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The effects of a glucose load and sympathetic challenge on autonomic function in obese women with and without type 2 diabetes mellitus

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Abstract

This study examined the effect of glucose ingestion on cardiac autonomic function in nonobese women and obese women with and without type 2 diabetes mellitus. Heart rate variability was measured via continuous electrocardiogram, and beat-by-beat blood pressure was recorded using finger photoplethysmography (Portapres, TNO Biomedical Instrumentation, Amsterdam, The Netherlands) in a fasted state and in response to a 75-g glucose load in 42 middle-aged women (40-60 years). Upright tilt was also used as an orthostatic stress to provide a clinically relevant challenge to the cardiovascular system. Significant main effects for log-transformed (Ln) total power (TP, square milliseconds) were observed with upright tilt ($P < .01$) and glucose challenge ($P < .05$). LnTP decreased in all groups in both the fasted and fed state with upright tilt ($P < .01$), but glucose ingestion resulted in higher LnTP in the supine position only ($P = .008$). Tilt resulted in a significant main effect for low-frequency (LFnu, calculated in normalized units) and high-frequency (HFnu, calculated in normalized units) power ($P < .000$), whereas the glucose challenge had no effect on LFnu or HFnu power. LFnu approached significance for group differences ($P = .07$), such that the nonobese had lower LF power than either of the obese groups. Sympathovagal balance (LnLF/HF ratio) was affected by position ($P < .000$) and group ($P < .05$), with a lower LnLF/HF in the nonobese than in the obese women. Baroreceptor sensitivity decreased ($P < .01$) during upright tilt but was not changed by the glucose challenge. In conclusion, basal sympathovagal balance is higher in obese individuals with and without type 2 diabetes mellitus. Women with type 2 diabetes mellitus showed no differences in autonomic function with an orthostatic challenge or glucose load than nondiabetic, obese women. The glucose load did alter total spectral power in all of these middle-aged women but had no impact on baroreceptor sensitivity.

1. Introduction

Heart rate (HR) and blood pressure (BP) are carefully regulated by the autonomic nervous system (ANS). Dysfunction of the autonomic system (eg, increased sympathetic and decreased parasympathetic activity) are related to increased mortality [1,2], and this dysfunction has been reported in obese individuals [3-6]. In addition, perturbations to the ANS occur with both changes in body position and with food ingestion [7]. In particular, carbohydrate ingestion, but not fat or protein ingestion, increases sympathetic nerve activity [8,9], whereas fasting has an opposite effect [9].

The alterations observed in ANS activity after carbohydrate ingestion are associated with hyperinsulinemia [10]. In healthy, nonobese individuals, physiologic hyperinsulinemia causes acute desensitization of sinus node activity, producing a shift in autonomic balance toward increased sympathetic modulation [4]. Rowe et al [11] has demonstrated that acute euglycemic hyperinsulinemia causes a dose-dependent increase in circulating norepinephrine levels

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Table 1
List of medications the subjects were taking during the study

	Obese, type 2 diabetic (n = 9)	Obese, nondiabetic (n = 22)	Nonobese (n = 11)
Glucose-lowering drugs			
Metformin	7	0	0
Sulfonylurea	0	0	0
Other	3	0	0
Lipid-lowering drugs			
Statin	1	4	0
Fibrate	1	0	0
Other	1	0	0
Antihypertensives			
HCTZ	1	1	0
ACE-I	4	0	1
Valsartan	0	2	1
Hormone replacement therapy	0	3	2
Antidepressants	6	4	2
Other drugs	9	7	2
No medications	0	8	5

HCTZ indicates hydrochlorothiazide; ACE-I, angiotensin-converting enzyme inhibitor.

in healthy volunteers, whereas others [12] have demonstrated no change in sympathetic nervous system activity.

Obesity is usually associated with increased sympathetic activity [2,13,14], which is probably related to insulin resistance, but may also be due to the elevated circulating insulin levels in these individuals. As many obese individuals are in a state of chronic hyperinsulinemia [4], the alterations in sympathetic activity are believed to be chronic. Healthy nonobese subjects show reductions in total spectral power of HR variability (HRV) and an increased low-frequency/high-frequency (LF/HF) ratio during an insulin infusion, whereas these changes do not occur in insulin-resistant individuals [15]. We speculate that although obese individuals with insulin resistance have sympathetic hyperactivity at rest, they respond with reduced sympathoexcitation to an immediate increase of insulin levels. Chronic hyperinsulinemia may prevent enhancement of cardiac sympathetic tone during an acute rise in insulin levels possibly due to an attenuated baroreflex response [15].

