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ORIGINAL RESEARCH

Cardiovagal Function Measured by the Deep Breathing Test: Relationships With Coronary Atherosclerosis

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BACKGROUND: The cardiovagal function can be assessed by quantification of respiratory sinus arrhythmia (RSA) during a deep breathing test. However, population studies of RSA and coronary atherosclerosis are lacking. This population-based study examined the relationship between RSA during deep breathing and coronary atherosclerosis, assessed by coronary artery calcium score (CACS).

METHODS AND RESULTS: SCAPIS (Swedish Cardiopulmonary Bioimage Study) randomly invited men and women aged 50 to 64 years from the general population. CACS was obtained from computed tomography scanning, and deep breathing tests were performed in 4654 individuals. Expiration–inspiration differences (E-Is) of heart rates were calculated, and reduced RSA was defined as E-I in the lowest decile of the population. The relationship between reduced RSA and CACS (CACS≥100 or CACS≥300) was calculated using multivariable-adjusted logistic regression. The proportion of CACS≥100 was 24% in the lowest decile of E-I and 12% in individuals with E-I above the lowest decile (P<0.001), and the proportion of CACS≥300 was 12% and 4.8%, respectively (P<0.001). The adjusted odds ratio (OR) for CACS≥100 was 1.42 (95% CI, 1.10–1.84) and the adjusted OR for CACS≥300 was 1.62 (95% CI, 1.15–2.28), when comparing the lowest E-I decile with deciles 2 to 10. Adjusted ORs per 1 SD lower E-I were 1.17 (P=0.001) for CACS≥100 and 1.28 (P=0.001) for CACS≥300.

CONCLUSIONS: Low RSA during deep breathing is associated with increased coronary atherosclerosis as assessed by CACS, independently of traditional cardiovascular risk factors. Cardiovagal dysfunction could be a prevalent and modifiable risk factor for coronary atherosclerosis in the general population.

Key Words: autonomic function ■ coronary atherosclerosis ■ heart rate variability

The autonomic nervous system continuously modulates heart rate and peripheral vascular tones to maintain homeostasis of the cardiovascular system.[1] The high-frequency respiratory fluctuations in heart rate during normal breathing, that is, respiratory sinus arrhythmia (RSA), are mainly controlled by the parasympathetic part of the autonomic nerve system.[2,3] The deep breathing test (DBT) increases the parasympathetic signal during registration of RSA and is a measure of the cardiovagal function.[2,3] Low RSA during deep breathing has been associated with increased mortality in patients with high cardiovascular risk.[4]

Although population-based studies of RSA during deep breathing are unusual, heart rate variability (HRV) during normal breathing is widely used as measure of autonomic function. Reduced HRV is an adverse prognostic factor in patients with coronary

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CLINICAL PERSPECTIVE

What Is New?

- The cardiovagal function can be assessed by quantification of respiratory sinus arrhythmia during a deep breathing test.
- This population-based study found that individuals with low respiratory sinus arrhythmia during deep breathing had substantially higher coronary calcium scores than those with higher degrees of respiratory arrhythmia.

What Are the Clinical Implications?

- Cardiovagal dysfunction is a prevalent risk marker for coronary atherosclerosis in the general population, which potentially could be modified by lifestyle interventions.

Nonstandard Abbreviations and Acronyms

CACS	coronary artery calcium score
DBT	deep breathing test
E-I	expiration–inhalation difference
RSA	respiratory sinus arrhythmia
SD_{HR}	SD of heart rate

artery disease and a risk factor for incident cardiovascular disease in the general population.[5–8] Possible causal pathways between reduced HRV and cardiovascular disease include cardiac arrhythmias[9,10] and metabolic or proinflammatory consequences of autonomic dysfunction.[11–13] However, experimental and clinical data also suggest a pathophysiological link between autonomic dysfunction and atherosclerosis.[14–16] Studies of patients with diabetes or patients with high cardiovascular risk have reported associations between low HRV and carotid[17,18] or coronary atherosclerosis.[15,19,20] It is unclear whether autonomic dysfunction is a risk factor for coronary atherosclerosis in the general population.

To the best of our knowledge, there are no previous studies of the association between RSA during deep breathing and coronary atherosclerosis. Coronary artery calcium score (CACS) is widely used as a measure of the burden of coronary atherosclerosis and is a strong predictor of acute coronary events.[21] The aim of this population-based study was to examine the relationship between cardiovagal function by quantifying RSA during deep breathing and prevalence of coronary atherosclerosis measured by CACS.

METHODS

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers may be sent to the SCAPIS (Swedish Cardiopulmonary Bioimage Study) steering committee at https://www.scapis.org.

Study Population

SCAPIS is a collaboration between 6 Swedish universities with the purpose of studying cardiopulmonary diseases in a large population-based cohort.[22] Randomly selected individuals from the general population aged 50–64 years and living in 6 urban areas surrounding the university hospitals received an invitation letter. The study participants should be able to understand instructions and complete questionnaires, as judged by the study staff, but no other exclusion criteria were applied. The participants were examined at the screening center from 2014 to 2018. A total of 30 154 men and women participated in the study. Participation rate was ≈50% overall, and was 53% in the Malmö catchment area.

The participants attended the screening center at 3 different days, 1 to 2 weeks apart. A 12-lead ECG registration with DBT was performed in Malmö in 5136 individuals (of 6251) who were examined at this screening center. The main reason for not doing the DBT was lack of time or shortage of staff at the screening center. There were no significant differences in mean age (57.5 versus 57.5 years), proportion of men (46.6% versus 48.2%) or prevalence of CACS≥100 (13.6% versus 15.1%) between those who did and did not perform the DBT, respectively.

We excluded individuals with missing information about coronary calcium and ECGs with artefacts or multiple premature beats (see ECG and DBT). The final study population comprised 4654 individuals. A flowchart of the study population and a description of excluded individuals are presented in Figure 1 and Table S1.

Written informed consent was obtained from all participants. The project was approved by the ethics committee at Lund University (2016/1031).

