CLINICAL STUDY

Risk of elevated resting heart rate on the development of type 2 diabetes in patients with clinically manifest vascular diseases

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Abstract

Objective: Sympathetic nerve activation is causally related to insulin resistance as both a cause and a consequence. Resting heart rate (RHR) reflects sympathetic nerve activity. We investigated the effect of RHR on the incidence of type 2 diabetes mellitus (T2DM) in patients with clinically manifest vascular diseases.

Design: Data were used from the second manifestations of arterial disease (SMART) study: a prospective cohort study of patients with clinically manifest vascular diseases ($n = 3646$).

Methods: RHR was obtained using an electrocardiogram. Patients were followed up for incident type 2 diabetes ($n = 289$) during a median period of 5.5 (interquartile range 3.2–8.4) years. The relation between RHR and incident T2DM was estimated by Cox proportional hazard analysis. As age was an effect modifier ($P = 0.048$), analyses were stratified for age.

Results: Patients in quartile 4 (Q4) of RHR had a 65% increased risk of T2DM compared with those in Q1 (reference; hazard ratios (HR), 1.65; 95% confidence interval (95% CI), 1.15–2.36) adjusted for age, gender, smoking, estimated glomerular filtration rate, systolic blood pressure, location of vascular disease, and antihypertensive medication. Every 10 beats per minute (bpm) increase in RHR increased the risk for T2DM with 10% (HR, 1.10; 95% CI, 1.00–1.21) in the total population. This risk was particularly high in subjects aged 55–63 years (per 10 bpm: HR, 1.22; 95% CI, 1.04–1.43) and was independent of the location of vascular disease and beta-blocker use.

Conclusions: Increased RHR, an indicator of sympathetic nerve activity, is associated with an increased risk for T2DM in patients with manifest vascular diseases, particularly in middle-aged patients.

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Introduction

Insulin resistance and elevated sympathetic nerve activity are closely related, but the precise pathophysiological relation is complex and only partially understood. Visceral obesity is associated with a more pronounced sympathetic nerve activity than subcutaneous obesity (1), probably to a large extent caused by elevated serum levels of insulin and leptin (2). Administration of insulin in humans increases muscle sympathetic nerve activity, the reference standard of measuring sympathetic nerve activity (3), by a direct effect on insulin receptors in the CN (4, 5). Plasma leptin levels are independently associated with resting heart rate (RHR) in healthy male subjects (6). Furthermore, the infusion of leptin in rats increases the heart rate, through leptin receptors in the CN (7, 8).

The causal relation between sympathetic nerve activity and insulin resistance could also be the other

way around. Acute reflex activation of the sympathetic nerve system is reported to induce acute insulin resistance in humans (9). Moreover, chronically increased sympathetic nerve activity can precede the development of insulin resistance and obesity (10, 11). RHR reflects sympathetic tone, and correlates with muscle sympathetic nerve activity and noradrenaline serum levels (12). An increased RHR identifies subjects at higher risk for developing cardiovascular disease and mortality (13, 14). Furthermore, an increased RHR at baseline is independently associated with an increased risk for developing type 2 diabetes mellitus (T2DM) in healthy populations (15, 16, 17, 18, 19) and in subjects with obesity or impaired glucose tolerance (20) but this has not been shown in patients with vascular diseases.

Identifying subjects at high risk for developing T2DM is important, as diabetes is a strong risk factor for cardiovascular morbidity and mortality (21, 22) and patients with diabetes have a reduced quality of life (23).

Furthermore, the risk for T2DM can be reduced through lifestyle interventions and medication (24). Patients with clinically manifest vascular diseases are at particularly high risk of developing new vascular events and T2DM given shared pathophysiological pathways ('common soil' hypothesis) of diabetes and atherosclerosis, such as insulin resistance and low-grade inflammation (25). As increased sympathetic nerve activity, reflected by an elevated RHR, is associated with increased risk for vascular diseases and mortality on the one hand (13, 26), and diabetes on the other hand (15, 16, 17, 18, 19, 20), increased sympathetic tone may also be part of the shared pathophysiology in the development of vascular diseases and T2DM. Patients with vascular diseases who have a particular high risk for the development of diabetes are an ideal group for preventive measures, given their high risk and the fact that they are already receiving medication and lifestyle advice, in contrast to the general population. Therefore, in this study we investigated the relation between RHR and incidence of T2DM during follow-up in a cohort of patients with clinically manifest vascular diseases.