Although acute euglycemic hyperinsulinemia alters the HRV response in healthy individuals [3,4], little research on autonomic function has been conducted in the obese population during an acute glucose load and a physiologic challenge [15,16], and no information is available on obese individuals with type 2 diabetes mellitus. Quantifying the ANS response to a physiologic challenge can provide important information on autonomic dysfunction not evident at rest [17]; such information is important to elucidate the autonomic response to common everyday challenges in these populations.

The primary purpose of this study was to establish the effect of a glucose load on autonomic function in nonobese women, obese women, and obese women with type 2 diabetes mellitus. We also used upright tilt as an orthostatic stress, which provides a clinically relevant challenge to the cardiovascular system [18]. We hypothesized that the obese women with and without type 2 diabetes mellitus would have reduced HRV and greater sympathetic activation at rest but an attenuated response to tilt and to the glucose challenge compared with the nonobese women. Furthermore, we hypothesized that the obese women with type 2 diabetes mellitus would have greater autonomic dysfunction than the obese women without type 2 diabetes mellitus.

2. Methods

2.1. Subjects

Forty-two healthy women volunteered for the present study. Subjects were classified into 3 groups based on their body mass index (BMI) and metabolic status: nonobese (BMI $\leq$24 kg/m^2, n = 9, fasting glucose $<$100 mg/dL), obese (BMI $\geq$30 kg/m^2, n = 22, fasting glucose $<$100 mg/dL), and obese, type 2 diabetic (BMI $\geq$30 kg/m^2, n = 11, fasting glucose $\geq$126 mg/dL, and 2-hour oral glucose tolerance test [OGTT] glucose $\geq$200 mg/dL). All subjects were physically inactive and had not been involved in any regular exercise during the past 6 months. Diabetic subjects did not have peripheral neuropathy. Subjects were nonsmokers and had no signs or history of overt heart disease, verified by a physician-supervised maximal exercise stress test. Both pre- and postmenopausal women were included, but subjects were excluded if they reported missed menstrual periods. Postmenopausal women on hormone replacement therapy (n = 5) were included. Premenopausal women were studied in the first 10 days of their menstrual cycle. Subjects should not be taking β-blockers or any medication that could alter their HR or BP responses. The list of medications is presented in Table 1. The institutional review boards at Syracuse University and State University of New York Upstate Medical University approved the protocol. Written informed consent was obtained from all subjects.

2.2. Experimental design

Subjects came to our laboratory on 2 occasions. Initially, the subjects had an exercise stress test that was used to determine cardiovascular fitness and to screen for heart disease. On the second visit, the subject came to the laboratory at 7:00 AM after a 12-hour overnight fast. Autonomic function was measured at rest and during upright tilt, and before and during an OGTT. A venous catheter was inserted into an anticubital vein and kept patent with normal saline. This was followed by 30 minutes of rest in a supine position. Heart rate variability was measured for the final 10 minutes in the supine position and 5 minutes in the upright tilt position. Upon completion of the upright tilt in the fasted state, the OGTT was initiated and blood samples were drawn for 4 hours. Thirty minutes after the glucose ingestion, the period during which blood glucose peaked, the nondiabetic and obese subjects repeated the upright tilt. On the basis of previous studies [19,20], we repeated the autonomic function

testing 1 hour after glucose ingestion in the women with type 2 diabetes mellitus, as we have found the peak glucose levels occur at this time in this population. The subjects then rested quietly for the remainder of the study day. Anthropometric measures were obtained during this quiet period.

2.3. Anthropometric testing

Height and weight were taken and BMI was calculated. Percentage of body fat was measured using the Bod Pod (Life Measurements, Concord, CA), according to the guidelines established by the manufacturer.

2.4. Oral glucose tolerance test

Upon completion of the fasting measures of autonomic function, subjects were given 75 g of glucose to be consumed in less than 5 minutes (NERL Diagnostics, East Providence, RI). Blood samples were drawn before and every half hour for 4 hours during the OGTT. During the second autonomic function testing, which occurred either at 30 minutes or 1 hour after glucose ingestion, blood samples were taken every 10 minutes to establish the peak glucose levels.