Basic Examination

Computed tomography was performed using equipment from Siemens (Definition Flash 2×128 slice, stellar detector, 4D-Care dose, Care-kV, and sinogram-affirmed iterative reconstruction, Forchheim, Germany). CACS was calculated as the sum of calcium content in each coronary artery with the scoring system according to Agatston.[23] In accordance with previously proposed cutoffs, we used CACS≥100 and CACS≥300 as outcome measures, with CACS≥100 as our main outcome.[24–26]

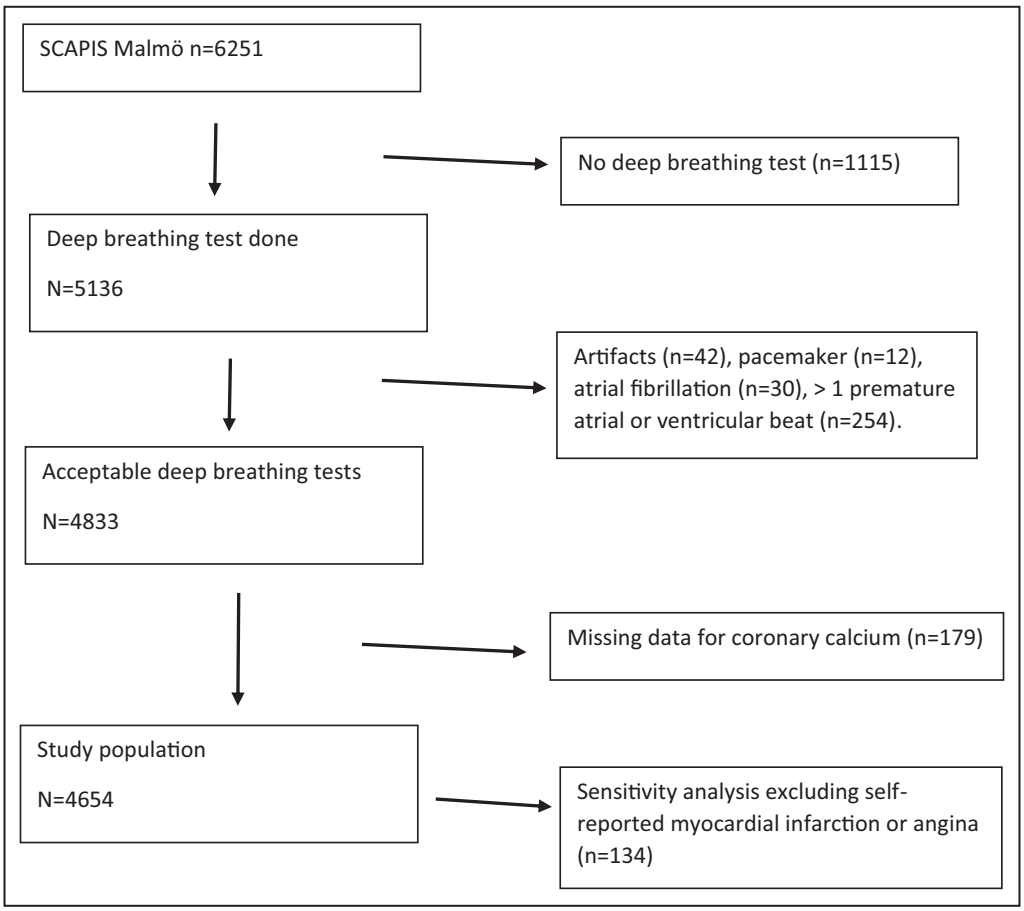

Figure 1. Flowchart of study population.
SCAPIS, Swedish Cardiopulmonary Bioimage Study.

Smoking, physical activity, and treatment for hypertension were derived from the questionnaire. Smoking status was categorized as current smoker, former smoker, and never smoker. Physical activity in spare time during the past 12 months was assessed using the Saltin–Grimby scale of leisure time physical activity.[27] The scale consists of a question ("How much do you move around and exert yourself physically during your leisure time during past 12 months?") with 4 response alternatives with examples of activities (1, mostly sedentary [eg, reading, watching television]; 2, some light physical activity [eg, walking or cycling to workplace >4 h/week]; 3, moderate and regular training [eg, running, swimming at least 2–3 h/week]; and 4, regular hard physical training [eg, hard training or competition in running, swimming, skiing at least 3 times per week]). Individuals who reported "mostly sedentary" or "some light physical activity" were considered to have low physical activity. Participants were classified as having diabetes based on responses in the questionnaire and a blood test for capillary glucose. Participants with elevated capillary p-glucose (≥7.0 mmol/L) had a repeated measurement during a second visit to confirm a new diagnosis of diabetes.

Body weight was measured on a digital scale with participants dressed in light indoor clothing without shoes. Body height was measured to the nearest centimeter. Body mass index was calculated as body weight/height^2 (kg/m^2). Systolic blood pressures were measured in the supine position twice in both arms, supported at heart level, with an Omron M10-IT blood pressure reader (Omron Corp, Kyoto, Japan). Mean systolic blood pressure from the arm with the highest mean systolic blood pressure was used in the analysis.

A fasting venous blood sample was collected for analysis of lipids, creatinine, and CRP (C-reactive protein). The analyses were performed using standard methods at the laboratory of the university hospital. Estimated glomerular filtration rate was calculated according to the creatinine-based Chronic Kidney Disease Epidemiology Collaboration formula.[28]

ECG and DBT

The participants rested in a supine position and breathed normally for 5 minutes before the DBT. The participants were then guided by a nurse to inhale

for 5 seconds and exhale for 5 seconds. The nurse watched a clock with a second hand counting "in, in, in, in, in, out, out," and so on. This was repeated during 6 breathing cycles while ECGs were recorded with a sampling rate of 500 Hz.

Artefacts and ectopic beats could substantially distort assessment of RSA. The ECGs from all registrations were therefore visually scanned by 2 of the authors (G.E., A.P.). We excluded ECGs with atrial fibrillation, artefacts, and ECGs with >1 premature ventricular or atrial contraction. One premature contraction on a 1-minute recording was accepted, and the median-based expiration–inhalation difference (E-I_{median}) was used for the primary analysis. This measure has previously been shown to be robust to single artefacts or premature contractions.[29]

A total of 3 measures of RSA were calculated from the ECG files: the median-based expiration–inhalation difference (E-I_{median}), mean-based expiration–inhalation difference (E-I_{mean}), and expiration–inhalation ratio (E/I). In addition, we calculated measures of HRV in the time domain from the same ECGs, SD of heart rate (SD_{HR}), root mean square of successive differences (RMSSD), and mean circular resultant (MCR).[29] MCR is a vector-based measure originally introduced to reduce the effects of premature ventricular contractions and differences in mean heart rate between individuals when assessing HRV.[30] Low RSA or HRV was defined as the lowest 10% of the distribution in the study population.

