

## Autonomic Markers of Impaired Glucose Metabolism: Effects of Sleep-Disordered Breathing

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### Abstract

#### Background:

The association between diabetes and abnormalities in autonomic function is well-known, but it is not clear if this association can be extended to subjects with prediabetic impaired glucose metabolism (IGM). Sleep-disordered breathing (SDB), which commonly occurs in this population, is often overlooked. We sought to determine how autonomic function, monitored in an overnight sleep study setting, may be impaired in subjects with IGM and/or SDB.

#### Methods:

Polysomnograms (PSGs) selected from the Cleveland Family Study database were categorized into four groups: normal, SDB (respiratory disturbance index > 5/h), IGM, and both SDB and IGM. Impaired glucose metabolism was defined as an oral glucose tolerance test (OGTT) level > 140 mg/dl. Time-domain and frequency-domain indices of heart rate variability were used to quantify autonomic impairment. Baroreflex sensitivity determined using pulse transit time (BRS<sub>PTT</sub>), an indirect measure of baroreflex sensitivity based on spontaneous pulse transit time fluctuations, was used as a surrogate measure of baroreflex sensitivity.

#### Results:

Based on 31 PSGs from subjects (16 males, 15 females) ages 20.8–61.2 years, both SDNN and BRS<sub>PTT</sub> were found to be 20–25% lower in SDB and ~40% lower in IGM and SDB + IGM as compared to subjects without either condition. In analyses of continuous measures, mean standard deviation of 5 min R–R intervals (SDNN) and BRS<sub>PTT</sub> were found to be negatively correlated with OGTT following adjustment for age and body mass index. Oral glucose tolerance test and age were the two most significant factors for predicting SDNN and BRS<sub>PTT</sub>.

#### Conclusions:

Our analyses suggest that cardiac autonomic control is impaired in IGM, regardless of whether SDB is present. The abnormal autonomic function involves degradation of baroreflex regulation.

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**Abbreviations:** (ANOVA) analysis of variance, (BMI) body mass index, (BRSPTT) baroreflex sensitivity determined using pulse transit time, (CFS) Cleveland Family Study, (ECG) electrocardiogram, (FBG) fasting blood glucose, (HRV) heart rate variability, (IFG) impaired fasting glucose, (IGM) impaired glucose metabolism, (IGT) impaired glucose tolerance, (mRRI) mean R–R interval, (OGTT) oral glucose tolerance test, (PLETH) photoplethysmograph, (PSG) polysomnogram, (PTT) pulse transit time, (RDI) respiratory disturbance index, (REM) rapid eye movement, (RRIHF) high-frequency power of R–R interval variability, (RRILF) low-frequency power of R–R interval variability, (RRILHR) ratio of low-frequency power to high-frequency power of R–R interval variability, (SDB) sleep-disordered breathing, (SDANN) standard deviation of the average R–R intervals calculated over 5 min periods, (SDNN) mean standard deviation of 5 min R–R intervals

**Keywords:** autonomic nervous system, baroreflex sensitivity, glucose tolerance, heart rate variability, pulse transit time, sleep apnea

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## Introduction

The association between diabetes and cardiovascular disease, in particular, coronary heart disease and stroke, has been demonstrated in many epidemiological studies.<sup>1</sup> Type 2 diabetes is also associated with cardiac autonomic dysfunction, detected via stress tests or using heart rate variability (HRV).<sup>2–4</sup> These findings therefore underscore the need to determine whether subjects who are prediabetic or who are at high risk for developing diabetes show early signs of autonomic impairment. Establishing such an association would also be clinically significant in suggesting that one could use noninvasive autonomic biomarkers to monitor nonintrusively the long-term progression of metabolic dysfunction in high-risk individuals. However, the existence of an association between prediabetic impaired glucose metabolism (IGM) and reduced HRV remains unclear because there are studies that support and some that refute this notion.<sup>5,6</sup> Limitations of prior work include the lack of adequate control for the effect of overweight or obesity as well as the use of different measures of abnormal glucose metabolism. Another limitation has been the lack of consideration of possible confounding influences associated with the presence of occult sleep-disordered breathing (SDB) in subjects with IGM, because obesity or overweight is a risk factor for both disease entities. Sleep-disordered breathing has been shown to be an independent risk factor for development of IGM,<sup>7–15</sup> with a report that treatment of SDB improves metabolic function.<sup>16</sup> Several studies have demonstrated an association between reduced HRV and SDB,<sup>17–19</sup> but the concurrent presence of IGM in the subjects with SDB was not tested. In this study, we report on the contribution of SDB to alterations in cardiac autonomic function in subjects who have normal or impaired glucose tolerance (IGT).

Due to the ubiquity of instrumentation and software for monitoring heart rate or pulse interval, HRV has been employed extensively as a means of quantifying cardiac autonomic function. However, there are important limitations with HRV that are often overlooked. One is the potential “contamination” of the high-frequency components of HRV with variations in ventilatory pattern.<sup>20</sup> Delineating sympathetic from vagal contributions to cardiac control using indices derived from HRV remains a controversial issue.<sup>21,22</sup> Similar problems arise when the low-frequency power of blood pressure variability

is blindly employed as a marker of sympathetic tone.<sup>23</sup> Some of these limitations can be circumvented by employing baroreflex sensitivity, the gain with which the baroreflexes translate changes in blood pressure into changes in heart rate, in addition to HRV to assess cardiac autonomic control. On the other hand, determination of baroreflex sensitivity in the clinical setting requires noninvasive measurement of continuous blood pressure, which is highly expensive and not practicable for deployment in large-scale clinical studies. However, pulse plethysmography is routinely measured. The speed at which the arterial pressure pulse travels is directly proportional to blood pressure, assuming constant elasticity of the arterial wall. As such, pulse transit time (PTT) variability has been shown to be correlated with blood pressure variability.<sup>24</sup> In this study, we introduce a surrogate measure of baroreflex sensitivity, (baroreflex sensitivity determined using pulse transit time [BRS<sub>PTT</sub>]), in which PTT variability is used in place of blood pressure variability. BRS<sub>PTT</sub> is employed alongside the conventional indices of HRV to assess cardiovascular autonomic function, as well as to provide complementary knowledge about the potential mechanisms that may be responsible for abnormalities in HRV. In this study, we sought to determine how BRS<sub>PTT</sub> and other accepted measures of HRV during wakefulness and various stages of sleep are altered by IGM and SDB.

## Methods

### Data Source

Polysomnogram (PSG) data were selected from the Cleveland Family Study (CFS) database, representing standardized PSG studies on well-phenotyped subjects. A detailed description of cohort assembly is provided elsewhere.<sup>25</sup> In brief, the cohort consists of probands with known sleep apnea, their family members, and neighborhood controls. In the last examination, subjects were studied with detailed metabolic and cardiovascular examination. Polysomnogram data were collected using a 14-channel monitor in a dedicated clinical research facility, along with measures of blood pressure, anthropometry, and glucose testing performed after an overnight fast and after a 2 h glucose challenge test. All PSG recordings were at least 6 h in duration, with typical signal channels as nasal/oral thermistery, chest wall impedance, finger pulse oximetry, and electrocardiogram (ECG).

