

# Cardiac autonomic nervous dysfunction detected by both heart rate variability and heart rate turbulence in prediabetic patients with isolated impaired fasting glucose

Akif Serhat Balcioğlu, Sinan Akıncı, Davran Çiçek, Ali Çoner, Uğur Abbas Bal<sup>1</sup>, İbrahim Haldun Müderrisoğlu<sup>1</sup>

Department of Cardiology, Medical and Research Center of Alanya, Başkent University, Alanya, Antalya-Turkey

<sup>1</sup>Department of Cardiology, Faculty of Medicine, Başkent University, Ankara-Turkey

## ABSTRACT

**Objective:** Cardiac autonomic nervous dysfunction (CAND), a severe complication of diabetes, has also been shown to affect prediabetic patients. The role of isolated impaired fasting plasma glucose (IFG), a subtype of prediabetes, is not clear in the pathogenesis of CAND. The aim of this study was to examine the relationship between isolated IFG and cardiac autonomic function using heart rate variability (HRV) and heart rate turbulence (HRT) indices derived from 24-h Holter–electrocardiogram recordings.

**Methods:** This observational, prospective, cross-sectional study examined 400 consecutive subjects divided into three groups according to oral glucose tolerance test results: the control group [Group I, fasting plasma glucose (FPG) <100 mg/dL and normal glucose tolerance, n=193], the isolated IFG group (Group II, FPG ≥100 and <126 mg/dL, n=134), and the isolated impaired glucose tolerance (IGT), both IFG and IGT, or newly diagnosed diabetes' group (Group III, n=73). Patients with non-sinus rhythm, known diabetes mellitus, coronary artery disease, heart failure, severe valvular disease, or receiving medical therapy that may affect HRV and HRT indices were excluded. Time domain HRV parameters, turbulence onset (TO), turbulence slope (TS), and HRT category were examined. Chi-square, one-way analysis of variance, Kruskal–Wallis H, and Mann–Whitney U tests were used to compare variables where appropriate. The correlation between Holter data and FPG levels was analyzed using the Spearman's test. Multiple linear regression analysis was performed to identify independent predictors of the HRV and HRT parameters.

**Results:** Median (interquartile range 25–75) FPG levels in Groups I, II, and III were 89 (83/93) mg/dL, 109 (104/116) mg/dL, and 174 (150.5/197) mg/dL, respectively. There were significant differences in HRV and HRT parameters between and among all groups. While HRV parameters and TS decreased from Group I to Group III, TO and HRT category gradually increased. Additionally, FPG level was significantly correlated with SDNN,  $r=-0.220$ ; SDNN index,  $r=-0.192$ ; SDANN,  $r=-0.207$ ; RMSSD,  $r=-0.228$ ; pNN50,  $r=-0.226$ ; TO,  $r=0.354$ ; and TS,  $r=-0.331$  (all  $p<0.001$ ).

**Conclusion:** CAND, as detected by both HRV and HRT, appear to be present in the isolated IFG subtype of prediabetes.

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**Keywords:** autonomic function, glucose, heart rate variability, prediabetes

## Introduction

Cardiac autonomic nervous dysfunction (CAND) is a frequent chronic complication of diabetes with potentially life-threatening effects such as silent myocardial ischemia and infarction, arrhythmia, sudden death, perioperative cardiovascular instability, orthostatic hypotension, and cardiomyopathy (1–3). CAND is caused by the impairment of the autonomic nerve fibers regulating heart rate, myocardial contractility, cardiac electrophysiology, and blood vessel constriction and dilatation (2). Hyperglycemia is the leading cause of this pathogenic process (4, 5). Because the clinical signs associated with CAND do not generally occur until late in the disease process and reversal of

cardiovascular denervation is thought to be possible in the early stages of the disease (1, 3), screening tests are useful for the early detection of CAND.