The 1-year reproducibility of the RSA and HRV measures was examined in 84 individuals (49 men, 35 women, mean age 57.2±3.7 years at first visit) who were randomly invited and reexamined with deep breathing after 1 year (±1 month) using the same procedures. Paired-sample *t* tests, Spearman test–retest correlations, and 2-way mixed-effects intraclass correlation coefficients were used to assess reproducibility after 1 year (Table S2).

Statistical Analysis

Measures of RSA (E-I_{median}, E-I_{mean}, E/I) and HRV (SD_{HR}, RMSSD, MCR) were divided into deciles, and the prevalence of cardiovascular risk factors and CACS was examined across the distribution of RSA or HRV.

Missing data for covariates (0.6% of all data points) were handled using multiple imputation with fully conditional specification assuming missingness at random.[31] Missing information for smoking, antihypertensive medication, or physical activity (≈2%–5% of the study population) and missing laboratory values (0.3%–0.5%) was imputed using all outcome and predictor variables used in the subsequent multivariate analysis.[31] The estimates were pooled across 5 imputation sets using the Rubin rules. The robustness of the imputation model was confirmed by a complete cases analysis.

CRP was log-transformed in all analyses because of a positively skewed distribution. A 1-way ANOVA and Pearson chi-square test were used, as appropriate, to assess the distribution of risk factors across categories of RSA.

To assess the risk factors for cardiovagal dysfunction in the population, a backward stepwise logistic regression model was applied with low E-I_{median} as a dependent variable. The predictor variables (ie, age, sex, smoking [current, former, never], antihypertensive treatment [yes, no], systolic blood pressure, waist circumference, diabetes, high-density lipoprotein cholesterol, CRP, heart rate, and physical inactivity [yes, no]) were entered into the logistic regression model, and variables with $P>0.10$ were removed one by one.

The relationship between low RSA (or low HRV) values and CACS was assessed using a logistic regression model with adjustments in 2 models. Model 1 was adjusted for age, sex, and mean heart rate; model 2 also included cardiovascular risk factors, that is, smoking status (current, former, never), antihypertensive medication (yes, no), use of β-blockers (yes, no), systolic blood pressure, waist circumference, diabetes, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log CRP, estimated glomerular filtration rate, and physical inactivity. The RSA and HRV values were modeled as per 1 SD decrease and as dichotomous variables (lowest decile versus deciles 2–10). To examine whether the results were driven by patients with clinical coronary heart disease, we performed a sensitivity analysis in which participants with self-reported histories of myocardial infarction or angina pectoris were excluded. Finally, we performed a subgroup analysis for individuals with and without diabetes. Interactions between RSA (or HRV) and diabetes were performed using a multiplicative interaction term in the logistic regression model with model 2 adjustments. The IBM SPSS Statistics (version 27; Armonk, NY) software was used for all statistical calculations.

RESULTS

The characteristics of the study population by E-I_{median} are presented in Table 1 and in Table S3. Individuals with low E-I_{median} were older, more often men, and smokers and had higher systolic blood pressure and higher CRP levels with a higher prevalence of diabetes than those with higher E-I_{median}. These factors were also significantly associated with low E-I_{median} after adjustment in a backward stepwise logistic regression (Table 2).

Coronary Calcium in Relation to RSA

The proportion of CACS≥100 was ≈2 times higher in the lowest decile of E-I_{median} compared with those with

Table 1. Characteristics of the Study Population in Relation to E-I_{median} Below and Above the 10th Percentile

	E-I_{median}		
	<10th percentile	>10th percentile	*P* value
No.	473	4181	
E-I_{median}, bpm	3.7±0.91	11.5±5.5	
E-I_{median}, range	0.2–4.8	4.9–58.2	
E/I	1.06±0.02	1.20±0.11	<0.001
E-I_{mean}, bpm	4.5±2.0	12.2±5.4	<0.001
SD_{HR}, bpm	2.1±1.1	4.8±2.0	<0.001
MCR	0.84±0.4	2.1±0.42	<0.001
RMSSD, ms	26.0±24	57.5±37	<0.001
Heart rate, bpm	61.6±9.7	63.8±8.8	<0.001
Age, y	59.1±4.1	57.2±4.2	<0.001
Women	43	56	<0.001
Smoking			
Never	35	45	0.001
Former	45	39	
Current	20	17	
Diabetes	13	7.4	<0.001
Physical activity			0.25
Low	66	63	
High	34	37	
Systolic blood pressure, mm Hg	128±17	123±16	<0.001
Diastolic blood pressure, mm Hg	77±10	75±9.7	0.002
Blood pressure medication			<0.001
No	72	81	
Yes	28	19	
Body mass index, kg/m^2	27.9±4.7	27.1±4.5	0.002
Height, cm	173±9.4	171±9.7	<0.001
Waist, cm	98.0±14	94.6±13	<0.001
C-reactive protein, mg/L†	1.2 (0.62–2.9)	1.1 (0.42–2.4)	0.003
eGFR, mL/min per 1.73 m^2	84±13	85±12	0.032
LDL cholesterol, mmol/L	3.6±1.0	3.6±0.93	0.41
HDL cholesterol, mmol/L	1.58±0.52	1.68±0.53	<0.001
Coronary calcium scores			
<1	44	58	<0.001
1–99	32	30	
100–299	12	7.2	
≥300	12	4.8	

Values are presented as mean±SD or percentage unless otherwise stated. eGFR indicates estimated glomerular filtration rate; E/I, expiration–inhalation ratio; E-I_{mean}, mean-based expiration–inhalation difference; E-I_{median}, median-based expiration–inhalation difference; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCR, mean circular resultant; RMSSD, root mean square of successive differences; and SD_{HR}, SD of heart rate.

†Medians and interquartile ranges are presented as a result of skewed distribution. Log-transformed values used for significance testing.

higher E-I_{median} (24% versus 12%; *P*<0.001). A similar relationship was observed for CACS scores ≥300 (12% versus 4.8%; *P*<0.001). The proportion with no coronary calcification was 44% versus 58%, respectively, for those below and above the lowest decile (Table 1).