2.5. Upright tilt test

Subjects were supine on the tilt table for 20 minutes before any measurements. Heart rate and hemodynamic data were measured during 5 minutes of quiet rest with spontaneous breathing, 5 minutes of paced breathing (12 breaths per minute), and 5 minutes of 80° head-up tilt with paced breathing. Subjects were instructed not to move their hands or feet throughout the testing. This same procedure was repeated in the glucose-challenged state.

2.6. Autonomic measurements

We continuously recorded electrocardiographic R-R intervals via a modified CM5 lead interfaced with a digital acquisition system (Biopac, Santa Barbara, CA). Data were sampled at 1000 Hz and saved to a personal computer for off-line analysis. Also, beat-by-beat finger plethysmographic arterial pressure (Portapres, TNO Biomedical Instrumentation, Amsterdam, The Netherlands) was collected at a sampling rate of 100 Hz. The subject's arm with the cuffed finger was placed in a sling with the hand at heart level. Electrocardiographic R-R intervals and beat-by-beat arterial pressure were measured in the supine position at rest and during tilt before and during an OGTT. The breathing pace was set at 12 breaths per minute (0.2 Hz) and was set by a metronome.

2.7. Data analysis

2.7.1. Heart rate variability

Heart rate variability analysis was conducted using Heart Signal software (Oulu, Finland), and data were analyzed in 5-minute epochs. The electrocardiographic signal was filtered via visual and automatic editing and only periods with no ectopic beats were analyzed. After filtering, only data with fewer than 2% of beats removed were included in the HRV analysis. The filtering and analysis of the R-R intervals were conducted according to procedures described by Huikuri et al [21].

The total area under the curve was taken as the overall variability—total power (TP)—and was obtained by integrating the spectral band from 0.004 to 0.400 Hz. The power spectra were divided into bands of 0.040 to 0.150 Hz as LF and 0.150 to 0.400 Hz as the HF band [23] using an autoregressive model (fixed model order of 10) [22]. The very low-frequency power was obtained for the region below 0.040 Hz and was subtracted from the total power when the data were normalized. Sympathetic modulation of the heart has been associated with the LF spontaneous oscillations of the spectral period [24], whereas the HF component has been shown to be primarily influenced by the efferent vagal activity [6]. As an indirect measure of sympathovagal balance the LF/HF ratio has been used. HF and LF power spectral densities were calculated in both absolute and normalized units (normalized to total spectral power), as suggested by the task force [23].

2.7.2. Analysis of beat-by-beat arterial pressure

R-R intervals and systolic BP (SBP) derived from the finger arterial pressure waveform were used to determine the coupling between fluctuations in HR and SBP (arterial baroreflex sensitivity [BRS]). Baroreflex sensitivity was determined via time domain (sequence technique) and frequency domain analyses using the WinCPRS software (Absolute Aliens Oy, Turku, Finland).

2.7.3. Time domain analysis

We estimated BRS using the sequence technique (WinCPRS software), which searches for runs of 3 or more consecutive beats characterized by a progressive increase or a decrease in SBP of at least 1 mm Hg. Baroreflex sequences were selected from the changes in SBP if R-R intervals concurrently changed in the same direction with SBP for 3 or more consecutive beats for at least 4 milliseconds. Sequences of increasing SBP and R-R intervals were defined as "up sequences," whereas decreasing SBP and R-R intervals were defined as "down sequences." Baroreflex sensitivity was calculated as the slope of the regression line between SBP and R-R intervals. Only sequences with correlations of 0.80 or higher were accepted.