Prediabetes is a continuum between normoglycemia and diabetes mellitus consisting of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and indicates an increased risk for cardiovascular diseases and the future development of type 2 diabetes mellitus (T2DM) (6). While patients with IFG and IGT both suffer from insulin resistance and deficiencies in insulin secretion, the two conditions show some differences such as those in associated cardiovascular risk (7, 8). There is a strong correlation between cardiovascular events and IGT, whereas this relation is less clear in IFG (8). Studies investigating CAND

**Address for correspondence:** Dr. Akif Serhat Balcioğlu, Başkent Üniversitesi Alanya Uygulama ve Araştırma Merkezi, Kardiyoloji Anabilim Dalı, Saray Mh., Yunus Emre Cd. No: 1, 07400, Alanya, Antalya-Türkiye  
Phone: +90 242 510 25 25 Mobile: +90 532 628 46 97 Fax: +90 242 511 55 63 E-mail: serhatbalcioglu@gmail.com

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and prediabetes show conflicting results, particularly in terms of the relation between CAND and isolated IFG. It has been reported that altered cardiac autonomic function is present in both IGT and diabetic subjects but not in patients with IFG (9). However, in the KORA S4 survey, the prevalence of CAND increased not only in individuals with T2DM and IGT but also in a lesser degree in those with isolated IFG (10).

Heart rate variability (HRV) and heart rate turbulence (HRT) are dependable Holter–electrocardiogram (ECG) parameters that indicate cardiac autonomic function (11, 12). The most commonly used methods for the diagnosis of CAND are based on HRV assessment (the physiological variation in the time interval between heartbeats) and enable the independent measurement of the parasympathetic and sympathetic components of the autonomic nervous system (11). Another technique for evaluating CAND is the HRT, a reliable index of baroreceptor sensitivity, referring to variations in the sinus rhythm cycle length following isolated premature ventricular beats (PVBs) (12). A decrease in HRV is the first finding of CAND (13). Similarly, HRT has been found to be disturbed in patients with CAND (14).

This is the first study in the literature that aims to examine whether there is a relation between isolated IFG and cardiac autonomic function using both HRV and HRT parameters.

## Methods

This observational, prospective, cross-sectional study examined 400 consecutive subjects who underwent an oral glucose tolerance test (OGTT) in the endocrinology outpatient department and were referred to the cardiology outpatient department for Holter–ECG assessments. The exclusion criteria were as follows: non-sinus rhythm, known T2DM, the use of any antidiabetic medication, history of coronary artery disease defined as a stenosis of more than 50% in at least one epicardial coronary artery in a past coronary angiography, acute coronary syndrome or a previous myocardial infarction, typical stable angina pectoris, cardiomyopathies, heart failure (left ventricular ejection fraction <50%), severe valvular disease, hyperthyroidism, hypothyroidism, ventricular tachycardia on Holter–ECG, and use of medicine, including beta blockers, non-dihydropyridine calcium channel blockers, or antiarrhythmic drugs that may affect HRV and HRT indices.

The study was approved by the Local Ethics Committee and was performed in accordance with the Helsinki Declaration. All subjects gave informed consent prior to enrollment.

A total of 481 individuals underwent a 24-h Holter recording to obtain HRV and HRT parameters between January 2013 and June 2014. Eighty-one subjects were excluded because of the absence of PVBs needed for HRT analysis on the Holter recording. A total of 400 subjects were enrolled in the study.

The study population was divided into three groups according to the results of the 75-g OGTT using American Diabetes Association criteria (6). The control group (Group I) included 193 subjects with a fasting plasma glucose (FPG) of less than 100

mg/dL and normal glucose tolerance (a 2-h OGTT glucose level below 140 mg/dL). The isolated IFG group (Group II) included 134 patients with an FPG of 100–125 mg/dL and a 2-h OGTT glucose level below 140 mg/dL. Group III included 73 patients with either isolated IGT (FPG level below 100 mg/dL and a 2-h OGTT glucose level between 140 and 200 mg/dL), both IFG and IGT (FPG level between 100 and 125 mg/dL and a 2-h OGTT glucose level between 140 and 200 mg/dL), or newly diagnosed T2DM (a 2-h OGTT glucose level above 200 mg/dL).

Holter–ECG recordings were acquired using three-channel digital recorders (Cardioscan Premier Version 12, DM Systems Co., Ltd. Beijing, China). Recordings lasting more than 20 h and of sufficient quality for evaluation were analyzed. A physician completely blind to the study assessed the Holter–ECG records. Before analysis, data were manually reviewed to check all complexes marked as true PVBs. HRV parameters included the standard deviation of the normal-to-normal (NN) interval (SDNN), the standard deviation of the average NN interval (SDANN) calculated over 5-min periods, the mean of the 5-min standard deviation of the NN interval (SDNN index) calculated over 24 h, the square root of the mean squared differences of successive NN intervals (RMSSD), and the division of the number of interval differences of successive NN intervals of more than 50 ms by the total number of NN intervals (pNN50). All analysis were made according to the standards determined by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (11).

