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Metabolism Clinical and Experimental 54 (2005) 966–974

Metabolism
Clinical and Experimental

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Impaired response of cardiac autonomic nervous system to glucose load in severe obesity

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Received 4 September 2004; accepted 24 February 2005

Abstract

Objective: This study was undertaken to further analyze the response of the cardiovascular autonomic nervous system (ANS) to changes in plasma insulin concentration induced by an oral glucose load. We hypothesized that, as a consequence of insulin resistance, an inability of insulin to increase the sympathetic modulation of heart rate (HR) and blood pressure (BP) would be observed in normotensive obese patients.
Methods: We used spectral analysis to measure simultaneously the short-term variability of HR and BP in 23 never-obese subjects and in 70 normotensive overweight or obese patients subdivided into 3 subgroups: (1) overweight group (body mass index [BMI], 25-29.9 kg/m^2), n = 23; (2) class I-II obese group (BMI, 30-39.9 kg/m^2), n = 23; (3) class III obese group (BMI, $\geq$40 kg/m^2), n = 23.
Results: Oral glucose ingestion and the related increased insulinemia caused significant changes in the indices of sympathetic modulation (low-frequency [LF] power and LF/high-frequency ratio) of both HR and BP in normal weight, overweight, and obese subjects. However, the LF increments gradually decreased with the BMI classes, suggesting that sympathetic nervous system modulation in these subjects may be insulin-resistant.
Conclusion: Obesity could develop resistance to the sympatho-excitatory effects of insulin that might play a role in the etiology of obesity. Spectral analysis of BP and HR can be used in research to evaluate the reactivity of the sympathetic nervous system in a manner that represents another feature of the obesity/insulin-resistance syndrome.

1. Introduction

Obesity is associated with increased peripheral sympathetic nervous system (SNS) activity [1-4]. However, peripheral sympathetic tone may vary with complications associated with obesity, such as hypertension [5,6] or sleep apnea disease [7], and can differ between different ethnic groups [8]. On the other hand, low central SNS activity, reactivity, or sensitivity may be a cause of obesity. Using the power spectral analysis of both heart rate (HR) and blood pressure (BP) variability, a noninvasive and sensitive method for the evaluation of cardiovascular autonomic modulation, we recently showed [9] that the indexes of the sympathetic modulation of HR and BP (low-frequency [LF] spectral power of both HR and BP) were lower in normotensive overweight or obese subjects than in controls, at rest and in a situation of increased sympathetic activity (standing position). This is in accordance with the findings of 2 previous studies [10,11]. Furthermore, we demonstrated that changes in SNS modulation were strongly correlated to insulin resistance, suggesting that insulin resistance is associated with a decreased responsiveness of the cardiovascular system to sympathetic activation in normotensive obese subject.

The present study was undertaken to further analyze the response of the cardiovascular autonomic nervous system (ANS) to changes in plasma insulin concentration induced by an oral glucose load. We hypothesized that, as a consequence of insulin resistance, an inability of insulin to acutely increase the sympathetic modulation of HR and BP would be observed in normotensive obese patients and could be influenced by the degree of obesity. Moreover, investigating glucose-

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doi:10.1016/j.metabol.2005.03.002

load–induced cardiovascular ANS modulation may be an interesting physiological approach to identify possible abnormalities in the responsiveness of obese subjects to insulin. Finger arterial pressure measurement, analyzing both HR and BP, provides a noninvasive tool for investigating autonomic cardiovascular regulation.

In this study, we measured HR and BP variability at rest in the supine and standing positions, and in response to an oral glucose load in the supine position.

2. Methods

2.1. Subjects

Two groups of subjects, experimental and control, were compared in this study. The inclusion criteria were weight change of less than 1% during the month before inclusion in the study, no hypertension as defined by the World Health Organization criteria (systolic BP [SBP] <140 mm Hg and diastolic BP [DBP] <80 mm Hg), physically and socially active, and a fasting plasma glucose of less than 7 mmol/L (or <11 mmol/L at the second hour of the oral glucose tolerance test).

Exclusion criteria were hyper- or hypothyroidism, chronic diseases, medication except contraceptive, supraventricular extrasystoles or nonstationary recordings.

The experimental group consisted of 22 male and 48 female volunteers, all of whom were classified as overweight (body mass index [BMI] of 25 kg/m^2 or higher) or obese (BMI of 30 kg/m^2 or higher). There were 23 subjects in the control group (5 men and 18 women) (BMI <25 kg/m^2), all of whom were in good health, with normal clinical and routine laboratory data. All obese and overweight subjects had been recruited before an ongoing program for dietary education and weight loss. Seven subjects had fasting moderate hyperglycemia (6 mmol/L < fasting plasma glucose < 7 mmol/L).

The study was carried out in accordance with the World Medical Association Declaration of Helsinki (1989). The experimental protocol followed was approved by the hospital ethics committee, and all subjects gave their written informed consent.

