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Influence of blood glucose on heart rate and cardiac autonomic function.

The D.E.S.I.R. Study

Paul Valensi

Fabrice Extramiana

Céline Lange

Martine Cailleau

Abdeddayem Haggui

Pierre Maison Blanche

Jean Tichet

Beverley Balkau

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Abstract

Aims To evaluate in a general population, the effect of dysglycaemia, insulin resistance and metabolic parameters, on heart rate (HR), HR recovery and HR variability (HRV).

Methods 447 participants in the D.E.S.I.R. study were classified according to glycaemic status over the preceding nine years. All were free of cardiac antecedents and were not taking drugs which alter HR. During five consecutive periods: rest, deep-breathing, recovery, rest and lying-to-standing, HR and HRV were evaluated and compared by ANCOVA and trend tests across glycaemic classes. Spearman correlation coefficients quantified the relations between cardio-metabolic risk factors, HR and HRV.

Results HR differed between glycaemic groups, except during deep-breathing. Between rest and deep-breathing periods, diabetic patients had a lower increase in HR than others ($P_{\text{trend}} < 0.01$); between deep-breathing and recovery, the HR of diabetic patients continued to increase, for others, HR decreased ($P < 0.009$). HR was correlated with capillary glucose and triglycerides during the five test periods. HRV (SDRR) differed according to glycaemic status, especially during the recovery period. In contrast to HR, HRV was negatively correlated with age. After age, sex and BMI adjustment, HRV was correlated with triglycerides at two test periods. Change in HR between recovery and deep-breathing was negatively correlated with HRV at rest, ($r = -0.113, P < 0.05$): lower resting HRV was associated with HR acceleration.

Conclusions HR, but not HRV, was associated with glycaemic status and capillary glucose. After deep-breathing, HR recovery was altered in known diabetic patients, and was associated with reduced HRV. Over-weight was a major correlate of HRV.

Keywords: diabetes, epidemiology, heart rate, heart rate variability, heart rate recovery

Introduction

Elevated heart rate (HR) predicts cardiovascular morbidity and mortality in both diabetic [1,2] and non-diabetic individuals [3,4]. HR is the result of fitness, neurohormonal factors and the autonomic nervous system function. In type 2 diabetic patients, HR is faster [5] and this may be related to cardiac autonomic neuropathy (CAN). Fasting plasma glucose is associated with higher resting HR [6] and in the Insulin Resistance Atherosclerosis Study, HR was associated with insulin sensitivity and with the acute insulin response to glucose [7].

The HR profile during exercise and recovery, which may be modulated by an impaired ability to increase vago-sympathetic activity rapidly, is a predictor of sudden death in those without clinically detectable cardiovascular disease [8]. Fasting plasma glucose, even at non-diabetic levels, is strongly associated with abnormal HR recovery after exercise [6].

In diabetic patients, a lower HR variability (HRV) has been recognized as an early hallmark of CAN, and it predicts mortality [5,9,10]. In asymptomatic diabetic individuals, HRV is a predictor of major cardiac events and of silent myocardial ischemia [11]. CAN may impair prognosis through silent coronary disease [9], respiratory function, impaired ability to recognise hypoglycaemia, impaired recovery from hypoglycaemic episodes, hypertension [12], nephropathy [9], and changes in ventricular repolarization [13].

In type 2 diabetes, CAN is associated with age, obesity, systolic blood pressure, glycaemic control, and duration of diabetes [14] and in non-diabetic individuals, with age, overweight, pre-diabetes, HR, diminished HRV, treatment for hypertension, metabolic syndrome and insulin resistance [3,15]. Published data do not identify the temporal sequence between autonomic impairment and insulin resistance, and autonomic dysfunction could influence glucose dysregulation. Both insulin resistance and autonomic dysfunction might have shared precursors, such as physical inactivity or obesity. Interestingly, a longitudinal study has shown that autonomic nervous system dysfunction (high HR, low HRV), may be associated with incident diabetes [1].

We hypothesized that HR and HRV may be influenced by long-term glycaemic status per se, independently of confounding factors cross-correlated with glycaemic status, HR and HRV. We examined the influence of glucose status over the previous nine years, on HR and HRV measured during a standardized testing procedure - during rest, deep-breathing, recovery, rest and lying-to-standing. The secondary aims were to study the relations of HR and HRV with fasting insulin, the HOMA insulin resistance index and the metabolic syndrome, and to see whether a simple forced breathing test is adequate to detect reduced parasympathetic activity.

Methods

Participants

Men and women, aged 30-65 years, consultants in French Social Security health examination centers, were recruited in 1994-1996 into the study: Data from an Epidemiological Study on the insulin Resistance syndrome (D.E.S.I.R.) [16]; they were invited to health examinations three, six and nine years after inclusion. After the year-9 examination, all 321 individuals who had been treated for diabetes or who had fasting plasma glucose (FPG) ≥ 7.0 mmol/l at some time during the study, were invited to a year-10 examination on micro-vascular complications of diabetes; 237 agreed to participate, 17 had died. Control groups were also invited: diabetic individuals were matched by age, sex and examination centre to individuals with (1) impaired fasting glucose (IFG) at any time during the study (FPG 6.1-6.9 mmol/l) and (2) normal glucose levels (FPG < 6.1 mmol/l) during the entire study. The D.E.S.I.R. study was approved by the ethics committee of Kremlin Bicêtre Hospital, and participants signed an informed consent.

All people included in this analysis were free of cardiac disease (22 individuals excluded), and respiratory disease (24) and they were not taking medications which could change heart rate: neuroleptics (4 individuals excluded), anti-ischemics (29), anti-arrhythmics (43), cardiotonics and cardiac analeptics (1), thyroid hormone therapy (27), anti-histaminics (8), anti-glaucoma betablockers (1), tricyclic anti-depressant (0) or anti-hypertensive treatment other than diuretics, ACE inhibitors and angiotensin-2 receptor antagonists (108).

We have defined four glucose status groups:

1. known diabetic patient - treated by drugs for diabetes at some time during the study, n=70;
2. screened as diabetic - FPG ≥ 7.0 mmol/l at least once over the 9 years of the study, n=55;
3. intermediate, impaired fasting glucose - at least one FPG between 6.1 and 6.9 mmol/l, but not known as diabetic, n=157;
4. normal glucose levels during the preceding 9 years of the study - FPG always < 6.1 mmol/l, n=165.

Clinical assessment at baseline and year-10

Blood pressure was measured with a sphygmomanometer, in a supine position, after 5 minutes of rest and one minute after standing. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or taking antihypertensive medications. Waist circumference (smallest circumference between lower rib and iliac crests), weight and height were measured in lightly clad participants, and the BMI was calculated.

Participants completed a self-questionnaire, detailing smoking habits, habitual alcohol intake, and drug treatment.

Biological measurements at year-10

HbA_{1c} was measured on a DCA 2000 (Bayer, Puteaux, France) and capillary blood glucose on a glucometer (Bayer Diagnostics, Ascensia Glucodisc) just before the autonomic tests.