HRT is the fluctuation in the sinus rhythm cycle length following isolated PVBs. After an initial acceleration, the sinus rate decelerates after a PVB. There are two components of HRT: turbulence onset (TO) and turbulence slope (TS). A transient vagal inhibition triggers the mentioned initial acceleration in the heart rate as a reaction to the missed baroreflex afferent input due to hemodynamically ineffective ventricular contraction. The successive deceleration in heart rate is caused by a sympathetically mediated overshoot of blood pressure through vagal recruitment (15). After manual review of the Holter–ECG recordings, TO and TS were calculated as stated by Bauer et al. (12). A negative value of TO signifies early sinus acceleration and is considered normal, while a TS value over 2.5 ms/R-R interval indicates normal expected late deceleration (12). HRT values are generally classified into three categories: HRT category 0 indicating normal TO and TS, HRT category 1 indicating an abnormal TO or TS, and HRT category 2 indicating abnormal TO and TS (12).

## Statistics

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). The Kolmogorov–Smirnov test was used to determine whether continuous variable distributions were normal. Categorical variables were presented as the number of cases plus percentage and continuous variables as mean±standard deviation (SD) or median and 25/75% interquartile ranges, where applicable. The chi-square test was used for

**Table 1. Baseline characteristics and echocardiographic and laboratory findings**

Variables	Group I (n=193)	Group II (n=134)	Group III (n=73)	P
Age, years	54 (42/68)	58 (48/71)	56 (50.5/63)	0.126
Gender, male/female, numbers (%)	84/109 (43.5/56.5)	55/79 (41/59)	32/41 (43.8/56.2)	0.886
Current smoking, numbers (%)	46 (23.8)	31 (23.1)	22 (30.1)	0.494
Hypertension, numbers (%)	57 (29.5)	47 (35.1)	29 (39.7)	0.249
Body mass index, kg/m <sup>2</sup>	28.4 (26.1/31.6)	28.7 (25.7/32.1)	29 (25.4/31.6)	0.912
FPG, mg/dL	89 (83/93)	109 (104/116)	174 (150.5/197)	<0.001
HbA1c*, %	5.1±0.3	5.9±0.2	6.9±0.9	<0.001
Total cholesterol, mg/dL	189.1 (159.5/226.4)	194.6 (163.6/221)	200.7 (174/228.3)	0.251
HDL cholesterol, mg/dL	46 (39/54)	45.5 (39.3/52)	44 (38.5/53.5)	0.642
LDL cholesterol, mg/dL	114 (92/146)	120 (96/146)	126 (101/149)	0.263
Triglycerides, mg/dL	116 (90/158)	122.5 (88/178)	134 (95.5/187.5)	0.159
Systolic blood pressure, mm Hg	120 (112.5/132.5)	120 (110/140)	120 (110/130)	0.432
Diastolic blood pressure, mm Hg	80 (70/80)	75 (70/80)	80 (70/80)	0.502
Mean heart rate*, beats/min	75.2±10.8	75.4±10.9	73.8±10.1	0.545
Left ventricular diastolic diameter, mm	46 (42/48)	46 (40/49)	46 (43/49)	0.444
Left ventricular systolic diameter, mm	29 (26/32)	29 (26/33)	29 (27/32)	0.658
Interventricular septum thickness, mm	11 (10/12)	11 (10/12)	11 (10/12)	0.961
Posterior wall thickness, mm	10 (9/12)	10 (9/11)	10 (9/11.8)	0.878
Ejection fraction, %	65 (60/67)	65 (60/67)	64 (59/67)	0.175
Left atrial diameter, mm	35 (31/38)	35 (31/38)	34 (32/38)	0.738

