Is There a Glycemic Threshold for Impaired Autonomic Control?

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OBJECTIVE — Although hyperglycemia has been recognized as a predictor of cardiovascular autonomic neuropathy in diabetic patients, the glucose threshold at which autonomic control begins to become impaired has not been evaluated. This study examined whether fasting plasma glucose (FPG) or fasting plasma insulin (FPI) is associated with reductions in baroreflex sensitivity (BRS) in healthy volunteers.

RESEARCH DESIGN AND METHODS — FPG and FPI were measured after an overnight fast in 162 healthy volunteers (91 men, 71 women) who were 25–44 years of age. BRS was measured with power spectral analysis.

RESULTS — Univariate analyses showed that FPG was negatively correlated with BRS ($r = -0.25$, $P \leq 0.001$) with significant reductions observed in volunteers with FPG in the upper 2 quintiles (i.e., 93–124 mg/dl). However, after adjustment for other predictors of BRS (e.g., age, blood pressure, and BMI), the relationship between FPG and BRS was no longer significant. In contrast, FPI was negatively correlated with BRS in univariate analyses ($r = -0.32$, $P < 0.0001$) as well as after covariate adjustment, with close to a 50% reduction in BRS observed in the volunteers with insulin values in the highest quintile (i.e., 16–36 μU/ml).

CONCLUSIONS — These findings suggest that high normal levels of FPG are associated with reduced autonomic control secondary to the effects of aging, obesity, and elevated blood pressure on FPG levels and that elevations in FPI are associated with substantial reductions in autonomic cardiac control independent of other covariates.

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Recent findings that the risk for cardiac events is increased even at fasting plasma glucose (FPG) levels below the diagnostic threshold for diabetes (1,2) have challenged the criteria of defining diabetes with a FPG level of ≥126 mg/dl, a value based on the increased prevalence of microvascular complications at or above this concentration. Cardiovascular autonomic neuropathy has also been shown to predict cardiac mortality (3,4) and is often present at the time of diabetes diagnosis (5,6), which raises the question of whether impaired autonomic nervous system control may occur even at clinically normal glucose levels.

The purpose of the present study was to test whether the FPG level is related to low baroreceptor-mediated control of heart rate over the normoglycemic range of FPG found in healthy subjects. In addition, because some evidence suggests that elevated fasting plasma insulin (FPI) level predicts the development of parasympathetic neuropathy in newly diagnosed type 2 diabetic patients (7), we also evaluated whether FPI level was related to impaired autonomic control in these volunteers.

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Abbreviations: BRS, baroreflex sensitivity; DBP, diastolic blood pressure; ECG, electrocardiogram; FPG, fasting plasma glucose; FPI, fasting plasma insulin; MAP, mean arterial pressure; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

Subjects

A total of 162 men and women volunteers 25–44 years of age were recruited using regional postings and newspaper advertisements. Recruitment criteria excluded individuals with a history of psychiatric disorders, diabetes, or obesity (i.e., 30% above the ideal body weight defined by 1983 Metropolitan Life Insurance tables). Individuals were also excluded if they were smokers, if they had a systolic blood pressure (SBP) level of >160 mmHg or a diastolic blood pressure (DBP) level of >105 mmHg, or if they had a history of taking antihypertensive medications. All subjects read and signed a consent form approved by the Duke University Medical Center Institutional Review Board. Demographic characteristics of the subjects are presented in Table 1.

Baroreceptor reflex assessment procedures

All measurements were taken while subjects were in a supine posture and at least 6 h after their last consumption of caffeine. Blood pressure was measured continuously with the Finapres (Ohmeda, Madison, WI) noninvasive blood pressure monitor with the appropriate size cuff applied to the middle finger of the left hand. This instrument, which uses the vascular unloading technique to measure SBP, DBP, and mean blood pressure level on a beat-by-beat basis, has been validated against intra-arterial measures under various conditions (8). The arm was supported at or close to the level of the heart with precise placement to equal the Finapres mean arterial pressure (MAP) with MAP in the brachial artery measured using the auscultatory technique. An electrocardiogram (ECG) was recorded with 3 disposable ECG chest electrodes, which permitted measurement of the R-R interval as the time interval between successive R-waves.