2.7.4. Frequency domain analysis

We calculated the squared coherence for the R-R interval and arterial pressure signals derived from the finger arterial pressure waveform dividing the cross-spectral densities of systolic pressure and R-R interval by the product of the individual spectral densities. The mean value of the square root of the ratio of the spectral powers of R-R interval and SBP was calculated when squared coherence was greater than 0.5. We considered only those frequency components in the LF range (αLF). Finally, we calculated the cross-

Table 2
The physical characteristics of the subjects by group

	Obese, type 2 diabetic (n = 9)	Obese, nondiabetic (n = 22)	Nonobese (n = 11)
Age (y)	49.7 ± 1.8	48.7 ± 1.1	48.2 ± 1.6
Height (cm)	160.3 ± 1.8	164.8 ± 1.2	164.2 ± 1.7
Weight (kg)	89.4 ± 3.9	98.3 ± 2.5	61.0 ± 3.6*
BMI (kg/m^2)	34.8 ± 1.4	36.8 ± 0.8	22.6 ± 1.2*
% Fat	43.5 ± 1.6	46.5 ± 1.0	32.4 ± 1.4*
$\dot{V}O_2$ (mL kg LBM^{-1} min^{-1})	39.2 ± 1.4	40.8 ± 3.7	45.1 ± 5.5*
HbA_{1c} (%)	6.9 ± 0.3	6.1 ± 0.3	5.4 ± 0.1
Waist circumference (cm)	111.9 ± 3.6	107 ± 2.3	79.1 ± 3.2*
Fasting glucose (mmol/L)	7.5 ± 1.8†	5.1 ± 0.2	4.7 ± 0.4
Glucose area under the curve (min · mmol/L)	2653 ± 137.7†	1387 ± 38.8	1297 ± 63.1
Fasting insulin (pmol/L)	113.0 ± 32.8	135.4 ± 16.5	53.4 ± 18.7‡
Peak insulin concentrations (pmol/L)	466.0 ± 87.9	662.2 ± 104.0	339.7 ± 78.1
Insulin sensitivity	4.5 ± 2.5	6.1 ± 1.9	19.6 ± 2.5*

Data are expressed as mean ± SE. $\dot{V}O_2$ indicates oxygen consumption per unit time; LBM, lean body mass; HbA_{1c}, hemoglobin A_{1c}.
*$P < .01$, nonobese vs obese and type 2 diabetic.
$^{\dagger}P < .01$, type 2 diabetic vs nonobese and obese.
$^{\ddagger}P < .01$, nonobese vs type 2 diabetic.

spectral transfer function at LF ranges (LFgain) by dividing the cross-spectra of the 2 signals by the power spectrum of systolic pressure. Both αLF and LFgain were used as indices of baroreflex gain.

2.7.5. Blood analyses

Plasma glucose concentrations were determined using the glucose oxidase method with the YSI 2300 Stat (Yellow Springs Instruments, Yellow Springs, OH). Blood samples for insulin were placed in EDTA tubes, centrifuged (2300 rpm), aliquoted, and stored at −80°C for later analysis. All samples for 1 subject were analyzed in the same assay, and commercially available radioimmunoassay kits were used (Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation for the insulin assays were 7.6% and 8.9%, respectively. Hemoglobin A_{1c} was determined with kits from Diabetes Technologies (Thomasville, GA). Insulin sensitivity was calculated using the Mari equation [25].

2.8. Statistical analysis

A 1-way analysis of variance for differences in descriptive characteristics between the nonobese group, the obese group, and the obese group with type 2 diabetes mellitus was conducted. All data are expressed as mean ± SE. Significance was set at $\alpha = .05$.

By creating a histogram of the data, normal distribution of the data was checked and skewness was calculated. Natural log transformation (Ln) was used if the data were not normally distributed. The data were analyzed using a 3-way analysis of variance with repeated measures (3 [groups] × 2 [supine, tilt] × 2 [fasted, glucose challenge]). If significant interactions were found, we followed up with post hoc analyses using the Tukey test and applied Bonferroni corrections to the post hoc analyses. A correlation analysis was conducted to determine if there was an association between glucose and insulin levels and HRV. We assumed significance with $\alpha = .05$ and used 2-tailed statistical analyses so that results in either direction could be interpreted.

3. Results

The subject characteristics are presented in Table 2. As expected, the BMI was higher in both of the obese groups, but there was no difference in BMI between the obese with and without type 2 diabetes mellitus. No group differences were found for age and hemoglobin A_{1c}, but we found significantly higher percentage of body fat and lower aerobic fitness ($P < .01$) in the obese women than in the nonobese women. Fasting glucose levels and glucose area under the curve were higher in the women with type 2 diabetes mellitus than either group of nondiabetic women ($P < .01$). Fasting insulin concentrations were higher in the women with type 2 diabetes mellitus than in the nonobese women ($P < .01$). As expected, insulin sensitivity was higher ($P < .01$) in the nonobese group than in either of the obese groups.

