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Heart rate, autonomic function and future changes in glucose metabolism in non-diabetic individuals: the Whitehall II cohort study

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Abbreviations

CAN: cardiovascular autonomic neuropathy

HF power: high-frequency power

HR: heart rate

HRV: heart rate variability

LF power: low-frequency power

LH/HF ratio: low frequency / high frequency power ratio

hsCRP: high-sensitivity C-reactive protein

IL-6: Interleukin 6

IL-1Ra: interleukin 1 receptor antagonist

SDNN: the standard deviation of all normal-to-normal R-R intervals

RMSSD: the root mean square of the sum of the squares of differences between consecutive normal-to-normal R-R intervals

ABSTRACT

Objective

Autonomic nervous system dysfunction is associated with impaired glucose metabolism, but the temporality of this association remains unclear in non-diabetic individuals. We investigated the association of autonomic function (AF) with 5-year changes in glucose metabolism in individuals without diabetes.

Research Design and Methods

Analyses were based on 9.000 person-examinations for 3,631 non-diabetic participants in the Whitehall II cohort. Measures of AF included 5-minute resting heart rate and six heart rate variability (HRV) indices. Associations between baseline AF measures and 5-year changes in fasting and 2-hour plasma glucose, serum insulin concentrations, insulin sensitivity (ISI_{0-120} and HOMA-IS) and beta-cell function (HOMA-β) were estimated in models adjusting for age, sex, ethnicity, metabolic factors and medication.

Results

Ten beat per minute higher in resting heart rate was associated with 5-year changes in fasting and 2-hour insulin and ISI_{0-120} of (% change (95%CI)) 3.3(1.8;4.8)%,p<0.001, 3.3(1.3;5.3)%,p=0.001 and -1.4(-2.4;-0.3)%,p=0.009, respectively.

In models adjusted for age, sex, and ethnicity higher baseline values of several HRV indices were associated with a 5-year decrease in fasting and 2-hour insulin and ISI_{0-120}. However, significance was lost by full adjustment. A majority of HRV indices exhibited a trend toward higher values being associated with lower insulin levels and higher insulin sensitivity.

Conclusions

Resting heart rate in non-diabetic individuals is associated with a future changes in insulin levels and insulin sensitivity. Associations may be mediated via autonomic function however results are inconclusive. Resting heart rate may be a risk marker of future changes in glucose metabolism linked to other mechanisms.

People with diabetes have an increased prevalence of autonomic dysfunction. The prevalence of cardiovascular autonomic neuropathy (CAN) ranges from 20% in the general diabetes population(1; 2) to 65% in people with long-standing diabetes(3). In addition to established cardiometabolic risk factors, CAN is an independent determinant of cardiovascular morbidity, progression of diabetic nephropathy and overall mortality(4-6). Autonomic imbalance has been associated with reduced insulin sensitivity and β-cell function in individuals with recent onset of type 1 and type 2 diabetes. Findings have been suggested to be mediated by changes in the innervation of the endocrine pancreas(7) where pancreatic islets are innervated by both parasympathetic and sympathetic nerves and are involved in pancreatic hormone secretion(8). In addition, peripheral insulin sensitivity may be associated with autonomic function as hyperinsulinemic euglycemic clamp studies have demonstrated that insulin sensitivity can be affected by autonomic blockade in obese insulin-resistant individuals(9). In concert, autonomic dysfunction may affect both β-cell function and insulin resistance.

Autonomic imbalance has been observed in individuals with prediabetes(10) and the metabolic syndrome(11; 12) and is associated with incident diabetes(13; 14), suggesting that alterations in autonomic function may contribute to the pathogenesis of diabetes. Similar associations have been found in the offspring of type 2 diabetes patients(15) implying that a pathological interplay between autonomic neuropathy and glucose metabolism exists not only in diabetes but also in individuals with increased risk of the disease. Indeed, some cross-sectional studies have shown that CAN assessed by heart rate variability (HRV) is associated with lower insulin sensitivity in non-diabetic cohorts(16; 17). Furthermore, observational data suggest that autonomic function may be affected prior to development of hyperglycemia. This indicates that autonomic dysfunction may precede glycemic dysfunction. Thus, substantial evidence links autonomic dysfunction with impaired glucose metabolism. However, as the above-mentioned findings are based on cross-sectional studies, large prospective studies are needed to elucidate if autonomic dysfunction precedes glycemic dysfunction in non-diabetic individuals.

We hypothesize that autonomic dysfunction in non-diabetic individuals precedes future changes in glucose metabolism that may lead to diabetes.

In this cohort study with repeated measurements of glucose metabolism, we aimed to investigate the prospective associations of measures cardiac autonomic status as a proxy for autonomic function with subsequent 5-year changes in fasting and 2-hour plasma glucose and serum insulin concentrations, insulin sensitivity and beta-cell function in non-diabetic individuals.

Research Design and Methods

Study participants

Study participants are from the Whitehall II study, an occupational cohort of 10,308 British civil servants (6,896 men and 3,412 women aged 35–55 years) of mainly white ethnicity recruited between 1985 and 1988 (phase 1)(18). The cohort has been followed at eight subsequent phases, approximately 2.5 years apart. All study phases included a questionnaire, and every second phase (5 years apart) also included a clinical health examination (phases 1, 3, 5, 7 and 9). Phase 5 (1997-1999) was the first phase where resting heart rate and HRV were measured and was the baseline for the current analyses. A total of 7,870 participated at phase 5; 6,967 at phase 7 (2002–2004) and 6,761 at phase 9 (2007–2009).