Subjects from the experimental group were further subdivided into 3 groups based on BMI category: (1) overweight group (BMI, 25-29.9 kg/m^2), n = 23; (2) class I-II obese group (BMI, 30-39.9), n = 24; (3) class III obese group (BMI, ≥40), n = 23.

2.2. Measurements

Consumption of alcoholic or caffeinated beverages and any heavy work or sports activity were avoided for the 12 hours preceding the study.

Blood pressure and HR were monitored noninvasively and continuously at the finger using photoplethysmography (Finapres 2300, Ohmeda, Louisville, Colo). To avoid hydrostatic errors, all subjects held the cuffed finger at the level of the heart (fourth intercostal space in the midaxillar line). Recordings were carried out while the subjects were fasting, at the same time of the day (8-9 AM) at room temperature (20°C-22°C) and using the same devices.

2.3. Blood pressure and HR variability [12]

Finger arterial pressure was recorded and converted to a digital signal, which was used to obtain values for SBP, DBP, and HR. The data were collected for a constant period at a rate of 500 Hz. The overall variability of SBP, DBP, and HR was calculated and expressed in absolute terms as the individual standard deviation. Equal time interval sampling of SBP, DBP, and HR at a rate of 1 Hz made it possible to carry out direct spectral analysis of each distribution using a fast Fourier transform algorithm on a 256-point stationary time series using the computer program Anapres 3.0 (Notocord Systems, Croissy sur Seine, France). Only stationary periods with no ectopic beats or missing data were analyzed. The total area under the curve (AUC) was taken as the overall variability (total power) and was obtained by integrating the spectral band from 0.004 to 0.500 Hz. The power spectra were divided [12] into an LF band (0.040-0.150 Hz), to include the Mayer waves, and an HF band (0.150-0.400 Hz).

Very low frequency (VLF) spectral power was obtained by integrating the spectral band below 0.040 Hz. Very low frequency values were evaluated after the glucose load, on a 1024-point stationary time between 30 and 60 minutes.

Table 1
Characteristics of subjects

BMI categories	Controls	Overweight	Obese subjects		*P* (ANOVA)
	18.5-24.9 (n = 23)	25-29.9 (n = 23)	30-39.9 (n = 24)	≥40 (n = 23)	
Age (y)	31.8 ± 9.5	38.3 ± 8.7	37.9 ± 13.6	35.3 ± 1.0	NS
Sex ratio (F/M)	18/5	18/5	17/7	15/8	NS
BMI (kg/m^2)	22.5 ± 2.3	28.2 ± 1.7*	35.7 ± 2.5*	48.4 ± 8.2*	<.001
Blood glucose (mg/dL)	90 ± 12	88 ± 12	96 ± 10*	102 ± 15*	.004
Fasting insulin (mU/L)	6.0 ± 2.6	9.4 ± 4.9	17.2 ± 9.8*	41.5 ± 31.1*	<.0001
SBP (mm Hg)	108.7 ± 16.4	112.4 ± 24.0	114.1 ± 13.0	112.2 ± 15.0	NS
DBP (mm Hg)	59.0 ± 9.0	63.6 ± 13.9	59.9 ± 8.9	59.4 ± 7.8	NS
HR (bpm)	64.3 ± 10.5	67.0 ± 9.5	68.1 ± 9.8	70.0 ± 11.0	NS

Values are mean ± SD. $P < .05$ was taken to be the threshold of statistical significance for ANOVA.

* $P < .05$ for differences between overweight or obese groups and control group (Fisher PLSD test).