3.1. Heart rate variability

Resting HR was similar between the nonobese and obese women with and without type 2 diabetes mellitus. Mean resting HR was 68.7 ± 2.1 beats per minute in all groups, and the subjects responded with an increase in HR (82.6 ± 2.9 beats per minute) with upright tilt ($P < .05$). There was no significant change in the mean supine or tilt HR in response to the glucose challenge.

Fig. 1 shows the HRV data expressed in square milliseconds and normalized units, and log transformed if necessary. When all groups were pooled together, total power (square milliseconds, Fig. 1A) significantly decreased from the supine to upright position during fasting ($P < .01$) and glucose challenge ($P < .05$). Both of the main effects remained when total power was log transformed ($P < .01$ and $P < .05$, respectively, Fig. 1B). There

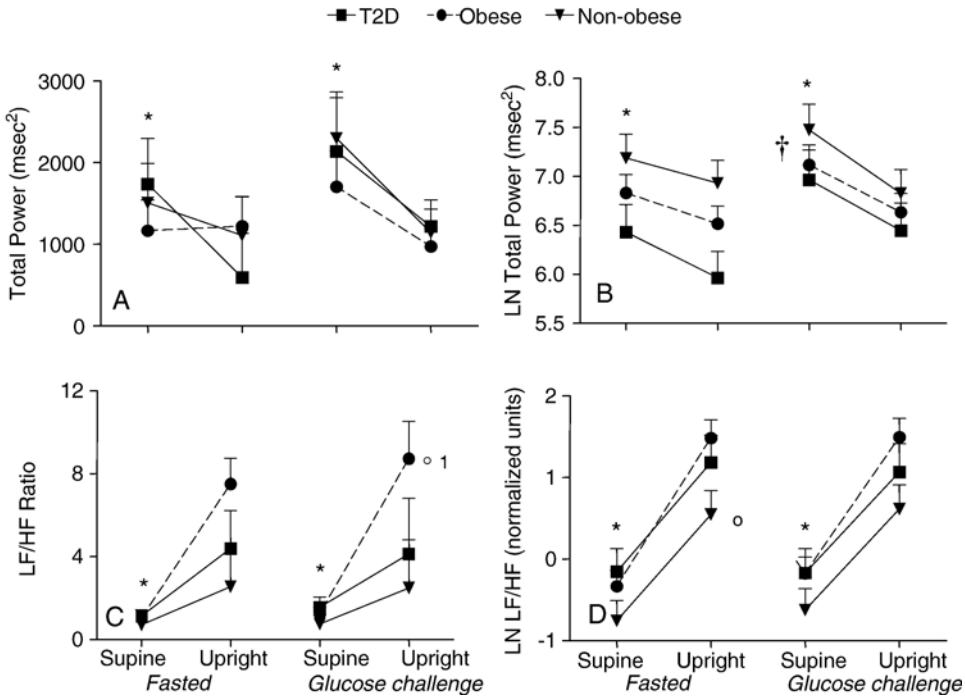

Fig. 1. Total power and the LF/HF ratio for each group in the supine position and in response to tilt. The data are also presented in the fasted and glucose-challenged condition. The left panel represents the raw data and the right panel is the natural log. Data are expressed as mean ± SE. *$P < .01$, supine vs upright; $^{1}P < .05$, tilt-by-group interaction; $^{\circ}P < .05$, between groups; $^{\dagger}P < .000$, interaction between glucose challenge and position.

was an interaction between glucose challenge and position ($P < .000$). With upright tilt, there was a decrease in log-transformed total power (LnTP) in all groups in both the fasted and fed state. Glucose ingestion however resulted in a significantly higher LnTP in the supine position ($P = .008$) when compared with the fasted condition, but this was not significant in the tilted state. There was no significant difference between the 3 groups for LnTP.