At phases 5, 7 and 9, a standard 2-hour 75 g OGTT was performed in the morning after an overnight fast (≥8 h of fasting). For around one-third of the examinations, the OGTT was administered in the afternoon after a light fat-free breakfast (≥5 h of fasting) and data from these examinations were not included in the analysis. Diabetes was diagnosed by the treating physician (outside the study) or during screening ascertained throughout follow-up by OGTTs administered every 5 years with diabetes defined according to the World Health Organization(19). Prediabetes was defined by fasting glucose between or equal to 6.1 and 7.0 mmol/L and/or a 2-hour glucose level between or equal to 7.8 and 11.1 mmol/L. Diabetes was defined by measures above these levels.

Autonomic function was assessed in a subset of the participants: in 54% at phase 5, 64% at phase 7 and in 83% at phase 9.

During follow-up, participants were censored if they died, were lost to follow-up, were diagnosed with diabetes and/or developed heart disease, such as ischemic heart disease and arrhythmias either diagnosed at study examination or by register based follow-up. We excluded 7,183 (35.8%) person-examinations for which the participant had been fasting <8 h (OGTTs administered in the afternoon or known diabetes) and another 951 person-examinations because the participants had developed heart disease. Up to a total of 9000 person-examinations for 3,631 participants without known diabetes, ischemic heart disease and arrhythmias were analysed (5,709 person-examination for 2,519 participants for the analyses of HRV).

The UK NHS Health Research Authority London-Harrow Ethics Committee reviewed and approved the study. Written informed consent was obtained from each participant at each examination phase. The study was conducted according to the principles of the Helsinki Declaration.

Measurements

Plasma glucose, serum insulin, HbA_{1c}, serum lipids and blood pressure at phases 5, 7 and 9 were measured as described previously(20; 21). Whole-body insulin sensitivity was estimated by the insulin sensitivity index ($ISI_{0,120}$) based on fasting and 2-hour values of glucose and insulin(22). Insulin sensitivity in the fasting state was assessed using HOMA-IS(23). Beta-cell function was estimated based on fasting plasma glucose and serum insulin concentrations using the homeostasis model assessment of beta-cell function (HOMA-β) (23).

HRV indices characterizing autonomic status were derived from 5-minute resting 12-lead ECG recordings obtained subsequent to 5-minute of rest in the supine position at phases 5 and 7. Recordings were filtered through an automated algorithm, allowing the analyses of suitable normal-to-normal sinus rhythm R–R intervals without the presence of arrhythmias, ectopic beats and branch blocks (N-N intervals). Indices of HRV were analyzed both in the time domain: the standard deviation of all N-N intervals (SDNN) and the root mean square of the sum of the squares of differences between consecutive N-N intervals (RMSSD), and also in the frequency domain by utilizing a Blackman-Tukey algorithm: low-frequency (LF) power (in the 0.04–0.15 Hz frequency band), high-frequency (HF) power (in the 0.15–0.4 Hz frequency band) and total power (in the ≤ 0.4 Hz frequency band). The ratio between LF and HF power (LF/HF ratio) was calculated. Resting heart rate (rHR) was calculated from the ECG recordings. RMSSD and HF power outcomes are associated mainly with parasympathetic modulations, whereas the remaining measures characterize mixed sympathetic and parasympathetic influences.

Information on smoking habits (never/ex/current), physical activity (hours per week of mild, moderate and vigorous physical activity) and medication use were collected using self-administered questionnaires at phases 5, 7 and 9(24).

Statistical analysis

In the main analysis, the following glycemic outcomes were studied: fasting and 2-hour serum insulin, fasting and 2-hour plasma glucose, HOMA-IS and $ISI_{0,120}$, HOMA-β, and HbA_{1c}. Outcomes with a skewed distribution (fasting and 2-hour serum insulin, HOMA-IS, $ISI_{0,120}$ and HOMA-β) were log-transformed before analysis in order to fulfil the assumption of normally distributed model residuals. Heart rate and the six HRV indices (SDNN, RMSSD, HF power, LF power, LF/HF ratio and total power) were included as exposures. All exposures except heart rate were $\log_2$-transformed prior to analysis.

In the analyses of the six HRV indices, the subset of participants with autonomic function assessed was used. For the analysis of heart rate, the total study population was included. HbA_{1c} was only measured at phase 7

and 9 so for this outcome phase 5 was not used. For most of the covariates, less than 5% of the values were missing, but with up 9% for physical activity. To avoid exclusion of patients with missing values, missing data on the covariates at each phase were imputed by using the Multivariate Imputations by Chained Equations method(25) with a missing-at-random assumptions (25 imputations) and including a number of auxiliary data on the participants not used in the analyses. Estimates of parameters of interest were averaged across the imputation copies according to Rubin rules (26).

In each 5-year observation window of two consecutive phases, we studied the associations of baseline levels of heart rate and HRV indices and follow-up levels of the different outcomes, adjusting for baseline level of the outcome. Because the same individual may contribute with up to three observations to the analyses, associations were estimated using linear mixed-effects models with a participant-specific random intercept and slope to account for the correlation of repeated measurements within participants.