Group I - subjects with FPG <100 mg/dL and normal glucose tolerance test; Group II - patients with isolated IFG; Group III - patients with isolated IGT/both IFG and IGT/newly diagnosed T2DM; FPG - fasting plasma glucose; IFG - impaired fasting glucose; IGT - impaired glucose tolerance. Data presented as median (25/75% interquartile range). \*Data presented as mean±standard deviation. Kruskal–Wallis or one-way ANOVA test was used for continuous variables. Chi-square test was performed for the comparison of categorical variables

inter-group comparisons. Continuous variables were compared between Groups I, II, and III using the one-way analysis of variance (ANOVA) or Kruskal–Wallis H tests as appropriate. The Mann–Whitney U test was used to compare continuous variables in terms of hypertension, gender, and smoking status. The correlation between Holter findings and FPG was analyzed using the Spearman’s test. Multiple linear regression analysis was used to determine the predictor(s) with the greatest effect on the HRV and HRT parameters after adjustment for all possible confounding factors. Variables with a p value of <0.10 in the univariable test as well as all variables of known clinical importance were accepted as a candidate for the multivariable model. Standardized coefficient of regression and levels of significance for each independent variable were also calculated. Logarithmic transformation was used for SDNN, SDNN index, SDANN, RMS-SD, pNN50, TO, and TS in regression analysis as data were not normally distributed. All tests of significance were two-tailed. Statistical significance was defined as p<0.05.

## Results

Baseline characteristics and clinical, laboratory, and echocardiographic findings are presented in Table 1. Although the ratio of patients with hypertension was higher in Groups II and

III, this difference was not significant. As expected, median FPG levels were significantly higher in Groups II and III than in the control group and higher in Group III than in Group II (p<0.001, p<0.001, and p<0.001, respectively).

Holter data are given in Table 2. The duration of Holter–ECG recordings and mean R-R intervals were similar in all groups. HRV and HRT parameters were significantly different between Groups I and II (SDNN: p=0.013, SDNN index: p=0.018, SDANN: p=0.032, RMSSD: p=0.012, pNN50: p=0.005, TO: p<0.001, TS: p<0.001); Groups I and III (SDNN: p<0.001, SDNN index: p<0.001, SDANN: p<0.001, RMSSD: p<0.001, pNN50: p<0.001, TO: p<0.001, TS: p<0.001); Groups II and III (SDNN: p=0.017, SDNN index: p=0.032, SDANN: p=0.010, RMSSD: p=0.005, pNN50: p=0.018, TO: p<0.001, TS: p=0.001); and among all groups (Table 2). SDNN, SDNN index, SDANN, RMSSD, pNN50, and TS gradually decreased from Group I to Group III, while TO and HRT category increased (Table 2). HRV and HRT measurements according to gender, hypertension, and smoking status are presented in Table 3. SDNN (p<0.001), SDNN index (p=0.001), SDANN (p<0.001), TO (p=0.005), and TS (p<0.001) were significantly different in patients with hypertension.

Correlation analyses revealed that FPG was significantly associated with SDNN (r=–0.220, p<0.001), SDNN index (r=–0.192, p<0.001), SDANN (r=–0.207, p<0.001), RMSSD (r=–0.228, p<0.001),

**Table 2. Comparison of 24-h Holter electrocardiographic findings, heart rate variability, and heart rate turbulence parameters**

