Modified Autonomic Balance in Offsprings of Diabetics Detected by Spectral Analysis of Heart Rate Variability

Claudio De Angelis, Pietro Perelli, Roberto Trezza, Maria Casagrande, Roberto Biselli, Gaetano Pannitteri, Benedetto Marino, and Stefano Farrace

This study was performed to evaluate the influence of family history for non–insulin-dependent diabetes mellitus (NIDDM) on autonomic balance. The latter was assessed by spectral analysis of heart rate variability (SA-HRV) and by analyzing the relative contribution of low-frequency (LF) and high-frequency (HF) components. Twenty glucose normotolerant offsprings of NIDDM parents and 20 controls underwent a 1-hour continuous electrocardiogram (ECG). LF and HF (mean ± SEM in normalized units [NU]), respectively increased and decreased in offspring versus controls. The LF/HF ratio (mean ± SEM) significantly increased (LF/HF = 3.25 ± 0.7 *v* 1.45 ± 0.5, *P* < .0001 offsprings *v* controls). To test a stimulated response, a passive tilting (+ 90°) after 30 minutes of bed rest (0°) was performed in a subsample of subjects (10 offsprings *v* 10 controls). During bed rest, we found significantly higher values of the LF/HF ratio in offsprings versus controls (1.93 ± 0.3 *v* 1.08 ± 0.2, *P* < .05), whereas in the head-up position, the LF/HF ratio value increased to the same levels in the 2 groups (6.48 ± 1.3 *v* 6.89 ± 1.4, not significant [NS]). NIDDM family history is characterized in the basal condition by an imbalance of the autonomic system, which, compared with controls, is expressed by a higher weight of sympathetic and a lower weight of parasympathetic components. No significant differences can be found under stimulated conditions.

HEART FUNCTION is accurately modulated by the neurovegetative system, namely the sympathetic and parasympathetic efferences. These 2 components establish an integrated network, which regulates heart activity beat by beat. Spectral analysis of heart rate variability (SA-HRV) has been successfully introduced in physiology and medicine to investigate the relative influence of sympathetic and parasympathetic neuromodulation in a number of pathophysiologic conditions.[1-4] In both humans and animal models, a high-frequency component (≈ 0.25 Hz) has been established as being determined by vagal activity, whereas both vagal and sympathetic outflows are represented by the low-frequency (LF) component (≈ 0.1 Hz). Alterations of the physiologic patterns of HRV have been reported in both insulin and non–insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively) at all ages,[5-8] and these alterations have been recognized as markers of an underlying condition of autonomic neuropathy.

Nonetheless, the autonomic nervous system plays a rather important physiologic role in the regulation of glucose homeostasis. The stimulatory effects of acetylcholine on insulin secretion[9-11] and, conversely, the inhibitory action displayed by catecholamines at the same level,[12-15] has been well documented. Furthermore, several previous studies have demonstrated the antagonist role played by catecholamines on insulin-mediated glucose metabolism[16-19] and the in vivo effects of vagotomy on glucose disposal.[20,21] Considering these remarkable effects of the autonomic nervous system on insulin-mediated glucose metabolism, it may be argued that alterations of autonomic balance could also be part of the pathophysiologic mechanism, which leads to the onset of glucose intolerance. Thus, the aim of the present study was to evaluate HRV in healthy offsprings of diabetic patients on a continuously recorded electrocardiogram (ECG) in baseline and stimulated conditions (after a head-up tilt).

From the C.S.V. Department of Medicine, Aeroporto Pratica di Mare, Pomezia, Rome; and the Istituto di Chirurgia del Cuore e dei Grossi Vasi, Università degli Studi di Roma "La Sapienza", Rome, Italy.

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Address reprint requests to Claudio De Angelis, MD, C.S.V., Department of Medicine, Aeroporto Pratica di Mare, 00040 Pomezia, Rome, Italy.