All analyses were adjusted for age, sex, ethnicity and baseline value of the outcome studied (model 1). HRV indices were further adjusted for the simultaneously measured heart rate. Additional adjustments included BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers (model 2). In model 1, we tested for a modifying effect of baseline glycemic state (normal glucose tolerance (NGT) or pre-diabetes) on the association with heart rate and HRV indices. Glycemic state was treated as a time-varying confounder which allowed the same participant to contribute with person-examinations first to the NGT group and later to the pre-diabetes group (and vice versa).

To allow direct comparisons of effect sizes between the exposure variables, we further calculated standardized regression coefficients for the subset of the population for whom heart rate and all six HRV indices were available at the same time points, i.e. the subset with autonomic function assessed. HRV indices were log-transformed before standardization and the adjustments used in model 2 were applied.

Statistical analyses were performed in R version 3.3.3 (The R Foundation for Statistical Computing) and SAS, version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Median (IQR) time between phases 5, 7 and 9 was 5.1 (5.0; 5.5) years. All measures of HRV diminished during the study period. Characteristics of the study population by study phase are shown in Table 1.

We found no statistically significant modifying effect of pre-diabetes status for any associations p >= 0.051, so associations were estimated for normal glucose tolerance and pre-diabetes combined.

Associations of heart rate and HRV indices with 5-year changes in serum insulin concentrations

Higher levels of resting heart rate were significantly associated with a 5-year increase in levels of fasting serum insulin in all statistical models. Higher values of total power were associated with a reduction in fasting serum insulin levels 5 year after baseline in model 1, but associations lost significance after further adjustment. Similar trends for the HRV indices SDNN, RMSSD and total power were seen (Table 2). Overall, heart rate and HRV indices showed similar direction of the associations with 2-hour insulin as for fasting insulin. A higher baseline heart rate was associated with a five-year increase in 2-hour insulin in both models. Low frequency power and Total Power were associated with a five-year drop in 2 hour-serum insulin in model 1, however significance was lost in Model 2. Similar trends for the HRV indices SDNN and high frequency power were identified (Table 2) (Figure 1).

Associations between heart rate and HRV indices and 5-year changes in insulin sensitivity

In model 1, higher heart rate was significantly associated with a decrease in insulin sensitivity assessed by $ISI_{0,120}$ five years after baseline. Higher levels of SDNN, low frequency power and total power was significantly associated with higher insulin sensitivity. After full adjustment, only heart rate remained significantly associated with $ISI_{0,120}$. The trends toward higher SDNN and low frequency power being associated with higher insulin sensitivity were seen only in model 1. (Table 2) (Figure 1).
When insulin sensitivity was assessed by HOMA-IS similar associations were seen but of smaller magnitude, and only significant for heart rate (Supplementary Table 1). (Supplementary Figure 1).

Associations between heart rate and HRV indices and 5-year changes in beta-cell function

Higher baseline heart rate were associated with a 5-year increase in HOMA-β in all models. The opposite associations were seen for high frequency power, but the associations lost statistical significance in model 2. (Supplementary Table 1, Supplementary figure 1).

Associations between heart rate and HRV indices and changes in glycemic measures

Higher heart rate was significantly associated with very small increases in fasting and 2-hour glucose and HbA_{1c} in model 1 but lost significance in model 2. Higher LF/HF ratio was associated with small decreases in 2-hour fasting glucose in both model 1 and model 2 (Figure 1) (Supplementary Table 2).

Effect size

When comparing the standardized regression coefficients, heart rate was overall strongly associated with the outcomes. However, all effect sizes were relatively small (Figure 1).

DISCUSSION

The present study investigated the temporal associations of resting heart rate and HRV with 5-year changes in glucose metabolism in a large population of non-diabetic individuals. Our results show that higher resting heart rate was associated with subsequent increases in fasting insulin, 2-hour insulin and β-cell function and with a decrease in insulin sensitivity but was not associated with glycemia in a clinically significant manner. Few HRV indices reflecting both parasympathetic modulations and a mixture of sympathetic and parasympathetic modulations were significantly associated with insulin measures only in models adjusted for age, sex and ethnicity. More favorable baseline values of these HRV indices were associated with decreases in fasting insulin, 2-hour insulin, β-cell function and insulin sensitivity. Similar trends toward the same association was seen for the majority of all HRV outcomes in fully adjusted models, however only few estimates had p values close to 0.05. No associations of HRV measures with HbA_{1c}, fasting glucose or 2-hour glucose were observed in the fully adjusted models.

Insulin

Our results show that higher and less favorable levels of resting heart rate were associated with future increases in both fasting and 2-hour insulin levels. These associations may in part be medicated through autonomic function as higher and favorable levels HRV in heart rate adjusted analyses in several cases seemed to trend toward an association with higher future levels of insulin in the fasting state and during glucose administration. It is known that insulin itself can influence the autonomic nervous system(27) by reducing parasympathetic function and potentiating sympathetic drive. This insulin-induced change in autonomic tone is however attenuated in insulin-resistant individuals(28), suggesting that alterations in autonomic tone in insulin-resistant individuals are due to pathological mechanisms less closely related to hyperinsulinemia. As the endocrine pancreas is innervated by the autonomic nervous system(8) it is plausible that autonomic dysfunction may elicit future dysinsulinemia by an imbalance in nervous control of the pancreatic islet. However, due to the paucity of significant associations between HRV indices and insulin measures it cannot be concluded that autonomic function is associated with insulin levels in non-diabetic individuals. The associations found between heart rate and insulin levels may be due to residual confounding not addressed in the present study such as inflammation and physical fitness.