Biological measurements at baseline and year-9

At baseline and year-9, biology was from one of four health centre laboratories at La Riche, Blois, Chartres or Orleans. Fasting plasma glucose, was measured by the glucose-oxidase method, using a Technicon RA100 (Bayer Diagnostics, Puteaux, France) or a Specific or a Delta (Konelab, Evry, France). HbA_{1c} was measured using an automated HPLC ion-exchange analyser from Hitachi/Merck-VWR or a DCA 2000 automated immunoassay system from Bayer Diagnostics, Puteaux, France. To adjust for differences between laboratories, both glucose and HbA_{1c} data were standardized by age and sex with respect to reference data assayed in the La Riche laboratory. Serum insulin was quantified by Micro particle Enzyme Immunoassay with an IMX automated analyser from Abbott (Rungis, France). Total cholesterol, HDL-cholesterol and triglycerides were assayed by a DAX 24 (Bayer Diagnostics, Puteaux, France) or a KONE (Evry, France); HOMA insulin-resistance index (HOMA-IR) was calculated, and the AHA/NLBI definition of the metabolic syndrome used [17].

Heart rate and Heart Rate Variability assessment

HR and HRV have been measured during static conditions (at rest) and dynamic conditions (deep-breathing and lying-to-standing):

- 5 minutes resting in recumbent position,
- 1 minute deep-breathing in recumbent position: 6 deep respiratory cycles during 1 minute [14],
- 1 minute recovery,
- 1 minute resting,
- during a lying-to-standing test: participants were asked to stand up quickly [14].

A 12 derivation ECG was registered at 25 mm/s on a CardioPlug Cardionics ECG Computer Systems (Brussels, Belgium) with a program specifically written for this study to time precisely the tests. ECG recordings have been edited on a beat-to-beat basis to ensure accurate detection of QRS complexes and identification and labeling of premature beats. Mean RR interval, SDNN (standard deviation of normal R-R intervals (NN) in ms), RMSSD (root mean square of differences between successive R-R intervals in ms), PNN50 (percent normal R-R intervals > 50 ms different from previous interval), RR max/RR min, (RR max - RR min), were calculated according to consensus guidelines [18], and the RR standardized SDNN: SDNN/NN [19].

Statistical Methods

SAS (version 9.1.3, SAS Institute, Cary, NC). was used for analyses

Descriptive data are shown as means (standard deviations) or number of participants (percentages). HR across glucose groups are shown graphically and compared by analysis of covariance, with adjustments for age, sex and additionally for BMI, as well as linear trend tests across groups. HRV parameters are shown graphically according to glucose groups, and compared by analysis of covariance after appropriate transformation - $\log(y)$, $-1/y^2$, \sqrt{y} , $1/\sqrt{y}$, following inspection of residuals from linear models; PNN50 was used as a binary variable ($<3^{\text{rd}}$ quartile, $\geq 3^{\text{rd}}$ quartile), and analyzed by logistic regression; strictly normal individuals and the group (IFG and detected diabetic individuals) were also compared. Spearman partial correlation coefficients, adjusted on age and sex, were determined between HR, HRV (SDNN/NN) and cardio-metabolic risk factors; additional adjustment was made for BMI. Relations between HRV (SDNN/NN) at different periods and change in HR between recovery and deep-breathing were quantified by Spearman correlation coefficients, before and after adjusting on age, sex and BMI.

Results

Characteristics of population studied

There were 70 patients with known diabetes, mean diabetes duration: 6.4 years; they were well controlled: mean HbA1c 6.7% (Table 1). BMI, HbA1c and capillary glucose varied over glucose groups; systolic blood pressure and pulse pressure differed significantly, but there was no difference in blood pressure changes between lying-to-standing. More known and detected diabetic individuals smoked. Known diabetic participants had a more severe cardio-vascular risk profile: 75% had the metabolic syndrome in comparison to only 15% in normoglycemic individuals.

Heart rate, heart rate recovery: glycaemic status and factors associated

HR was related with glycaemic status (Figure 1), particularly during recovery after deep-breathing, and during subsequent rest ($P < 0.04, 0.03$), and known diabetic patients had the highest HR. During deep-breathing in comparison to during rest, mean HR increased less in the known diabetic participants than in those always normoglycemic ($P < 0.008$), a relation attenuated after BMI adjustment ($P < 0.1$) (Fig. 1, 2A). Further, there was a trend across the glycaemic groups ($P < 0.01$). At recovery after deep-breathing, the HR in known diabetic individuals continued to increase, while in all other groups it decreased (Fig. 1, 2B). The trend across groups was highly significant ($P < 0.009$) and remained after BMI adjustment ($P < 0.04$).

Capillary glucose, triglycerides and supine blood pressures were positively correlated with HR at almost all of testing periods, after age and sex adjustment (Table 2), and these results were little

attenuated after additional BMI adjustment. The correlation between blood pressure and HR was significant in the normoglycemic subjects, even after BMI adjustment (data not shown). Insulin concentrations and the HOMA-IR index correlated positively with HR at some time periods, but these correlations disappeared after adjusting on BMI. HR was higher in those with the metabolic syndrome for all test periods (Table 2), but was attenuated after adjustment on BMI with a significantly different HR remaining only during deep-breathing.

Heart rate variability and glycaemic status

HRV was more closely related with glycaemic status at deep-breathing and particularly at recovery, than at other periods; patients with known diabetes had the lowest HRV (Fig. 3 A-F). At recovery, the detected diabetic and IFG individuals had similar HRV, and all six HRV indices were significantly lower than in those with normal glucose. After adjustment on BMI the relations were attenuated, and were all non significant $P < 0.1$.

Age was always associated with HRV, as measured by SDNN/NN, at all test periods. Capillary glucose and HbA1c were negatively correlated with SDNN/NN at recovery, after age and sex adjustment, (Table 3). BMI, waist circumference, HOMA-IR, triglycerides, HDL-cholesterol and HR were significantly correlated with HRV at several time periods, particularly at recovery. (Table 3). Supine blood pressure was only related with HRV parameters during lying-to-standing, and this was attenuated but remained associated after BMI adjustment.

In those with the metabolic syndrome, HRV was significantly lower in deep-breathing and recovery periods (Table 3), but not after adjustment on BMI.

The change in HR between recovery and deep-breathing (recovery-[deep-breathing]) was correlated with HRV (SDNN/NN), inversely at rest ($r = -0.117$), positively at deep-breathing ($r = 0.210$) and inversely at recovery ($r = -0.326$), and these correlations were little changed after adjusting on age, sex and BMI.

Discussion

HR and HRV were assessed during five consecutive periods: rest, deep-breathing, recovery, rest, lying-to-standing, our results showed:

1. significant HR difference between the four glycaemic groups; treated diabetic patients had the highest HR, those with screened diabetes or IFG had slightly elevated HR in all test periods; HR was correlated with capillary glucose and triglycerides during all test periods;
2. lower increase in HR between rest and deep-breathing in diabetic patients than in others;

3. HR which continued to increase between deep-breathing and recovery in diabetic patients in contrast to other groups;
4. differences for HRV according to glycaemic status, especially during deep-breathing and recovery periods, attenuated but no longer significant after BMI adjustment;
5. negative correlation between change in HR between deep-breathing and recovery and HRV at rest, and recovery.