Subjects and methods

Study design and patients

In this study, data were used from patients enrolled in the second manifestations of arterial disease (SMART) study, an ongoing single-center prospective cohort study carried out at the University Medical Center Utrecht. This study started in September 1996. Patients aged 18–80 years, referred to our institution with clinically manifest atherosclerotic vascular diseases, were included. Patients with terminal malignant disease, those not independent in daily activities (Rankin scale > 3), and not sufficiently fluent in Dutch were not included.

The aims of the SMART study were to determine i) the risk factors for atherosclerosis, ii) prevalence of additional vascular disease, and iii) incidence of future cardiovascular events and type 2 diabetes. The study complied with the Declaration of Helsinki Principles; the Medical Ethics Committee of the UMC Utrecht, The Netherlands, approved the study and all patients gave written informed consent. Patients were asked to complete a health questionnaire covering medical history, risk factors, smoking habits, and medical treatment. A standardized diagnostic protocol consisting of physical examination and laboratory testing in a fasting state was followed. A more detailed description of the design of the study has been published previously (27).

For this study, data were used from patients ($n=5280$) enrolled in the SMART cohort from September 1996 to March 2010 with a recent diagnosis or history of coronary artery disease (CAD),

cerebrovascular disease (CVD), peripheral arterial disease (PAD), or abdominal aortic aneurysm (AAA).

Patients who had died ($n=480$) or were lost to follow-up ($n=91$) before the assessment of incident T2DM started in 2006 (see Follow-up and assessment of T2DM) were excluded (Fig. 1). Patients with diabetes mellitus (type 1 or 2) at study inclusion ($n=809$), defined as a referral diagnosis of diabetes, self-reported diabetes (use of glucose-lowering agents), or a known history of diabetes, were excluded. To make sure that all patients with diabetes at baseline were excluded, subjects without a history of diabetes, but with the combination of a fasting plasma glucose level ≥ 7.0 mmol/l at baseline and receiving treatment with glucose-lowering agents within 1 year after baseline were considered as having diabetes at baseline and were excluded ($n=50$). Furthermore, patients without a study electrocardiogram (ECG; $n=85$) or without sinus rhythm on ECG ($n=169$) were excluded, leaving a total of 3646 patients for analyses (Fig. 1).

Figure 1 Flow chart.

Measurement of RHR

At study inclusion, a 12-lead ECG was obtained after the patient had rested in supine position for 5 min. RHR was calculated using the digitally stored 12-lead 10-s data, by dividing the number of R–R intervals (number of QRS-complexes minus one) by the time difference between the first and last beat, and the result was converted to beats per minute (bpm). This calculation was performed using the Marquette 12SL analysis program (General Electric Healthcare, Hoevelaken, The Netherlands).

Follow-up and assessment of type 2 diabetes

The main outcome of interest for this study was incident T2DM. All participants that had been included until June 2006 without diabetes at baseline received a questionnaire in the period between June and December 2006 to assess the incidence of T2DM after study inclusion. After 2006, they were biannually asked to complete this questionnaire. Patients were asked whether they had diabetes and if 'yes', patients received a supplementary questionnaire regarding date of diagnosis, initial treatment (diet, oral medication, or insulin), current treatment, and family history of diabetes. If the answers were incomplete or unclear, patients and/or their general practitioner were called on the phone for further information. To validate the diagnosis of diabetes, two independent physicians audited and classified all diabetes cases. Furthermore, cross-validation with the hospital diagnosis registry revealed that none of the patients who reported not to have diabetes had a physician's diagnosis of diabetes.

Follow-up duration (years) was defined as the period between study inclusion and date of diagnosis of T2DM, date of death, date of loss to follow-up, or the preselected date of 1 March 2010. From 1996 until 1 March 2010, a total of 72 (2%) patients were lost to follow-up.