Insulin sensitivity

Higher heart rate was associated lower whole-body insulin sensitivity in all models. As for insulin, higher and more favorable levels of several HRV indices were associated increased insulin sensitivity. However, only trends of this association remained after full adjustment. Similar bust less significant associations were found when insulin-sensitivity was assessed by HOMA-IS. Cross-sectional studies have reported autonomic imbalance to be associated with insulin resistance in obese individuals(29). Also, intervention studies demonstrating improvements in insulin sensitivity by weight loss, training or ACE inhibition have shown improvements in parasympathetic activity (28; 30; 31). In concert with the above-mentioned studies, our results may indicate that autonomic dysfunction could be associated with future loss of insulin sensitivity. However, only heart rate remained significantly associated to future changes in insulin sensitivity in fully adjusted models which could imply that other pathways than autonomic dysfunction may mediate the associations or that heart rate is just a risk marker of other pathogenic mechanisms as stated above.

Beta-cell function

Our findings suggest that less favorable levels of heart rate are associated with improved future beta-cell function. This seems to contradict our finding in respect to levels of insulin and insulin sensitivity, where more favorable measures of heart rate were associated with improvements in glucose control. It is possible that these changes are a result of compensatory improvement in beta-cell function in non-diabetic states, however it is more likely that our association on beta-cell function may be a spurious finding and an artefact caused by improvements in insulin sensitivity and unaffected measures of glycemia. Low peripheral insulin sensitivity is associated with increased secretion of insulin (32; 33). If insulin sensitivity is improved and glucose levels remain stable, this would lead to reduced levels of insulin and a false reduction in beta-cell function estimated by the HOMA-β equation.

Glycemia

No clinically meaningful associations of resting heart rate or HRV indices with measures of glycemia were observed. Fasting glucose levels are mainly determined by hepatic glucose production, whereas increased 2-hour glucose concentrations mainly reflect peripheral glucose uptake(34). The autonomic nervous system is associated with both systems innervating the liver (35; 36), affecting hepatic glucose production and release(37) and possibly regulating muscle glucose-uptake in a non-insulin dependent manner(37) (38). It is possible that we did not find any association between heart rate and HRV-based indices of autonomic dysfunction and glycemic measures due to compensatory mechanisms in this cohort of non-diabetic adults enabling preserved glucose homeostasis. This compensation could be mediated through e.g. increased insulin levels as seen in our results discussed above.

Strengths and weaknesses

Our study benefits from its large sample size, the comprehensive measurements of outcomes and exposures assessed simultaneously, and the extensive adjustment for confounders. Importantly, the prospective design allowed us to assess the temporal association between heart rate and HRV and changes in glucose metabolism, whereas associations in previous cross-sectional studies may be attributable to reverse causality.

However, resting heart rate is not a gold standard measure of autonomic function, as it may be affected by several factors such as physical fitness, hemoglobin levels and inflammation. Therefore, any conclusions about the associations of heart rate and autonomic function should be drawn with caution. The study holds no pure sympathetic measures. The lack of associations between autonomic function measures and glucose metabolism may be due this limitation, as sympathetic activation is associated with insulin secretion. Further limitations include the observational design that precludes causal inferences. However, the present study provides evidence for temporal associations.

$ISI_{0,120}$ and HOMA-IS correlate only moderately well with the euglycemic–hyperinsulinemic clamp(39). Thus, our assessment of insulin sensitivity was less precise than the gold standard. In addition, our study did not include dynamic assessments of beta-cell function, thus we could not examine beta-cell function relative to insulin sensitivity using the disposition index. The association between autonomic function and glucose regulation may have been underestimated due to censoring of study participants with comorbidities in whom the associations may have been more pronounced.

The study population was predominantly of European descent, so the results may not be generalized to other ethnic groups. Also, the cohort is based on people employed as civil servants at the study start excluding e.g. unemployed people and people employed in the private sector. The study may therefore not be fully generalizable to the general population.

Conclusion

In summary, our study demonstrates that more favorable baseline levels of heart rate and autonomic function assessed by HRV indices were associated with improvements in insulin sensitivity, and lower serum insulin concentrations in non-diabetic individuals. Associations were found for both parasympathetic and mixed sympathetic and parasympathetic measures. However, only trends of these association were found for HRV indices in fully adjusted models implying that association between heart rate and future changes in glucose metabolism may be mediated via other mechanisms than autonomic dysfunction or that heart rate is merely a risk marker of other pathological mechanisms. Effect sizes were moderate, but on a population level these results may suggest that elevated heart rate marks subsequent deterioration of glucose metabolism. No

corresponding associations were observed for HbA_{1c}, fasting or 2-hour plasma glucose. Prospective studies following individuals from non-diabetic states to overt diabetes are needed to elucidate if autonomic dysfunction is indeed associated with adverse changes in glucose metabolism leading to the disease.

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Authors' contributions

CSH, KF, MEJ, DRV, EJB, AGT, MK and DV contributed to the study concept and design. DRV, EJB, AGT, MK, MM contributed the data. CSH, DV planned the statistical analysis. DV conducted the statistical analysis. CSH, KF and DV drafted the paper. All authors contributed to, critically revised and approved the final version of the manuscript.

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Competing interests

All the authors declare that there is no duality of interest associated with their contribution to this manuscript. The funders of the study had no role in study design, data collection, analysis, interpretation or writing of the report.