Table 2
Heart rate and BP variability in the supine and standing positions

BMI categories (kg/m^2)	Controls	Overweight	Obese subjects		*P* (ANOVA)
	18.5-24.9 (n = 23)	25-29.9 (n = 23)	30-39.9 (n = 24)	≥40 (n = 24)	
Baseline (supine)					
Total spectral power					
SBP (mm Hg/VHz)	137.5 ± 20.0	143.0 ± 24.7	150.3 ± 19.2	145.1 ± 18.1	NS
DBP (mm Hg/VHz)	77.7 ± 10.4	81.7 ± 13.4	81.7 ± 19.9	78.3 ± 10.6	NS
HR (bpm/VHz)	91.0 ± 19.7	95.0 ± 14.4	96.7 ± 20.8	97.5 ± 24.0	NS
Normalized LF					
SBP	62.4 ± 9.1	62.1 ± 8.4	59.8 ± 8.8	53.8 ± 9.9*	.006
DBP	63.9 ± 8.4	65.1 ± 7.7	60.9 ± 7.6	56.6 ± 9.9*	.004
HR	47.9 ± 11.1	49.2 ± 9.1	46.3 ± 9.5	43.8 ± 9.4	NS
LF/HF					
SBP	1.79 ± 0.62	1.71 ± 0.60	1.59 ± 0.54	1.28 ± 0.54*	.020
DBP	1.09 ± 0.64	2.02 ± 0.74	1.64 ± 0.48	1.42 ± 0.54*	.006
HR	1.02 ± 0.48	1.04 ± 0.44	0.92 ± 0.33	0.83 ± 0.32	NS
Standing					
Total spectral power					
SBP (mm Hg/VHz)	154.1 ± 26.9	164.2 ± 32.6	169.3 ± 22.0	165.0 ± 17.4	NS
DBP (mm Hg/VHz)	96.7 ± 20.6	102.2 ± 16.3	99.2 ± 11.2	94.6 ± 16.1	NS
HR (bpm/VHz)	112.1 ± 20.3	114.0 ± 19.6	113.1 ± 23.7	112.3 ± 21.0	NS
Normalized LF %					
SBP	64.1 ± 7.8	62.0 ± 7.9	63.8 ± 4.3**	57.8 ± 11.2*	.040
DBP	69.8 ± 7.6	67.0 ± 7.6	67.0 ± 5.4**	64.1 ± 9.0**	NS
HR	63.2 ± 7.3**	59.2 ± 10.7**	54.7 ± 8.6*·**	48.5 ± 10.9*·**	<.001
LF/HF					
SBP	1.9 ± 0.64	1.74 ± 0.58	1.80 ± 0.33	1.55 ± 0.71	NS
DBP	2.53 ± 0.98	2.47 ± 1.50**	2.11 ± 0.50**	1.96 ± 0.71**	NS
HR	1.82 ± 0.57**	1.61 ± 0.65**	1.28 ± 0.38*·**	1.04 ± 0.47*·**	<.001

LF (% total power) indicates normalized low frequency. Values are mean ± SD. $P < .05$ was taken to be the threshold of statistical significance for ANOVA and $P < .05$ for differences between overweight or obese groups and control group (Fisher PLSD test).

* For comparison with control group.

** For the comparison between orthostatism and baseline.

To avoid respiratory events that might influence LF power, we checked that subjects breathed at a rate of at least 8 breaths/min (0.130 Hz).

Heart rate and BP spectral power were expressed as normalized units (nu) relative to the total spectral power for LF and HF [LF or HF power × 100 / (total power − VLF)] and in absolute terms (mm Hg min^{-1} Hz$^{-0.5}$ or beat min^{-1} Hz$^{-0.5}$). To facilitate the reading, the values are presented only in relative values in the tables, except if the results were significantly different.

The reproducibility of our measurements was tested in 10 healthy subjects with 2 subsequent evaluations at weekly intervals. The intraclass correlation coefficient during controlled breathing was more than 0.5 for BP variability and more than 0.7 for HR variability. The reproducibility of the method has been established previously [13] for measurements carried out under steady-state conditions in healthy subjects, as was the case in this study.

Spectral analysis of HR and BP variabilities was determined by end-organ ANS responses and could be interpreted as an integrated index of cardiovascular modulation by the ANS. Innervated organ responsiveness does not depend only on SNS activity, but also on receptor function and on the ability of the organ to promote the signal transduction.

The LF spontaneous oscillations of heart period have been attributed to and correlated with the sympathetic efferent control of the heart and to baroreflex sensitivity [1]. However, the concept of a central oscillator has been

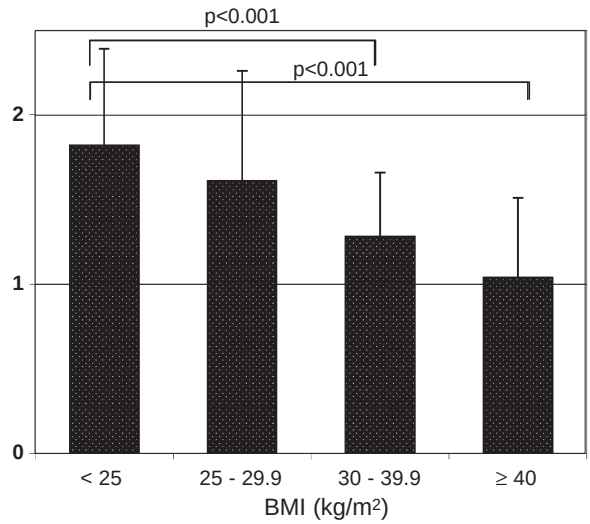

Fig. 1. Comparison of the LF/HF ratio of HR in the standing position between the 4 groups of subjects (n = 93).