Data analyses

Continuous variables are expressed as mean $\pm$ s.d. when normally distributed or as median (interquartile range (IQR)) in case of skewed distribution. Categorical variables are expressed as numbers (percentage). Single imputation methods were used to reduce missing covariate data for smoking status ($n=15$; 0.4%), body mass index (BMI; $n=5$; 0.1%), systolic blood pressure (SBP; $n=5$; 0.1%), and estimated glomerular filtration rate (eGFR, calculated using the modification of diet in renal disease formula; $n=18$; 0.5%), as this method reduces the chance for bias compared with complete case analysis.

The incidence rate of T2DM was calculated as the number of new T2DM patients divided by total amount of person-years during the study follow-up. Corresponding 95% confidence intervals (95% CIs) were calculated

using Fisher's exact test. The relation between RHR and incident type 2 diabetes was quantified with Cox proportional hazard analysis. Results are expressed as hazard ratios (HR) with 95% CI. Patients were censored if they died or were lost to follow-up. The proportional hazard assumption was confirmed by testing the correlations between scaled Schoenfeld residuals for RHR and time. No significant nonproportionality ($P<0.05$) was observed.

HR on incident T2DM were calculated per quartile RHR, using patients in quartile 1 (Q1) as reference category. Second, linear regression analyses were performed to estimate the risk of incident T2DM per 10 bpm increase in RHR, for the whole study population and after stratification for age in tertiles (<55 , 55–63 and >63 years). Third, to explore if associations between RHR and incident T2DM were different in patients with different locations of vascular diseases, analyses were performed per 10 bpm increase in RHR after stratifying patients according to the vascular diagnosis at study inclusion, being CAD, CVD, PAD, or AAA. If inclusion in the SMART study was not due to a recent vascular diagnosis, the first vascular diagnosis in their medical history was used to classify them as CAD, CVD, PAD, or AAA.

All regression analyses were conducted with a crude model (I) and three models to adjust for potential confounding factors. In model II, we adjusted for age and gender and in model III, additional adjustments were carried out for current smoking, SBP, eGFR, the use of medication with possible effect on the RHR and on incident T2DM (beta-blockers, diuretics, calcium channel-blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers) and the location of vascular disease at study inclusion (CAD, CVD, PAD, or AAA). The study conclusions are based on this fully adjusted model. Finally, in an exploratory model (IV) we additionally adjusted for variables that may be in the causal pathway between RHR and incident T2DM, being BMI and fasting glucose. Current smoking, gender, the vascular diagnosis, and the use of medication were included as categorical variables and age, eGFR, SBP, BMI, and fasting glucose were entered as continuous variables.

Analyses were repeated after exclusion of patients with baseline fasting glucose values ≥ 7.0 mmol/l ($n=212$) and patients with extreme heart rates (≤ 50 ($n=436$) or ≥ 100 bpm ($n=35$)), to avoid a large effect of patients with impaired fasting glucose or extreme heart rates. Additional exploratory correction for thyroid-stimulating hormone (TSH), another possible factor in the causal pathway between RHR and incident T2DM, was also carried out. Finally, besides correcting for beta-blocker use, analyses were conducted in patients on beta-blocker ($n=1934$) and not on beta-blocker ($n=1712$) treatment separately, as beta-blockers influence both the RHR and the risk for diabetes. SPSS version 15.0.1 was used for all analyses.

Results

Baseline characteristics

Baseline characteristics per quartile of RHR are presented in Table 1. The average RHR increased from 50 ± 4 bpm in Q1 to 79 ± 9 bpm in Q4. The mean age of the study population was 59 ± 10 years and the percentage of male participants decreased from 81 in Q1 to 66 in Q4. Over the quartiles with increasing RHR, slight increases were observed in fasting serum glucose levels (Q1, 5.7 ± 0.7 –Q4, 5.8 ± 0.8 mmol/l) and BMI (Q1, 26.2 ± 3.2 –Q4, 26.8 ± 4.2 kg/m²).

Follow-up and incident type 2 diabetes

The total follow-up time in this study was 21 535 person-years, with a median follow-up of 5.5 (IQR, 3.2–8.4) years. During this follow-up, 289 patients were diagnosed with incident T2DM and 188 patients died. The unadjusted incidence rate (new cases of T2DM per 1000 person-years of follow-up) increased across quartiles of RHR (from 9.6 (95% CI, 7.3–12.5) in Q1 to 16.0 (95% CI, 13.0–19.6) in Q4; Fig. 2A) and

with increasing age (from 11.9 (95% CI, 9.7–14.5) for subjects < 55 years to 14.2 (95% CI, 11.6–17.2) for subjects > 63 years (Fig. 2B)).