The distribution of CACS≥100 and CACS≥300 in relation to deciles of RSA and HRV is presented in Figure 2A through 2F. For all measures of RSA, there was an inverse relationship with CACS, with substantially higher CACS for those in the lowest decile. The distribution of CACS≥100 and CACS≥300 in relation to deciles of SD_{HR} and MCR were similar to those observed for RSA (Figure 2A through 2F).

The multivariable-adjusted relationships between RSA and HRV, respectively, and CACS are presented in Table 3. E-I_{median} in the lowest decile was associated with CACS≥100 after model 2 adjustment (odds ratio [OR], 1.42; 95% CI, 1.10–1.84; *P*=0.007). The corresponding adjusted OR for CACS≥300 was 1.62 (95% CI, 1.15–2.28; *P*=0.006). Per 1 SD reduction of E-I_{median} and after adjustments for risk factors, the OR for CACS≥100 was 1.17 (95% CI, 1.06–1.30; *P*=0.002), and the OR for CACS≥300 was 1.28 (95% CI, 1.10–1.48; *P*=0.002). The relationships were largely the same for

Table 2. Factors Associated With Low E-I_{median} During the Deep Breathing Test

	Odds ratio (95% CI), full model*	Odds ratio (95% CI), reduced model†
Age, per 1 y	1.10 (1.07–1.12)	1.10 (1.07–1.13)
Women (vs men)	0.71 (0.56–0.89)	0.66 (0.54–0.80)
Current smoking (vs never)	1.49 (1.13–1.97)	1.47 (1.12–1.93)
Diabetes (yes vs no)	1.45 (1.05–2.0)	1.56 (1.15–2.13)
Systolic blood pressure (per 10 mm Hg)	1.09 (1.03–1.16)	1.11 (1.04–1.17)
Blood pressure medication (yes vs no)	1.21 (0.96–1.53)	
Waist (1 cm)	1.005 (0.99–1.015)	
Log C-reactive protein (1 unit)	1.08 (0.97–1.21)	1.12 (1.02–1.24)
HDL cholesterol (1 mmol/L)	0.93 (0.72–1.15)	
eGFR (1 mL/min per 1.73 m^2)	1.00 (0.99–1.01)	
Heart rate (1 bpm)	0.97 (0.95–0.98)	0.97 (0.95–0.98)
Low physical activity (vs high)	1.02 (0.81–1.28)	

Low E-I_{median} is defined as lowest 10% of the distribution. eGFR indicates estimated glomerular filtration rate; E-I_{median}, median-based expiration–inhalation difference; and HDL, high-density lipoprotein.

*Full model: all risk factors in the table were entered into the logistic regression model.

†Reduced model: final results after backward stepwise elimination.

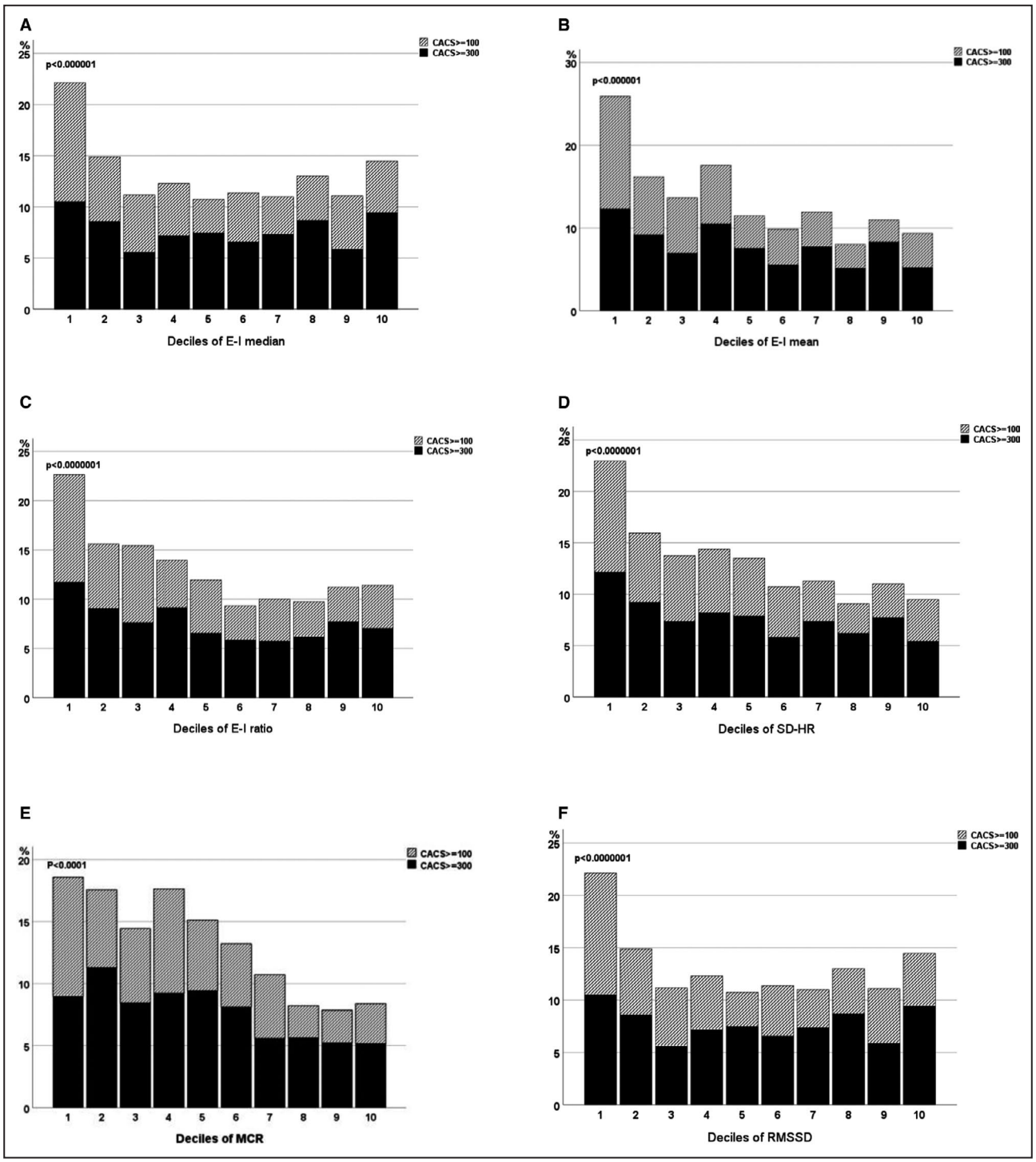

Figure 2. Percentages with high CACS values (≥100, shaded bars; and ≥300, black bars) in deciles of respiratory sinus arrhythmia and heart rate variability during deep breathing.
(**A**) Deciles of E-I_{median}, (**B**) deciles of E-I_{mean}, (**C**) deciles of E-I ratio, (**D**) deciles of SD-HR, (**E**) deciles of MCR, and (**F**) deciles of RMSSD. The *P* values refer to the proportion of CACS≥100 in decile 1 vs deciles 2 to 10. CACS indicates coronary artery calcium score; E-I_{median}, median-based expiration–inhalation difference; E-I_{mean}, mean-based expiration–inhalation difference; E/I, expiration–inhalation ratio; E-I, expiration–inhalation; MCR, mean circular resultant; RMSSD, root mean square of successive differences; and SD-HR, SD of heart rate.