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MATERIALS AND METHODS

Subjects

The experimental subjects, whose characteristics are reported in Table 1, were selected from a population of 145 male trainees of the Italian Air Force academy. The whole population was previously screened and found to match the physical and psychological standards adopted by the Italian Air Force and required in order to be licensed as fighter pilots. All of the subjects with a family history of diabetes were enrolled in the study. Family history was assessed by a questionnaire on the clinical status of parents and/or grandparents. From the same population, 20 additional subjects with no family history of diabetes (both IDDM and NIDDM) or other metabolic and endocrine disturbances, matched for age and body mass index, were randomly enrolled as a control group. A written and informed consent to take part in the study was given by all subjects, and a physical examination performed just prior to the study was negative in all of them. No one was under medication.

The subjects with a family history of NIDDM had 1 of the parents affected (average duration from onset 10 ± 4 years). Eight of 20 subjects also had 1 of the grandparents with NIDDM. All affected relatives were in treatment with sulphonylureas, and no one was under insulin treatment. For first-degree relatives, no one reported clinical evidence of complications.

Evaluation of Glucose Tolerance

This was performed by evaluating the plasma glucose, insulin, and C-peptide response to a 120-minute oral glucose tolerance test. After 3 days of a diet with not less than 250 g of carbohydrates/day, all subjects (8:00 AM) underwent a 120-minute oral glucose tolerance test (OGTT), performed according to World Health Organization (WHO) criteria[22] as previously reported.[23]

Table 1. Characteristics of the Experimental Subjects

	Offsprings (n = 20)	Controls (n = 20)
Age (yr)	20.0 ± 1.5	19.8 ± 0.8
Gender	All male	All male
Mean arterial pressure (mm Hg)	95.6 ± 7.4	89.6 ± 7.1
Body mass index (kg/m^2)	24.0 ± 1.2	23.4 ± 1.6
Waist/hip ratio	0.904 ± 0.02	0.898 ± 0.04

Analytical Procedure

Each sample was placed in tubes combined with EDTA and 500 U/mL of aprotinine, centrifuged (10 minutes, 3,000 rpm at 4°C), and stored at -70°C for subsequent hormone assays. After centrifugation, a small volume (10 μL) of plasma was used to determine glucose concentration using the glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma insulin and C-peptide levels were determined by double-antibody radioimmunoassay (Ares Serono, Milan, Italy). All samples were run in triplicate and interassay and intra-assay coefficient of variation (CV) were less than 10%.

HRV and Blood Pressure Data Collection

On a different day, all subjects (3 at a time) underwent a 1-hour continuous ECG (Del Mar Avionics, Model 563, StrataScan Holter Analysis System, Irvine, CA) and ambulatory arterial pressure (Takeda TM 2420, A&D Co, Tokyo, Japan) recordings. After a 12-hour overnight fast, all subjects were admitted to a quiet environment at 8:00 AM. They were requested to remain seated, breath normally and avoid coughing, sighing, moving, or sleeping throughout the study. ECGs and blood pressure recorder devices were applied to all subjects. Forearm cuff blood pressure was measured at 5-minute intervals. On another day, 2 smaller samples of 10 subjects (1 at a time) for each group were evaluated for autonomic response to tilting. All subjects underwent 30 minutes of bed rest (0°) and then a passive tilt to +90°. This body position was maintained for an additional 30 minutes. The tilting maneuver was performed in less than 10 seconds. Throughout the study, ECG Holter and ambulatory blood pressure were recorded by using the same instrumentation described above.

HRV Analysis

Identification of the spectral components was performed by decomposing the signal into a series of sine waves of different amplitudes and sequences using the Fast Fourier Transform mathematical device. This operates on short period (5-minute) segments of data identified on the basis of a stationary signal at the end of each experimental condition (seated, supine, and head-up position). The computer program first calculated the interval tachogram. From tachogram sections of 256 interval values (epochs), time and frequency domain measures of heart rate (as R-R intervals in milliseconds) variability were analyzed in accordance with the European Society of Cardiology/North American Society of Pacing and Electrophysiology (ESC/NASPE) recommendation[24]: the mean of all intervals between adjacent QRS (mean RR), standard deviation of RR intervals (SDNN), the variance of RR intervals over the temporal segment, total power (TP) (0.00 to 0.40 Hz), absolute and normalized LF band power (0.04 to 0.15 Hz) and high-frequency (HF) band power (0.15 to 0.40 Hz), LF/HF ratio. LF and HF in normalized units were calculated with the following formula: [(LF)/(TP − VLF)] × 100 and [(HF)/(TP − VLF)] × 100.