RHR and incident T2DM

Subjects in the highest quartile of RHR (Q4) had a 65% higher risk of incident T2DM compared with those in the reference Q1 (HR, 1.65; 95% CI, 1.15–2.36) based on the fully adjusted model (III; Fig. 3). Every 10 bpm increase in RHR was related to a 10% increase in incident T2DM (HR, 1.10; 95% CI, 1.00–1.21) in the total population. The point estimates in this paragraph should be interpreted with caution as there is effect modification by age.

Effect modification of age on the relation between RHR and incident T2DM

Before conducting the analyses, we investigated potential effect modification on a multiplicative scale of the relationship between RHR and incident T2DM for factors that may act as effect modifiers on pathophysiological grounds, by entering cross-products of RHR and the

Table 1 Baseline characteristics according to quartiles of resting heart rate ($n=3646$). The study population is presented in quartiles according to their resting heart rate.

	Quartile 1 ($n=889$)	Quartile 2 ($n=965$)	Quartile 3 ($n=838$)	Quartile 4 ($n=954$)
RHR (mean; bpm)	50 ± 4	58 ± 2	65 ± 3	79 ± 9
RHR (range; bpm)	31–54	55–61	62–69	70–122
Male gender (n , %)	723 (81)	711 (74)	578 (69)	628 (66)
Age (years)	58.9 ± 9.9	59.0 ± 10.3	58.2 ± 10.6	58.7 ± 10.8
Current smoking (n , %)	246 (28)	289 (30)	313 (37)	395 (41)
Body mass index (kg/m ²)	26.2 ± 3.2	26.7 ± 3.7	26.7 ± 3.9	26.8 ± 4.2
Waist circumference (cm)	94 ± 10	95 ± 11	94 ± 12	95 ± 13
Systolic BP (mmHg)	138 ± 22	140 ± 22	142 ± 22	144 ± 21
Diastolic BP (mmHg)	80 ± 11	82 ± 11	84 ± 12	85 ± 12
Laboratory parameters				
Total cholesterol (mmol/l)	4.82 ± 1.14	4.89 ± 1.14	5.09 ± 1.30	5.19 ± 1.28
Triglycerides (mmol/l)	$1.29 (0.96–1.80)$	$1.37 (1.00–1.97)$	$1.43 (1.04–2.04)$	$1.49 (1.09–2.14)$
HDL-cholesterol (mmol/l)	1.24 ± 0.36	1.25 ± 0.37	1.27 ± 0.40	1.27 ± 0.40
LDL-cholesterol (mmol/l)	2.89 ± 1.01	2.90 ± 1.00	3.05 ± 1.05	3.09 ± 1.07
ApoB (g/l)	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.3
Glucose (mmol/l)	5.7 ± 0.7	5.7 ± 0.7	5.8 ± 0.9	5.8 ± 0.8
eGFR (ml/min per 1.73 m ²)	76 ± 16	76 ± 16	78 ± 17	77 ± 19
hsCRP (mg/l)	$1.5 (0.7–3.1)$	$1.6 (0.7–3.2)$	$1.9 (1.0–4.0)$	$2.5 (1.1–4.9)$
TSH (mU/l)	$1.70 (1.20–2.40)$	$1.60 (1.10–2.30)$	$1.60 (1.10–2.30)$	$1.70 (1.20–2.50)$
Vascular disease at inclusion (n , %)				
CAD	605 (68)	565 (59)	401 (48)	344 (36)
CVD	160 (18)	224 (23)	239 (29)	309 (32)
PAD	96 (11)	131 (14)	144 (17)	226 (24)
AAA	28 (3)	45 (5)	54 (6)	75 (8)
Medication use (n , %)				
Platelet aggregation inhib	721 (81)	769 (80)	616 (74)	624 (65)
Oral anticoagulation	58 (7)	79 (8)	74 (9)	106 (11)
Lipid-lowering med	596 (67)	639 (66)	519 (62)	559 (59)
BP-lowering med	705 (79)	722 (75)	578 (69)	611 (64)
Beta-blocker	604 (68)	580 (60)	403 (48)	347 (36)
Diuretic	92 (10)	154 (16)	135 (16)	195 (20)
ACE-i/ARB	251 (28)	294 (31)	271 (32)	336 (35)
Calcium channel blocker	188 (21)	204 (21)	157 (19)	166 (17)