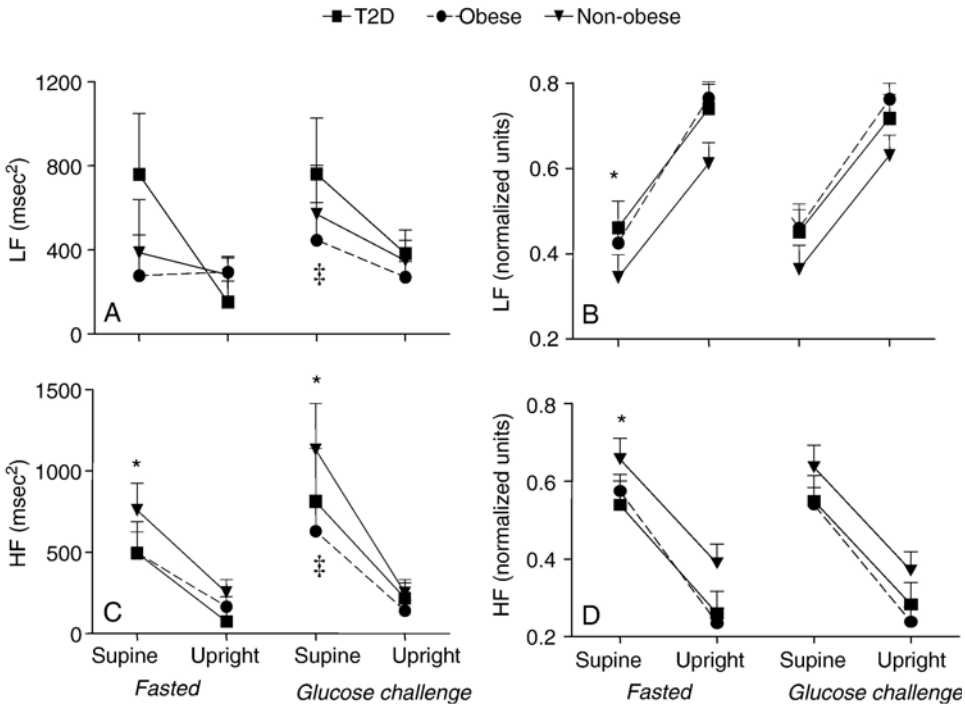

Fig. 2. HF and LF power for each group in the supine position and in response to tilt. The data are also presented in the fasted and glucose-challenged condition. The left panel represents the raw data and the right panel is the normalized units. Data are expressed as mean ± SE. *$P < .01$, supine vs upright; $^{\ddagger}P < .01$, fasted vs glucose challenge.

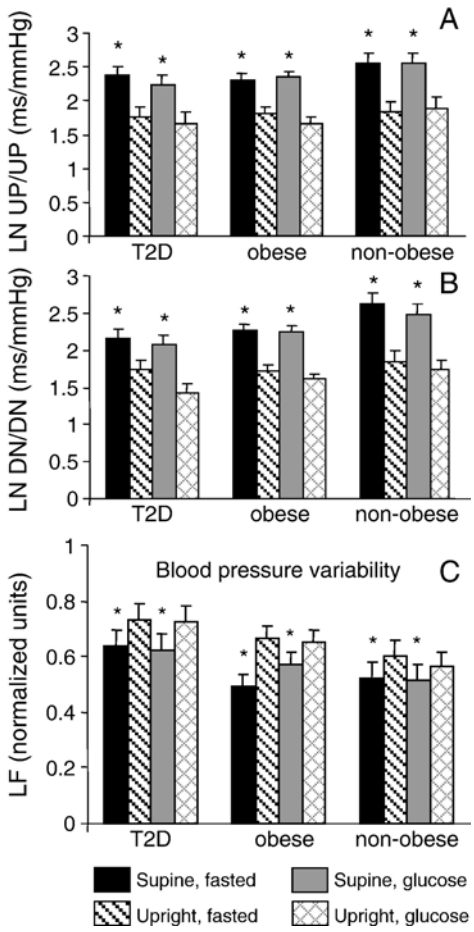

Fig. 3. Spontaneous baroreflex sequences expressed as natural logarithms (LN) for each group. (A,B) Sequences of concurrent increases in SBP and R-R intervals were defined as "up sequences" (UP/UP), whereas sequences of concurrent decreases in SBP and R-R intervals were defined as "down sequences" (DN/DN). UP/UP and DN/DN were calculated as the slopes of the regression lines between SBP and R-R intervals. (C) LF data for BPV for each group and each condition. Data are expressed as mean ± SE. *P < .01, supine vs upright.