HR was highest in known diabetic patients and slightly higher in dysglycemia (screened diabetes or IFG), at all test periods, as seen in other studies [5,6]. HR in non-diabetic individuals, has been found to be associated with insulin resistance, with the acute insulin response to glucose and with proinsulin after adjustment for demographic characteristics, glucose tolerance status, and smoking [7]. In addition a high HR has been shown to predict incident diabetes [1]. Sex, age and BMI may influence HR. In the current study the HR during all five periods, correlated significantly with capillary glucose measured at the time of examination and with triglycerides even after sex, age and BMI adjustment. HR was slightly faster in subjects with metabolic syndrome, significantly during all periods. Higher sympathetic tone can cause insulin resistance [20]. Conversely insulin resistance and hyperinsulinemia can cause sympathetic overactivity and depress vagal activity in healthy subjects [21] and in insulin-resistant non-diabetic offspring of type 2 diabetes patients [22]. In our population, HR correlated with HOMA-IR and fasting insulin, but these correlations were no longer significant after BMI adjustment. The present data suggest that glucose is a determinant of HR.

The plasticity of HR to changes provides further information. The heart-rate profile during exercise and recovery (high resting HR, low HR increase during exercise, low HR decrease after exercise) predicts sudden death [23]. Fasting blood glucose has been reported to be strongly associated with abnormal HR recovery after exercise [6]. In our study, in the known diabetic patients, HR increased less between rest and deep-breathing and continued to increase during the recovery time after deep-breathing while in all other groups, HR decreased, the differences remaining significant after BMI adjustment. Interestingly, this response in diabetic patients was observed despite the smaller HR increase induced by deep-breathing (Fig. 1); individuals detected as diabetic also displayed an abnormal response, and there was a significant trend across glycaemic groups. These results suggest that this relatively simple test has good sensitivity, and could be a new marker, worthy of evaluation.

Defects in HR recovery after exercise may result from an impaired ability to increase vagal and sympathetic activity rapidly [23]. The mechanism cannot be inferred from our data. However, we found a negative correlation between the difference in HR between recovery and deep-breathing and HRV at rest, which was modestly attenuated after adjusting on age, sex and BMI. This suggests that alterations of the vago-sympathetic balance responsible for abnormal HRV, is involved in the abnormal HR recovery

after a minor effort, and that abnormal HR recovery after exercise is a marker of reduced parasympathetic activity.

Lower HRV has been reported in fasting hyperglycemia [3], glucose intolerance [14]; the metabolic syndrome has been implicated in reduced HRV in non-diabetic individuals, independently of glucose [3]. Some data suggest that HRV impairment is associated with more severe insulin resistance in non-diabetic obese individuals [24]. Obesity is a major contributor to CAN in type 2 diabetic patients [17]. A reduced HRV has been reported in 40% of non-diabetic obese patients with a negative correlation between HRV during deep-breathing and fasting glycemia [25], but this may be reversed after weight loss [26]. In adults with normal fasting glucose, impaired baroreflex sensitivity, another marker of autonomic cardiac control, was associated with higher fasting plasma insulin, independent of BMI, physical activity, or blood pressure, while there was no multivariable association between fasting glucose and baroreflex sensitivity [27]. In our study, using a variety of parameters measuring HRV, we found, in particular during deep-breathing and recovery, a relation between HRV and glycaemic status; known diabetic patients had the lowest HRV. For the SDNN/NN HRV index, which is effectively HR adjusted, there was a large difference between the known diabetic patients and the normoglycaemic during deep-breathing. HRV during the five test periods correlated negatively with current capillary blood glucose, BMI, insulin, HOMA-IR and triglycerides, and was lower in those with metabolic syndrome; most associations were greatly attenuated after adjustment on BMI, which strongly suggests that over-weight may be the determinant of HRV and that weight should be included in models when evaluating HRV parameters.

Strength and limitations

In this study glycaemic status was well defined during the previous nine years, with a three-yearly fasting plasma glucose measures. Further HR and HRV were evaluated during five test periods and results were often concordant. A limitation of this study is that there was no oral glucose tolerance test - it appears that autonomic function is altered in impaired glucose tolerance but not in isolated impaired fasting glucose [28]. In our study, some of those with IFG would also have impaired glucose tolerance. Even the known diabetic patients in our study were well controlled, whereas HRV abnormalities are usually explored in poorly controlled patients.

Clinical implications

Our results are clinically relevant: elevated HR cardiovascular morbidity and mortality [4], in particular in hypertensive patients [29], in diabetic patients [1,2] and in patients with coronary artery disease [30]; reduced HRV is predictive of mortality in diabetic patients [5,10]; the HR profile during exercise and recovery is associated with sudden death [8,23]. This study extends the clinical utility of HRV in overweight and obese people and in individuals with fasting hyperglycemia.

In conclusion glycaemic status and glucose are associated with HR but not with HRV, while over-weight seems to be associated with HRV. After deep-breathing, HR recovery is altered in known diabetic patients, with a trend across glucose groups. HR recovery is associated with reduced HRV in periods before and after deep-breathing. Alterations of the vago-sympathetic balance responsible for abnormal HRV are involved in abnormal HR recovery after a minor effort such as forced breathing. The value of this simple forced deep-breathing test warrants further evaluation in diabetic as well as in pre-diabetic patients.

Competing Interests Nothing to declare

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References

1. Carnethon MR, Yan L, Greenland P, Garside DB, Dyer AR, Metzger B *et al.* Resting heart rate in middle age and diabetes development in older age. *Diabetes Care* 2008; **31**: 335-339.
2. Stettler C, Bearth A, Allemann S, Zwahlen M, Zanchin L, Deplazes M *et al.* QTc interval and resting heart rate as long-term predictors of mortality in type 1 and type 2 diabetes mellitus: a 23-year follow-up. *Diabetologia* 2007; **50**: 186-194.
3. Stein PK, Barzilay JI, Domitrovich PP, Chaves PM, Gottdiener JS, Heckbert SR *et al.* The relationship of heart rate and heart rate variability to non-diabetic fasting glucose levels and the metabolic syndrome: the Cardiovascular Health Study. *Diabet Med* 2007; **24**: 855-863.
4. Palatini P. Elevated heart rate as a predictor of increased cardiovascular morbidity. *J Hypertens Suppl* 1999; **17**: S3-S10.
5. Ziegler D, Zentai CP, Perz S, Rathmann W, Haastert B, Döring A *et al.*; KORA Study Group. Prediction of mortality using measures of cardiac autonomic dysfunction in the diabetic and nondiabetic population: the MONICA/KORA Augsburg Cohort Study. *Diabetes Care* 2008; **31**: 556-561.
6. Panzer C, Lauer MS, Brieke A, Blackstone E, Hoogwerf B. Association of fasting plasma glucose with heart rate recovery in healthy adults: a population-based study. *Diabetes* 2002; **51**: 803-807.
7. Festa A, D'Agostino R Jr, Hales CN, Mykkanen L, Haffner SM. Heart rate in relation to insulin sensitivity and insulin secretion in nondiabetic subjects. *Diabetes Care* 2000; **23**: 624-628. 339 2001
8. Cole CR, Foody JM, Blackstone EH, Lauer MS. Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 2000; **132**: 552-555.
9. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care* 2003; **26**: 1553-1579.
10. Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 2003; **26**: 1895-1901.
11. Valensi P, Sachs RN, Harfouche B, Lormeau B, Paries J, Cosson E *et al.* Predictive value of cardiac autonomic neuropathy in diabetic patients with or without silent myocardial ischemia. *Diabetes Care* 2001; **24**: 339-343.
12. Ayad F, Belhad M, Paries J, Attali JR, Valensi P. Association between cardiac autonomic neuropathy and hypertension and its potential influence on diabetic complications. *Diab Med* 2010 in press
13. Valensi PE, Johnson NB, Maison-Blanche P, Extramania F, Motte G, Coumel P. Influence of cardiac autonomic neuropathy on heart rate dependence of ventricular repolarization in diabetic patients. *Diabetes Care* 2002; **25**: 918-923.