BP, blood pressure; bpm, beats per minute; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; TSH, thyroid-stimulating hormone; inhib, inhibitors; med, medication; ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Figure 2 Incidence rates for T2DM in patients with clinically manifest vascular diseases. Incidence rates are expressed as cases per 1000 person-years of study follow-up.

possible effect modifier in the Cox proportional hazard model. Gender was not a significant effect modifier ($P=0.38$), nor was beta-blocker use ($P=0.16$). Only age appeared to modify the relation between RHR and incident T2DM significantly (P for interaction = 0.048). Stratification for age in three groups (tertiles) showed that in subjects aged between 55 and 63 years, every 10 bpm increase in RHR increased the risk of incident T2DM by 22% (HR, 1.22; 95% CI, 1.04–1.43), while there was no increased risk of T2DM with increasing RHR in patients aged <55 years (HR, 1.08; 95% CI, 0.91–1.30) and patients aged >63 years (HR, 1.00; 95% CI, 0.84–1.18; Table 2). Further exploratory analyses in smaller age groups (quintiles) confirmed the finding that the relation between increased RHR and incident T2DM is absent in the youngest and oldest groups of patients, and is present in middle-aged patients (between 51 and 68 years in this analysis).

Exploratory analyses

In an exploratory analysis (model IV), the relation between RHR and incident T2DM was attenuated to a large extent by adding BMI and fasting serum glucose to the fully adjusted model, indicating that BMI and serum glucose levels are part of the causal pathway

between sympathetic nerve activity measured with RHR and incident T2DM (Table 2).

Furthermore, in separate analyses after exclusion of patients with a baseline fasting plasma glucose level ≥ 7.0 mmol/l or after exclusion of patients with an RHR ≤ 50 or ≥ 100 bpm, the results were not essentially different. Adjustment for levels of TSH did not change the results either. Each 10 bpm increase in RHR was associated with a 19% higher risk for incident T2DM in patients on beta-blocker treatment (HR, 1.19; 95% CI, 1.04–1.37) and only with a 3% higher risk in patients without beta-blocker use (HR, 1.03; 95% CI, 0.90–1.18); however, this difference was not statistically significant (P for interaction = 0.16).

RHR and incident T2DM according to location of vascular disease

The relation between RHR and incident T2DM was not significantly different between patients with different locations of vascular disease (P for interaction = 0.72 for CAD, 0.69 for CVD, 0.82 for PAD, and 0.84 for AAA); every 10 bpm increase in RHR was associated with a 13% higher risk for T2DM in patients with CVD and PAD (HR, 1.13; 95% CI, 0.93–1.37 and HR, 1.13; 95% CI, 0.93–1.37 respectively), an 8% higher risk in patients with CAD (HR, 1.08; 95% CI, 0.92–1.26), and a 5% higher risk in patients with AAA (HR, 1.05; 95% CI, 0.78–1.41; Fig. 4).

Discussion

In this study it is shown that an increased RHR is associated with an increased risk for developing T2DM in patients with clinically manifest vascular diseases,

Figure 3 The risk of increasing RHR on incident T2DM in patients with clinically manifest vascular diseases. Results are expressed as hazard ratios (HR) with 95% CI per quartile of RHR, relative to quartile 1 (reference). Model I: crude; model II: age and gender; model III: age, gender, beta-blocker use, diuretic use, calcium-blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking, estimated glomerular filtration rate, systolic blood pressure, location of vascular disease (CAD, CVD, PAD or AAA).

Table 2 The risk of RHR per 10 bpm on incident T2DM in patients with clinically manifest vascular diseases, after stratification in three age groups. Results are expressed as hazard ratios (HR) with 95% CI per 10 bpm increase in RHR.