the E-I_{mean} and E/I (Table 3). SD_{HR} and MCR were similarly significantly associated with CACS. However, the RMSSD was not associated with CACS when modeled as per 1 SD decrease (Table 3). These results were similar in a complete cases analysis of 4288 individuals with full information for all covariates (Table S4).

Table 3. Logistic Regression Analysis of Deep Breathing Test and Presence of High CACS (CACS≥100 or CACS≥300)

	CACS≥100		CACS≥100		CACS≥300		CACS≥300	
	Lowest 10% vs deciles 2 to 10		Per 1 SD decrease		Lowest 10% vs deciles 2 to 10		Per 1 SD decrease	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Respiratory sinus arrhythmia								
E-I_{median}	1.65 (1.29–2.11)*	1.42 (1.10–1.84)*	1.23 (1.11–1.36)*	1.17 (1.06–1.30)*	1.97 (1.42–2.74)*	1.62 (1.15–2.28)*	1.38 (1.18–1.61)*	1.28 (1.10–1.48)*
E-I_{mean}	1.58 (1.22–2.03)*	1.36 (1.04–1.76)*	1.18 (1.07–1.30)*	1.13 (1.02–1.24)*	1.74 (1.24–2.45)*	1.38 (0.97–2.00)	1.30 (1.12–1.50)*	1.21 (1.05–1.40)*
E/I	1.72 (1.36–2.18)*	1.48 (1.16–1.89)*	1.21 (1.09–1.33)*	1.16 (1.05–1.28)*	1.95 (1.43–2.66)*	1.56 (1.12–2.17)*	1.33 (1.14–1.56)*	1.24 (1.06–1.45)*
Time domain HRV								
SD_{HR}	1.63 (1.28–2.08)*	1.39 (1.08–1.79)*	1.17 (1.06–1.28)*	1.11 (1.01–1.22)*	1.82 (1.31–2.54)*	1.43 (1.01–2.02)*	1.30 (1.12–1.50)*	1.20 (1.04–1.38)*
MCR	1.30 (1.00–1.70)*	1.21 (0.92–1.59)	1.25 (1.13–1.38)*	1.20 (1.08–1.33)*	1.70 (1.20–2.51)*	1.56 (1.09–2.25)*	1.34 (1.15–1.56)*	1.25 (1.07–1.46)*
RMSSD	1.72 (1.31–2.26)*	1.45 (1.09–1.92)*	1.05 (0.95–1.15)	1.02 (0.93–1.13)	2.00 (1.40–2.86)*	1.57 (1.08–2.29)*	1.11 (0.96–1.29)	1.08 (0.93–1.26)

Values are provided as OR (95% CI). Model 1: adjusted for age, sex, and heart rate. Model 2: model 1+smoking status (current, former, never), antihypertensive medication (yes, no), use of β-blocker (yes, no), systolic blood pressure, waist circumference, diabetes, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log C-reactive protein, physical activity, and estimated glomerular filtration rate. CACS indicates coronary artery calcium score; E/I, expiration–inhalation ratio; E-I_{mean}, mean-based expiration–inhalation difference; E-I_{median}, median-based expiration–inhalation difference; HRV, heart rate variability; MCR, mean circular resultant; OR, odds ratio; RMSSD, root mean square of successive differences; and SD_{HR}, SD of heart rate.

*Significant ORs (P<0.05).

Table 4. Logistic Regression Analysis of Deep Breathing Test and Presence of High CACS (CACS≥100 or CACS≥300) in Individuals With and Without Diabetes

	No diabetes		Diabetes		No diabetes		Diabetes	
	CACS≥100		CACS≥100		CACS≥300		CACS≥300	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Respiratory sinus arrhythmia								
E-I_{median}	1.18 (1.06–1.32)*	1.13 (1.01–1.26)*	1.44 (1.10–1.87)*	1.38 (1.06–1.79)*	1.27 (1.07–1.51)*	1.20 (1.01–1.43)*	1.64 (1.17–2.31)*	1.53 (1.09–2.16)*
E-I_{mean}	1.13 (1.02–1.26)*	1.08 (0.97–1.20)	1.40 (1.08–1.81)*	1.36 (1.06–1.76)*	1.20 (1.01–1.41)*	1.12 (0.95–1.33)	1.54 (1.12–2.12)*	1.46 (1.06–2.01)*
E/I	1.16 (1.05–1.29)*	1.12 (1.00–1.24)*	1.43 (1.08–1.91)*	1.39 (1.05–1.84)*	1.23 (1.03–1.46)*	1.16 (0.98–1.38)	1.64 (1.13–2.37)*	1.54 (1.06–2.23)*
Time domain HRV								
SD_{HR}	1.12 (1.01–1.23)*	1.06 (0.96–1.18)	1.40 (1.09–1.80)*	1.36 (1.06–1.76)*	1.19 (1.01–1.40)*	1.11 (0.95–1.31)	1.53 (1.13–2.08)*	1.46 (1.07–2.00)*
MCR	1.20 (1.08–1.34)*	1.16 (1.04–1.29)*	1.53 (1.13–2.07)*	1.48 (1.08–2.04)*	1.21 (1.03–1.43)*	1.15 (0.97–1.36)	1.83 (1.25–2.69)*	1.74 (1.17–2.60)*
RMSSD	1.04 (0.93–1.15)	1.01 (0.91–1.12)	1.06 (0.80–1.40)	1.10 (0.82–1.48)	1.11 (0.94–1.31)	1.07 (0.90–1.26)	1.08 (0.78–1.50)	1.11 (0.79–1.56)

Values are provided as odds ratios (95% CI) per 1 SD decrease of respiratory sinus arrhythmia or HRV. Model 1: adjusted for age, sex, and heart rate. Model 2: model 1+smoking status (current, former, never), antihypertensive medication (yes, no), use of β-blocker (yes, no), systolic blood pressure, waist circumference, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log C-reactive protein, physical activity, and estimated glomerular filtration rate. CACS indicates coronary artery calcium score; E/I, expiration–inhalation ratio; E-I_{mean}, mean-based expiration–inhalation difference; E-I_{median}, median-based expiration–inhalation difference; HRV, heart rate variability; MCR, mean circular resultant; RMSSD, root mean square of successive differences; and SD_{HR} SD of heart rate.