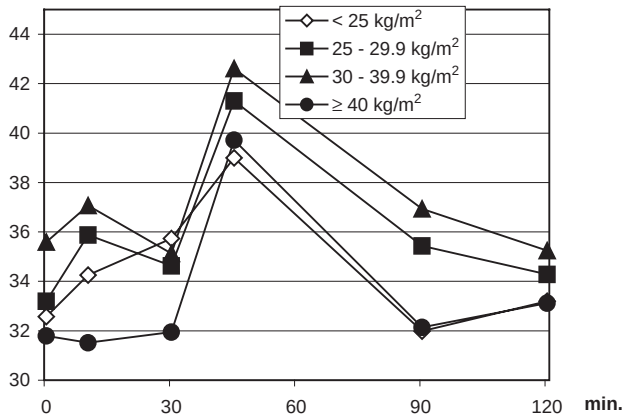

Fig. 2. Evolution of the LF (nu) component of HR after the glucose load.

suggested in previous human and animal studies [14-17]. For the LF oscillations of BP, the sympathetic contribution has been convincingly demonstrated [18]. The ratio of LF to HF (LF/HF) signals can be considered, with some precautions, as an indirect index of sympathovagal balance [19]. The efferent vagal activity is a major contributor to the HF component [20,21], whereas the physiological explanation of the VLF component is much less defined.

2.4. Experimental procedure

Three recordings, each lasting at least 10 minutes, were obtained after the signal had stabilized. After a period of rest in the supine position, a recording was done while the subject was breathing spontaneously. For the second recording, the subjects were in the standing position, with the analysis performed after stabilization of the curve, at least 5 minutes after commencement of active orthostatism. The effects of controlled breathing were then tested in the supine position with metronome breathing at a fixed rate (12 breaths/min). After 10 minutes, the subjects ingested the glucose solution (75 g in 200 mL) over a 5-minute period, after which the recording of BP and HR was made without delay.

We observed in a preliminary study that the LF and HF components of power spectra rose during the first hour and then declined throughout the test. Consequently, we analyzed HR and BP variability before ($t = 0$) and at 5, 10, 30, 35, 40, 45, 50, 55, 60, 120 minutes after the glucose load. Insulin concentrations were determined at $t = 0$, 30, 60, 120 minutes. The ingestion of water alone had no effect on the HR and BP modulation.

The AUC, together with the minimal and the maximal values of each spectral component, was then calculated. The AUC was approximated using the trapezoidal rule: first by dividing the area into a number of strips, then, approximating the area of each strip by the area of the trapezium formed when the upper end is replaced by a chord. The sum of these approximations gives the final numerical result of the AUC as described elsewhere [22].

Plasma glucose was measured by the glucose oxidase method (Beckman Instruments Inc, Fullerton, Calif), whereas plasma insulin was determined by immunoassay (Insulin IMX, Abbott laboratories, Tokyo, Japan). The intra-assay coefficient of variation was 4% and the interassay coefficient was 6%. Cross-reactivity with proinsulin was 0.05%.

2.5. Statistical analysis

Values are expressed as means (± SD). The BMDP statistical software (University of California Press, Berkeley, Calif, 1992) was used to perform the statistical analysis. Normal frequency distribution was verified by skewness and kurtosis tests. Analysis of variance (ANOVA) tests were

Table 3
Area under the curve in response to an oral glucose load (AUC, 0-120 minutes) for the HR (bpm min^{-1} $Hz^{-0.5}$) and blood pressure (mm Hg min^{-1} $Hz^{-0.5}$) spectral components

	Controls	Overweight	Obese subjects		*P* (ANOVA)
BMI categories (kg/m^2)	18.5-24.9	25-29.9	30-39.9	≥40	
AUC ins (mU min/mL)	1904 ± 1118	5711 ± 2144	11743 ± 7762	13904 ± 5665	<.001
Total					
SBP	3507 ± 883	3818 ± 888	5385 ± 1178*	3858 ± 895**	<.001
DBP	2108 ± 528	2301 ± 493	2985 ± 674***	2271 ± 671****	.009
HR	3132 ± 1202	2962 ± 945	4187 ± 1501	3431 ± 1851	NS
LF					
SBP	1134 ± 269	1446 ± 416	2033 ± 624*	1359 ± 428**	.018
DBP	792 ± 189	986 ± 282	1202 ± 331*	870 ± 300**	<.001
HR	969 ± 362	102 ± 405	1558 ± 541***	1138 ± 670****	.049
LF %					
SBP	3631 ± 507c	4320 ± 760***	4443 ± 606	3821 ± 762****	.005
DBP	4267 ± 839***	4909 ± 784	4807 ± 555	4179 ± 784****	.004
HR	3412 ± 786	3931 ± 558	4437 ± 595*	3574 ± 779**	<.001

Values are mean ± SD. $P < .05$ was taken to be the threshold of statistical significance for ANOVA and $P < .01$ for differences between overweight or obese groups and control group (Fisher PLSD test).

* $P < .01$ for comparison with control group.

** $P < .01$ for comparison with obese group (BMI, 30-39.9 kg/m^2).

*** $P < .05$ for comparison with control group.

**** $P < .05$ for comparison with obese group (BMI, 30-39.9 kg/m^2).

used to compare the mean values. The Fisher PLSD test was used to compare overweight and obese groups to the control group when ANOVA testing was significant ($P < .05$). Linear regression analysis was used to evaluate correlations between variables, whereas multiple regression analysis was used to assess the effects of several independent variables on spectral components. A value of $P < .05$ was taken to be the threshold of statistical significance.