After 20 min of resting in the supine posture, 5 min of continuous blood pressure and R-R interval measurements were recorded for noninvasive assessment of baroreflex sensitivity (BRS). The automatic

Table 1—*Demographic characteristics of study group*

n	162
Sex (M/F)	91/71
Age (years)	33 ± 6 (25–44)
BMI (kg/m^2)	26.0 ± 3.4 (18.8–38.4)
SBP (mmHg)	120 ± 13 (91–152)
DBP (mmHg)	79 ± 10 (59–102)
Heart rate (beats/min)	66 ± 11 (42–103)
FPG (mg/dl)	91 ± 8 (72–124)
FPI (μU/l)	11 ± 7 (0–36)

Data are *n* or means ± SD (ranges).

servo-adjustment option of the Finapres was disabled for the 5-min recording period. Beat-by-beat SBP and R-R interval were then edited for artifacts, interpolated, and resampled at a frequency of 4 Hz to obtain equally spaced events. Power spectra were estimated with the Welch algorithm (9). Specifically, a fast Fourier transform was applied to the data after detrending and application of a Hanning filtering window. Power spectra were derived as the average of 60-s data segments overlapping by half. For each 60-s segment, 256 points were analyzed, which included 240 sampled points with zero padding. SBP oscillations in the frequency range between 0.070 and 0.129 Hz represent rhythmic fluctuations in vasomotor activity, which are also known as Mayer waves. These blood pressure waves most often show a 10-s periodicity and are substantially reduced after pharmacological blockade of α-adrenergic receptors (10), which suggests an underlying involvement of sympathetic vasomotor activity. The R-R interval oscillations at the baroreflex frequency are reduced after sinoaortic denervation (11) and, when collected under supine conditions, are mediated entirely by vagal control mechanisms (12).

Cross-spectral analysis was performed to assess the magnitude of the R-R interval changes associated with SBP oscillations. Coherence between the changes in SBP and in the R-R interval was required to be at least 0.5 to accept points as baroreflex estimates. BRS was estimated from the modulus of the cross-spectrum of the R-R interval and the SBP for frequencies ranging from 0.070 to 0.129 Hz. Spectral analysis–derived estimates of BRS are significantly correlated with BRS estimated with phenylephrine to stimulate baroreceptor pathways (13).

Measurement of physical activity

An overall fitness score was derived with aerobic activity points based on a system developed by Cooper (14). Subjects reported the number of minutes they engaged in various aerobic activities, such as jogging, biking, tennis, and aerobics class, during the past 7 days. Aerobic activity points were then calculated for each activity on each day of the week, and a total score was obtained by summing all of the points together.

Measurement of FPG and FPI

All blood samples were drawn between 7:00 and 9:00 A.M. after an overnight fast and before the baroreflex assessment test. FPG was measured with the glucose oxidase method, and FPI was determined with radioimmunoassay (Laboratory Corporation of America, Burlington, NC).

Statistics

The relationship between FPG or FPI and each vagal control measure was examined with regression analysis. Analysis of variance was used to compare continuous variables, and χ^2 tests were used to compare categorical variables between groups. In addition, analysis of covariance was used to examine the relationship between BRS and glucose or insulin, with glucose and insulin values used to separate groups into quintiles.

The association between glycemic control and baroreflex control was evaluated using hierarchical multiple regression. Predictors of BRS were entered in the model in 2 steps, with age, blood pressure, and BMI entered in step 1 and FPG and/or FPI entered in step 2.

RESULTS

Predictors of baroreflex cardiac control

The level of BRS was reduced with increasing age ($\beta = -0.29$, $P < 0.0001$), blood pressure ($\beta = -0.20$, $P < 0.01$), and BMI ($\beta = -0.16$, $P < 0.05$). With the addition of physical activity level, BMI was no longer significantly related to BRS (age: $\beta = -0.29$, $P < 0.0001$; blood pressure: $\beta = -0.17$, $P < 0.05$; BMI: $\beta = -0.14$, $P = 0.08$; physical activity: $\beta = 0.21$, $P < 0.005$). BRS could not be estimated in 2 of the volunteers because of poor coherence between R-R interval and SBP.