Variables	Group I	Group II	Group III	P
Recording time, h	23.1 (22.5/23.6)	23.3 (22.6/23.7)	23.1 (22.6/23.5)	0.128
Mean RR interval, ms	797.9 (722.6/908.6)	799.6 (731.3/872.3)	779.1 (728.4/874.1)	0.649
<b>Heart rate variability parameters</b>				
SDNN, ms	125 (100.5/153)	117 (94.5/134.5)	102 (84/119)	<0.001
SDNN index, ms	50 (41/62)	44 (36/58)	42 (33/51)	<0.001
SDANN, ms	113 (89.5/137.5)	102.5 (83.8/123.5)	90 (72/109.5)	<0.001
RMSSD, ms	29 (21/37)	24 (19/33)	22 (17/25)	<0.001
pNN50, %	7 (2/12.5)	3.5 (1.8/9)	2 (1.1/4)	<0.001
<b>Heart rate turbulence parameters</b>				
Turbulence onset, %	-1.879 (-3.843/-0.46)	-0.610 (-2.033/0)	-0.003 (-0.008/0.003)	<0.001
Turbulence slope, ms/RR	7.8 (3.4/13)	4.3 (2.5/8.5)	3 (2.1/4.4)	<0.001
<b>Heart rate turbulence category</b>				
0, numbers (%)	141 (73.1)	81 (60.4)	39 (53.4)	<b>0.001</b>
1, numbers (%)	42 (21.8)	41 (30.6)	20 (27.4)	
2, numbers (%)	10 (5.2)	12 (9)	14 (19.2)	
Group I - subjects with FPG <100 mg/dL and normal glucose tolerance test; Group II - patients with isolated IFG; Group III - patients with isolated IGT/both IFG and IGT/newly diagnosed T2DM; IGT - impaired glucose tolerance; IFG - impaired fasting glucose; FPG - fasting plasma glucose; pNN50 - the proportion of adjacent RR intervals differing by >50 ms in the 24-h recording; RMSSD - the square root of the mean squared differences of successive normal-to-normal intervals; SDANN - the standard deviation of the average normal-to-normal intervals calculated over the 5-min period of the entire recording; SDNN - the standard deviation of all normal-to-normal intervals; SDNN index - the mean of the deviation of the 5-min normal-to-normal intervals over the entire recording. Data presented as median (25/75% interquartile range). The Kruskal-Wallis and chi-square tests were used				

**Table 3. Heart rate variability and heart rate turbulence measurements according to gender, hypertension, and smoking status**

Variables	SDNN	SDNN index	SDANN	RMSDD	pNN50	TO	TS
<b>Gender</b>							
Female (n=229)	116 (96/141)	46 (38/56)	106 (84/128)	27 (19.5/34)	5 (2/11)	-0.750 (-2.913/-0.001)	4.8 (2.8/11)
Male (n=171)	118 (90/141)	49 (35/60)	102 (77/127)	24 (18/33)	4 (1.2/9)	-0.867 (-2.637/-0.003)	4.7 (2.4/9.5)
P	0.809	0.464	0.372	0.195	0.164	0.796	0.273
<b>Hypertension</b>							
No (n=267)	120 (98/150)	49 (39/60)	109 (89/132)	27 (19/34)	4.1 (2/11)	-1.045 (-3.310/-0.003)	5.5 (2.9/12.4)
Yes (n=133)	110 (87.5/132)	43 (35/52)	99 (72/119.5)	24 (17.5/33)	4 (1.1/8)	-0.420 (-1.979/0.008)	3.9 (2.1/6.9)
P	<0.001	0.001	<0.001	0.259	0.099	0.005	<0.001
<b>Smoking</b>							
No (n=301)	117 (92/140.5)	46 (37/58)	105 (80.5/128)	27 (19/34)	4 (2/10)	-0.967 (-2.836/-0.001)	4.8 (2.7/10.9)
Yes (n=99)	119 (98/141)	49 (37/60)	103 (89/128)	24 (18/34)	3.8 (1.3/9)	-0.500 (-2.807/-0.002)	4.7 (2.7/10.2)
P	0.543	0.802	0.427	0.492	0.485	0.399	0.879
pNN50 - the proportion of adjacent RR intervals differing by >50 ms in the 24-h recording; rMSSD - the square root of the mean squared differences of successive normal-to-normal intervals; SDANN - the standard deviation of the average normal-to-normal intervals calculated over 5-min period of the entire recording; SDNN - the standard deviation of all normal-to-normal intervals; SDNN index - the mean of the deviation of the 5-min normal-to-normal intervals over the entire recording; TO - turbulence onset; TS - turbulence slope. Data presented as median (25/75% interquartile range). Analyzed using the Mann-Whitney U test							

pNN50 ( $r=-0.226$ ,  $p<0.001$ ), TO ( $r=0.354$ ,  $p<0.001$ ), and TS ( $r=-0.331$ ,  $p<0.001$ ). TO had the strongest power among all HRV and HRT parameters regarding correlation with FPG level (Table 4).

Multiple linear regression analysis was performed to determine the independent predictors of HRV and HRT measures. Age had an independent relation with SDNN, SDNN index, SDANN, TO, and TS. The presence of hypertension was another independent predictor

of SDANN and TS. In addition, FPG level was an independent determinant of all examined HRV and HRT parameters (Table 5).