3. Results

3.1. Main characteristics of subjects

Age, sex ratio, baseline HR, and DBP and SBP values were not statistically different between the obese, overweight, and control groups (Table 1). In the present study, no significant differences were found between obese subjects and controls for HR and BP (Table 1). There were

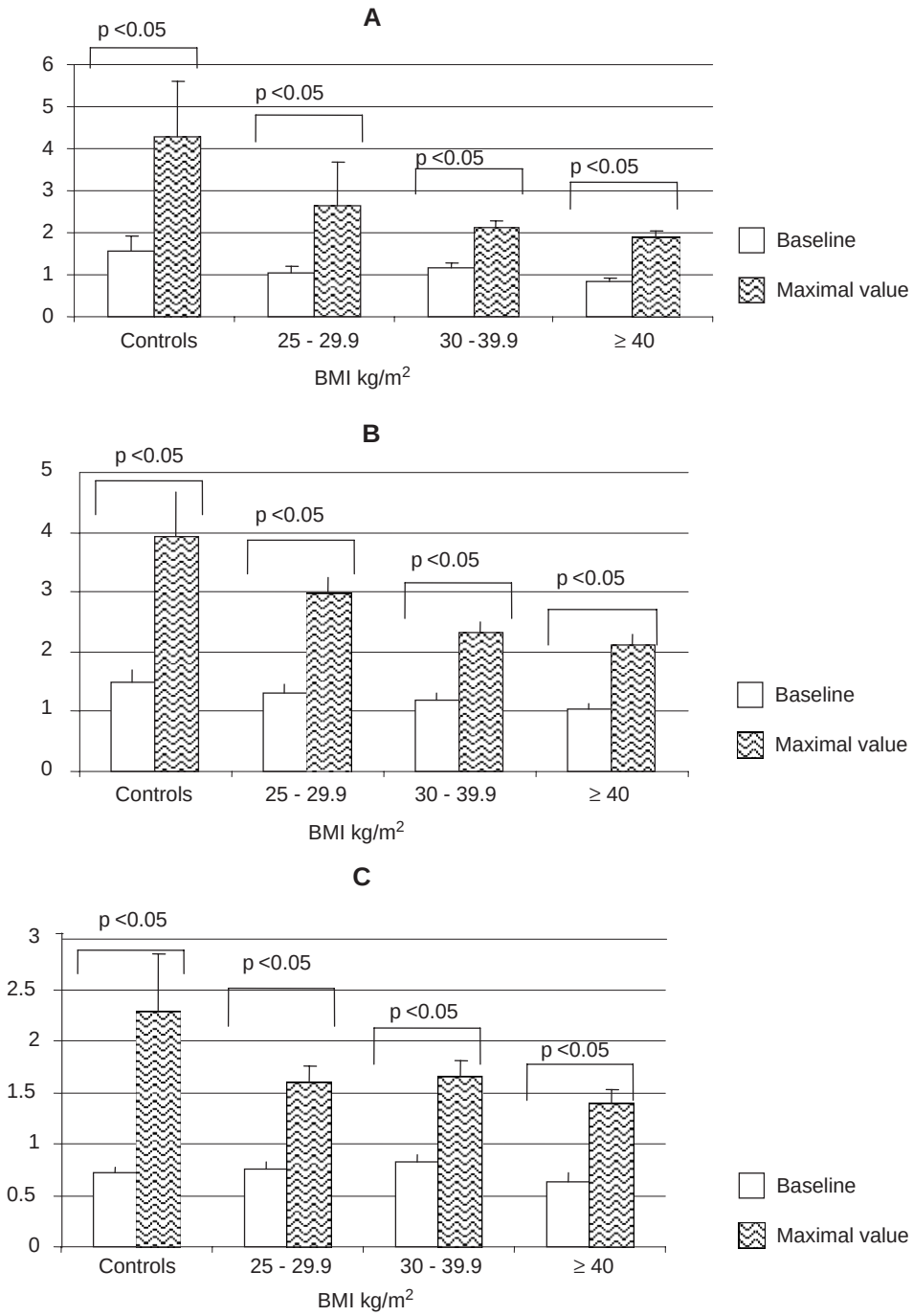

Fig. 3. Comparison of the maximal values of LF/HF ratio for (A) SBP, (B) DBP, and (C) HR, between baseline and after the glucose load, in the control and obese groups. Results are expressed as mean ± SD for each group. *$P < .05$. Values were significantly different between the 4 groups (ANOVA) and Fisher PLSD test (‡$P < .05$).