Effects of FPG

Glucose was significantly related to the physiological variables known to affect BRS (age: $\beta = 0.27$, $P < 0.0005$; blood pressure: $\beta = 0.16$, $P < 0.05$; BMI: $\beta = 0.25$, $P < 0.005$) with the exception of physical activity. Correlational analysis showed that increased FPG levels were significantly related to low levels of BRS ($r = -0.25$, $P \leq 0.001$). As shown in Fig. 1, BRS gradually decreased with increasing FPG through the fourth quintile (upper limit of 97 mg/dl); further increases in FPG produced no further reduction in BRS. The reduction in BRS was largely because of the increased amplitude of the Mayer blood pressure waves ($\beta = 0.31$, $P < 0.0001$), which are thought to reflect sympathetic vasoconstrictor effects (10). Although the R-R interval power at the Mayer wave frequency also increased with increasing FPG level ($\beta = 0.22$, $P < 0.01$), these changes were insufficient to maintain baroreflex control. The observed reductions in BRS associated with FPG were not observed after covariate adjustment for the explanatory variables, which suggests that the relationship between FPG and BRS is largely mediated by the co-occurrence of high blood pressure, elevated BMI, and older age in the volunteers with higher FPG levels.

Effects of FPI

FPI was related to increased BMI ($\beta = 0.37$, $P < 0.00001$) but, unlike FPG, was not related to blood pressure or aging. FPI was also unrelated to level of physical activity. FPI was significantly related to low BRS in both univariate analysis ($r = -0.32$, $P < 0.0001$) and in covariate-adjusted multivariate analysis ($\beta = -0.18$, $P < 0.05$). With the addition of FPI to the model, BMI was no longer significantly related to BRS ($P = 0.34$), which suggests that the relationship between BMI and BRS may be secondary to the effects of insulin. At levels of FPI greater than ~15 μU/ml, BRS levels dropped precipitously (Fig. 2). However, even in a subset restricted to quintiles 1–4 (i.e., in volunteers with an FPI level of <15.7 μU/ml), the relationship between FPI and BRS was maintained ($\beta = -0.17$, $P < 0.05$). In contrast with the effects of FPG, FPI's effects on BRS were mediated entirely by reduced R-R interval power at the Mayer wave frequency ($\beta = -0.17$, $P < 0.05$); no relationship existed between FPI and the amplitude of Mayer waves in blood pressure.

To examine physiological differences that exist among individuals with lower and higher FPI levels, comparisons were made between individuals showing the highest and lowest levels of FPI defined by the

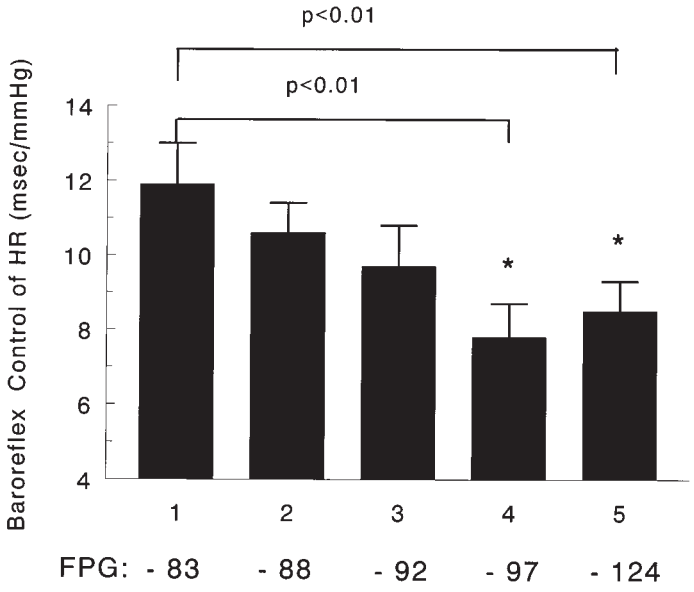

Figure 1—*Baroreflex control of heart rate in healthy volunteers (*n = *160) as a function of quintile of FPG.* *P < *0.01; HR, heart rate.*

upper and lower quintiles of FPI, respectively (lower quintile: $n = 33$, FPI 3.5 ± 2.0 μU/l; upper quintile: $n = 30$, FPI 21.8 ± 5.1 μU/l). BRS was reduced by almost 50% in the volunteers with the higher FPI levels [$F(1, 61) = 24.5$, $P < 0.0001$]. In addition, BMI was significantly higher [$F(1, 62) = 20.5$, $P < 0.0001$] and physical activity was significantly lower [$F(1, 62) = 7.4$, $P < 0.01$] in the group with elevated FPI levels. Although FPG levels tended to be higher in the high FPI group, the elevation was not statistically significant ($P = 0.16$). No differences were evident in age or blood pressure between the 2 groups.