## Discussion

According to our knowledge, this is the first study in the literature investigating the relation between HRT and prediabe-

**Table 4. Correlation coefficients and significance levels between both heart rate variability and also heart rate turbulence parameters with baseline characteristics, echocardiography, and laboratory measurements**

Variables	SDNN	SDNN index	SDANN	rMSSD	pNN50	TO	TS
<b>Age</b>							
<i>R</i>	<b>-0.268**</b>	<b>-0.300**</b>	<b>-0.267**</b>	-0.079	<b>-0.143**</b>	<b>0.238**</b>	<b>-0.334**</b>
<i>P</i>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.113	<b>0.004</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Body mass index</b>							
<i>R</i>	-0.064	-0.055	-0.047	0.032	0.044	0.079	0.039
<i>P</i>	0.199	0.274	0.353	0.524	0.376	0.114	0.434
<b>FPG</b>							
<i>R</i>	<b>-0.220**</b>	<b>-0.192**</b>	<b>-0.207**</b>	<b>-0.228**</b>	<b>-0.226**</b>	<b>0.354**</b>	<b>-0.331**</b>
<i>P</i>	<b>&lt;0.001</b>						
<b>Systolic BP</b>							
<i>R</i>	-0.078	-0.088	-0.063	-0.021	-0.045	0.084	-0.062
<i>P</i>	0.118	0.077	0.212	0.669	0.368	0.093	0.217
<b>Diastolic BP</b>							
<i>R</i>	-0.086	-0.063	-0.079	-0.034	-0.028	0.030	-0.092
<i>P</i>	0.087	0.211	0.113	0.493	0.580	0.547	0.067
<b>HDL cholesterol</b>							
<i>R</i>	0.095	0.048	0.093	0.027	0.025	-0.059	<b>0.126*</b>
<i>P</i>	0.059	0.346	0.066	0.595	0.624	0.248	<b>0.012</b>
<b>LDL cholesterol</b>							
<i>R</i>	0	-0.035	0.020	-0.078	<b>-0.100*</b>	0.002	0.001
<i>P</i>	0.993	0.483	0.690	0.121	<b>0.046</b>	0.972	0.989
<b>Triglycerides</b>							
<i>R</i>	-0.096	-0.077	-0.077	-0.058	-0.036	-0.027	<b>-0.121*</b>
<i>P</i>	0.057	0.127	0.126	0.253	0.482	0.596	<b>0.017</b>
<b>LVEF</b>							
<i>R</i>	<b>0.112*</b>	0.038	<b>0.133**</b>	0.070	0.080	-0.065	<b>0.135**</b>
<i>P</i>	<b>0.026</b>	0.446	<b>0.008</b>	0.160	0.111	0.194	<b>0.007</b>
<b>IVS thickness</b>							
<i>R</i>	-0.052	-0.011	-0.066	0.014	0.026	0.080	-0.048
<i>P</i>	0.302	0.833	0.189	0.775	0.599	0.111	0.340
<b>PW thickness</b>							
<i>R</i>	-0.071	-0.017	-0.094	0.009	0.027	0.085	-0.088
<i>P</i>	0.156	0.741	0.060	0.850	0.589	0.091	0.080
<b>Left atrial diameter</b>							
<i>R</i>	-0.049	0.002	-0.078	0.048	0.034	0.048	<b>-0.151**</b>
<i>P</i>	0.332	0.975	0.118	0.336	0.501	0.341	<b>0.003</b>

BP - blood pressure; FPG - fasting plasma glucose; IVS - interventricular septum; LVEF - left ventricular ejection fraction; pNN50 - the proportion of adjacent RR intervals differing by >50 ms in the 24-h recording; R - coefficient of correlation; PW - posterior wall; rMSSD - the square root of the mean squared differences of successive normal-to-normal intervals; SDANN - the standard deviation of the average normal-to-normal intervals calculated over the 5-min period of the entire recording; SDNN - the standard deviation of all normal-to-normal intervals; SDNN index - the mean of the deviation of the 5-min normal-to-normal intervals over the entire recording; TO - turbulence onset; TS - turbulence slope. Spearman's test was used. \**P*<0.05; \*\**P*<0.01

tes. In addition, our study clarifies the conflicting data regarding CAND and IFG. The principal findings of this study were as follows: 1) Patients with isolated IFG were likely to have CAND;