Ethics

The study was approval The UK NHS Health Research Authority London-Harrow ethics committee approved the study which was conducted in accordance to the Helsinki Declaration with written informed consent from all participants.

Availability of data and materials

Whitehall II data, protocols, and other metadata are available to bona fide researchers for research purposes. Please refer to the Whitehall II data sharing policy at http://www.ucl.ac.uk/whitehallII/data-sharing

Guarantor's statement

Dr. Christian Stevns Hansen is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1 Characteristics of the study population at each study phase

	Phase 5	Phase 7	Phase 9
N	4271	4294	3343
Men (%)	70.7 (69.3;72.0)	72.0 (70.6;73.3)	71.7 (70.2;73.3)
White ethnicity (%)	92.0 (91.1;92.8)	92.6 (91.8;93.4)	93.0 (92.1;93.9)
Age (years)	55.0 (5.9)	60.4 (5.8)	65.1 (5.8)
Height (cm)	172.2 (9.1)	171.4 (9.1)	171.3 (9.1)
BMI (kg/m^2)	26.1 (3.9)	26.6 (4.3)	26.5 (4.3)
Waist circumference (cm)	90.5 (11.6)	93.2 (12.0)	94.0 (11.8)
Current smoker (%)	10.6 (9.7;11.5)	8.6 (7.8;9.5)	5.8 (5.0;6.6)
Moderate to vigorous exercise (hours/week)	11.3 (4.5;19.8)	11.8 (4.5;20.3)	-
Alcohol intake (units/week)	10.0 (3.0;20.0)	9.0 (3.0;18.0)	7.0 (2.0;16.0)
Systolic blood pressure (mmHg)	122.2 (16.3)	127.8 (16.7)	125.4 (15.8)
Diastolic blood pressure (mmHg)	77.3 (10.5)	74.6 (10.5)	71.7 (10.0)
Medication			
Tricyclic antidepressants (%)	2.2 (1.8;2.7)	2.6 (2.1;3.1)	3.4 (2.8;4.0)
Diuretics (%)	2.9 (2.4;3.5)	7.4 (6.6;8.2)	11.2 (10.1;12.3)
Beta-blockers (%)	4.6 (4.0;5.3)	7.9 (7.1;8.7)	6.5 (5.7;7.4)
Blood measurements			
Fasting plasma glucose (mmol/L)	5.1 (0.7)	5.3 (0.7)	5.2 (0.6)
2-hour plasma glucose (mmol/L)	5.8 (1.7)	6.3 (1.9)	6.4 (1.9)
HbA_{1c} (mmol/mol)	-	38.0 (5.4)	38.0 (4.9)
HbA_{1c} (%)	-	5.6 (0.5)	5.6 (0.4)
Fasting serum insulin (pmol/L)	7.0 (4.9;10.1)	7.0 (4.7;10.6)	6.5 (4.3;10.1)
2-hour serum insulin (pmol/L)	32.6 (19.8;53.2)	37.8 (23.4;63.6)	41.0 (25.2;68.7)
ISI_{0-120}	38.1 (29.7;49.0)	34.4 (26.4;44.4)	33.8 (25.7;43.9)
HOMA Insulin resistance	1.6 (1.1;2.4)	1.6 (1.1;2.6)	1.5 (1.0;2.4)
HOMA β cell function	92.1 (65.2;131.4)	80.0 (55.3;118.9)	81.5 (55.4;120.0)
Total cholesterol (mmol/L)	5.9 (1.1)	5.8 (1.0)	5.4 (1.0)
HDL cholesterol (mmol(L)	1.5 (0.4)	1.6 (0.4)	1.6 (0.4)
LDL cholesterol (mmol/L)	3.9 (0.9)	3.6 (0.9)	3.2 (1.0)
Triglycerides (mmol/L)	1.3 (0.9)	1.3 (0.9)	1.2 (0.7)
Hear rate measurements			
Resting heart rate from ECG (bpm)	67.2 (11.1)	67.8 (11.6)	65.5 (11.1)
SDNN (ms)	34.6 (26.3;44.8)	33.8 (25.6;44.9)	30.4 (22.6;41.3)
RMSSD (ms)	20.5 (13.9;29.5)	20.2 (13.5;30.2)	18.0 (12.3;26.6)
Low frequency power (ms^2)	315.8 (170.0;572.8)	286.4 (160.0;518.0)	234.7 (121.4;468.1)
High frequency power (ms^2)	132.2 (63.1;262.5)	116.0 (55.5;234.1)	92.1 (45.2;189.8)
LF/HF ratio	2.56 (1.52;4.04)	2.62(1.59;4.06)	2.69 (1.59;4.14)
Total power (ms^2)	1068 (619;1795)	1010 (577;1778)	815 (453;1513)

Data are means (SD), medians (25th; 75th percentiles) or proportions (95% CI); N: number of participants

Table 2 Effect sizes (with 95% CI) of baseline heart rate or HRV on 5-year changes in serum insulin concentrations and insulin sensitivity

