured 24-h UFC, the PREVEND study has some important methodological strengths. Firstly, in our study, in the majority of cases, 24-h UFC was measured on two consecutive days thus giving a more reliable estimate of 24-h UFC levels, whereas in the INCHIANTI study only one day of 24-h urine was available. Secondly, we were able to verify completeness of the samples by measuring urinary creatinine

excretion. We had to exclude 16% of the samples based on this analysis. In the INCHIANTI study, compliance was assessed by self-report and therefore only 2.7% of samples was excluded. Thirdly, in the INCHIANTI study 24-h UFC was measured by Bayer's ADVIA-Centaur immunoassay system which has poor specificity due to cross-reactivity with cortisone (44%) and is sensitive to drug interference [29]. In PREVEND, 24-h UFC was measured by means of LC-MS/MS which is free of interferences from cortisol metabolites and conjugates, and also eliminates drug interferences [30].

4.3. Cortisol levels and cardiovascular outcomes

Three studies investigated the relationship between cortisol levels and cardiovascular mortality [11,12,31] Two found cortisol levels to be predictive of cardiovascular mortality (a flatter slope of salivary cortisol levels over the day [12] in the WHITEHALL II, or higher levels of 24-h UFC [11] INCHIANT study respectively). Whereas one, the Vietnam Experience study, did not find an association between serum cortisol levels and cardiovascular mortality [31]. None of these studies took non-fatal events into account as we did. Yet, if higher cortisol levels really do increase the risk of CVD one would expect to also find an association with non-fatal events, which we did not. Our negative results cannot be explained by a lack of events. In the WHITEHALL II study and the CHIANTI study 32 and 41 fatal cardiovascular events took place respectively, whereas in PREVEND 121 MACE took place. In general 10 events per predictor variable are needed to get reliable regression estimates [32]. Lacking events does not just decrease power to detect a true effect, but also biases regression coefficients, and can also lead to an overestimation of the hazard [32]. The PREVEND study differs from WHITEHALL II and the INCHIANTI study on several other aspects. In terms of average age (52 years at T2) PREVEND has a relatively young population compared to the aforementioned studies, where the average participant was older than 65 years. Furthermore, PREVEND is a general population based cohort, whereas participants in WHITEHALL II were white collar workers, and the participants of the INCHIANTI study were all retired. It might thus be that cortisol forms a risk for cardiovascular events only in an elderly population. We tested for this possibility by including and interaction term between 24-h UFC and age into our model, but found no statistical support for this hypothesis.

An explanation for our null findings might be that within-individual stability of cortisol levels are low. The few studies that assessed intra-individual stability of cortisol levels over longer

periods of time showed perplexingly high intra-individual variability [33,34]. This would make it difficult to detect a true effect of 24-h UFC on MACE. Perhaps longer term indices of within-person levels of cortisol, such as cortisol measured in scalp hair [35], are more suitable to study the relationship between HPA-axis functioning and the occurrence of MACE.

4.4. Resting heart rate and cardiovascular outcomes

In patients with CVD, higher RHR has consistently been found to predict cardiovascular mortality [18,20,36,37]. In the current study, we demonstrated in a relatively young and healthy population that RHR increases the risk of fatal and nonfatal MACE while adjusting for various conventional risk factors. Our study adds to the accumulating epidemiological evidence from general population cohorts that RHR is a significant predictor of fatal and non-fatal cardiovascular outcomes in populations without preexisting CVD [13—15,17,38,39]. In patients with stroke, RHR has been demonstrated to increase the risk of myocardial infarction [37]. Several other studies failed to demonstrate a significant relationship between RHR and non-fatal cardiac events in patients with a history of CVD [18—20]. From the literature it is unclear whether the relationship between RHR and cardiovascular outcomes is linear. Some studies have demonstrated a J-shaped relationship [40] or threshold effect [36], whereas others showed a clear linear relationship [18,41]. Our study suggests that there might be threshold effect because, when examining quintiles of RHR, only people with a RHR larger than 77 beats per minute had an increased risk of MACE after adjusting for conventional risk factors. New results from the Multi-Ethnic Study of Atherosclerosis point in the same direction, with only people who have a RHR larger than 80 beats per minute having a higher risk of MACE [42]. Based on these results a cut-off value for RHR of 80 beats per minute in the general population seems reasonable to classify a low and a high-risk group. Future studies should examine in other cohorts whether adding RHR to existing risk scores improves its discriminative power.

The question arises whether RHR is a modifiable risk factor or epiphenomena, meaning it is only a risk marker. For certain patient groups RHR seems to be a modifiable risk factor, as reduction of heart rate has proven to be protective against cardiovascular mortality in patients with left ventricular failure. For instance, in the BEAUTIFUL study, heart rate reduction with Ivabradine, a selective inhibitor of the I_f current, was shown to decrease the risk of cardiovascular death in patients with coronary artery disease and left ventricular dysfunction who had a RHR > 70 beats per minute [41]. Likewise, in the SHIFT study, in patients with systolic heart failure, heart rate reduction with Ivabradine led to a decrease in cardiovascular deaths and hospitalizations [43]. In contrast, results from the SIGNIFY study make it clear that for patients with stable coronary artery disease without clinical heart failure, RHR seems to merely be a risk marker, as lowering heart rate did not improve clinical outcomes [44].

In conclusion, RHR is a strong risk marker of MACE in the general population without pre-established CVD. This might have clinical implications, as the measurement of heart rate is a non-invasive low-cost procedure that could easily be used to detect people that are at heightened risk for a cardiovascular event. Finally, we did not find any evidence that higher urinary cortisol levels increase the risk of MACE.

Disclosure statement

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.02.030.

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