Respiratory events, such as hypopnea or apnea, were defined as partial reductions or complete cessations in airflow or chest wall impedance of duration  $\geq 10$ , with associated drop in oxygen saturation  $\geq 3\%$  from baseline (referred to the plateau saturation level at the beginning of the hypopnea/apnea). The severity of SDB was quantified in terms of the respiratory disturbance index (RDI), defined as the total number of respiratory events divided by total sleep duration (hours).

The detailed description of the experimental procedures for measuring blood glucose and carrying out the oral glucose tolerance tests (OGTTs) is given in Sulit and coauthors.<sup>26</sup> Briefly, for each patient, fasting blood glucose (FBG) was sampled and OGTT was performed in the morning following PSG. Fasting blood glucose was measured by venipuncture around 7:00 AM. The OGTT was conducted right after the FBG, in which 75 g anhydrous glucose was orally administered with venipuncture performed 2 h later for the OGTT value.

Height and weight were measured in stocking feet using calibrated stadiometers and scales. Body mass index (BMI) was computed as the ratio of weight to the square of the height ( $\text{kg}/\text{m}^2$ ).

### *Polysomnography*

From the CFS database of adult subjects (>18 years) who were not on treatment with continuous positive airway pressure, 60 overnight recordings were found to be eligible for detailed analysis after subjecting all PSGs to a rigorous screening process based on medical background and signal quality. The following inclusionary criteria were applied: subjects had to have (1) a photoplethysmograph (PLETH) channel in the PSG recording, (2) relatively large segments of data with good quality signals (>50% of total recording duration or >4 h) in both ECG and PLETH channels, (3) all sleep stages were represented in the good-quality segments, (4) no previous diagnosis of chronic cardiac or pulmonary disease, or (5) other exclusionary criteria as listed in **Table 1**, including known diabetes and hypertension. From the 60 PSGs that were deemed eligible for further analysis, 31 PSGs were selected for analysis to produce 4 subgroups ("disease status"; see Statistical Analysis section) of similar sizes and roughly matched for age, BMI, and gender composition for comparison.

For each subject, the PSG was recorded from a single-night sleep study in a sleep laboratory and later scored for wake/sleep stage [W, wakefulness; N1, N2, light sleep;

**Table 1.**  
Exclusionary Criteria of Cardiovascular,  
Respiratory, and Metabolic Conditions

Participants are excluded if they have

- Ever been diagnosed with myocardial infarction
- Ever been diagnosed with diabetes
- Ever been diagnosed with angina pectoris
- Ever had coronary bypass surgery
- Ever had coronary angioplasty
- Ever been diagnosed with congestive heart failure
- Ever had implant of cardiac pacemaker
- Ever been diagnosed with other heart disease
- Ever been diagnosed with stroke (cerebrovascular accident)
- Ever been diagnosed with carotid surgery (endarterectomy)
- Ever been diagnosed with transient ischemic attack
- Ever been diagnosed with asthma
- Ever been diagnosed with chronic bronchitis
- Ever been diagnosed with emphysema
- Ever been diagnosed with pneumonia

N3, deep sleep; R, rapid eye movement (REM) sleep], and severity of SDB quantified by the RDI, the number of apnea or hypopnea events per hour overnight. A bipolar ECG and finger photoplethysmogram from the PSG were used for further analysis.