2) While time domain HRV parameters and TS decreased from Group I to Group III, TO and HRT category gradually increased; 3) FPG level was significantly correlated with time domain HRV

**Table 5. The results of multiple linear regression analysis for determining the best predictors that affect heart rate variability and heart rate turbulence parameters**

Variables	SDNN	SDNN index	SDANN	rMSDD	pNN50	TO	TS
<b>Age</b>							
Beta (standardized)	-0.206	-0.257	-0.181	–	-0.106	0.245	-0.295
<i>P</i>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	–	<b>0.034</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Fasting plasma glucose</b>							
Beta (standardized)	-0.133	-0.152	-0.161	-0.214	-0.217	0.226	-0.257
<i>P</i>	<b>0.007</b>	<b>0.002</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Presence of hypertension</b>							
Beta (standardized)	-0.098	-0.063	-0.134	–	-0.035	0.057	-0.102
<i>P</i>	0.050	0.220	<b>0.007</b>	–	0.487	0.256	<b>0.032</b>
<b>Constant</b>							
B	2107	1883	2040	1541	1351	0.748	1255
<i>P</i>	<b>&lt;0.001</b>						
Adjusted R <sup>2</sup>	0.111	0.099	0.109	0.043	0.065	0.132	0.232
<p>pNN50 - the proportion of adjacent RR intervals differing by &gt;50 ms in the 24-h recording; rMSDD - the square root of the mean squared differences of successive normal-to-normal intervals; SDANN - the standard deviation of the average normal-to-normal intervals calculated over the 5-min period of the entire recording; SDNN - the standard deviation of all normal-to-normal intervals; SDNN index - the mean of the deviation of the 5-min normal-to-normal intervals over the entire recording; TO - turbulence onset; TS - turbulence slope. The independent predictors of SDNN, SDNN index, SDANN, rMSDD, pNN50, TO, and TS were identified by multiple linear regression analysis</p>							

parameters, TO, and TS; and 4) FPG and age were significant independent predictors of almost all impaired HRV and HRT parameters. Accordingly, altered sympathovagal function as revealed by impaired HRV and HRT was present in patients with isolated IFG and this influence was independently associated with the FPG level.

Prediabetes, a clinical condition standing on the continuum between a normal glycemic state and overt T2DM, is related with increased risk of the similar macrovascular and microvascular complications of overt diabetes (16). The overall prevalence of prediabetes was 36.5% among individuals older than 18 years in the National Health and Nutritional Examination Survey (17). According to the Turkish Diabetes Epidemiology Study II, which included 26,499 adults aged ≥20 years (mean age 45.8±15.3 years), the prevalence of prediabetes was 30.8% in Turkey (isolated IFG: 14.7%, isolated IGT: 7.9%, both IFG and IGT: 8.2%) (18).

CAND is one of the most important microvascular complications because of its association with significantly increased cardiovascular morbidity and mortality (19, 20). Clinical symptoms generally occur late in the disease process (1). However, the first finding of CAND is a decrease in HRV, which is apparent even at the subclinical stage (21). SDNN represents both the sympathetic and parasympathetic modulation of HRV, RMSSD, and the pNN50 parasympathetic system (11). As with HRV, HRT has also been determined to be applicable in the diagnosis of CAND (12). Two components of HRT, TO and TS, are critically vagal dependent and reflect the status of the parasympathetic system (22).

Although the relation between CAND and IGT is well established in several studies (23–26), previous studies have shown