	Age groups		
	1	2	3
<i>n</i>	1255	1127	1264
Age (median; range)	49 (19–54)	59 (55–63)	69 (64–80)
RHR (median; range)	63 ± 12 (31–117)	63 ± 12 (39–114)	63 ± 13 (36–122)
No. of events	94	94	101
Model HR (95% CI)			
I	1.13 (0.95–1.33)	1.32 (1.14–1.53)	0.94 (0.80–1.10)
II	1.13 (0.96–1.34)	1.31 (1.13–1.51)	0.92 (0.79–1.08)
III	1.08 (0.91–1.30)	1.22 (1.04–1.43)	1.00 (0.84–1.18)
IV	1.01 (0.83–1.23)	1.03 (0.86–1.22)	0.92 (0.76–1.10)

Model I: crude; model II: age and gender; model III: age, gender, beta-blocker use, diuretic use, calcium blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking, estimated glomerular filtration rate, systolic blood pressure, and inclusion diagnosis (CAD, CVD, PAD or AAA); model IV (exploratory): model III and body mass index and fasting glucose.

particularly in middle-aged (55–63 years) patients, independently of the location of vascular disease or beta-blocker use. These results are in accordance with previous findings in healthy subjects and in subjects with increased BMI or impaired glucose tolerance (15, 16, 17, 18, 19, 20). As patients with clinically manifest vascular diseases have an increased sympathetic nerve activity (26), reflected by an increased RHR, increased sympathetic tone can be considered to be part of shared pathophysiological pathways for developing diabetes and atherosclerotic vascular diseases, next to insulin resistance and low-grade inflammation as proposed in the 'common soil' hypothesis (25).

The overall 10% risk of developing T2DM (HR, 1.10; 95% CI, 1.00–1.21) for every 10 bpm increase in RHR in the present study fits within the range of risks in other study populations (15, 16, 17, 18, 19, 20). The reported risks in various populations are remarkably similar although population characteristics vary widely. Also, the method for recording RHR varied across studies including ECG, heart rate meter, or pulse palpation.

In this study it is shown that age is an important effect modifier in the relation between RHR and incident T2DM. The risk for incident T2DM is particularly high in middle-aged patients (age range 55–63 years), while there is no clear relation in younger and older patients. Main analyses were performed in tertiles of age. This was an arbitrary decision balancing statistical power and contrast between patient groups. The results were confirmed in exploratory analyses in smaller age groups (quintiles). In the other studies there was no statistically significant interaction of age on the relation between RHR and incident diabetes (15, 16, 18) or at least this was not reported (17, 19, 20). In a study conducted in healthy Chinese women, stratified analyses for age revealed that the effect of RHR was larger in women <55 years of age compared with women >55 years of age, although this was not a statistically significant

difference (18). Furthermore, the study conducted in the youngest population (mean age 44 ± 7 years) reports the highest risks of developing diabetes per s.d. increase in RHR: HR 1.37 (95% CI, 1.29–1.45) for men and HR 1.46 (95% CI, 1.31–1.62) for women (19) compared with the HR ranging from 1.10 to 1.27 in the other populations (mean age ranging from 48 to 54 years) (15, 16, 18, 20). Although drawing a firm conclusion on the basis of these data is not appropriate, an elevated RHR seems to be associated with a higher risk of incident T2DM in younger subjects or subjects at middle age than in older subjects.

Different pathophysiological explanations for the relation between RHR and incident T2DM may be considered. RHR is an indicator of sympathetic activity (12) and an increased sympathetic nerve system induces both acute and chronic insulin resistance (9, 10, 11). The major organs involved in insulin

Figure 4 The risk of RHR per 10 bpm on incident T2DM in patients with clinically manifest vascular diseases, stratified for the location of vascular disease. Results are expressed as hazard ratios (HR) with 95% CI per 10 bpm increase in RHR for patients with different locations of vascular diseases. Model I: crude; model II: age and gender; model III: age, gender, beta-blocker use, diuretic use, calcium-blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking, estimated glomerular filtration rate, systolic blood pressure, location of vascular disease (CAD, CVD, PAD, or AAA).

secretion, glucose production and glucose metabolism, including the pancreas, liver and skeletal muscle, are innervated by autonomic nerves (28, 29, 30). Stimulation of β -adrenergic receptors causes a reduction in the insulin-stimulated uptake of glucose (31), an increase in the proportion of insulin-resistant fast-twitch muscle fibers in rats (32), and most importantly, vasoconstriction leading to a decreased skeletal muscle blood flow, with impaired glucose uptake into the muscle cells as a result (33).