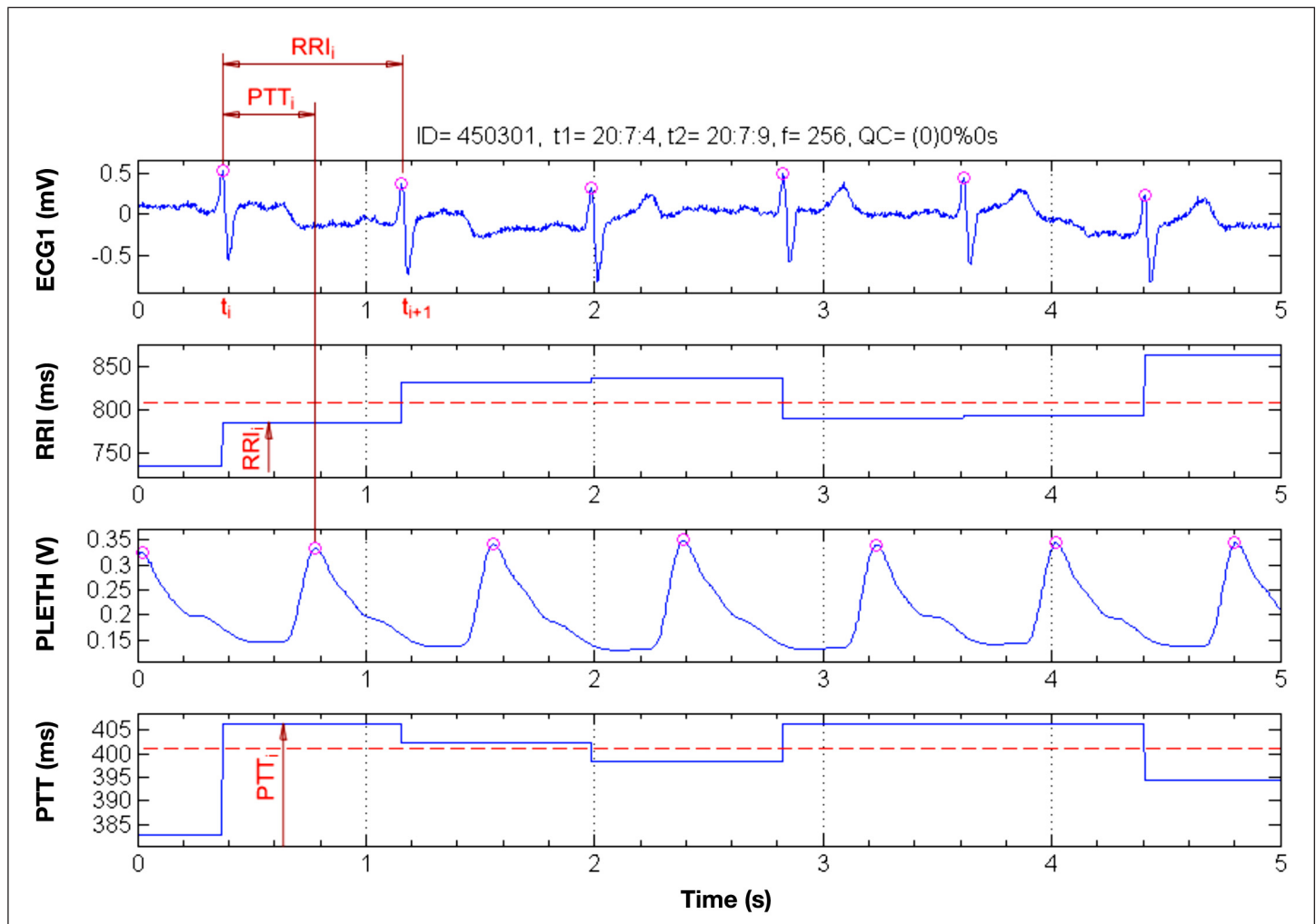
### *Heart Rate Variability Measures*

Mean R-R interval (mRRI) and the power spectrum of the fluctuations of RRI around the mean were derived from successive 5 min segments of the ECG signal over the entire duration of each PSG. Spectral analysis was performed using the Welch method with Hanning windowing.<sup>27</sup> The areas in the low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.4 Hz) bands of each HRV spectrum were calculated to obtain low-frequency power of R-R interval variability (RRILF) and high-frequency power of R-R interval variability (RRIHF), respectively.<sup>28</sup> From RRILF and RRIHF, the ratio of low-frequency power to high-frequency power of R-R interval variability (RRILHR) was also computed for each segment. Since RRILHR contains information about RRILF, we will only report values of RRIHF and RRILHR here.<sup>22</sup> As well, two time-domain HRV measures, mean standard deviation of 5 min R-R intervals (SDNN) and standard deviation of the average R-R intervals calculated over 5 min periods (SDANN), were calculated. SDNN is the mean standard deviation of 5 min normal-to-normal intervals, corresponding to the HRV within 5 min, and is equivalent to the square root of the total HRV power.<sup>28</sup> SDANN is the standard deviation of the average normal-to-normal intervals calculated over 5 min periods, reflecting the overnight heart rate cycles longer than 5 min.

### Pulse Transit Time Variability Measures

Pulse transit time is the duration taken for a pulse wave to travel between two arterial sites. Assuming the constant elasticity of arterial wall, the speed at which the arterial pressure pulse travels is directly proportional to blood pressure and can be used as an indirect measure of blood pressure.<sup>29</sup> As a simple inverse of the pulse travel velocity, PTT was also proposed to be an indicator of arterial blood pressure.<sup>30</sup> The ideal measure of PTT (or the velocity) requires two monitoring sites located at the same artery. However, implementation of this way of measuring PTT is confounded by sensitivity to motion artifacts and the extremely short intervals. A modified version of PTT, also known as pulse arrival time, was proposed to overcome these disadvantages by taking the apex of R-wave of ECG as the starting reference point.<sup>31</sup> Despite its advantages, the authors suggested

that this R-triggered PTT reflects a sum effect from both the pre-ejection period of a cardiac cycle and the true PTT. The relationship between PTT and arterial blood pressure is no longer linear under certain situations such as nonisovolumic pre-ejection. The PLETH channel recorded using pulse oximetry was used to obtain the ending reference. The ending reference of PTT is conventionally set at the baseline of PLETH cycle or the 25% point of the maximum amplitude; nonetheless, in situations that do not permit frequent calibration, it is nearly impractical to obtain, such as in long-term monitoring and overnight sleep recording. In contrast, rather than use the PTT value itself, the PTT variability can be calculated and derived using the peak of PLETH as the ending point. The corresponding derivations of PTT and RRI are shown in **Figure 1**. For each 5 min segment of PTT, the spectrum of the fluctuations of PTT



**Figure 1.** Derivation of RRI and PTT during 5 se.  $RRI_i$ , the  $i$ th R-R interval, is defined as the interval between  $t_i$  and  $t_i + 1$ , also the interval between the  $i$ th and  $(i + 1)$ th R waves.  $PTT_i$ , the  $i$ th PTT, is defined as the interval between the  $i$ th R peak and  $i$ th peak of PLETH. QC, quality control.



around its mean was calculated. The areas in the low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.4 Hz) bands of each PTT spectrum were calculated.

### Pulse-Transit-Time-Based Surrogate Measure of Baroreflex Sensitivity

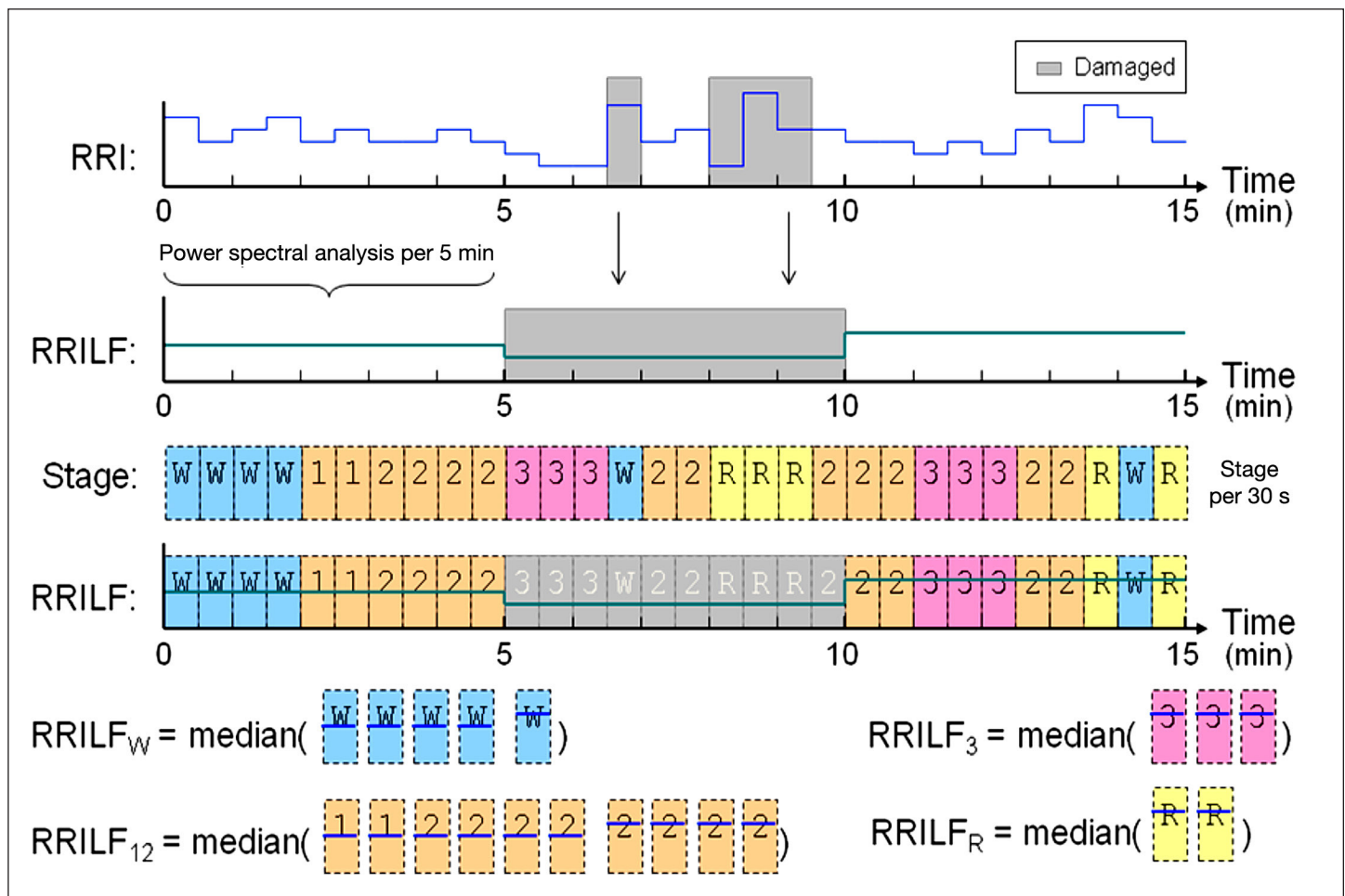
Baroreflex sensitivity is generally estimated using the spontaneous beat-by-beat fluctuations in systolic arterial blood pressure and RRI through either the sequence method<sup>32</sup> or the spectral method.<sup>33</sup> Both methods have been shown in humans to produce estimates of baroreflex sensitivity that are quantitatively similar and strongly correlated to one another.<sup>34</sup> For computational simplicity, we selected the spectral method of baroreflex slope estimation. However, instead of using spontaneous fluctuations in systolic blood pressure, we used spontaneous fluctuations in PTT. The surrogate measure of  $BRS_{PTT}$  was defined as