incompatible results with each other regarding the relation between CAND and isolated IFG. The FPG levels were classified as normal (<110 mg/dL, n=1779), IFG (110–125 mg/dL, n=56), and T2DM (≥126 mg/dL or receiving antidiabetic therapy, n=84) in the Framingham Heart Study (27). Although their IFG subjects had higher FPG criterion than our subjects (110 mg/dL vs. 100 mg/dL), SDNN and low-frequency and high-frequency power were inversely associated with plasma glucose levels and those parameters were reduced in both the diabetic and the IFG groups. However, in the Framingham Heart Study, classification of patients into normal glucose tolerance, IFG, and T2DM groups by solely FPG without OGTT may cause confusion because patients with IFG may also have IGT or diabetes. Therefore, using such a method, it is impossible to determine isolated IFG. Wu et al. (9) reported that altered cardiac autonomic function, as examined by SDNN, 30/15 ratio, and frequency-domain HRV parameters, was present in both IGT and diabetic subjects, but was not different in patients with isolated IFG from those with normal glucose tolerance. As in Wu's report, the Atherosclerosis Risk in Communities (ARIC) Study used a FPG level of <100 mg/dL as its IFG criterion and showed that there was no difference in SDNN between those with IFG and normal glucose tolerance (28). However, similar to the Framingham Heart Study, the ARIC study could not examine the influence of IGT in IFG subjects. The current study may be considered more reliable because of our use of 2-h OGTT glucose levels for the determination of true isolated IFG patients. Additionally, the difference in results between the current study and that of Wu et al. (9) may be caused by glycemia differences between normal glucose tolerance and isolated IFG groups. While mean HbA1c

values were quite close between the normal glucose tolerance and isolated IFG groups ( $4.9\% \pm 0.5\%$  vs.  $5.1\% \pm 0.5\%$ ) in Wu et al.'s study (9), they were  $5.1\% \pm 0.3\%$  vs.  $5.9\% \pm 0.2\%$  in our study. Therefore, the IFG subjects in Wu et al.'s (9) study may be less hyperglycemic compared with our isolated IFG patients, which could result in a similarity in HRV parameters between the normal glucose tolerance and isolated IFG groups.

In the recent Kora S4 survey of 1202 participants (the number of individuals with normal glucose tolerance, isolated IFG, isolated IGT, both IFG and IGT, and newly diagnosed T2DM was 565, 336, 72, 151, and 78, respectively), the prevalence of CAND increased in individuals with T2DM and IGT as well as in those with isolated IFG (10). Similarly, we observed that SDNN, SDNN index, SDANN, RMSSD, and pNN50 values were lower in the isolated IFG group than in the control group but higher than in the isolated IGT, both IFG and IGT, or newly diagnosed T2DM group. Different from the previous HRV studies, a novel finding of our study was the demonstration of the relation between disturbed HRT indices and prediabetes. We observed that TO and TS values and HRT category were impaired in patients with isolated IFG compared with the subjects with normal glucose tolerance, reflecting mainly parasympathetic CAND. In addition, correlation analysis revealed the continuous relation between FPG and HRV and HRT parameters. Our study also demonstrated that FPG was an independent determinant of almost all HRV and HRT parameters.

Although only parasympathetic involvement is expected in the early stages of CAND (4), disturbance in all studied HRV and HRT parameters indicates the impairment of both parasympathetic and sympathetic limbs of the autonomic nervous system, even in an isolated IFG state. These findings support the fact that patients may have subclinical CAND that is more serious than is believed for several years before it becomes clinically apparent (29). Accordingly, because the progression of cardiovascular denervation is partly reversible or can be slowed down in the early stages of the disease (30), screening for CAND may be recommended for all prediabetic patients in addition to all newly diagnosed T2DM patients. Assessment of CAND is possible through a variety of methods, such as cardiovascular autonomic reflex tests, HRV, and imaging modalities (3). The criteria for diagnosis and staging are published elsewhere (31).

## Study limitations

The first limitation of this study was the absence of Ewing's cardiovascular autonomic reflex tests, which are the gold standard for the determination of CAND. However, studies comparing Holter-based HRV analysis and cardiovascular autonomic reflex tests found a high correlation between both techniques. Additionally, frequency domain methods were not performed for HRV analysis. However, many time- and frequency-domain variables obtained over the 24-h period were found to be highly correlated with each other.

## Conclusion

Findings of this study revealed that HRT disturbance, considered a sign of sympathovagal imbalance, is already present in the prediabetic stage. Our study also verified recent data regarding the existence of a relation between CAND and isolated IFG and demonstrated the independent relation between FPG and almost all HRV and HRT parameters. It seems important to be aware of CAND in all prediabetic patients, including those with isolated IFG. Because previous data suggests that the reversal of cardiovascular denervation may occur in the early stages of the disease; screening of all subgroups of prediabetic patients for CAND may be considered.

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