*Significant odds ratios (P<0.05).

Sensitivity Analysis

A total of 58 individuals reported a history of myocardial infarction or angina pectoris, and information was unknown for another 76 participants. In a sensitivity analysis that excluded these individuals, low E-I_{median} was still significantly associated with CACS≥100 (OR, 1.38; 95% CI, 1.06–1.80; *P*=0.016) and CACS≥300 (OR, 1.62; 95% CI, 1.14–2.32; *P*=0.007) after an adjustment for risk factors (model 2).

Individuals With and Without Diabetes

The proportions with CACS≥100 were 27.3% and 12.0%, respectively, in individuals with and without diabetes (*P*<0.001), and the proportions with CACS≥300 were 17.5% and 4.4%, respectively (*P*<0.001).

The relationship for individuals with and without diabetes is presented in Table 4. All RSA measures were strongly associated with CACS in those with diabetes. For individuals without diabetes, the ORs tended to be weaker. The E-I_{median} and E/I remained significantly associated with CACS≥100 in individuals without diabetes after an adjustment for risk factors. The E-I_{median} was also associated with CACS≥300 after multivariate adjustments. There was a significant interaction between diabetes and SD_{HR} with respect to CACS≥100 (*P* interaction=0.04).

DISCUSSION

Low E-I difference during deep breathing was associated with high coronary calcium scores in this study of middle-aged men and women from the general population. This relationship remained significant after adjustment for multiple cardiovascular risk factors and was found both in individuals with and without diabetes. The results show that reduced cardiovagal activity could be a prevalent risk factor that associates with a substantially increased risk for coronary atherosclerosis in the general population.

Low E-I difference was associated with an increased prevalence of several risk factors associated with atherosclerosis, most notably with smoking, diabetes, high CRP, and high blood pressure. These results are in accordance with studies of HRV during normal breathing.[8,32] We also observed stronger relationships between RSA and CACS in individuals with diabetes. Some previous studies indicate that low HRV could be associated with the development of unfavorable metabolic risk factors, such as diabetes and hypertension.[13,33] The link between parasympathetic dysfunction and diabetes is supported by studies of vagal nerve function and glucose control.[34,35] For example, a recent study of diabetic rats showed that vagal nerve stimulation reduced blood glucose by enhancing vagal efferent activity and the release of glucagon-like peptide-1.[35] As such, autonomic dysfunction could be both cause and consequence of diabetes or hyperglycemia, which could explain the relatively strong relationships with CACS in the diabetic group.

The vagal system has local anti-inflammatory effects in atherosclerotic lesions and in acute inflammatory models mediated via α7nAChR (α7 nicotinic acetylcholine receptor).[12,16] It has been proposed that parasympathetic dysfunction could promote atherosclerosis.[36] Indeed, studies of mice have shown that a lack of α7nAChR accelerates atherosclerosis, whereas stimulation of α7nAChR decreases disease development, possibly by modulating immunity and inflammation.[16,37]

The respiratory variation in heart rate is mainly controlled by the parasympathetic part of the autonomic nerve system.[2,3] Another possible link between RSA and atherosclerosis is through the impaired fine-tuning of heart rate and blood pressure as a consequence of cardiovagal dysfunction. Hypertension is a major risk factor for atherosclerosis, and it is likely that suboptimal regulation of blood pressure could promote atherosclerosis.

One important question is whether cardiovagal dysfunction is preventable or modifiable. A recent systematic review of physical activity and HRV concluded that higher training intensities and frequencies are likely to improve HRV.[38] Because physical activity and physical fitness have been associated with lower CACS,[39] this could have important implications for prevention. A longitudinal cohort study reported that the number of unhealthy lifestyle factors (physically inactive, smoking, high alcohol consumption, overweight or obese) was associated with subsequently lower vagally mediated HRV and that those with a decreasing number of healthy lifestyle practices had lower subsequent vagally mediated HRV in comparison with those with an unchanged number of healthy lifestyles.[40] Poor sleep, stress, and inflammation are other reasons for autonomic dysfunction that potentially could be modified.[14,32] Hence, cardiovagal dysfunction is a prevalent risk factor for atherosclerosis that perhaps could be prevented.

The current study has a number of important limitations. This is a cross-sectional study and cannot assess any temporal or causal relationships. Although our main hypothesis is that impaired cardiovagal function could promote atherosclerosis, it is also possible that advanced atherosclerosis could reduce the response of the sinus node to vagal stimulation or reduce the cardiovagal function by inducing ischemic or mechanical stress on perivascular autonomic nerve fibers.[20] If so, this would further increase the relationships between cardiovagal dysfunction and atherosclerosis.

The large population-based study with information about DBT and coronary calcification is a unique

strength of the study. CACS was assessed by computed tomography, which provides a reliable and specific measure of coronary atherosclerosis burden. However, because noncalcified plaques were not analyzed in this study, CACS does not give the full picture of the coronary atherosclerotic plaque burden and risk of coronary events.

Although the participation rate of 53% in our study is acceptable, it is still unclear whether the results could be generalized to older age groups. The DBT was performed once over 1 minute. This is a common procedure for this test and a feasible registration time in clinical settings. Repeated measurements would most likely increase the precision of the E-I estimates and average values from several repeated DBTs would have been preferable. A 1-year follow-up of 84 participants in this study showed intraclass correlation coefficient values in the range 0.53 to 0.58 for RSA and 0.52 to 0.68 for measures of HRV. This is comparable with the long-term variability of other commonly used biomarkers in population studies, such as CRP, but somewhat higher than the variability of, for example, total cholesterol.[41] We used E-I_{median} as our main exposure variable because this measure is very robust for effects of single ectopic beats or artifacts.[29] Measures of E-I could be affected by deviances in the respiration rates, especially if the frequency is lower than the expected 6 cycles per minute. However, SD_{HR} is robust to deviances in respiratory frequency,[29] and the results were essentially the same for this measure. Finally, we currently do not have any prospective data of incident coronary events. However, as cardiovascular events accrue in the cohort, the relationship between the DBT and incident cardiovascular events could be explored in future studies.