Both insulin resistance and autonomic dysfunction are the result of, and can be influenced by, physical inactivity and obesity, which are established risk factors for the development of T2DM (34, 35), and are also known to influence autonomic function (36, 37). Obesity and fasting serum glucose levels are very likely to be part of the causal pathway between RHR and incident T2DM, as shown in the present study by attenuation of the results after adding BMI and fasting serum glucose levels to the regression model. However, obesity and elevated fasting glucose levels could have been the result of autonomic dysfunction (elevated RHR) during the patients' lives, or could have been caused by the high RHR. Obesity is a cause of insulin resistance, which in turn leads to increased sympathetic nerve activity, and to increased glucose levels progressing to overt T2DM. However, cause and effect could also be the other way around. Increased sympathetic nerve activity, as measured by RHR, could also lead to higher BMI and elevated plasma glucose and insulin levels with progression to T2DM. Which of these two explanations is true cannot be concluded from our study. As discussed before, sympathetic nerve activation can be both the cause and the consequence of insulin resistance. The recent finding that reducing sympathetic nerve activity, by renal sympathetic denervation (38), causes improvements in glucose metabolism and insulin sensitivity further underlines the pathophysiological role of the sympathetic nerve system in the development of insulin resistance.

The overall association between RHR and incident T2DM in the present study is found despite a high prevalence of beta-blocker use (53%). It seems that the predictive role of RHR in patients on beta-blocker treatment is greater (HR, 1.19; 95% CI, 1.04–1.37) than those without beta-blocker use (HR, 1.03 95% CI, 0.90–1.18), although this was not statistically significant. This is not surprising considering the fact that beta-blockers favor insulin resistance and increase the risk of developing diabetes (39).

The results of the present study are in line with previous results obtained in the general population (15, 16, 17, 18, 19, 20), but are of potentially greater clinical significance because of the possible therapeutic implications. RHR is a noninvasive, nonexpensive, and easily obtainable parameter, and therefore easily applicable in clinical practice, in contrast to other measurements that reflect sympathetic nerve activity

such as MSNA or plasma noradrenaline levels. Prevention of diabetes in patients with vascular diseases is important, as patients with diabetes have an increased risk for (cardiovascular) morbidity and mortality (21, 22) and a reduced quality of life (23). Reducing the risk of T2DM is feasible with lifestyle interventions, such as increasing physical activity and reducing body weight, and with medical treatment (24, 40).

Strengths of this study include the prospective cohort design and the large sample size of a clinically relevant and well-characterized group of patients with various locations of clinically manifest vascular diseases. Furthermore, RHR was accurately and consistently measured with ECG and the incidence of T2DM was thoroughly assessed. Assessment of several clinical and laboratory parameters allowed for identification of possible confounding factors.

Study limitations need to be considered. As we excluded subjects who died or were lost to follow-up before the assessment of diabetes started in 2006, and diabetes is associated with a higher mortality, this could have affected the results of this study toward the null hypothesis. This effect is possibly of greatest influence in the older age group, as older persons are at the highest risk of death, which could be an explanation for the absence of a relation between RHR and incident diabetes in this group. Unfortunately, serum insulin levels were not routinely measured in our study, so we could not adjust our exploratory model for insulin resistance expressed as homeostasis model assessment of insulin resistance (HOMA). However, by adjusting for BMI and fasting serum glucose, the role of insulin resistance can be explored as both parameters are strong determinants of HOMA. Finally, physical activity and cardiorespiratory fitness, factors in the causal pathway of RHR and incident T2DM, were not assessed in this study. In the exploratory analyses, we adjusted for other factors in the causal pathway between RHR and incident diabetes (BMI and fasting serum glucose levels). As not only BMI and fasting serum glucose levels but also variables such as age, gender, and smoking are closely related to physical activity and cardiorespiratory fitness, we believe the residual confounding effect in the exploratory analysis of physical activity and cardiorespiratory fitness is limited.

In conclusion, increased RHR, an indicator of increased sympathetic nerve activity, is associated with an increased risk for incident T2DM in patients with clinically manifest vascular diseases. This risk was particularly elevated in middle-aged patients and was irrespective of the location of vascular disease and of the use of beta-blockers.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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