Data Analysis

All data are presented as mean ± SEM. HRV data represent the mean of the values recorded during the 3 epochs ± SEM. The continuous variables were tested for normal distribution using the Kolmogorov-Smirnov 1-sample test, and values (d-max ranging from 0.06 to 0.13) were never statistically significant ($P = .20$). This confirms that the observed data follow the hypothesized normal distribution. To evaluate whether the control and experimental groups showed normal and similar responses to the glucose tolerance test, 1 multivariate analysis of variance (MANOVA) for the glucose tolerance test data: *group* (offsprings *v* controls) by *time* (0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes) was performed considering plasma glucose, insulin, and C-peptide as dependent variables. To evaluate differences in HRV between control and experimental groups, 1 MANOVA was performed for the seated HRV data (n = 20/group) and considered only 1 between factor: *group* (offsprings *v* controls). Although, the *t* test for independent samples is the most commonly used method to evaluate differences between 2 groups, the MANOVA gives a global multivariate measure of between group differences and also allows a univariate analysis of variance (providing the same results as the *t* test), thus enabling identification of the specific dependent variables that contribute to the overall significant effect. One MANOVA was performed for the head-up tilt data (n = 10/group), considering the *group* (offsprings *v* controls) as between factor and the *position* (supine *v* head-up tilt) as within factor. Finally, to identify the specific contribution of each dependent variable to the significant multivariate effects, univariate F tests for each dependent variable were examined. Tukey honest significant difference (HSD) post hoc comparisons were used to evaluate univariate *group* by *position* interactions. The Statistica 4.1 software package (StatSoft, Tulsa, OK) was used for statistical analysis. A P value of .05 was considered statistically significant.

RESULTS

Evaluation of Glucose Tolerance

The multivariate effect of *group* was not significant (Rao's $R = 2.1$; degree of freedom [df] = 3.36; $P = .15$), as was the multivariate interaction of *group* and *time* (Rao's $R = 3$; $df = 12.27$; $P = .07$). Univariate results confirm nonsignificant differences between groups for each dependent variable (glucose, $F_{1.38} = 4.2$; $P = .06$; insulin, $F_{1.38} = 5.1$; $P = .08$; C-peptide, $F_{1.38} = 2.8$; $P = .11$) and also for each *group* by *time* interaction (glucose, $F_{4.152} = 1.7$; $P = .17$; insulin, $F_{4.152} = 1.5$; $P = .21$; C-peptide, $F_{4.152} < 1$). Figure 1 summarizes glucose, insulin, and C-peptide response curves, respectively. In both groups, glucose, insulin, and C-peptide responses to glucose load were within the physiologic range, with no signs of glucose intolerance, according to WHO evaluation criteria.[22]

Seated HRV Analysis

MANOVA showed a significant multivariate effect (Rao's $R = 6.8$; $df = 8.31$; $P = .0001$). Table 2 reports time and frequency domain measures (mean ± SEM) and ANOVA results of HRV in offsprings and controls in the seated position.

Supine and Head-Up Tilt Data HRV Analysis

Table 3 reports time and frequency domain measures (mean ± SEM) and ANOVA results of HRV in offsprings and controls in both supine and head-up positions.