CONCLUSIONS — Impaired autonomic control of heart rate is an early event in the progression of diabetes and is often present at the time of diagnosis (5,7). The present findings of an association between FPG or FPI levels and reduced BRS in healthy nondiabetic volunteers suggest that even modest elevations of FPG or FPI are sufficient to reduce autonomic cardiac control. Furthermore, the finding that elevated FPG levels are not associated with impaired BRS when the effects of age, blood pressure, and BMI are accounted for suggests that glucose may be less directly related to reduced autonomic control than insulin in this population.

These findings are consistent with earlier findings that high FPI more strongly predicts parasympathetic neuropathy in newly diagnosed type 2 diabetic patients than FPG (7). However, other studies have found a more prominent role for glucose level in the development of parasympathetic neuropathy (15,16) in diabetic patients. For example, newly diagnosed type 1 diabetic patients with good glycemic control (as indexed by lower levels of HbA_{1c}) showed higher levels of heart rate variability 1 year after diagnosis when compared with newly diagnosed type 1 diabetic patients with consistently poor glycemic control (15). Similarly, newly diagnosed type 2 diabetic patients who showed a sustained reduction in fasting blood glucose (i.e., maintained for 1 year after an educational program) showed significant improvements in the E/I ratio (defined typically as the ratio between the maximum and minimum R-R intervals averaged across several consecutive cardiac cycles during breathing paced to 6 cycles/min), whereas the patients whose glucose levels were resistant to this intervention showed a reduction in the E/I ratio (16). Failure to control for changes in blood pressure or BMI over time may partially explain these discrepancies. This interpretation is supported by the present findings of a significant relationship between FPG level and reduced BRS only when the effects of blood pressure, age, and BMI are not accounted for. Interventions that reduce blood pressure or body fat may therefore be associated with improved autonomic control independently of parallel improvements in glycemic control. Another source of the discrepancies may be differences in the techniques used to assess autonomic control. For example, although the E/I ratio is a commonly used measure of parasympathetic neuropathy, failure to monitor the rate and depth of the breaths used to derive the E/I ratio can influence the magnitude of respiratory sinus arrhythmia.

In the present study, we used the level of baroreceptor-mediated R-R interval

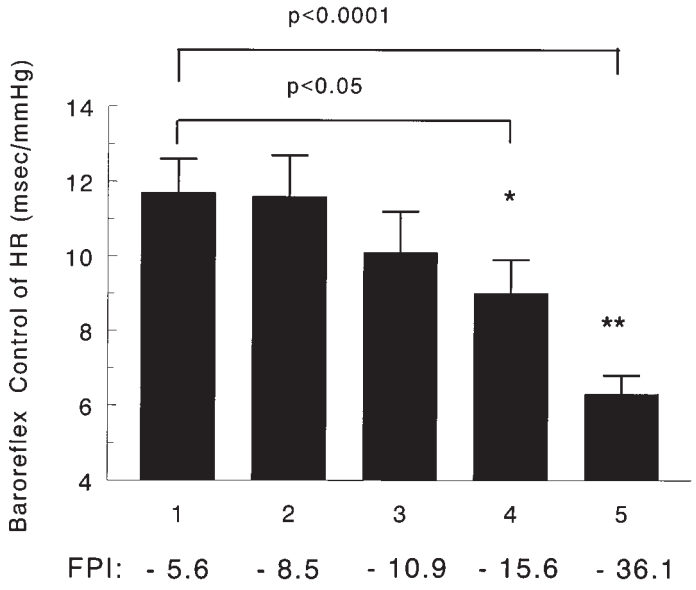

Figure 2—*Baroreflex control of heart rate in healthy volunteers (*n = *160) as a function of quintile of FPI.* *P < *0.05;* **P < *0.0001; HR, heart rate.*

oscillations as our primary measure of autonomic control. Attenuated baroreceptor-mediated heart rate control predicts the risk of sudden cardiac death in experimental animal models (17) and predicts life-threatening arrhythmias and cardiac mortality in patients after myocardial infarction (18,19). Impaired BRS is also reportedly a sensitive measure of cardiovascular autonomic neuropathy (20) and occurs even in diabetic patients with no physical symptoms of autonomic neuropathy (21,22). Until recently, however, measurement of BRS was cumbersome and required intravenous injection of vasoactive drugs to activate or deactivate the baroreflex pathways. In the current study, we measured BRS with a noninvasive procedure involving cross-spectral analysis that derives BRS from the oscillations in R-R interval coincident with the blood pressure oscillations under resting conditions. Spectral analysis–derived estimates of BRS are significantly correlated with BRS estimated with more invasive measures (13,23) and show satisfactory short-term reproducibility (24,25).