14. Valensi P, Pariès J, Attali JR; French Group for Research and Study of Diabetic Neuropathy. Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and microangiopathic complications-the French multicenter study. *Metabolism* 2003; **52**: 815-820.
15. Gerritsen J, Dekker JM, Ten Voorde BJ, Bertelsmann FW, Kostense PJ, Stehouwer CD *et al*. Glucose tolerance and other determinants of cardiovascular autonomic function: the Hoorn Study. *Diabetologia* 2000; **43**: 561-570.
16. Balkau B, Eschwege E, Tichet J, Marre M. Proposed criteria for the diagnosis of diabetes: evidence from a French epidemiological study (D.E.S.I.R.). *Diabetes Metab* 1997; **23**: 428-434.
17. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA *et al*; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752.
18. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996; **93**: 1043-1065.
19. Schönauer M, Thomas A, Morbach S, Niebauer J, Schönauer U, Thiele H. Cardiac autonomic diabetic neuropathy. *Diab Vasc Dis Res* 2008; **5**: 336-344.
20. Deibert DC, DeFronzo RA. Epinephrine-induced insulin resistance in man. *J Clin Invest* 1980; **65**: 717-721.
21. Van De Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson EA. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. *Am J Physiol* 1999; **276**: R178-R183.
22. Laitinen T, Vauhkonen IK, Niskanen LK, Hartikainen JE, Länsimies EA, Uusitupa MI *et al*. Power spectral analysis of heart rate variability during hyperinsulinemia in nondiabetic offspring of type 2 diabetic patients: evidence for possible early autonomic dysfunction in insulin-resistant subjects. *Diabetes* 1999; **48**: 1295-1299.
23. Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, Ducimetière P. Heart-rate profile during exercise as a predictor of sudden death. *N Engl J Med* 2005; **352**: 1951-1958.
24. Valensi P, Nguyen TN, Idriss S, Cazes P, Karam G, Pariès J *et al*. Influence of parasympathetic dysfunction and hyperinsulinemia on the hemodynamic response to an isometric exercise in noninsulin dependent diabetic patients. *Metabolism* 1998; **47**: 934- 939.
25. Valensi P, Thi BN, Lormeau B, Pariès J, Attali JR. Cardiac autonomic function in the obese patients. *Int J Obes* 1995; **19**: 113-118.
26. Quilliot D, Böhme P, Zannad F, Ziegler O. Sympathetic-leptin relationship in obesity: effect of weight loss. *Metabolism* 2008; **57**: 555-562.

27. Watkins L, Surwit R, Grossman P, Sherwood A. Is there a glycemic threshold for impaired autonomic control? *Diabetes Care* 2000; **23**: 826-830.
28. Wu JS, Yang YC, Lin TS, Huang YH, Chen JJ, Lu FH *et al*. Epidemiological evidence of altered cardiac autonomic function in subjects with impaired glucose tolerance but not isolated impaired fasting glucose. *J Clin Endocrinol Metab* 2007; **92**: 3885-3889.
29. Gillman MW, Kannel WB, Belanger A, D'Agostino RB. Influence of heart rate on mortality among persons with hypertension: the Framingham Study. *Am Heart J* 1993; **125**: 1148-1154.
30. Diaz A, Bourassa MG, Guertin MC, Tardif JC. Long-term prognostic value of resting heart rate in patients with suspected or proven coronary artery disease. *Eur Heart J* 2005; **26**: 967-974.

Figure Legends

FIGURE 1 Heart rate (beats/minute) according to glucose status across the five test periods; *P*-values for age and sex-adjusted ANOVA and for age and sex-adjusted trend test across the four glucose classes at the various test periods.

FIGURE 2 Change in Heart rate (HR) (beats/minute), with SE bars shown, by glycaemic status, between **A** rest period and deep breathing period, **B** deep breathing period and recovery period; ANOVA and trend test comparisons.

FIGURE 3 A - F. Various measures of heart rate variability (HRV) according to glucose status across the five test periods; age and sex-adjusted ANCOVA comparison across the 4 glucose classes: * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001; and between normal and (IFG+detected diabetes): + *P* < 0.05, ++ *P* < 0.01, +++ *P* < 0.001

Table 1 Characteristics (means (standard deviation) or number (percentage)) of participants according to glucose status at examination. The D.E.S.I.R.

Study

	known diabetic n=70	detected diabetic n=55	intermediate n=157	always normal n=165	ANOVA <i>P</i> -value*	<i>P</i> trend*
Measures at the examination						
Age (years)	61 (9)	57 (9)	60 (8)	60 (8)		
Men (%)	48 (69%)	46 (84%)	124 (79%)	127 (77%)		
HbA1c (%)	6.7 (1.0)	5.7 (0.5)	5.4 (0.4)	5.2 (0.3)	0.0001	0.0001
Capillary glycaemia (mmol/l) **	8.80 (3.71)	6.37 (1.66)	5.83 (1.16)	5.38 (0.85)	0.0001	0.0001
Diabetes duration (years)	6.4 (0-35)					
BMI (kg/m ²)	28.7 (4.5)	26.8 (3.5)	26.9 (3.6)	25.2 (3.4)	0.0001	0.0001
Supine SBP (mmHg)	142 (20)	138 (16)	139 (17)	134 (18)	0.008	0.002
Supine DBP (mmHg)	82 (9)	83 (9)	81 (9)	80 (9)	0.3	0.07
Supine Pulse Pressure (mmHg)**	60 (16)	55 (12)	58 (14)	54 (13)	0.007	0.004
Lying-to-standing SBP (mmHg)	141 (19)	137 (16)	138 (18)	133 (18)	0.006	0.001
Lying-to-standing DBP (mmHg)	84 (9)	86 (9)	84 (10)	82 (9)	0.04	0.02
Alcohol (g/day)	16 (15)	21 (26)	20 (16)	17 (17)	0.2	0.7
Smoker (%)	11 (17%)	10 (20%)	16 (11%)	15 (9%)	0.2	0.04