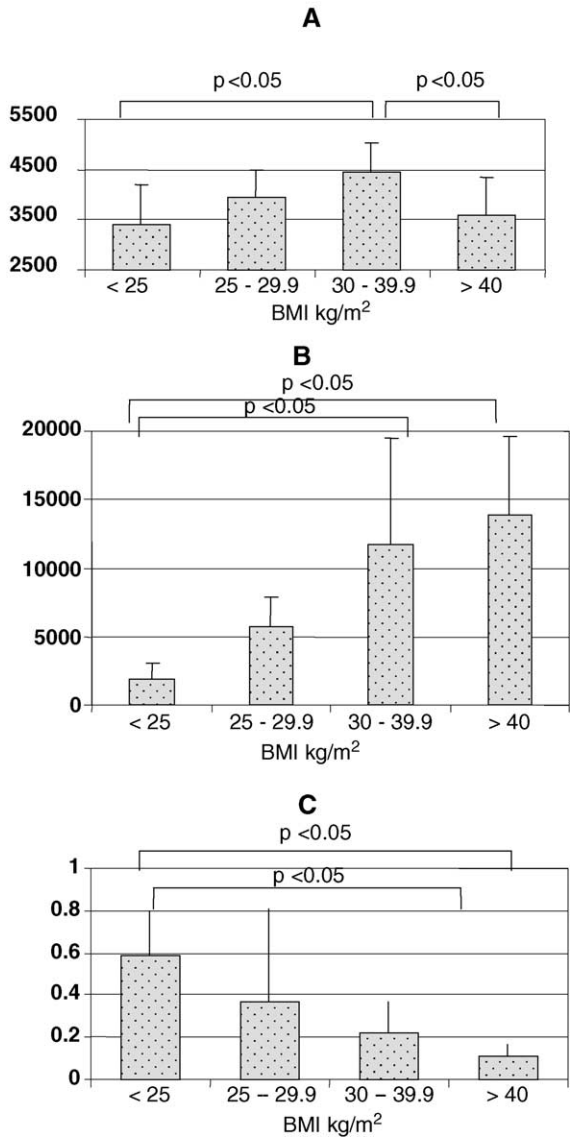

Fig. 4. Comparison of AUC LF for the HR (bpm min^{-1} $Hz^{-0.5}$) (A) of AUC insulin (mU min/mL) (B) and of AUC LF/AUC insulinemia (LF/insulin concentration), in the control, overweight, and obese groups. Results are expressed as mean ± SD for each group. *$P < .05$. Differences were significant between the 4 groups (ANOVA and Fisher PLSD test, ‡$P < .05$).

no statistically significant sex differences in HR or BP variability. As age and BMI distribution were similar in both sexes, data from both women and men were pooled.

3.2. Variation in the sympathetic modulation of LF and LF/HF ratio

In the supine position, total spectral power of HR and BP variability was not significantly modified in the 4 groups, whereas the LF (absolute and nu) of SBP and DBP was lower than that of control in both obese subject groups (Table 2). In the same way, the LF/HF ratio of SBP and DBP was significantly decreased in both groups of obese subjects.

In the orthostatic position, in comparison to the supine values, we observed a significant increase in the total and LF spectral power of HR (absolute and nu) in the 4 groups ($P < .05$; 2 asterisks in Table 2). This increase was lower in the class III obese group ($P < .01$). Heart-rate LF and SBP LF were lower for both groups of obese subjects compared to control ($P < .05$; asterisk in Table 2), whereas the LF spectral values for DBP were not significantly decreased. The LF/HF ratio (Fig. 1, Table 2) decreased significantly in both obese groups compared to control, but only for the HR. The total spectral power of both HR and BP was not different among the 4 groups.

After the oral glucose load, as compared to baseline data, LF power and normalized LF of both HR and BP rose significantly in the 4 groups ($P < .0001$) (Fig. 2). The AUC between 0 and 120 minutes (AUC, 0-120) of the LF (absolute or nu) of HR and BP was significantly higher in the class I-II obese group (30-39.9 kg/m^2) (Table 3). The values observed in the class III obese group (BMI, ≥40 kg/m^2) were not significantly different from the control group. The results were approximately the same if we calculated the AUC above the rest values.

The maximum values of the LF (nu and absolute) of both HR and BP were higher in the class I-II obese group (30-39.9 kg/m^2), whereas the values of the class III obese group were not different from the control group. When we considered the maximum value of the LF/HF ratio (Fig. 3), of both HR and BP, this ratio was less increased for the obese group than in the control group.

As proposed by Spraul et al [23], we have evaluated the increments in LF for an increment of 1 U of insulin concentration by the ratio of the AUC LF to AUC insulinemia. These ratios were highly significantly decreased in the obese groups for both HR and BP (Fig. 4).

3.3. High-frequency spectral values

During controlled breathing, the HF component increased significantly and was greater in both obese groups (Table 4).