$$BRS_{PTT} = \sqrt{\frac{RRILF}{PTTLF}}.$$

Note that  $BRS_{PTT}$  is unitless because both  $RRILF$  and low-frequency  $PTT$  have same the unit ( $ms^2$ ).

### Sleep-Stage-Adjusted Indices

Scoring of sleep stage was carried out in consecutive epochs of 30 s each [wakefulness (W), REM sleep, light non-REM sleep consisting of stage 1 and stage 2 (N1, N2), and deep non-REM sleep (N3)]. Because each 5 min segment of the PSG channel was used to produce values for  $PTT$  and  $HRV$ , the segment was divided into equal-length 30 s segments bearing the identical spectral index. For each sleep stage, an overnight median value of spectral indices was deduced according to the frequency-weighting algorithm. **Figure 2** shows an illustrative example of the computations used to derive the median  $RRILHR$  representative of each sleep stage in each individual subject. Employing the same method, each of the other indices was represented by the median of all the overnight 5 min segment values for each stage. Medians were adopted instead of means in order to minimize the effect of outlier values. Although the quiet



**Figure 2.** Schematic illustration of the method used to compute the median value of the index representative of each sleep-wake state.

wakefulness prior to sleep onset might represent “true wakefulness,” several recordings start with sleep stages, which led us to use nocturnal wakefulness to calculate the stage-specific index. In other words, the wakefulness stage in phase I of the study represents not only the waking period before the sleep onset, but also the nocturnal wakefulness. Damaged 5 min segments, either from the original ECG or RRI, were excluded from index derivation. A similar stage-adjusting algorithm has been successfully implemented in HRV analysis of the Sleep Heart Health Study data.<sup>19</sup>

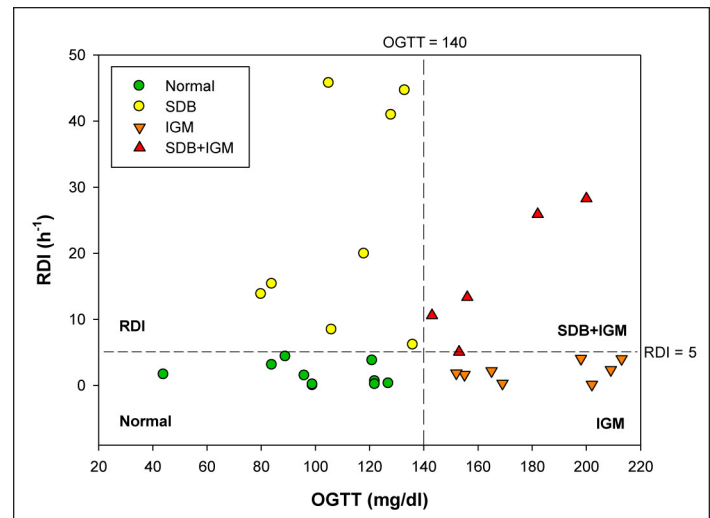
### Blood Glucose Measures

According to the American Diabetes Association diagnostic criteria, impaired fasting glucose (IFG) is defined as a FBG level  $\geq 100$  but  $< 126$  mg/dl. Impaired glucose tolerance is defined as an OGTT result  $\geq 140$  and  $< 200$  mg/dl. Subjects with IFG tend to have elevated hepatic glucose output and a defect in early insulin secretion, whereas those with IGT have peripheral insulin resistance.<sup>35</sup> Previous studies have also found a greater incidence of altered cardiac autonomic function in subjects with IGT but not isolated IFG.<sup>36</sup> Thus, for purposes of this study, we used the OGTT measurements as a continuous measure of the degree of IGM. Impaired glucose metabolism was defined as an OGTT level higher than 140 mg/dl in our data set.

### Statistical Analysis

The subjects studied were divided into four “disease status” categories based on severity of SDB (no SDB;  $RDI < 5 \text{ h}^{-1}$  versus SDB;  $RDI \geq 5 \text{ h}^{-1}$ ) and degree of impairment of glucose metabolism (normal glucose metabolism;  $OGTT < 140$  mg/dl versus IGM;  $OGTT \geq 140$ ). The IGM category included three subjects with OGTT slightly higher than 200 ( $\leq 213$ ) mg/dl. Thus the four “disease status” categories were (a) control ( $RDI < 5 \text{ h}^{-1}$ ,  $OGTT < 140$  mg/dl); (b) SDB only ( $RDI \geq 5 \text{ h}^{-1}$ ,  $OGTT < 140$  mg/dl); (c) IGM only ( $RDI < 5 \text{ h}^{-1}$ ,  $OGTT \geq 140$  mg/dl), and (d) SDB + IGM ( $RDI \geq 5 \text{ h}^{-1}$ ,  $OGTT \geq 140$  mg/dl). Except for sex (which was taken as a binary variable), age, BMI, RDI, and OGTT were considered as continuous variables. All variables used in the statistical analyses were first checked for normality and were log transformed if found to be not normally distributed. A scatterplot showing the distribution of the individual subjects across these four “disease status” groups is displayed in **Figure 3**.

Two-way repeated measures analysis of variance (ANOVA) with interaction was performed on each of the



**Figure 3.** Scatter plot of subject distribution across RDI and OGTT ( $n = 31$ ).

autonomic indices (SDNN, SDANN, RRIHF, RRIHR, mRRI, and  $BRS_{PTT}$ ) with “disease status” (SDB, IGM) as the independent variable and sleep–wake stage as the repeated variable.