DURING 5 MINUTES AT REST, heart rate and measures of heart rate variability

Heart rate (beats/minute)	71 (12)	68 (10)	69 (11)	67 (10)	0.07	0.01
SDNN (ms)**	30.1 (15.6)	35.6 (17.0)	33.4 (15.8)	35.5 (17.8)	0.07	0.02
RMSSD (ms)**	23.9 (16.2)	22.1 (8.2)	21.9 (13.6)	24.8 (17.3)	0.3	0.4
PNN50 (%)**	7.22 (11.36)	3.69 (4.09)	4.80 (8.58)	6.14 (10.22)	0.1	0.8
Max RR / Min RR***	1.24 (0.12)	1.27 (0.11)	1.28 (0.14)	1.28 (0.13)	0.1	0.08
Max RR - Min RR**	186 (94)	210 (72)	212 (101)	217 (97)	0.1	0.02
SDNN/NN**	0.034 (0.014)	0.040(0.02)	0.037 (0.016)	0.039 (0.017)	0.2	0.1

Measures at year 9 of the study

Supine systolic blood pressure (mmHg)	146 (21)	140 (17)	144 (19)	139 (19)	0.03	0.01
Supine diastolic blood pressure (mmHg)	84 (10)	84 (9)	84 (11)	82 (10)	0.4	0.2
Hypertension #	50 (75%)	28 (51%)	98 (62%)	80 (49%)	0.003	0.001
Waist circumference (cm) men	99 (11)	95 (9)	94 (9)	89 (9)	0.0001	0.0001
Waist circumference (cm) women	92 (10)	89 (14)	88 (12)	79 (8)	0.0002	0.0001
Fasting plasma glucose (mmol/l) **	8.37 (2.54)	6.6 (1)	5.86 (0.54)	5.12 (0.47)	0.0001	0.0001
Insulin (pmol/l) **	104.2 (115.78)	89.66 (52.02)	75.61 (46.28)	51.94 (26.6)	0.0001	0.0001
HOMA IR **	5.46 (6.29)	3.76 (2.43)	2.78 (1.77)	1.67 (0.9)	0.0001	0.0001
Triglycerides (mmol/l) **	1.65 (1.2)	1.72 (1.24)	1.33 (0.86)	1.14 (0.55)	0.0001	0.0001
HDL cholesterol (mmol/l) **	1.33 (0.32)	1.37 (0.3)	1.42 (0.31)	1.51 (0.32)	0.0001	0.0001
Metabolic syndrome (%)	51 (75)	25 (45)	65 (41)	25 (15)	0.0001	0.0001

* adjusted for age and sex ** log transformation for analysis *** $-1/y^2$ transformation for analysis # Hypertension : systolic /diastolic blood pressure \geq 140/90 mmHg or medication for hypertension

Table 2. Spearman partial correlation coefficients, adjusted for age and sex, between heart rate and cardio-metabolic risk factors among all participants: at rest, deep breathing, recovery, at rest and lying-to-standing The mean heart rate (SD) for those with and without the AHA/NHBLI defined metabolic syndrome is also given.

	at rest	deep breathing	recovery	at rest	lying-to-standing
Age #	0.010	-0.045	-0.031	-0.024	-0.008
Capillary glucose	0.144**	0.110*	0.140**	0.146**	0.159***
HbA1c	0.056	0.023	0.067	0.067	0.057
BMI	0.156***	0.074	0.118*	0.142**	0.092*
Waist circumference	0.128**	0.056	0.102*	0.118**	0.107*
Insulin	0.116**	0.053	0.091	0.100*	0.073
HOMA-IR	0.143**	0.074	0.120**	0.130**	0.099*
Triglycerides	0.211***	0.141**	0.193***	0.209***	0.129**
HDL-cholesterol	-0.032	0.016	-0.015	-0.029	-0.006
Supine systolic BP	0.177***	0.214***	0.171***	0.165***	-0.089
Supine diastolic BP	0.165***	0.191***	0.161***	0.150**	0.092*

Mean (SD) of Heart Rate (ms) †

Metabolic syndrome	70 (11) ++	73 (10) +	73 (11) ++	71 (11) ++	79 (12) +
No Metabolic syndrome	67 (11)	70 (10)	70 (11)	68 (11)	76 (12)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ tests are adjusted for age and sex

† adjusted for age and sex

adjusted for sex

+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.0001$: comparisons between individuals with and without the metabolic syndrome

Table 3. Spearman partial correlation coefficients, adjusted for age and sex between heart rate variability parameter, SDNN/NN and cardio-metabolic risk factors among all participants: at rest, deep breathing, recovery, at rest and lying-to-standing, and the mean SDNN/NN (SD) for those with and without the AHA/NHBLI defined metabolic syndrome.

	at rest	deep breathing	recovery	at rest	lying-to-standing
Age#	-0.296***	-0.426***	-0.322***	-0.216***	-0.310***
Capillary glucose	-0.086	-0.084	-0.103*	0.042	-0.006
HbA1c	-0.014	-0.070	-0.094*	0.030	0.004
BMI	-0.075	-0.145**	-0.224***	-0.040	-0.091*
Waist circumference	-0.059	-0.139**	-0.198***	0.016	-0.082
Insulin	-0.067	-0.085	-0.147**	-0.020	-0.077
HOMA-IR	-0.080	-0.099*	-0.171***	-0.006	-0.075
Triglycerides	-0.078	-0.084	-0.179***	-0.110*	-0.033
HDL-cholesterol	0.035	0.116*	0.101*	-0.026	0.058
Systolic BP	-0.030	-0.039	-0.030	0.020	-0.123**
Diastolic BP	-0.008	-0.045	-0.033	-0.000	-0.130**
Heart rate	-0.176***	-0.046	-0.143**	-0.122**	-0.111*

Mean (SD) SDNN/NN (ms), adjusted on age and sex †

Metabolic syndrome	0.036	0.077	0.050	0.034	0.043
	(0.016)	(0.032)++	(0.019) +	(0.017)	(0.018)
No Metabolic syndrome	0.039	0.087	0.054	0.034	0.044
	(0.016)	(0.032)	(0.019)	(0.016)	(0.018)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ tests are adjusted for age and sex

+ $P < 0.05$, ++ $P < 0.01$: comparisons between individuals with and without metabolic syndrome

FIGURE 1. Heart rate (beats/minute) according to glucose status across the five test periods; *P*-values for age and sex-adjusted ANOVA and for age and sex-adjusted trend tests, across the four glucose classes at the various test periods.