The present study found that increased BMI predicted high FPG and FPI levels in nondiabetic volunteers. Interestingly, high levels of FPG were also observed more often in the older volunteers (i.e., >35 years of age) and in volunteers with higher blood pressure levels. Neither age nor blood pressure, however, was associated with higher FPI levels. FPG and FPI also showed a different cardiovascular profile regarding the effects on the sympathetic and parasympathetic limbs of the autonomic nervous system. For example, increased FPG level was associated with both increased amplitude of the Mayer waves and increased amplitude of the R-R interval oscillations coherent with Mayer waves. In contrast, increased FPI was associated with significant reductions in the R-R interval power at the Mayer wave frequency. Under the supine conditions used in the present study, the R-R interval oscillations at the Mayer wave frequency (~1 Hz) are entirely parasympathetically mediated (12), which suggests that insulin may be exerting a vagolytic effect.

Some evidence suggests that high levels of FPI may reduce vagal cardiac control via the sympathoexcitatory effects of insulin. For example, intravenous infusion of insulin has been shown to simultaneously increase muscle sympathetic nerve activity and to reduce vagal control of heart rate (26). Given that sympathetics modulate the release of acetylcholine in an inhibitory fashion via a presynaptic mechanism, this could contribute to reduced cardiac vagal control observed under hyperinsulinemic states. Reduced baroreflex control with hyperinsulinemic states may also be related to the development of arterial stiffening and impaired baroreceptor afferent responses to arterial wall stretch (27) secondary to dyslipidemia and metabolic syndrome X. Insulin resistance has been associated with intra-abdominal fat, findings that have been confirmed by the relationship between FPI and BMI in the present study.

The present findings suggest that high normal levels of FPG or FPI are related to reduced BRS. The effects of FPG on BRS are explained by the association of high normal FPG levels with aging, obesity, and high blood pressure. In contrast, high FPI levels are associated with a decrease in BRS independent of other factors. Additional studies are needed to examine whether the hyperinsulinemia characteristic of type 2 diabetes is similarly associated with reduced BRS and to determine the prognostic significance of the insulin–BRS relationship.