In conclusion, reduced RSA during deep breathing is associated with increased coronary atherosclerosis as assessed by CACS, independently of traditional cardiovascular risk factors. Cardiovagal dysfunction could be a prevalent and preventable but so far largely neglected risk factor for coronary atherosclerosis in the general population.

ARTICLE INFORMATION

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Author Contributions: Engström, Persson, Hamrefors, Fedorowski, and Platonov planned the study. Ostenfeld, Goncalves, and Markstad were responsible for radiographic measurements and Persson, Carlson, and Platonov for analyses of ECGs. All authors interpreted the results. Engström and Hamrefors drafted the first manuscript and Fedorowski, Johnson, and Johansson gave critical input to the revision. All authors read and approved the final version. Engström is the guarantor for the overall content.

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Disclosures

Dr Fedorowsky reports lecture fees from Medtronic Inc., Biotronik, and Finapres Medical Systems. Dr Johnson reports research collaboration with MedicAlgorithmics SA outside this project. The remaining authors have no disclosures to report.

Supplemental Material

Tables S1–S4

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SUPPLEMENTAL MATERIAL

Table S1. Comparison of individuals included in the study and individuals excluded due to missing coronary calcium score or due to multiple ectopic beats, pacemaker, atrial fibrillation or artifacts.

	Included	Excluded	p-value
N	4654	482	
Age (years)	57.4±4.3	58.5±4.3	<0.001
Women n (%)	2510 (54)	234 (46)	<0.001
Smoking (%)			<0.001 (3 df)
Never	42	37	
Former	38	43	
Current	17	15	
Unknown	2	6	
Diabetes n (%)	364 (7.8)	79 (16)	<0.001
Physical activity (%)			<0.001 (2 df)
Low	60	61	
High	35	28	
Missing	5	11	
Systolic blood pressure (mmHg)	123±17	124±17	0.36
Diastolic blood pressure (mmHg)	75±9.8	75±10	0.75
Blood pressure medication (%)			<0.001 (2 df)
No	77	62	
Yes	20	28	
Unknown	4	9	
Body mass index (kg/m^2)	27.2±4.6	27.9±5.2	0.001
Height (cm)	172±9.7	172±10	0.07
Waist (cm)	94.9±12.9	98.2±14.5	<0.001
C-reactive protein ǂ (mg/L)	1.1 (0.60, 2.4)	1.1 (0.61, 2.4)	0.99
eGFR (ml/min/1.73 m^2)	85±12	84±13	0.32
LDL (mmol/L)	3.6±0.94	3.2±0.95	<0.001
Coronary calcium scores			
<1	57	50	0.008 (3 df)
1-99 (%)	30	29	
100-299 (%)	7.7	12	
≥300 (%)	5.5	8.9	

Coronary calcium scores was available for 292 out of 482 excluded individuals.

ǂ Medians and interquartile ranges are presented due to skewed distribution.

df degrees of freedom; eGFR estimated glomerular filtration rate; LDL low density lipoprotein cholesterol

Table S2. Long-term variability of respiratory sinus arrhythmia and heart rate variability measures for 84 subjects with repeated deep breathing test after one year.

	Baseline (mean±SD)	Re-exam, 1 year (mean±SD)	P (baseline vs 1 year)	Spearman r	ICC
E-I_{median} (bpm)	10.2±5.5	9.8±4.7	0.47	0.64	0.57
E-I_{mean} (bpm)	10.8±5.4	10.5±4.9	0.47	0.61	0.58
E/I	1.19±0.12	1.17±0.10	0.17	0.69	0.53
SD_{HR} (bpm)	4.34±2.0	4.15±1.8	0.35	0.59	0.54
MCR	2.24±1.1	2.12±1.0	0.30	0.59	0.52
RMSSD (ms)	54.3±46.7	52.0±43.6	0.57	0.66	0.68
Heart rate (bpm)	62.0±8.7	64.1±10.4	0.02	0.68	0.67

Of the 84 subjects, 46 had coronary calcium score (CACS) 0, 29 had CACS 1-99, 5 had CACS 100-299 and 3 had CACS >=300. One individual had no measurement of CACS from the baseline examination.

ICC Intra-class correlation coefficient; E-I_{median} median-based expiration-inhalation difference; E-I_{mean} mean-based expiration-inhalation difference; E/I expiration-inhalation ratio; MCR mean circular resultant; RMSSD root mean square of successive differences; SD standard deviation; SD_{HR} standard deviation of heart rate

Table S3. Cardiovascular risk factors by deciles of E-I_{median}.