The relationships between each of the autonomic indices and the potential explanatory variables (sex, age, BMI, RDI, and OGTT), following stratification for sleep stage, were explored further using multiple linear regression analysis. Four models were tested, in which the independent variables were

- (a) model A: sex, age, RDI
- (b) model B: sex, age, OGTT
- (c) model C: sex, age, RDI, OGTT
- (d) model D: sex, age, RDI, OGTT, BMI

Comparison of goodness of fit among the models was carried out using the adjusted  $r^2$  statistic ( $R^2_{adj}$ ). The relative strength of each explanatory variable on the dependent variable (autonomic index) was quantified using the standardized regression coefficient corresponding to that independent variable. The standardized regression coefficient  $\beta_i$  for the  $i$ th explanatory variable is defined as  $\beta_i = b_i(SD_i/SD_y)$ , where  $b_i$  is the  $i$ th regression coefficient estimated from the multiple regression analysis,  $SD_i$  is the standard deviation of the  $i$ th explanatory variable, and  $SD_y$  is the standard deviation of the dependent variable  $y$ .<sup>37</sup> We also performed a power analysis using G\*Power, version 3.0.10.<sup>38</sup> The analysis showed that, with a sample size of 30, the multiple regression model

with five explanatory variables was capable of detecting a partial  $r^2$  of 0.35 with a power of 80% and significance level of 0.05. However, with the sample size employed in the study, we were probably underpowered to detect more modest associations.

The default significance level for all tests was 0.05. All statistical procedures were conducted using SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL).

## Results

The characteristics of the subjects in this analytical sample, and in subgroups defined by “disease status,” are displayed in **Table 2**. One-way ANOVA identified no significant difference in sex, age, BMI, PSG percentage (percentage of PSG recording usable for analysis), and total PSG duration among the four disease status categories (**Table 2**). As expected, both the SDB and SDB + IGM groups had significantly higher RDI levels compared with the non-SDB groups ( $p < .001$ ). The IGM and SDB + IGM groups had significantly higher OGTT and higher levels of FBG than the groups who had normal glucose tolerance ( $p < .003$ ). However, because there was a strong correlation between OGTT and FBG among subjects ( $p < .001$ ), and due to our *a priori* hypothesis, only OGTT was used to represent the severity of IGM in subsequent analyses.

The results of the two-way repeated measures ANOVA (disease condition versus sleep stage) for all six autonomic indices that were tested are shown in **Table 3**. The values of each of these autonomic parameters across the various “disease status” categories and across sleep-wake states are displayed graphically in **Figure 4**. This analysis along with *post hoc* tests (Holm-Sidak) showed that  $BRS_{PTT}$  in the IGM and SDB + IGM groups were significantly lower relative to the control group in all sleep stages.  $BRS_{PTT}$  in the SDB group was lower than

**Table 3.**  
Associations between Autonomic Indices and Disease Status and Sleep Stage: *P* Values from Two-Way Repeated Measures ANOVA on Autonomic Indices with Disease Status as the Unrepeated Factor and Sleep Stage as Repeated Factor ( $n = 31$ )<sup>a</sup>

Autonomic index	Disease status	Sleep stage	Interaction
SDNN	<0.001	<0.001	0.351
SDANN	0.016	0.062	0.625
RRHF	0.007	0.028	0.613
RRLHR	0.625	<0.001	0.977
mRRI	0.045	<0.001	0.243
$BRS_{PTT}$	<0.001	0.010	0.347

<sup>a</sup> *P* values are shown in bold if  $<0.05$  or italics if  $0.05 \leq p < .1$ .

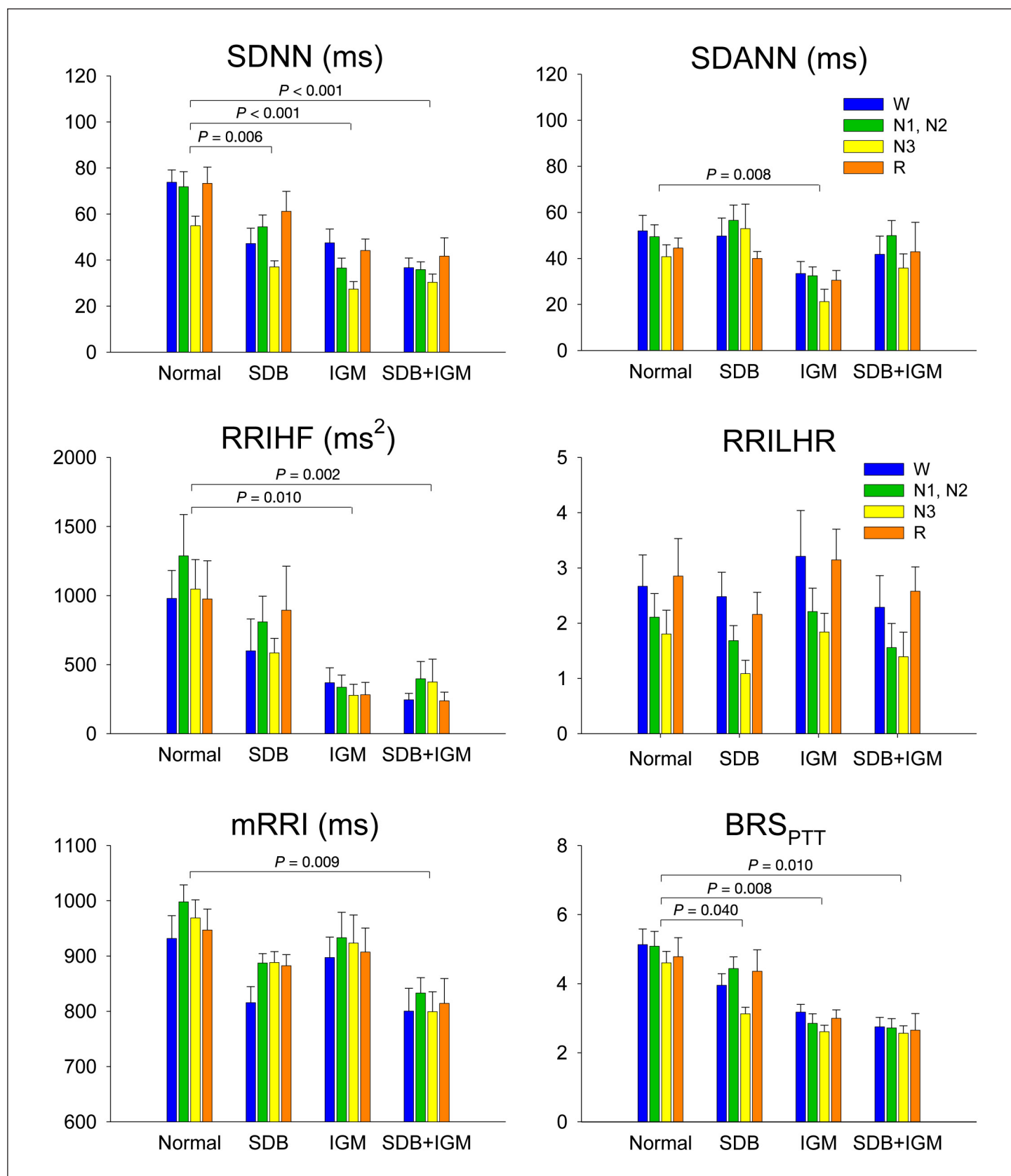
**Table 2.**  
Summary of Subject Characteristics ( $n = 31$ )<sup>a</sup>