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References

1. Bjornholt JV, Erikssen G, Aaser E, Sandvik L, Nitter-Hauge S, Jervell J, Eriksen J, Thaulow E: Fasting blood glucose: an underestimated risk factor for cardiovascular death: results from a 22-year follow-up of healthy nondiabetic men. *Diabetes Care* 22:45–49, 1999
2. Balkau B, Shipley M, Jarrett RJ, Pyörälä K, Pyörälä M, Forhan A, Eschwège E: High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic men: 20-year follow-up in the Whitehall Study, the Paris Prospective Study, and the Helsinki Policemen Study. *Diabetes Care* 21:360–367, 1998
3. O'Brien IA, McFadden JP, Corrall RJM: The influence of autonomic neuropathy on mortality in insulin-dependent diabetics. *Q J Med* 290:495–502, 1991
4. Rathmann W, Ziegler D, Jahnke M, Haastert B, Gries FA: Mortality in diabetic patients with cardiovascular autonomic neuropathy. *Diabet Med* 10:820–824, 1993
5. Lehtinen J, Uusitupa M, Siitonen O, Pyörälä K: Prevalence of neuropathy in newly diagnosed NIDDM and nondiabetic control subjects. *Diabetes* 38:1307–1313, 1989
6. Pfeifer MA, Weinberg CR, Cook D, Reenan A, Halter JB, Ensinck JW, Porte D: Autonomic neural dysfunction in recently diagnosed diabetic subjects. *Diabetes Care* 7: 447–453, 1984
7. Toyry JP, Niskanen LK, Mantysaari MJ, Lansimies EA, Uusitupa M: Occurrence, predictors, and clinical significance of autonomic neuropathy in NIDDM: ten-year follow-up from the diagnosis. *Diabetes* 45: 308–315, 1996
8. Hartikainen JEK, Tahvanainen KUO, Mantysaari MJ, Tikkanen OE, Lansimies EA, Airaksinen KEJ: Simultaneous invasive and noninvasive evaluations of baroreflex sensitivity with bolus phenylephrine technique. *Am Heart J* 130:296–301, 1995
9. Welch PD: The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short modified periodograms. *IEEE Trans Audio Electroacoust* 15:70–73, 1967
10. Japundzic N, Grichois M, Zitoun P, Laude D, Elghozi J: Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J Auton Nerv Syst* 30:91–100, 1990
11. DiRienzo M, Parati G, Castiglioni P, Omboni S, Ferrari AU, Ramirez AJ, Pedotti A, Mancia G: Role of sinoaortic afferents in modulating blood pressure and pulse interval spectral characteristics in unanesthetized cats. *Am J Physiol* 261:H1811–H1818, 1991
12. Pomeranz B, Macaulay RJB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, Benson H: Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 248:H151–H153, 1985
13. Watkins LL, Grossman P, Sherwood A: Noninvasive assessment of baroreflex control in borderline hypertension: comparison with the phenylephrine method. *Hypertension* 28:238–243, 1996
14. Cooper KH: *Aerobics.* New York, Bantam, 1968
15. Ziegler D, Mayer P, Muhlen H, Gries FA: The natural history of somatosensory and autonomic nerve dysfunction in relation to glycaemic control during the first 5 years after diagnosis of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 34: 822–829, 1991
16. Vanninen E, Uusitupa M, Lansimies E, Siitonen O, Laitinen J: Effect of metabolic control on autonomic function in obese patients with newly diagnosed type 2 diabetes. *Diabet Med* 10:66–73, 1993
17. Billman GE, Schwartz P, Stone HL: Barore-

ceptor reflex control of heart rate: a predictor of sudden cardiac death. *Circulation* 66: 874–880, 1982
18. Hohnloser SH, Klingenheben T, van de Loo A, Hablawetz E, Just H, Schwartz PJ: Reflex versus tonic vagal activity as a prognostic parameter in patients with sustained ventricular tachycardia or ventricular fibrillation. *Circulation* 89:1068–1073, 1994
19. De Ferrari GM, Landolina M, Mantica M, Manfredini R, Schwartz PJ, Lotto A: Baroreflex sensitivity, but not heart rate variability, is reduced in patients with life-threatening ventricular arrhythmias long after myocardial infarction. *Am Heart J* 130:473–480, 1995
20. Lloyd-Mostyn RH, Watkins PJ: Defective innervation of heart in diabetic autonomic neuropathy. *Br Med J* 3:15–17, 1975
21. Eckberg DL, Harkins SW, Fritsch JM, Musgrave GE, Gardner DF: Baroreflex control of plasma norepinephrine and heart period in healthy subjects and diabetic patients. *J Clin Invest* 78:366–374, 1986
22. Lawrence IG, Weston PJ, Bennett MA, McNally PG, Burden AC, Thurston H: Is impaired baroreflex sensitivity a predictor or cause of sudden death in insulin-dependent diabetes mellitus? *Diabet Med* 14:82–85, 1997
23. Robbe HWJ, Mulder LJM, Ruddel H, Langewitz WA, Veldman JBP, Mulder G: Assessment of baroreceptor reflex sensitivity by means of spectral analysis. *Hypertension* 10:538–543, 1987
24. Herpin D, Ragot S: Mid- and long-term reproducibility of noninvasive measurements of spontaneous arterial baroreflex sensitivity in healthy volunteers. *Am J Hypertens* 10:790–797, 1997
25. Lord SW, Clayton RH, Hall MC, Gray JC, Murray A, McComb JM, Kenny RA: Reproducibility of three different methods of measuring baroreflex sensitivity in normal subjects. *Clin Sci* 95:575–581, 1998
26. Van DeBorne P, Hausberg M, Hoffman RP, Mark AL, Anderson EA: Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. *Am J Physiol* 76:R178–R183, 1999
27. Chapleau MW, Cunningham JT, Sullivan MJ, Wachtel RE, Abboud FM: Structural versus functional modulation of the arterial baroreflex. *Hypertension* 26:341–347, 1995