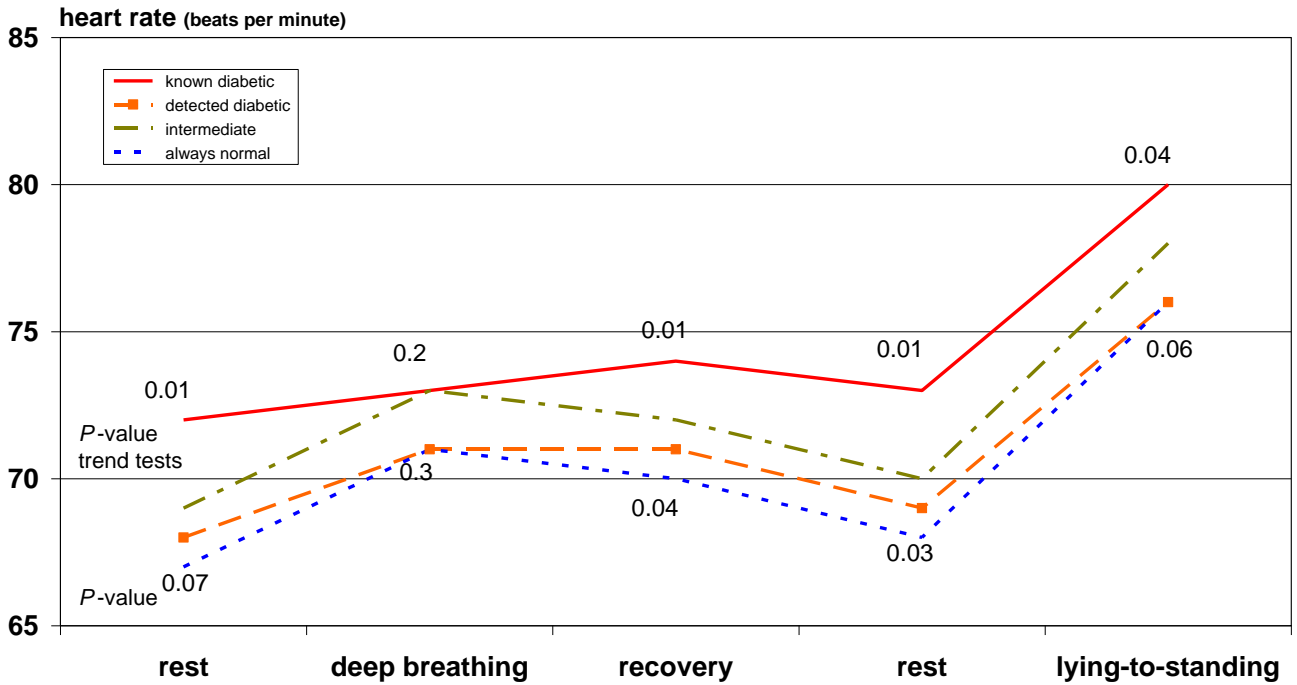


FIGURE 2. Change in heart rate (HR) (beats/minute), with SE bars shown, by glycaemic status, between **A** rest period and deep breathing period, **B** deep breathing period and recovery period; ANOVA and trend test comparisons.