	Control ( $n = 10$ )	SDB ( $n = 8$ )	IGM ( $n = 8$ )	SDB + IGM ( $n = 5$ )	<i>P</i> value <sup>b</sup>
Sex, male:female	5:5	5:3	4:4	2:3	0.89
Age, years	35.6 ± 12.9 (20.8–54.7)	42.6 ± 15.2 (20.8–65.2)	40.4 ± 9.4 (29.6–54.9)	48.9 ± 11.3 (33.0–61.2)	0.29
BMI, kg/m <sup>2</sup>	29.1 ± 6.7 (20.0–45.3)	35.4 ± 5.8 (28.4–42.2)	37.1 ± 11.0 (26.0–55.4)	34.7 ± 5.8 (26.3–42.5)	0.17
RDI, h <sup>-1</sup>	1.6 ± 1.6 (0.0–4.4)	24.4 ± 16.6 <sup>c</sup> (6.1–45.7)	2.1 ± 1.5 (0.2–4.1)	16.6 ± 10.0 <sup>c</sup> (5.1–28.3)	<0.001
OGTT, mg/dl	100.3 ± 25.1 (44–122)	111.3 ± 21.4 (80–136)	182.9 ± 25.1 <sup>c</sup> (152–213)	166.8 ± 23.5 <sup>c</sup> (153–200)	<0.001
FBG, mg/dl	85.4 ± 6.7	94.1 ± 5.6	100.6 ± 11.9 <sup>c</sup>	98.4 ± 5.6 <sup>c</sup>	0.003
PSG percentage	58.9 ± 15.6	62.6 ± 12.3	66.0 ± 3.9	58.1 ± 8.3	0.54
PSG total, h	5.7 ± 1.7	6.5 ± 1.5	6.4 ± 0.6	5.5 ± 1.0	0.42

<sup>a</sup> Values are counts for sex, mean ± standard deviation, with ranges in parentheses for continuous data. PSG percentage, percentage of PSG recording that was usable for analysis; PSG total, total duration of PSG recording. *P* values are shown in bold if  $<0.05$ .

<sup>b</sup> *P* values from one-way ANOVA.

<sup>c</sup>  $P < \text{critical level}$  from pairwise comparison versus control group (Holm-Sidak).



**Figure 4.** Grouped bar charts of SDNN, SDANN, RRIHF, RRILHR, mRRI, and BRSPTT across SDB/IGM categories and sleep stages ( $n = 31$ ). Note that  $p$  values are from a Holm-Sidak *post hoc* test comparing with normal group (overall significant level 0.05).



control in W and R stages only. When averaged over sleep stage,  $BRS_{PTT}$  was ~20% lower in SDB and ~40% lower in IGM and SDB + IGM relative to control (control,  $4.90 \pm 0.29$ ; SDB,  $3.97 \pm 0.32$ ; IGM,  $2.91 \pm 0.32$ ; SDB + IGM,  $2.67 \pm 0.41$ ). The SDNN was also lower in SDB, IGM, and SDB + IGM versus control in all sleep stages, except in R stage, where SDNN in SDB was not different from control. Averaged over sleep stages, SDNN was ~25% lower in SDB and over 40% lower in IGM and SDB + IGM versus control (control,  $68.5 \pm 4.1$  ms; SDB,  $50.0 \pm 4.6$  ms; IGM,  $38.9 \pm 4.6$  ms; SDB + IGM,  $36.2 \pm 5.8$  ms). RRIHF varied similarly, except that SDB was not significantly different from control in all sleep stages. The differences between the disease groups and control were less clear when autonomic function was quantified using mRRI and SDANN. There were no apparent differences across disease status when RRILHR was employed.

**Table 4** displays the results of the comparison among the four multiple linear regression models (A, B, C, and D) after stratification for sleep–wake stage. The results displayed are limited to the three autonomic indices that were most sensitive to disease status, based on the two-way repeated measures ANOVA described earlier;

these are RRIHF,  $BRS_{PTT}$ , and SDNN.  $R^2_{adj}$  was roughly twofold larger when OGTT was used as the third explanatory variable (after including sex and age as the first two) compared with RDI (model B versus model A). Moreover, even in model A, where RDI was forced to be the third explanatory variable, the associated coefficient never attained statistical significance in any of the cases considered. Including both RDI and OGTT as explanatory variables (model C) produced little change in  $R^2_{adj}$  compared with model B. Finally, including BMI in addition to RDI and OGTT as explanatory variables (model D) led to a small increment in  $R^2_{adj}$  in most but not all of the cases. In this sample, where matched groups were selected, RDI was not significantly correlated with BMI ( $r = 0.296$ ,  $p = .105$ ) but displayed a weak correlation with OGTT that was marginally significant ( $r = 0.352$ ,  $p = .052$ ). In virtually all cases displayed in **Table 4**, the regression coefficients corresponding to age and OGTT were statistically significant, whereas none of the autonomic indices displayed any dependence on RDI. The standardized regression coefficients associated with model 5 are displayed along with their corresponding  $p$  values in **Table 5**. Overall, for each of the three autonomic indices considered, OGTT and age each are

**Table 4.**  
**Results of Multiple Linear Regression Analysis Using the Four Models**

Dependent variable	Sleep–wake state	Models							
		A (sex, age, RDI)		B (sex, age, OGTT)		C (sex, age, RDI, OGTT)		D (sex, age, RDI, OGTT, BMI)	
		$R^2_{adj}$ <sup>a</sup>	Significant explanatory variables <sup>a</sup>	$R^2_{adj}$	Significant explanatory variables	$R^2_{adj}$	Significant explanatory variables	$R^2_{adj}$	Significant explanatory variables
ln(RRIHF)	W	0.084	Age	0.115	Age	0.124	Age	0.238	Age, BMI
	12	0.107	Age	0.349	Age, OGTT	0.349	Age, OGTT	0.374	Age, OGTT
	34	0.027	Age	0.301	Age, OGTT	0.278	Age, OGTT	0.311	Age, OGTT
	R	0.226	Age, sex	0.386	Age, OGTT	0.375	Age, OGTT	0.372	Age, OGTT
ln( $BRS_{PTT}$ )	W	0.222	Age	0.413	Age, OGTT	0.413	Age, OGTT	0.552	Age, RDI, OGTT, BMI
	12	0.218	Age	0.500	Age, OGTT	0.495	Age, OGTT	0.580	Age, OGTT, BMI
	34	0.180	Age	0.326	Age, OGTT	0.330	Age, OGTT	0.423	Age, OGTT, BMI
	R	0.295	Age	0.405	Age, OGTT	0.390	Age, OGTT	0.376	Age, OGTT
ln(SDNN)	W	0.151	Age	0.238	Age, OGTT	0.299	Age, OGTT	0.329	Age, OGTT
	12	0.134	Age	0.497	Age, OGTT	0.490	Age, OGTT	0.537	Age, OGTT
	34	0.005	—	0.395	Age, OGTT	0.373	Age, OGTT	0.426	Age, OGTT
	R	0.190	Sex	0.476	Age, sex, OGTT	0.462	Age, sex, OGTT	0.443	Age, OGTT

<sup>a</sup> The table entries include  $R^2_{adj}$  (adjusted  $R$  squared) and the names of the regression coefficients that were found to be statistically significant ( $p < .05$ ).

associated with the dependent variable by roughly the same degree. In some of the cases, BMI also exerts some influence, although less compared with OGTT and age. The signs of all the standardized regression coefficients for age, OGTT, and BMI are negative, implying that each autonomic index decreases with increases in each of the explanatory variables, consistent with our understanding of the underlying physiology.  $R^2_{adj}$  was always lowest in all the models tested when RRIHF was selected to be the autonomic index, compared with the cases where either  $BRS_{PTT}$  or SDNN were employed.