	Deciles of E-I_{median}										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**p-value**
N	473	440	441	519	453	480	453	462	464	469	
E-I_{median}, range	0.2-4.8	4.9-6.0	6.2-7.0	7.1-8.1	8.2-9.4	9.5-10.7	10.8-12.4	12.5-14.7	14.8-18.4	18.5-58.2	
E-I_{mean} (bpm)	4.5±2.0	6.5±1.7	7.8±1.9	8.5±1.5	9.6±1.6	10.9±1.7	12.3±1.7	14.2±1.8	17.0±2.0	23.2±4.5	<0.001
E/I	1.06±0.02	1.09±0.01	1.11±0.02	1.13±0.02	1.15±0.03	1.18±0.02	1.20±0.03	1.24±0.04	1.29±0.05	1.43±0.11	<0.001
SD_{HR} (bpm)	2.1±1.1	2.8±1.0	3.2±1.0	3.5±0.9	3.8±0.8	4.3±1.0	4.9±0.9	5.5±0.88	6.5±0.9	8.5±1.7	<0.001
MCR	0.83±0.4	1.11±0.5	1.22±0.5	1.41±0.6	1.75±0.6	1.77±0.8	2.17±0.8	2.50±0.9	3.01±1.0	3.87±1.6	<0.001
RMSSD (ms)	25.8±25	37.1±25	44.7±30	44.4±24	45.6±27	52.3±25	55.9±28	61.1±30	73.4±37	103±52	<0.001
Age (years)	59.1±4.1	58.0±4.2	58.0±4.1	57.5±4.4	57.5±4.2	57.1±4.2	57.0±4.2	56.8±4.2	56.6±4.1	56.3±4.2	<0.001
Women (%)	43	48	49	55	59	55	59	61	59	54	<0.001
Smoking (%)											<0.001
Never	35	42	42	43	41	45	49	47	44	47	
Former	45	41	38	38	41	40	38	37	39	37	
Current	20	17	20	19	19	15	13	16	17	16	
Diabetes (%)	13	8	8	8	8	6	6	6	6	9	0.003
Low physical activity (%)	66	66	66	63	62	61	63	63	63	64	0.12
Systolic BP (mmHg)	128±17	125±17	125±17	123±16	123±16	122±17	122±17	122±15	122±16	122±16	<0.001
Diastolic BP (mmHg)	77±10	76±10	76±10	75±10	74±9	75±10	75±10	75±9	75±9	75±9.5	0.001
BP medication yes (%)	28	23	21	20	17	19	19	19	18	17	<0.001
BMI (kg/m^2)	27.8±4.7	27.8±4.6	27.5±4.5	27.2±4.7	26.9±4.6	27.1±4.6	27.0±4.4	26.9±4.5	26.6±4.2	26.8±4.6	<0.001
Waist (cm)	98.0±14	96.9±12	96.4±13	94.9±12	93.7±12	94.4±13	94.0±13	93.4±13	93.4±12	94.4±14	<0.001
eGFR ($ml/min/1.73\ m^2$)	84±13	84±12	84±13	84±13	85±12	86±12	85±11	84±12	85±12	86±12	0.001
LDL (mmol/L)	3.6±1.0	3.6±1.0	3.7±1.0	3.6±0.9	3.5±0.9	3.6±0.9	3.6±0.9	3.6±0.9	3.7±1.0	3.6±1.0	0.55
HDL (mmol/L)	1.58±0.52	1.62±0.52	1.62±0.50	1.68±0.53	1.70±0.51	1.70±0.55	1.72±0.54	1.72±0.55	1.69±0.50	1.72±0.54	0.10

continued

	Deciles of E-I_{median}										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**p-value**
Coronary calcium scores											<0.001
<1 (%)	44	52	56	56	58	57	57	62	63	64	
1-99 (%)	32	32	28	30	30	32	31	31	27	27	
100-299 (%)	12	9	10	8	8	6	8	5	8	5	
>= 300 (%)	12	7	6	6	5	5	4	2	2	4	

Values are mean±standard deviation or %.

BMI body mass index; BP blood pressure; E-I_{median} median-based expiration-inhalation difference; E-I_{mean} mean-based expiration-inhalation difference; E/I expiration-inhalation ratio; eGFR estimated glomerular filtration rate; HDL high density lipoprotein cholesterol; LDL low density lipoprotein cholesterol; MCR mean circular resultant; RMSSD root mean square of successive differences; SD_{HR} standard deviation of heart rate

Table S4. Logistic regression analysis of deep breathing test results and presence of high coronary calcium scores (CACS≥100 or CACS≥300) in 4288 individuals with complete information on all covariates.

	CACS≥100		**CACS≥100**		**CACS≥300**		**CACS≥300**	
	Lowest 10% vs decile 2-10		**Per 1 SD decrease**		**Lowest 10% vs decile 2-10**		**Per 1 SD decreased**	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
Respiratory sinus arrhythmia								
E-I_{median}	**1.70 (1.32-2.20)**	**1.48 (1.14-1.94)**	**1.22 (1.10-1.35)**	**1.16 (1.05-1.29)**	**2.08 (1.48-2.94)**	**1.76 (1.23-2.51)**	**1.38 (1.17-1.62)**	**1.28 (1.09-1.512**
E-I_{mean}	**1.63 (1.26-2.13)**	**1.44 (1.10-1.89)**	**1.17 (1.06-1.29)**	**1.12 (1.01-1.23)**	**1.77 (1.24-2.54)**	**1.47 (1.01-2.14**)	**1.29 (1.10-1.50)**	**1.20 (1.03-1.40)**
E/I	**1.83 (1.39-2.40)**	**1.59 (1.20-2.11)**	**1.19 (1.08-1.32)**	**1.14 (1.03-1.27)**	**2.21 (1.55-3.16)**	**1.88 (1.29-2.74)**	**1.32 (1.12-1.56)**	**1.24 (1.05-1.46)**
Time domain heart rate variability								
SD_{HR}	**1.63 (1.26-2.11)**	**1.42 (1.08-1.85)**	**1.16 (1.06-1.29)**	**1.11 (1.00-1.22)**	**1.80 (1.27-2.56)**	**1.47 (1.02-2.13)**	**1.29 (1.10-1.50)**	**1.21 (1.03-1.42)**
MCR	1.23 (0.93-1.64)	1.17 (0.87-1.56)	**1.24 (1.12-1.38)**	**1.19 (1.07-1.32)**	**1.56 (1.06-2.72)**	**1.48 (1.00-2.20)**	**1.34 (1.15-1.56)**	**1.25 (1.07-1.46)**
RMSSD	**1.66 (1.25-2.22)**	**1.42 (1.05-1.90)**	1.05 (0.95-1.15)	1.03 (0.93-1.14)	**2.00 (1.37-2.93)**	**1.63 (1.10-2.43)**	1.13 (0.96-1.32)	1.09 (0.93-1.28)

Values are odds ratios (95% confidence intervals). Significant ORs ($p<0.05$) are indicated in bold

Model 1. Adjusted for age, sex, heart rate

Model 2 Model 1 + smoking status (current, former, never), anti-hypertensive medication (yes, no), use of beta-blocker (yes, no), systolic blood pressure, waist circumference, diabetes, high density lipoprotein cholesterol; low density lipoprotein cholesterol;, log C-reactive protein, physical activity, estimated glomerular filtration rate;

E-I_{median} median-based expiration-inhalation difference; E-I_{mean} mean-based expiration-inhalation difference; E/I expiration-inhalation ratio; MCR mean circular resultant; RMSSD root mean square of successive differences; SD_{HR} standard deviation of heart rate