Low-frequency power (square milliseconds) was altered with the glucose challenge (P < .05) but only approached significance for upright tilt (P = .065, Fig. 2A). High-frequency power (square milliseconds) declined with upright tilt (P < .000) during both fasting and the glucose challenge (Fig. 2C). The glucose challenge (P < .05) resulted in a higher HF in the supine position than was seen in the fasted state. Both HFnu (HF power calculated in normalized units) and LFnu (LF power calculated in normalized units) data revealed a significant change from the supine to the upright position (P < .000, Fig. 2B and D). LFnu approached significance for group differences (P = .07), such that the nonobese group had lower LF power than either of the obese groups (Fig. 2B). The glucose challenge had no effect on the LFnu or HFnu response to tilt. Sympathovagal balance (LF/HF ratio) exhibited a significant main effect for tilt (P < .000), a tilt-by-group interaction (P < .05), and a significant group effect (P < .05, Fig. 1C). Normalizing and log transforming the LF/HF ratio resulted in a significant effect with upright tilt (P < .000), and group effect (P < .05), such that there was a lower LnLF/HF (nu) in the nonobese women than in the obese women (Fig. 2D). There were no differences between the obese women with and without type 2 diabetes mellitus in LnLF/HF (nu).

3.2. Blood pressure variability and BRS

There was a significant change in LFnu of BP variability (BPV) with upright tilt (P < .001, Fig. 3C). There was no group effect or glucose challenge effect on BPV. Baroreflex sensitivity significantly decreased during upright tilt but was not changed with the glucose challenge (Fig. 3A and B). The gain of the baroreflex mechanism (α) was not found to be different between groups, nor with the glucose challenge or with the upright tilt (data not shown).

3.3. Correlation analysis

A significant negative correlation was found between the log of the blood glucose concentration at time 0 and LnTP in the supine position (r = −0.37, P < .05). No correlations were found between peak glucose or insulin concentrations, or insulin or glucose area under the curve with the glucose feeding and any measure of HRV.

4. Discussion

Both obesity and hyperinsulinemia [4,6,25,26] have been shown to cause alterations in HRV; however, the potential additive effects are unknown, and differences between obese women with and without type 2 diabetes mellitus are not clear. Consistent with earlier findings [4], we observed that sympathovagal balance (LnLF/HF) is higher in obese women than in nonobese women, but there was no difference between the obese women with and without type 2 diabetes mellitus. Upright tilt caused increased sympathetic and decreased parasympathetic modulation in all groups. The glucose load resulted in a higher total power in the supine position but not in the upright position regardless of the metabolic status of these women. There was a negative association between glucose levels and fasting TP.

4.1. Glucose challenge

Obesity is characterized by insulin resistance and compensatory hyperinsulinemia, which increase the risk of cardiovascular morbidity [27]. It has been postulated that the enhanced sympathetic nervous activity in obese subjects is related to chronic hyperinsulinemia [4,28]. Muscelli et al [4] has suggested that physiologic hyperinsulinemia causes acute desensitization of sinus node activity and that the autonomic balance is shifted toward sympathetic modulation. Our data support this finding as the nonobese women had ~22% lower LnLF/HF ratio in the supine position in both the fasted or glucose-challenged state. There was a trend in nonobese women for a higher TP and a higher parasympathetic modulation, which

supports previous reports [4,29]. These findings are consistent with an HRV profile associated with higher cardiovascular risk in the obese women, both with and without type 2 diabetes mellitus.

Glucose feeding had no substantial effect on HRV in any of the groups. Our findings differ slightly from those of Paolisso and colleagues [15] who noted a decrease in TP with insulin infusion and an increase in LF in healthy individuals but not in insulin-resistant individuals. They noted that insulin failed to stimulate cardiac ANS in insulin-resistant patients, independent of the cause of insulin resistance [15]. Although others [3,4] have indicated that hyperinsulinemia may cause the sympathetic overactivity in obese women, our data, as well as others [12], do not support this. In response to the glucose ingestion, the highest insulin levels were found in the obese women, followed by the women with type 2 diabetes mellitus and the nonobese women, yet the glucose challenge did not change indicators of sympathovagal balance in any of the groups regardless of position (Fig. 1). Only TP changed with the glucose ingestion (~25% increase), which was a somewhat unexpected finding, and not consistent with an increased sympathetic state. The increased glucose concentrations were not associated with HRV after glucose ingestion, suggesting that when administered a physiologic glucose load, other counterregulatory influences prevent autonomic dysfunction.