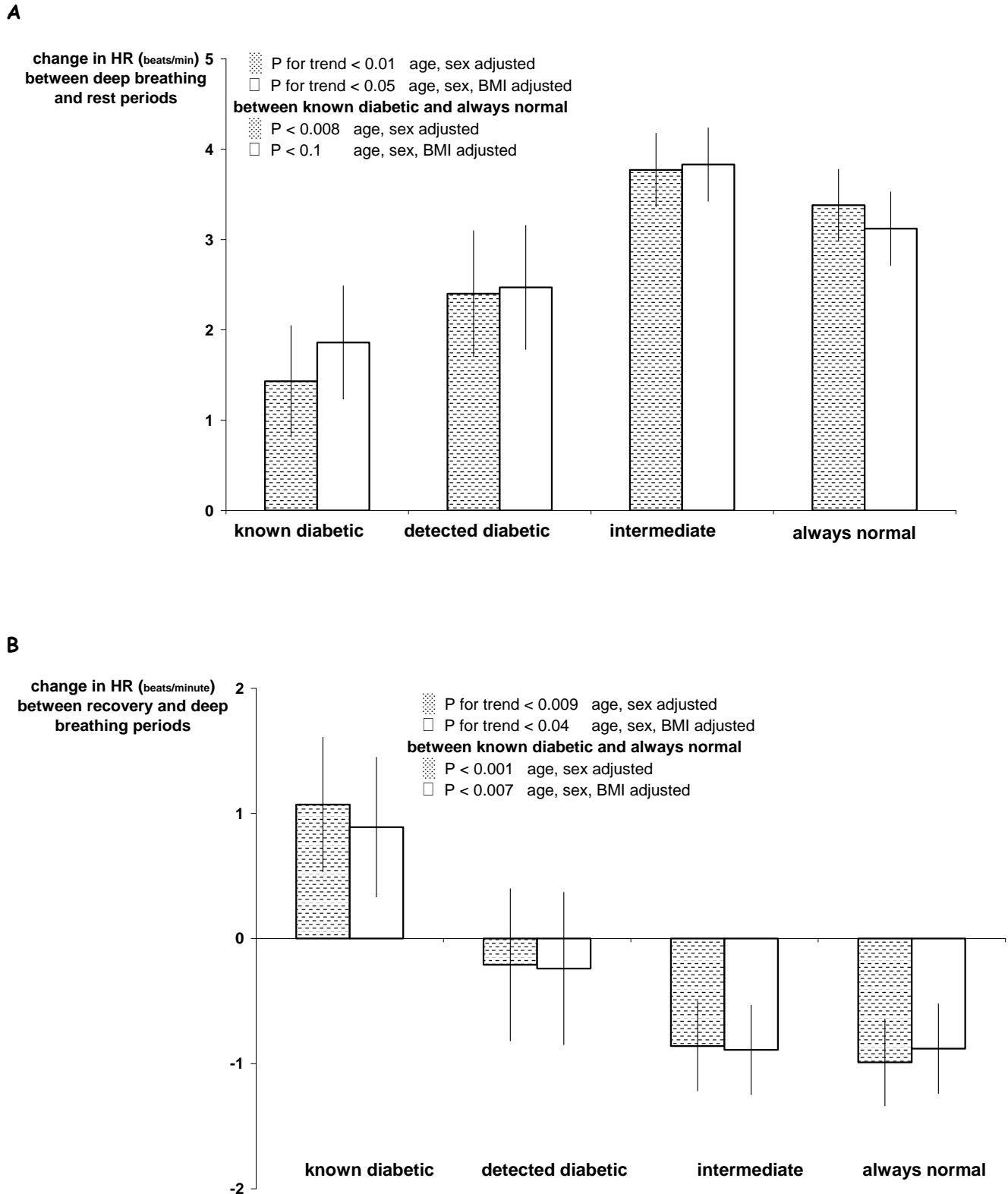
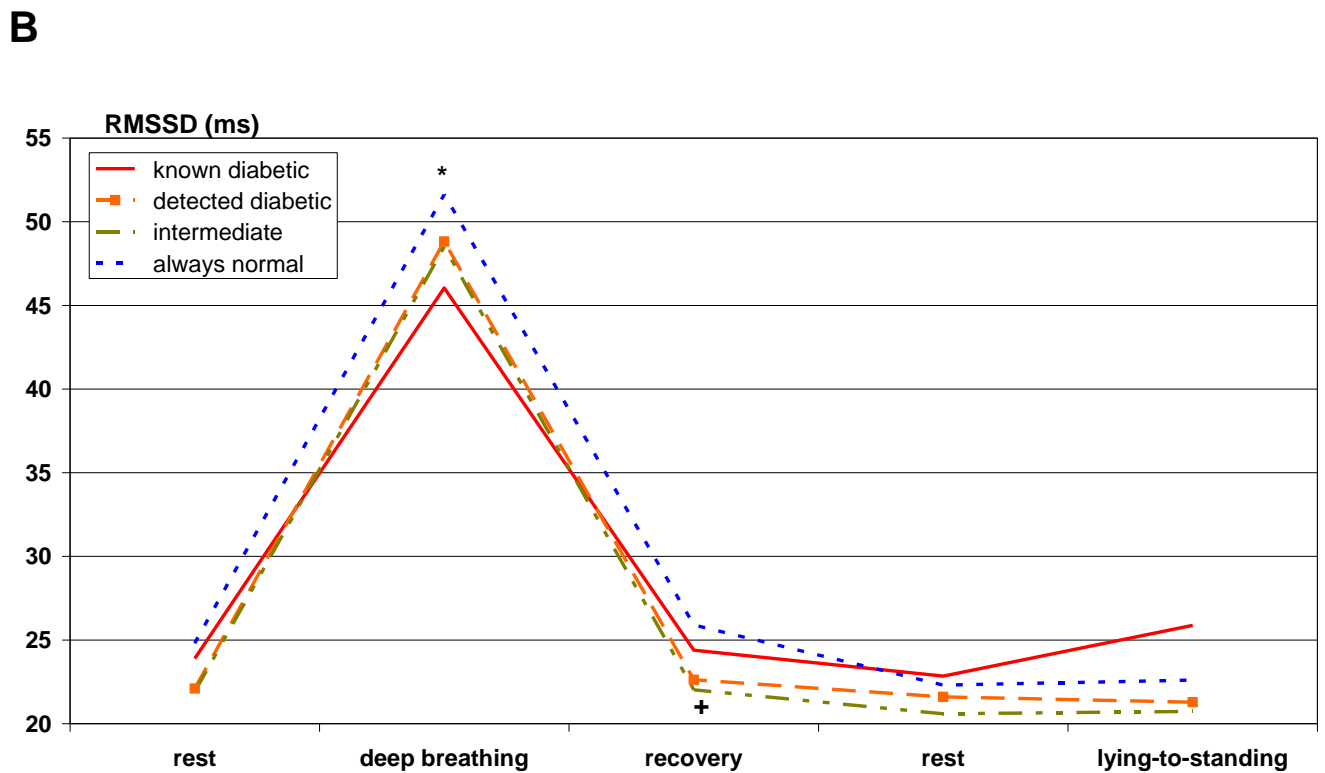
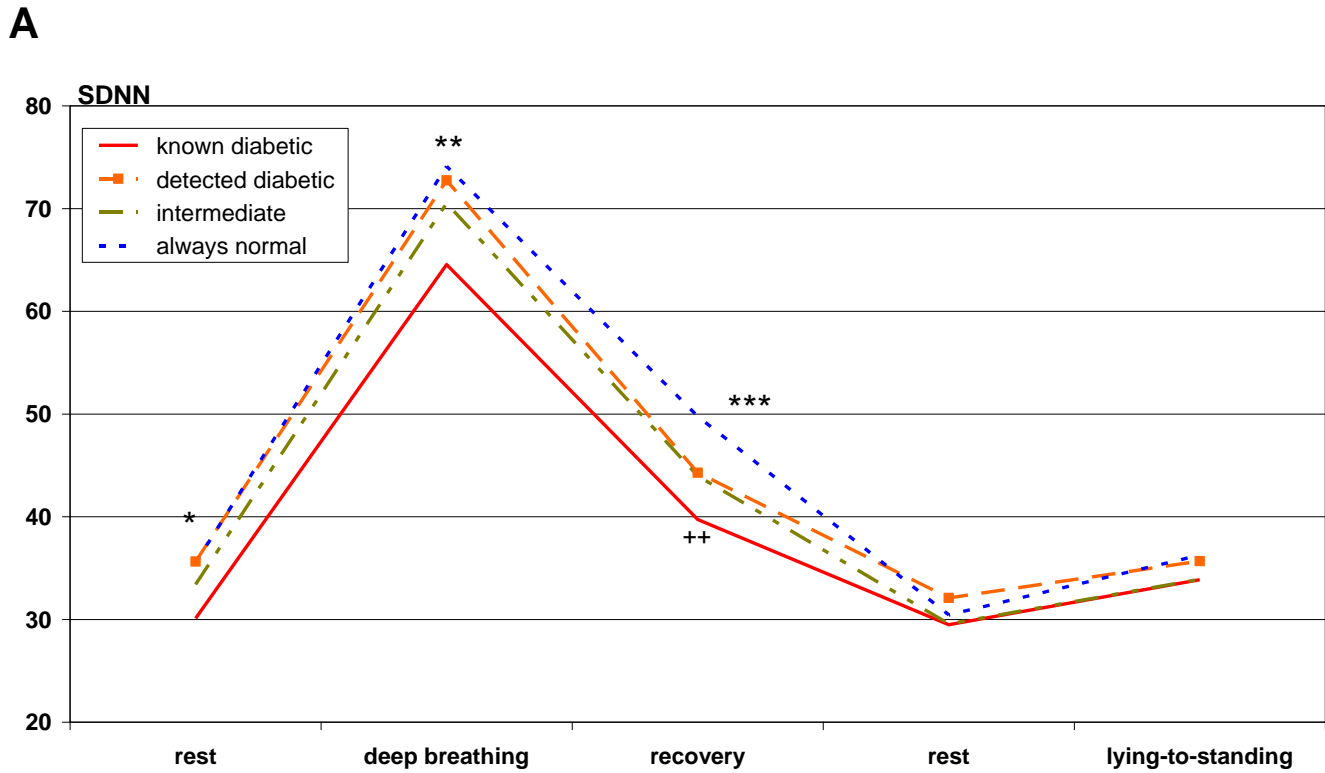
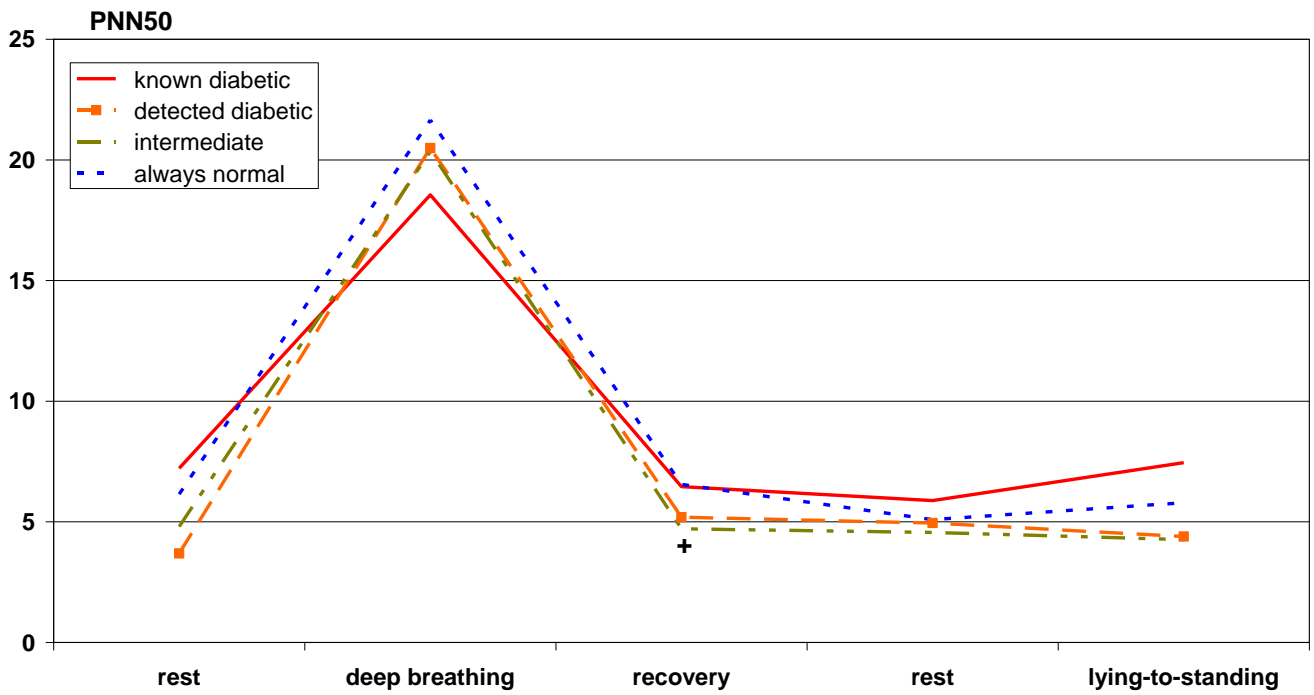