## Discussion

In this study, we derived cardiac autonomic indices in the various sleep–wake stages, using measurements of HRV from overnight PSGs in subjects who had varying degrees of SDB and IGM. We also introduced a novel surrogate measure of baroreflex sensitivity,  $BRS_{PTT}$ , that is derived from measurements of HRV and PTT variability and provides information that complements the results derived from HRV alone; in particular, this index provides an indication as to whether the detected differences in HRV could be the result of alterations in baroreflex function. The baroreceptors are known to exert a continuous inhibitory influence on sympathetic efferent activity; thus impaired baroreflex function can lead to sympathetic overactivity, elevated blood pressure,

and increased blood pressure variability.<sup>39</sup> In a separate study, we have shown that  $BRS_{PTT}$  correlates strongly with corresponding measures of spontaneous baroreflex sensitivity derived from noninvasive continuous measurement of arterial blood pressure.<sup>40</sup> Initial statistical analysis revealed substantial differences in SDNN and RRIHF (both HRV indices) as well as  $BRS_{PTT}$  in patients with SDB and/or IGM compared with normal controls, after adjusting for sleep–wake stage (**Figure 4**). However, further analysis using multiple linear regression showed that, after adjusting for sex, age, and BMI, SDNN and  $BRS_{PTT}$  were each significantly associated with OGTT (used as a continuous measure of IGM severity) but not with RDI (used as a continuous measure of SDB). This was the case for all sleep–wake stages except for REM sleep in  $BRS_{PTT}$ . Similar findings were obtained with RRIHF as the index representing autonomic function; however, there tended to be substantially more variability in the RRIHF estimates compared with SDNN and  $BRS_{PTT}$ . Taken together, our findings suggest that (1) cardiovascular autonomic function is substantially more sensitive to IGM than to the presence of SDB without IGM and (2) impaired baroreflex function is associated with and may be responsible in part for the reduction in HRV in IGM.

Autonomic nervous system dysfunction, including sympathetic overactivity, reduced parasympathetic drive,

**Table 5.**  
Standardized Regression Coefficients and *P* Values (Displayed within Parentheses) from Multiple Linear Regression Analysis on Stage-Specific Autonomic Indices with All Five Covariates: Male Gender, Age, BMI, RDI, and OGTT (*n* = 31)<sup>a</sup>

Log-transformed index	Stage	Sex	Age	BMI	RDI	OGTT	$R^2_{adj}$
RRIHF	W	-0.220 (0.185)	-0.449 ( <b>0.013</b> )	-0.431 (0.053)	-0.060 (0.817)	-0.188 (0.264)	0.247
	N1,N2	-0.149 (0.328)	-0.484 ( <b>&lt;0.001</b> )	-0.284 ( <b>0.031</b> )	0.229 (0.107)	-0.426 ( <b>0.046</b> )	0.430
	N3	-0.190 (0.248)	-0.416 ( <b>0.005</b> )	-0.290 ( <b>0.045</b> )	0.163 (0.262)	-0.442 ( <b>0.042</b> )	0.355
	R	0.044 (0.885)	-0.540 ( <b>&lt;0.001</b> )	-0.172 (0.109)	0.169 (0.207)	-0.360 ( <i>0.090</i> )	0.428
$BRS_{PTT}$	W	-0.156 (0.214)	-1.363 ( <b>&lt;0.001</b> )	-0.469 ( <b>0.037</b> )	0.035 (0.845)	-0.343 ( <b>0.002</b> )	0.569
	N1,N2	-0.024 (0.802)	-0.555 ( <b>&lt;0.001</b> )	-0.379 ( <b>0.032</b> )	0.257 ( <i>0.061</i> )	-0.441 ( <b>0.002</b> )	0.586
	N3	-0.004 (0.960)	-0.521 ( <b>0.002</b> )	-0.403 ( <i>0.071</i> )	-0.010 (0.894)	-0.315 ( <b>0.039</b> )	0.410
	R	0.204 (0.300)	-0.528 ( <b>0.001</b> )	-0.119 (0.319)	0.126 (0.360)	-0.302 (0.105)	0.392
SDNN	W	0.140 (0.465)	-0.464 ( <b>0.009</b> )	-0.282 (0.271)	-0.178 (0.269)	-0.340 ( <b>0.036</b> )	0.326
	N1,N2	-0.182 (0.281)	-0.526 ( <b>&lt;0.001</b> )	-0.303 ( <i>0.051</i> )	0.219 (0.132)	-0.520 ( <b>0.003</b> )	0.528
	N3	-0.182 (0.306)	-0.463 ( <b>0.003</b> )	-0.325 ( <i>0.062</i> )	0.087 (0.572)	-0.487 ( <b>0.009</b> )	0.426
	R	0.286 ( <i>0.071</i> )	-0.355 ( <b>0.010</b> )	-0.055 (0.443)	0.098 (0.524)	-0.503 ( <b>0.007</b> )	0.460

<sup>a</sup> *P* values are shown in bold if  $<0.05$  or italics if  $0.05 \leq p < 1$ .

and impaired baroreflex sensitivity, is known to be important in the pathogenesis of hypertension and cardiovascular disease. Subjects with SDB, who are subjected to repetitive episodes of nocturnal intermittent hypoxia and transient arousal from sleep, have been shown in numerous studies to display abnormalities in autonomic function, as represented by reduced HRV or autonomic stress tests,<sup>17–20,41–43</sup> as well as blunted baroreflex sensitivity.<sup>44–48</sup> However, there have been studies that have reported negative findings; for example, in a study involving PSGs from 61 male obstructive sleep apnea subjects and 43 controls, Coughlin and associates<sup>10</sup> did not find any significant association between baroreflex sensitivity and RDI. At the same time, other studies have reported finding reduced HRV and impaired baroreflex function in subjects with abnormal fasting glucose levels<sup>5</sup> and IGT.<sup>4,36,49</sup> Thus a major limitation in all these previous studies is that the subjects with SDB were not tested for IGM, while the studies that focused on HRV in IGM did not test the subjects for occult SDB. One exception is the study by Peltier and coworkers<sup>50</sup> that found that there were no significant differences in autonomic function, as determined through stress tests, between subjects with SDB and subjects without SDB; however, when the same pool of subjects was divided into those who had IGM versus those who had normal glucose metabolism, the IGM subjects demonstrated impaired autonomic responses. These results are consistent with the conclusion from our present study that autonomic function appears to be more sensitive to IGM than to the direct effects of SDB *per se*.