MANOVA showed a significant multivariate main effect for the *position* factor (Rao's $R = 38.1$; $df = 8,9$; $P = .0001$). The multivariate effect of *group* was not significant (Rao's $R = 2.4$;

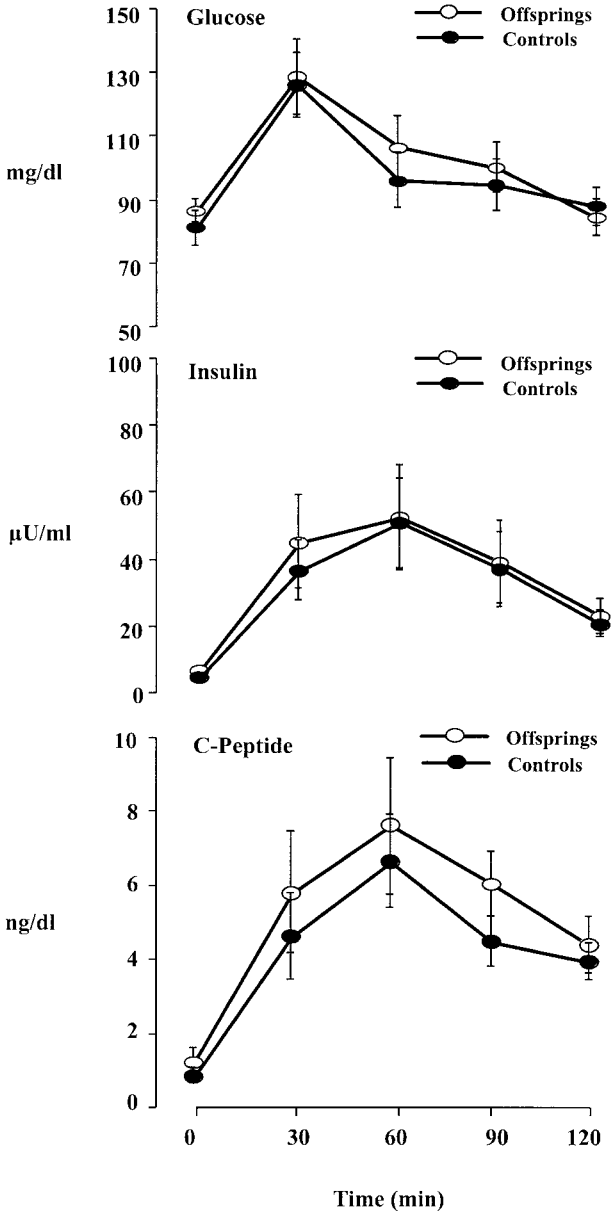

Fig 1. Plasma glucose (A), insulin (B), and C-peptide (C) response curves to a 75-g OGTT in diabetic offsprings (○) and matched controls (●).

df = 8.9; $P = .14$), while the multivariate interaction of *group* by *position* was significant (Rao's $R = 4.0$; *df* = 8.9; $P = .04$). Tukey HSD post hoc comparisons of the means for each of the 3 significant univariate interactions showed that in the supine position the absolute values of LF were higher ($P = .03$) in offsprings as compared with controls; the normalized values of LF were higher ($P = .002$) in offsprings as compared with controls; the normalized values of HF were lower ($P = .002$) in offsprings as compared with controls; the LF/HF ratio values were higher ($P = .05$) in offsprings as compared with controls. No significant differences were found for both systolic and diastolic blood pressure (SBP and DBP) between the groups.

DISCUSSION

Among the various indexes that are an expression of the vegetative control of the cardiovascular system, the LF/HF ratio of HRV is considered to be the most effective. In the present study, we found a significant increase in this index for the offspring of diabetic patients compared with controls in resting conditions (seated and supine). We also found a significant difference in normalized LF and HF. The expression of LF and HF in normalized units (NU) emphasizes the behavior of the 2 branches of the autonomic nervous system and, at the same time, minimizes the effect of changes in TP on the values of LF and HF components. Thus, while in absolute values LF is decreased or unchanged during head-up tilt, the same values are increased when normalized. Therefore, these are more effective in describing sympathovagal interaction and equilibrium.