After the glucose load, HF values for both HR and BP rose significantly in the 4 groups ($P < .0001$). The AUC between $t = 0$ minute and $t = 120$ minutes (AUC, 0-120) of the absolute and normalized HF of HR and BP was significantly higher in class I-II obese group (30-39.9 kg/m^2) and in the class III obese group (BMI, ≥40 kg/m^2).

Aging was associated with a significant decrease in the HR and DBP HF (absolute and relative) during the controlled breathing, independently of BMI (Table 5).

3.4. Very low frequency values

The baseline values of the VLFs of both HR and BP were not different between the 4 groups. In the standing position, the VLF of the HR variability increased significantly ($P < .05$) but was not significantly different among the 4 groups.

The VLF of both HR and BP increased significantly after the glucose load in the 4 groups. Maximal values were

Table 4
High-frequency spectral components of HR and BP during controlled breathing and in response to an oral glucose load (bpm min^{-1} $Hz^{-0.5}$ and mm Hg min^{-1} $Hz^{-0.5}$)

	Controls	Overweight	Obese subjects		*P* (ANOVA)
BMI categories (kg/m^2)	18.5-24.9	25-29.9	30-39.9	≥40	
Controlled breathing HF (nu)					
SBP	40.3 ± 11.8	43.0 ± 10.2	44.2 ± 7.1	49.2 ± 10.1*	.031
DBP	40.6 ± 10.6	42.1 ± 9.0	46.4 ± 8.2	51.9 ± 10.6*	<0.001
HR	56.8 ± 9.2	56.2 ± 9.4	58.7 ± 10.1	63.6 ± 9.2*	.039
AUC HF (0-120 min)					
SBP	597 ± 294	929 ± 280*	1328 ± 406**	1101 ± 339**	<.001
DBP	393 ± 232	523 ± 159	758 ± 267**	635 ± 267*	.011
HR	894 ± 631	993 ± 361	1373 ± 635	1271 ± 761	NS

Values are mean ± SD. $P < .05$ was taken to be the threshold of statistical significance for ANOVA and $P < .01$ for differences between overweight or obese groups and control group (Fisher PLSD test).

* $P < .05$ for comparison with control group.

** $P < .01$ for comparison with control group.

significantly different among the 4 groups only for SBP (the differences were 25.8 ± 7.9 for the controls, 39.2 ± 12.1 for the overweight, 39.0 ± 12.6 for class I-II obese group [30-39.9 kg/m^2], and 31.8 ± 8.5 for the morbid obesity; $P = 0.033$). The AUC of the VLF component of both HR and BP was significantly higher in the class I-II obese group (30-40 kg/m^2). The VLF was evaluated on a 1024-point stationary time between 30 and 60 minutes, with comparable results obtained with the maximal value of the 256-point series, thus giving a high correlation between these 2 methods ($r > 0.80$).

4. Discussion

The major finding of our study was that oral glucose ingestion and the related increased insulinemia caused a significant increase in LF power, HF power, and LF/HF ratio of both the HR and BP variability, in normal, overweight, or obese subjects. This suggests that the sympathetic and parasympathetic nervous system can be stimulated by this physiological meal even if subjects are obese. These findings provide evidence of insulin-induced changes in cardiac and BP autonomic regulation, highlighted by an increase in HR and BP variability. Very high insulin concentrations make it possible to stimulate sympathetic activity in common obesity (BMI, < 40 kg/m^2). However, class III obesity is associated with a blunted increase in sympathetic modulation in response to an oral glucose load despite a larger increase in plasma insulin levels. The LF increment gradually decreased with the BMI classes studied here, suggesting that the sympathetic system modulation in these subjects may be insulin-resistant. Moreover, glucose-induced changes in SNS indexes in the class III obese group (BMI, ≥40 kg/m^2) were not significantly different from those measured in the control group. Insulinemia in the class III obese group was not different from the common obese group (BMI, 30-40 kg/m^2), and, as such, a decreased capacity for insulin hypersecretion could explain the relative decrease in sympathetic reactivity seen in extreme obesity.

This blunted increased response to an oral glucose load despite a larger increase in plasma insulin levels may represent another feature of the obesity/insulin-resistance syndrome. Previous studies using euglycemic hyperinsulinemic glucose clamp have demonstrated that hyperinsulinemia [24-27] affects the indicators of peripheral sympathetic activity (microelectrode nerve recordings, noradrenaline levels). These observations suggest that in humans hyperinsulinemia per se, rather than insulin-induced stimulation of carbohydrate metabolism, is the main mechanism that triggers sympathetic activation. Insulin acts directly on the ventral medial hypothalamus and in a roundabout way on brainstem sympathetic centers [28-31]. Insulin has also a direct vasodilatator effect that could stimulate the baroreflex, contributing to sympathetic activation. However, it has been demonstrated that the sympatho-excitatory actions are not mediated by a local mechanism in humans [32,33]. Nevertheless, decreased LF oscillations in BP may result from the delayed sympathetic vasomotor reaction to BP changes. In fact, the vasodilatations substantially enhanced the variability of BP and HR at frequencies below approximately 0.100 Hz [34].