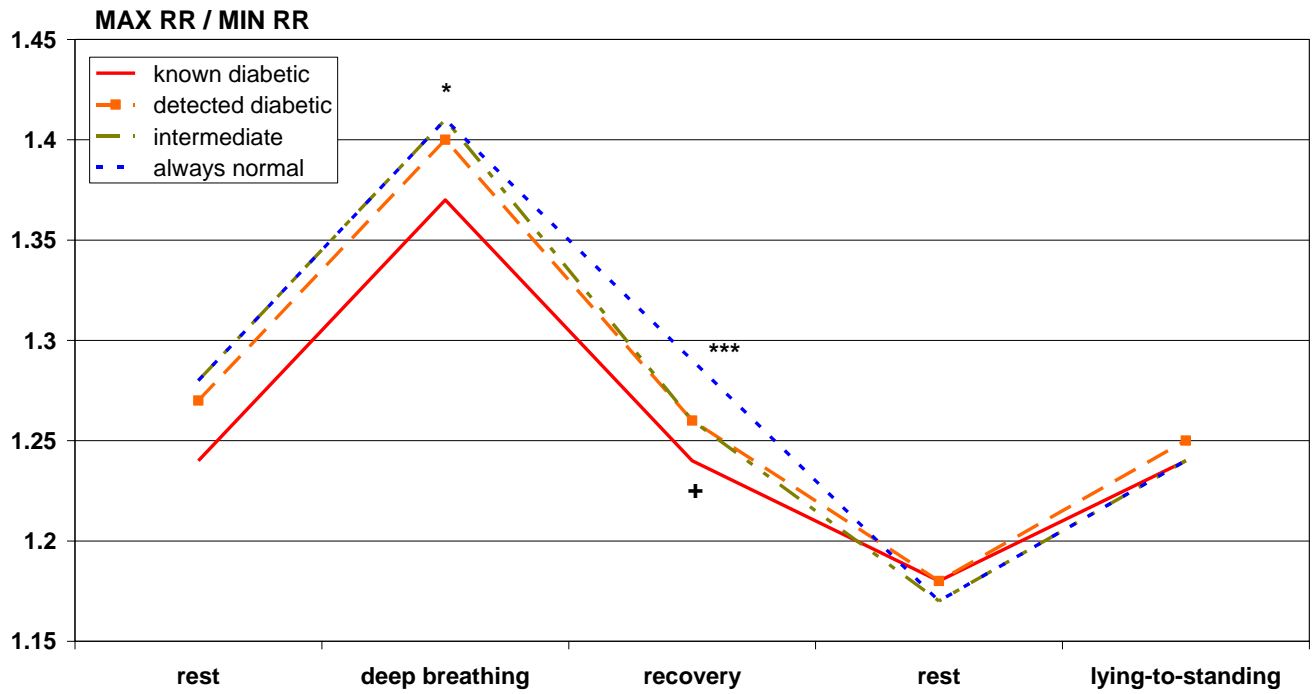
FIGURE 3. Various measures of heart rate variability (HRV) according to glucose status across the five test periods; age and sex-adjusted ANCOVA comparison across the 4 glucose classes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; between normal and (IFG+detected diabetes): + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$



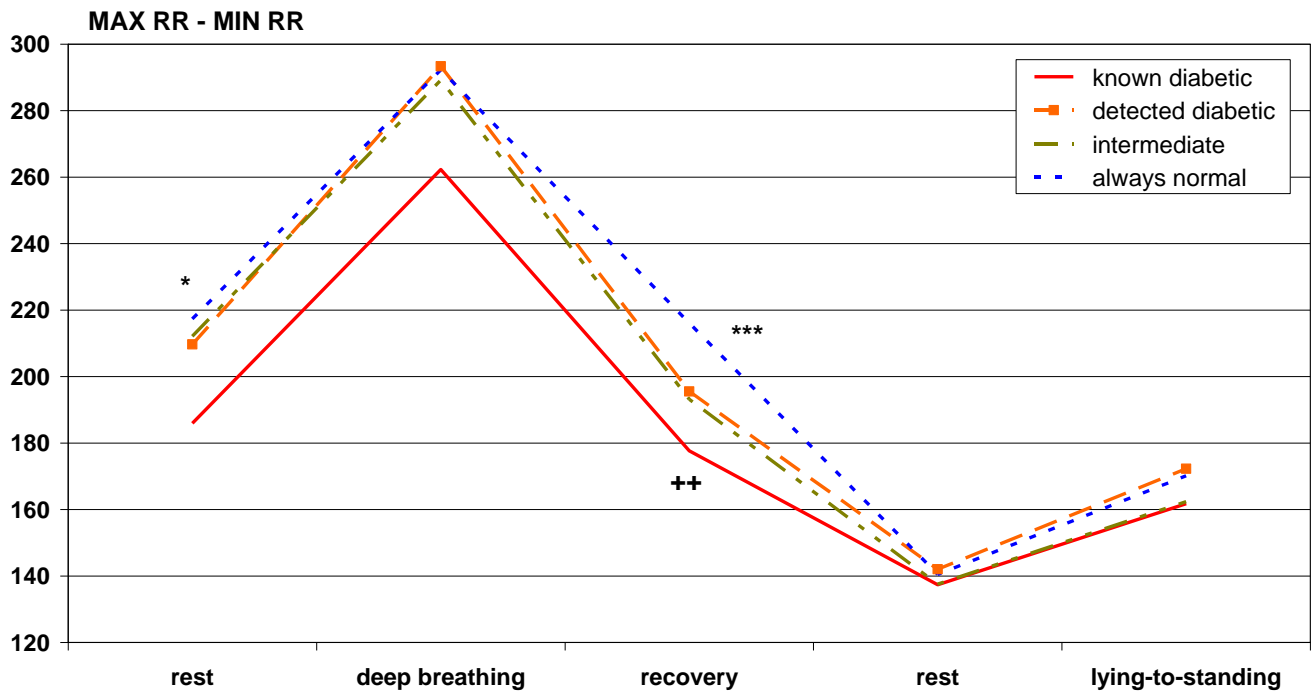
C



D



E



F