Our findings also contrast a recent report [16] that demonstrated that both the sympathetic and parasympathetic nervous systems were stimulated by a glucose challenge in obese subjects. Class III obesity (BMI >40 kg/m^2) was associated with a blunted increase in the sympathetic response to the glucose load, although they had a larger increase in plasma insulin levels. Likewise, others [30] have reported that basal LF/HF was significantly correlated with body fat content and distribution. It is possible that because our obese subjects had similar body fat content (~44% body fat), we did not observe differences in LF/HF (nu) between the nondiabetic and diabetic obese women.

There are several possible reasons for the differential findings between these studies. Some of the previous research reporting disturbances in HRV with hyperinsulinemia [3,4,30] used healthy volunteers who did not have underlying insulin resistance. These subjects would normally not be exposed to the insulin levels administered during the hyperinsulinemic clamp and would be expected to have greater insulin sensitivity than obese women or type 2 diabetic women. The OGTT used in the present work is more physiologically relevant. This 75 g of glucose is comparable to drinking ~22 oz of soda. In humans, hyperinsulinemia per se may be the main mechanism that triggers sympathetic activation and not the insulin-induced stimulation of carbohydrate metabolism [16,31].

Comparing individuals with various degrees of obesity, Quilliot et al [16] observed an increased LF power in BPV in all subjects, indicating that a physiologic meal stimulates sympathetic modulation in the peripheral arteries. In contrast, we found no change in the BPV with glucose ingestion. Insulin is also known to have a vasodilator effect that could stimulate the baroreflex to contribute to sympathetic activation [16], but in our study, glucose ingestion did not affect BRS. Using healthy subjects, Laitinen et al [18] found that counterregulation during hyperinsulinemic hypoglycemia does not influence cardiac parasympathetic regulation or baroreflex control of HR. During euglycemic hyperinsulinemia, they found no change in HRV, BPV, HR, or BP, and a similar BRS [18], which is in agreement with our findings that hyperglycemia had no impact on these variables.

Insulin resistance and elevated free fatty acid concentrations are thought to lead to the autonomic imbalance seen in individuals with type 2 diabetes mellitus. We had initially hypothesized there would be a greater disturbance in the autonomic function in the women with type 2 diabetes mellitus than in the obese women; however, contrary to our hypothesis, the women with type 2 diabetes mellitus exhibited similar responses to our obese nondiabetic women. This finding is consistent with a recent report by Manzella et al [32] who demonstrated that metformin treatment might improve cardiac sympathovagal balance in this population by lowering free fatty acid levels and improving insulin resistance. Our data are consistent with their findings as 7 of 9 women with type 2 diabetes mellitus were taking metformin, had excellent metabolic control, and had HRV responses that were not different than observed in the nonobese and obese women. Furthermore, the response to upright tilt in our study was not aligned with an earlier study that reported decreased HF power was a function of the severity of the neuropathy [29]. In response to tilt, Tanikawa et al [29] noted LF/HF increased significantly in the controls and in the patients with the least amount of neuropathy but was unchanged in those with more severe neuropathy. We observed little difference between type 2 diabetic women and obese nondiabetic women in response to tilt possibly because, overall, the women in our study had well-controlled blood glucose concentrations. As part of our screening criteria, we also excluded women who had known peripheral neuropathy. However, it is important to interpret our findings with caution because Eckberg [33] suggested that sympathetic and parasympathetic modulations do not change in a reciprocal manner; thus, the concept of sympathovagal balance may not be valid. Conversely, several others have shown that sympathovagal balance is a valid concept and adequately represented by the LF/HF ratio [15,34-36].

In conclusion, this study supports earlier work that basal sympathovagal balance is altered in obese individuals with and without type 2 diabetes mellitus compared with nonobese women. In contrast to a previous report [15] in which insulin infusion reduced the total spectra power and increased the LF/HF ratio in healthy subjects but not in insulin-resistant subjects, our findings demonstrate that there were no differences in cardiac autonomic regulation during a physiologic glucose load between healthy non-

obese women and obese women with and without well-controlled type 2 diabetes mellitus. The glucose load, however, did increase total spectral power in all of these middle-aged women.

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