Table 5
Partial coefficients of regression analysis between normalized HF component and BMI (kg/m^2) and age in the whole population (n = 93)

	Controlled breathing	
	r	*P*
Normalized SBP HF %		
BMI	0.310	.003
Age	−0.172	NS
Normalized DBP HF %		
BMI	0.413	<.0001
Age	−0.187	.056
Normalized HR HF %		
BMI	0.268	.008
Age	−0.307	.003

Our results are in accordance with those of Spraul et al [23] who evaluated sympathetic tone by microneurography (muscle sympathetic nerve activity) after a glucose load in white and Pima Indian subjects. Obesity is associated with a higher fasting sympathetic neural outflow to the muscle but a blunted increase in response to an oral glucose load despite a larger increase in plasma insulin levels. However, in our study the decreased baseline LF did not explain the lower LF in response to the glucose load.

Impaired sympathetic responsiveness in obesity has been reported using an insulin infusion test [27]. However, during this test the insulin concentrations are lower than what we observed after glucose load in obese subjects, and oral glucose tolerance test is closed to the physiological stimulation.

Our results are also in agreement with those of Paolisso et al [35] who showed that the rise in LF/HF ratio after glucose ingestion correlated negatively with BMI. However, we showed that in absolute terms the LF could increase in class I-II obese group (30-39.9 kg/m^2) after the glucose load, thus demonstrating that the SNS can still be stimulated with high insulin concentrations.

The unresponsiveness of the sympathetic system complies with the Mona Lisa hypothesis, suggesting that the ANS may be central in the regulatory system that maintains constant energy storage, and that the SNS assumes a major role in the pathophysiology of obesity [36,37]. At rest, the activity of the SNS in obesity has been assessed in several ways, with conflicting results [38]. However, 2 pivotal methods—the study of noradrenaline spillover from the heart and the analysis of nerve firing rates determined by microneurographic techniques—have shown that obesity is associated with increased SNS activity [1-4]. Increased plasma catecholamine levels may induce β-receptor downregulation via a tachyphylactic mechanism. This has been shown in patients with congestive heart failure [17,39]. Consequently, impaired β-receptor responsiveness, induced by insulin resistance and increased sympathetic tone, may contribute to the decreased variability of HR and BP observed in our obese subjects.

At rest, decreased HR variability in obese subjects had been reported in several studies [10,11]. However, Zahorska-Markiewicz et al [11] reported significant changes only in HF spectral power, whereas Piccirillo et al [10] reported significant changes only in the LF component. They also showed, contrary to our findings, that the BP power spectrum was increased. Hypertension and age can, however, influence the variability of both BP and HR [9,40-43], which is common in obese subjects. Gao et al [44] showed that cardiac autonomic function as assessed by HR spectral analysis could vary in women depending on their regional body fat distribution. Women with combined upper body obesity and visceral obesity had significantly higher cardiac sympathetic and parasympathetic activity than any other subgroup. In our study, patients were younger and only normotensive subjects were selected, which could explain the apparent discordance. For the LF oscillations of BP, the sympathetic contribution has been convincingly demonstrated [15]. The LF spectral power of BP variability increases with the BP level [18]. Thus, BP may be a confounding factor in obese subjects because of the high prevalence of hypertension in obesity. As a result, we selected normotensive subjects for our study.

In agreement with the study of Matsumoto et al [45], VLF measured in our study increased in the 4 groups after the glucose load. However, contrary to this previous study, the maximal values of the VLF evaluated on a 1024-point stationary time between 30 and 60 minutes, and the AUC of the VLF of both BP and HR were increased in obese subjects compared to controls, with the exception of class III obese subjects. It has been pointed out that the VLF of HR, centered on an average peak of 0.021 ± 0.0047 [45,46], might serve as an indicator of SNS activity associated with thermoregulation. Our selected obese subjects may have a magnitude of increase in the activity of the thermogenic component of the SNS, whereas the class III obese subjects might have an impaired activation of SNS related to thermoregulation. This could favor weight gain in the class III obese subjects. In contrast, previous studies have shown that VLF values were markedly reduced in obese young women after ingestion of a stimulatory compound, such as capsaicin, which increased thermogenesis without having an effect on insulin secretion [45,46].

In conclusion, these findings support the view that the study of BP and HR variability may be useful in research to evaluate the capacity of reactivity of the SNS in obesity. Hyperinsulinemia partly compensates for the blunted action of insulin on sympathetic activation in obese subjects. Obesity could result in development of resistance to the sympatho-excitatory effects of insulin that can be associated with low organ responsiveness. On the other hand, low SNS activity, reactivity, or sensitivity may be a cause of obesity. Reduced variability of HR and BP could also contribute to the higher risk of cardiovascular disease and sudden death in these subjects.

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