Of the other nonspectral indexes (eg, mean RR, SDNN), only the SDNN in the seated position was significantly lower in the offspring group. However, even when not significantly different from the control values, they were also all moving towards a decrease in the offspring group during rest conditions, which is consistent with the significant increase of the ratio to indicate an increase of sympathetic and/or a decrease of parasympathetic activity. Interestingly, when the autonomic nervous system was maximally stimulated by the head-up tilt, it showed the expected sympathetic activation in both groups at comparable levels. This seems to indicate that the response of the neurovegetative system to the orthostatic stress in offsprings of diabetics is still maintained and also suggests that resting experimental conditions are more appropriate to detect fine differences. A

Table 2. Time and Frequency Domain Measures (mean ± SEM) and ANOVA Results of HRV in Offsprings and Controls in the Seated Position

	Mean ± SEM		ANOVA Results	
	Offsprings	Controls	$F_{1,38}$	*P**
Mean RR (msec)	872.3 ± 43.0	936.5 ± 29.7	2.0	.17
SDNN (msec)	72.7 ± 5.8	93.9 ± 7.4	6.4	.01
Absolute LF ($msec^2$)	3034.6 ± 530.9	3,366.8 ± 570.7	<1	—
Absolute HF ($msec^2$)	956.8 ± 442.8	2,463.7 ± 637.6	5.0	.03
Total power ($msec^2$)	5,347.4 ± 1,046.0	9,135.4 ± 1,432.8	4.9	.03
Ratio LF/HF	3.2 ± 0.7	1.4 ± 0.5	36.7	.00001
Normalized LF values	76.4 ± 3.1	59.0 ± 2.8	39.1	.00001
Normalized HF values	24.2 ± 3.1	41.9 ± 2.3	29.4	.0001

NOTE. Normalized LF and HF are calculated as follows: [(LF)/(TP − VLF)] × 100 and [(HF)/(TP − VLF)] × 100.

Abbreviations: LF, low frequency; HF, high frequency; SDNN, standard deviation.

*Only $P < .20$ are reported.

Table 3. Time and Frequency Domain Measures (mean ± SEM) and ANOVA Results of HRV in Offsprings and Controls in Both Supine and Head-Up Positions

	Supine Mean ± SEM		Head-Up Mean ± SEM		ANOVA Results					
					Group		Position		Group by Position	
	Offsprings	Controls	Offsprings	Controls	$F_{1.18}$	*P**	$F_{1.18}$	*P*	$F_{1.18}$	*P*
Mean RR (msec)	1,082 ± 54.1	1,097.2 ± 23.7	830.5 ± 37.6	906.2 ± 33.4	<1	—	114.4	.00001	1.65	—
SDNN (msec)	71.7 ± 6.8	86.7 ± 7.7	58.9 ± 7.2	65.4 ± 6.1	3.6	.08	11.0	.005	<1	—
Absolute LF ($msec^2$)	2,686.1 ± 553.2	2,313.2 ± 711.1	3,059.1 ± 751.2	2,952.1 ± 331.2	<1	—	2.0	.18	<1	—
Absolute HF ($msec^2$)	1,380 ± 495.1	2,153.1 ± 600.6	473.4 ± 123.5	410.6 ± 107.9	1.88	.19	60.2	.00001	6.4	.02
Total power ($msec^2$)	5,489.7 ± 1,040.3	7,108.2 ± 1,320.4	4,908.8 ± 840.5	5,266.8 ± 957.3	<1	—	4.1	.06	1.1	—
Ratio LF/HF	1.9 ± 0.3	1.1 ± 0.2	6.5 ± 1.3	6.9 ± 1.4	<1	—	83.7	.00001	4.8	.05
Normalized LF values	61.9 ± 4.2	42.9 ± 3.7	84.9 ± 1.5	86.3 ± 4.2	3.0	.10	227.5	.00001	13.4	.003
Normalized HF values	31.3 ± 2.8	50.8 ± 2.9	15.2 ± 1.5	15.5 ± 3.2	8.2	.01	203.2	.0001	31.0	.0001

Abbreviations: LF, low frequency; HF, high frequency; SDNN, standard deviation.
*Only $P < .20$ are reported.