		Fasting serum insulin (% diff.)				2-hour serum insulin (% diff.)				$ISI_{0,120}$ (% diff.)			
	Model	**N**	**Npe**	**Estimate**	**P**	**N**	**Npe**	**Estimate**	**P**	**N**	**Npe**	**Estimate**	**P**
Resting heart rate (10 bpm)	1	3631	9000	3.4 (2.0;4.8)	<0.001	3631	9000	4.0 (2.2;5.9)	<0.001	3631	9000	-2.0 (-2.9;-1.0)	<0.001
	2	3631	9000	3.3 (1.8;4.8)	<0.001	3631	9000	3.3 (1.3;5.3)	0.001	3631	9000	-1.4 (-2.4;-0.3)	0.009
SDNN (doubling)	1	2519	5709	-2.4 (-5.2;0.4)	0.089	2519	5709	-5.1 (-9.1;-1.0)	0.016	2519	5709	3.1 (0.7;5.5)	0.010
	2	2519	5709	-0.5 (-3.3;2.3)	0.726	2519	5709	-3.1 (-7.1;1.1)	0.146	2519	5709	1.8 (-0.5;4.1)	0.134
RMSSD (doubling)	1	2519	5709	-1.4 (-3.5;0.7)	0.177	2519	5709	-2.3 (-5.2;0.7)	0.134	2519	5709	0.9 (-0.8;2.6)	0.304
	2	2519	5709	-0.5 (-2.5;1.6)	0.643	2519	5709	-1.0 (-4.0;2.0)	0.504	2519	5709	0.1 (-1.6;1.7)	0.932
Low frequency power (doubling)	1	2519	5709	-1.2 (-2.4;0.1)	0.069	2519	5709	-2.1 (-3.9;-0.2)	0.027	2519	5709	1.4 (0.4;2.4)	0.008
	2	2519	5709	-0.1 (-1.4;1.1)	0.822	2519	5709	-1.1 (-2.9;0.8)	0.256	2519	5709	0.7 (-0.3;1.7)	0.156
High frequency power (doubling)	1	2519	5709	-1.1 (-2.2;0.0)	0.046	2519	5709	-1.4 (-3.0;0.1)	0.073	2519	5709	0.7 (-0.1;1.6)	0.102
	2	2519	5709	-0.3 (-1.3;0.8)	0.601	2519	5709	-0.5 (-2.1;1.1)	0.521	2519	5709	0.1 (-0.7;1.0)	0.743
LF/HF ratio (doubling)	1	2519	5709	0.5 (-1.1;2.2)	0.556	2519	5709	-0.5 (-2.8;2.0)	0.699	2519	5709	0.8 (-0.5;2.0)	0.246
	2	2519	5709	0.4 (-1.2;2.0)	0.620	2519	5709	-0.7 (-3.1;1.6)	0.537	2519	5709	1.0 (-0.3;2.2)	0.140
Total power (doubling)	1	2519	5709	-1.3 (-2.7;0.2)	0.080	2519	5709	-2.5 (-4.6;-0.4)	0.018	2519	5709	1.6 (0.4;2.7)	0.008
	2	2519	5709	-0.2 (-1.6;1.2)	0.745	2519	5709	-1.5 (-3.6;0.6)	0.167	2519	5709	0.9 (-0.2;2.1)	0.117

N: number of participants used in the particular analysis; Npe: number of person-examinations used in the particular analysis; bpm: beats per minute;

P: p-value for the test of the effect being equal to zero. SDNN, standard deviation of normal-to-normal R–R intervals; RMSSD, the root mean square of the sum of the squares of differences between consecutive normal-to-normal R–R intervals; LF, low-frequency; HF, high-frequency.

Model 1: Adjusted for age, sex, ethnicity, study phase and baseline value of the outcome studied. For HRV indices, further adjustment for heart rate obtained as part of the HRV analyses; Model 2: further adjustment for BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers

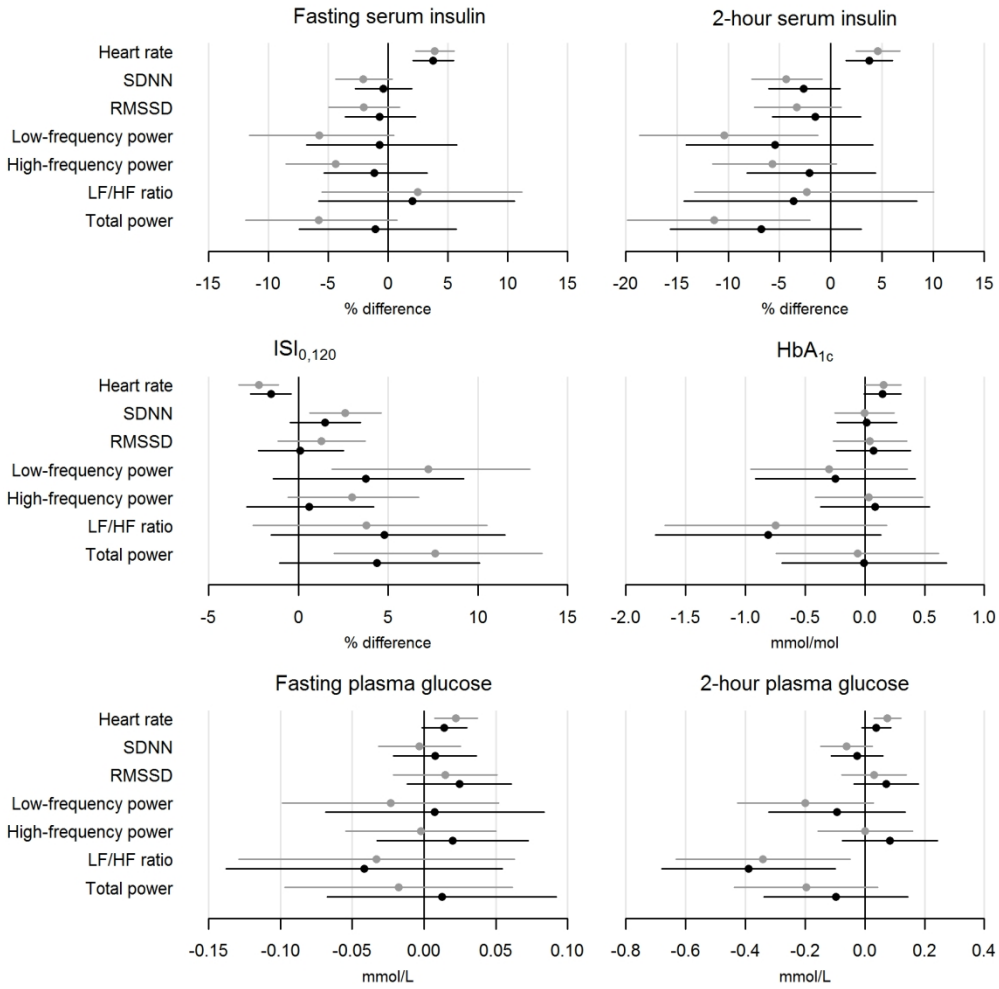

Figure 1 Effect (with 95% CI) of one population standard deviation difference in heart rate or in the Log of HRV indices at baseline on subsequent 5-year changes in markers of glucose regulation. The associations are adjusted for age, sex, ethnicity, study phase and baseline value of the outcome studied (grey) and further adjustment for BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers (black). For HRV indices, further adjustment for heart rate obtained as part of the HRV analyses.

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Supplementary Table 1 Effect sizes (with 95% CI) of baseline heart rate or HRV on 5-year changes in beta cell function and insulin sensitivity

		HOMA-β (% diff.)				HOMA-IS (% diff.)			
	Model	**N**	**Npe**	**Estimate**	**P**	**N**	**Npe**	**Estimate**	**P**
Resting heart rate (10 bpm)	1	3631	9000	3.8 (2.4;5.2)	<0.001	3631	9000	-3.4 (-4.8;-2.0)	<0.001
	2	3631	9000	3.0 (1.6;4.5)	<0.001	3631	9000	-3.4 (-4.8;-1.9)	<0.001
SDNN (doubling)	1	2519	5709	-2.5 (-5.3;0.4)	0.095	2519	5709	2.6 (-0.5;5.8)	0.107
	2	2519	5709	-0.9 (-3.7;2.1)	0.561	2519	5709	0.3 (-2.7;3.4)	0.835
RMSSD (doubling)	1	2519	5709	-2.0 (-4.1;0.1)	0.062	2519	5709	1.3 (-1.0;3.6)	0.270
	2	2519	5709	-1.1 (-3.2;1.0)	0.302	2519	5709	0.2 (-2.0;2.5)	0.841
Low frequency power (doubling)	1	2519	5709	-1.1 (-2.4;0.2)	0.094	2519	5709	1.2 (-0.1;2.6)	0.075
	2	2519	5709	-0.2 (-1.5;1.1)	0.746	2519	5709	0.1 (-1.2;1.4)	0.880
High frequency power (doubling)	1	2519	5709	-1.2 (-2.3;-0.1)	0.031	2519	5709	1.1 (-0.1;2.3)	0.065
	2	2519	5709	-0.5 (-1.5;0.7)	0.421	2519	5709	0.2 (-1.0;1.4)	0.728
LF/HF ratio (doubling)	1	2519	5709	0.9 (-0.8;2.6)	0.303	2519	5709	-0.4 (-2.1;1.4)	0.681
	2	2519	5709	0.7 (-1.0;2.4)	0.423	2519	5709	-0.3 (-2.0;1.5)	0.743
Total power (doubling)	1	2519	5709	-1.2 (-2.6;0.3)	0.105	2519	5709	1.3 (-0.2;2.9)	0.091
	2	2519	5709	-0.3 (-1.8;1.1)	0.643	2519	5709	0.2 (-1.3;1.7)	0.831

N: number of participants used in the particular analysis; Npe: number of person-examinations used in the particular analysis; bpm: beats per minute;

P: p-value for the test of the effect being equal to zero. SDNN, standard deviation of normal-to-normal R–R intervals; RMSSD, the root mean square of the sum of the squares of differences between consecutive normal-to-normal R–R intervals;

Model 1: Adjusted for age, sex, ethnicity, study phase and baseline value of the outcome studied. For HRV indices, further adjustment for heart rate obtained as part of the HRV analyses;

Model 2: further adjustment for BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers

Supplementary Table 2 Effect sizes (with 95% CI) of baseline heart rate or HRV on 5-year changes in plasma glucose and HbA_{1c} concentrations