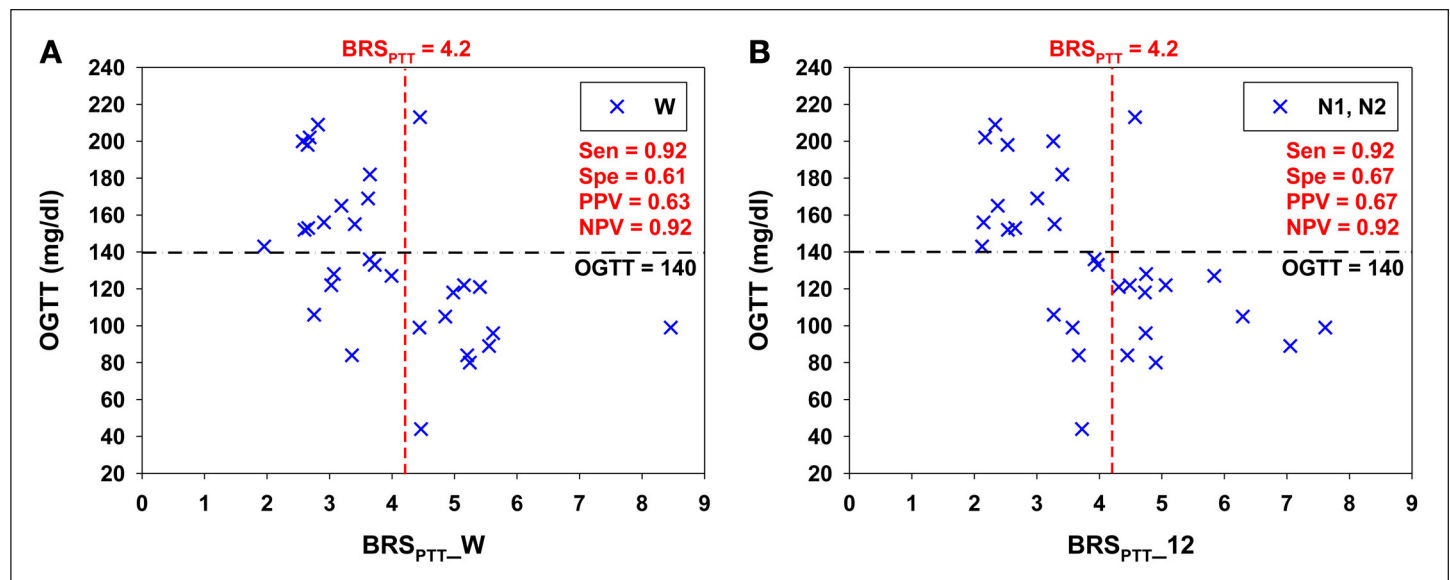
Since SDB itself may be an independent risk factor for IGM, as several studies<sup>7–10,13,14</sup> suggest, it may be argued that the direct effect of SDB on the HRV indices and  $BRS_{PTT}$  may have been masked by the association of SDB with IGM. However, in our study, we included several subjects who had SDB with normal glucose metabolism, and in this group of subjects, RDI was, on average, higher than RDI in the subjects with SDB + IGM. Indeed, RDI was not correlated with OGTT in our study. On the other hand, our study population included only a few subjects with severe SDB ( $RDI > 30 \text{ h}^{-1}$ ). Thus it is possible that including more subjects with severe SDB could affect our conclusion that autonomic function is more strongly linked to IGM than SDB. Another potential limitation of our study is that the vast majority of the subjects studied were overweight to obese ( $BMI > 25 \text{ kg/m}^2$ ). Therefore, it is possible that the relationship between autonomic function and IGM (with or without SDB) may be different in subjects who are not overweight. Indeed, in the subject pool studied here, there was a weak but

almost significant correlation between BMI and OGTT. A third limitation of this study is that the total number of subjects studied was relatively small. This constraint was offset to some extent by the fact that the screening procedure that we had applied to arrive at the pool of data sets that were ultimately analyzed was rigorous, thus assuring that the quality of the data we studied was high.

The strong association between the indices of autonomic function, SDNN,  $BRS_{PTT}$ , and OGTT suggests that the former measures may be useful, from a practical standpoint, as markers of IGM. We have conducted preliminary analyses to test this conjecture. **Figure 5** displays the OGTT values for all 31 subjects plotted against their corresponding values of  $BRS_{PTT}$  measured during wakefulness (**Figure 5**, left panel) and light non-REM sleep (**Figure 5**, right panel). Using a cutoff  $BRS_{PTT}$  value of 4.2, we find that the sensitivity of using  $BRS_{PTT}$  to detect IGM is 92% in both wakefulness and light sleep. The corresponding specificity is, however, less than 70% in both cases. On the other hand, the negative predictive value is 92% in both cases, indicating that subjects with high  $BRS_{PTT}$  are unlikely to have IGM. These results suggest that SDNN and  $BRS_{PTT}$ , derived in patients who have been referred for polysomnography or 24 h Holter monitoring, may be useful in providing complementary information about potential metabolic dysfunction. The ability of these indices to predict progression of metabolic dysfunction will require further evaluation in longitudinal large-cohort studies, such as the MONICA/KORA Augsburg Cohort Study.<sup>51</sup>

## Conclusions

In summary, we derived noninvasive measures of autonomic function from overnight recordings of spontaneous fluctuations in heart rate and finger pulse plethysmogram in 31 overweight subjects who had various degrees of SDB and IGM and all of whom had participated in an OGTT. The measures of autonomic function included time-domain and frequency-domain indices of HRV, as well as  $BRS_{PTT}$ , a surrogate index of cardiac baroreflex sensitivity based on measurements of PTT instead of blood pressure. Following statistical adjustment for age, BMI, and sex, we found that SDNN, one of the HRV indices, and  $BRS_{PTT}$  were correlated with the degree of IGM but not with the severity of SDB in all sleep stages. Our findings suggest that the impairment of cardiac autonomic function that has been reported in subjects with SDB is due more to the IGM that accompanies SDB than to direct effects of respiratory



**Figure 5.** Scatterplots displaying the OGTT and  $BRS_{PTT}$  values measured in all 31 subjects during wakefulness (A) and stage 1 and 2 sleep (B). The OGTT levels > 140 mg/dl represent IGT. Using a  $BRS_{PTT}$  cutoff value of 4.2, the sensitivity and specificity of detecting IGT using  $BRS_{PTT}$  values are as displayed. Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value.

disturbances. Moreover, the abnormal autonomic function involves degradation of baroreflex regulation. We further speculate that SDNN and  $BRS_{PTT}$  may be useful as complementary noninvasive and nonintrusive markers for the early detection of metabolic syndrome in patients who have been referred for PSG or 24 h Holter monitoring.

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