limitation of the present study was the lack of respiration monitoring, which should have been an important parameter in our experimental condition to confirm the behavior of vagal activity in the different body positions. On the whole, these data clearly indicate an autonomic nervous system imbalance in the offsprings, due to an increase in LF and a decrease in HF components, which may be interpreted, from a functional point of view, as an increase in sympathetic activity. This particular pattern of sympathovagal equilibrium observed in the offsprings was not associated with impaired glucose tolerance, because the glucose, insulin, and C-peptide response curves were all within the physiologic range, with no significant differences compared with controls. However, by performing an OGTT, we measured only glucose tolerance, which does not provide specific data on the level of insulin sensitivity, since the gold standard technique to measure insulin sensitivity is the euglycemic clamp study. Previous experimental evidence has demonstrated that genetically prone individuals maintain the same metabolic profile and glucose tolerance through a compensatory hyperinsulinemia[25] as a result of a reduced sensitivity. In the case of a significant increase in basal and/or stimulated insulin levels in the offsprings, the altered autonomic function could have been the result of an increase in norepinephrine output induced by hyperinsulinemia.[26] With regard to our experimental population, other than the normal trend of the response curves to the glucose load, the basal levels of plasma insulin and C-peptide were not even statistically different between the 2 groups, and the offspring were in any case certainly not hyperinsulinemic in the fasting state (fasting insulin, 4.6 ± 1.4 μU/mL in offspring *v* 3.7 ± 0.9 μU/mL in control). Alterations of autonomic patterns in a selected population of diabetic offsprings were recently reported in the study by Laitinen et al[27] in which the presence of autonomic alterations in diabetic offsprings was shown to be related to the type 2 diabetic fenotype of the probands population. Modified patterns of HRV were, in fact, detected in response to a dynamic metabolic challenge (ie, the hyperinsulinemic clamp). Our study is instead characterized by the finding of significant differences of autonomic patterns in the basal, not stimulated, condition in young subjects in whom, regardless of family history, the NIDDM fenotype should not yet have been expressed. It should also be stressed that under our experimental conditions many of the environmental confounding variables, which could influence the level of insulin sensitivity, such as the level of physical activity, lifestyle, and/or dietary habits, can be excluded. All of the experiments were, in fact, performed on highly selected individuals whose conditions must match considerably high physical and psychological standards because of their future work as fighter pilots. During the period in which the study was performed, all subjects were regularly attending the flight academy. This period is purposely characterized by a very standardized lifestyle, including a balanced diet, strong physical activity, and a daily schedule and workload. Under these conditions, it is likely that the main difference between the 2 groups was represented by the presence of a family history for NIDDM. Autonomic nervous system dysfunction can normally be found in clinically advanced diabetes mellitus. In this context, it is considered a complicance rather than an early feature of the disease. We have shown the presence of an alteration in autonomic balance which, as explained above, seems to be only related to the presence of a family history of NIDDM. From our data, it is still not possible to define what the pathogenetic relevance might be of these findings in the possible development of glucose intolerance and, later, of diabetes mellitus. However, it should be noted that our offspring were all in an excellent condition of physical fitness, with no signs of glucose intolerance, but with a significantly different pattern of their resting autonomic balance compared with controls. From data analysis, this different pattern suggests the presence of an increased adrenergic expression. Considering the physiologic role played by the sympathetic network in the regulation of the glucose metabolism, namely an antagonist action, we believe that our findings represent a useful background for further investigation to define the pathogenetic impact of a precocious autonomic imbalance (which, in turn, is probably the expression of a constitutional inherited factor) in the possible development of NIDDM.

In conclusion, this study demonstrates for the first time the presence of altered autonomic activity in healthy offsprings of NIDDM patients, both as an increased sympathetic tone and a

decreased parasympathetic tone. These results can be interpreted functionally as an increased sympathetic activity. This increment precedes clinical evidence for glucose intolerance and strongly suggests the need for further research into the autonomic balance of the offspring of diabetics. Furthermore, the family history of NIDDM should be taken into account whenever measures of power SA-HRV have to be performed in normal subjects.

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