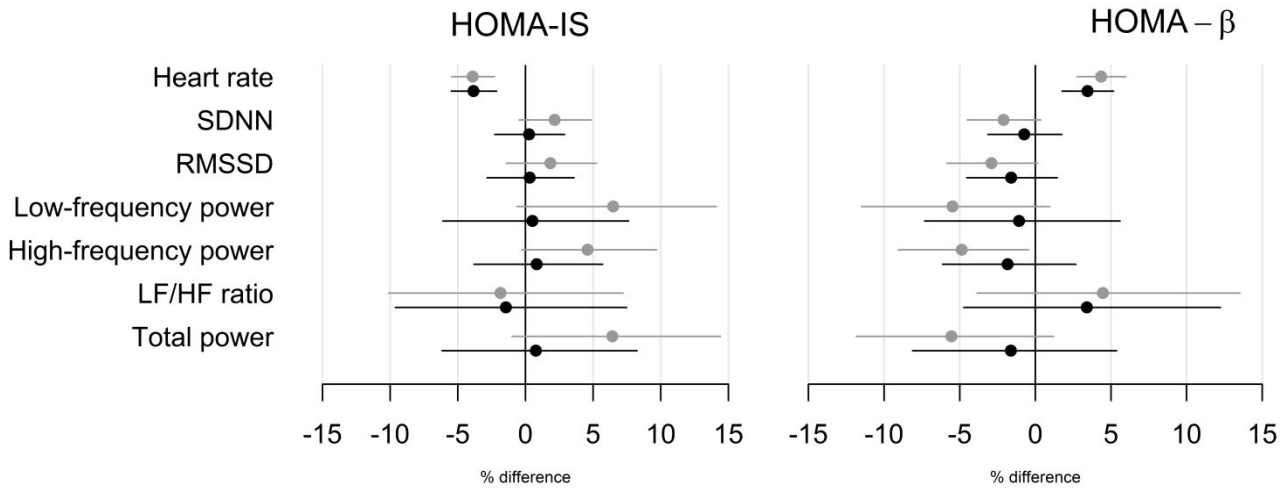
		Fasting plasma glucose (mmol/L)				2-hour plasma glucose (mmol/L)				HbA_{1c} (mmol/mol)			
	Model	**N**	**Npe**	**Estimate**	**P**	**N**	**Npe**	**Estimate**	**P**	**N**	**Npe**	**Estimate**	**P**
Resting heart rate (10 bpm)	1	3631	9000	0.0 (0.0;0.0)	0.003	3631	9000	0.1 (0.0;0.1)	0.001	2526	5052	0.1 (0.0;0.3)	0.041
	2	3631	9000	0.0 (0.0;0.0)	0.076	3631	9000	0.0 (0.0;0.1)	0.118	2526	5052	0.1 (0.0;0.3)	0.065
SDNN (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.828	2519	5709	-0.1 (-0.2;0.0)	0.161	1643	3286	0.0 (-0.3;0.3)	0.974
	2	2519	5709	0.0 (0.0;0.0)	0.609	2519	5709	0.0 (-0.1;0.1)	0.560	1643	3286	0.0 (-0.3;0.3)	0.896
RMSSD (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.427	2519	5709	0.0 (-0.1;0.1)	0.579	1643	3286	0.0 (-0.2;0.2)	0.788
	2	2519	5709	0.0 (0.0;0.0)	0.184	2519	5709	0.0 (0.0;0.1)	0.193	1643	3286	0.0 (-0.2;0.3)	0.653
Low frequency power (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.544	2519	5709	0.0 (-0.1;0.0)	0.087	1643	3286	-0.1 (-0.2;0.1)	0.369
	2	2519	5709	0.0 (0.0;0.0)	0.847	2519	5709	0.0 (-0.1;0.0)	0.426	1643	3286	0.0 (-0.2;0.1)	0.469
High frequency power (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.935	2519	5709	0.0 (0.0;0.0)	0.993	1643	3286	0.0 (-0.1;0.1)	0.880
	2	2519	5709	0.0 (0.0;0.0)	0.461	2519	5709	0.0 (0.0;0.1)	0.302	1643	3286	0.0 (-0.1;0.1)	0.714
LF/HF ratio (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.499	2519	5709	-0.1 (-0.1;0.0)	0.022	1643	3286	-0.2 (-0.3;0.0)	0.116
	2	2519	5709	0.0 (0.0;0.0)	0.396	2519	5709	-0.1 (-0.1;0.0)	0.009	1643	3286	-0.2 (-0.4;0.0)	0.092
Total power (doubling)	1	2519	5709	0.0 (0.0;0.0)	0.659	2519	5709	0.0 (-0.1;0.0)	0.108	1643	3286	0.0 (-0.2;0.1)	0.858
	2	2519	5709	0.0 (0.0;0.0)	0.762	2519	5709	0.0 (-0.1;0.0)	0.433	1643	3286	0.0 (-0.1;0.1)	0.985

N: number of participants used in the particular analysis; Npe: number of person-examinations used in the particular analysis; bpm: beats per minute;

P: p-value for the test of the effect being equal to zero. SDNN, standard deviation of normal-to-normal R–R intervals; RMSSD, the root mean square of the sum of the squares of differences between consecutive normal-to-normal R–R intervals; LF, low-frequency; HF, high-frequency.

Model 1: Adjusted for age, sex, ethnicity, study phase and baseline value of the outcome studied. For HRV indices, further adjustment for heart rate obtained as part of the HRV analyses; Model 2: further adjustment for BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers

Supplementary Figure 1



Supplementary Figure 1 Effect (with 95% CI) of one population standard deviation difference in heart rate or in the Log of HRV indices at baseline on subsequent 5-year changes in markers of insulin sensitivity and β-cell function. The associations are adjusted for age, sex, ethnicity, study phase and baseline value of the outcome studied (grey) and further adjustment for BMI, physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers (black). For HRV indices, further adjustment for heart rate obtained as part of the HRV analyses.