Effects of Different Insulin Infusion Rates on Heart Rate Variability in Lean and Obese Subjects

Giuseppe Paolisso, Daniela Manzella, Maria Rosaria Tagliamonte, Maria Rosaria Rizzo, Antonio Gambardella, and Michele Varricchio

The low-frequency to high-frequency ratio (LF/HF ratio) is an index of cardiac sympathovagal balance. We hypothesized that insulin might also stimulate the LF/HF ratio. Thus, 15 lean and 15 obese subjects were studied. Each subject underwent sequential hyperinsulinemic clamps (insulin infusion rate 0.50, 1, and 2 mU/kg · min) while the heart rate was recorded by the Holter technique continuously. Indirect calorimetry allowed determination of the respiratory quotient (Rq) and substrate oxidation. The leg blood flow (LBF), leg vascular resistance (LVR), and plasma norepinephrine concentration were also measured. In seven lean subjects, hyperinsulinemic clamps were repeated along with propranolol infusion (0.1 mg · kg^{-1} as an intravenous bolus dose followed by continuous intravenous infusion of 0.5 mg · kg^{-1} · min^{-1} throughout the study). Lean subjects had better insulin action than obese subjects. Insulin infusion was associated with an increase of the ΔLF/HF ratio in both lean ($P < .001$ for time-dependent changes) and obese ($P < .02$ for time-dependent changes) subjects; however, the extent of insulin-mediated stimulation of the LF/HF ratio was greater in lean versus obese subjects. Insulin infusion did not significantly affect HF values in both groups. Independently of gender, body fat, changes in the plasma norepinephrine concentration, LBF, and LVR, the ΔLF/HF ratio at the end of the fastest insulin infusion (0.8 ± 0.2 *v* 0.3 ± 0.2, $P < .04$) was still greater in lean versus obese subjects. The ΔLF/HF ratio was also more stimulated during insulin versus insulin + propranolol infusion in lean subjects. In conclusion, insulin stimulates the LF/HF ratio in both lean and obese subjects and thus produces a shift in the cardiac autonomic nervous system activity toward sympathetic predominance.

SYMPATHETIC NERVOUS SYSTEM (SNS) activity has been reported to be the link between insulin resistance and hypertension.[1] Several techniques have been used to investigate SNS activity. Plasma norepinephrine turnover (PNT) and muscle sympathetic nerve activity (MSNA) recordings are most frequently used.[1] Both techniques have provided evidence for a stimulatory effect of insulin on SNS activity. Nevertheless, PNT and MSNA have several limitations that greatly reduce their feasibility in large epidemiologic studies.

Power spectral analysis of RR variability (heart rate variability [HRV]) is a useful and safe tool to evaluate SNS and parasympathetic nervous system activity.[2] In particular, the low-frequency to high-frequency ratio (LF/HF ratio) is considered an index of cardiac sympathovagal balance.[2-4] Indeed, specific interventions to increase or decrease the LF/HF ratio may produce a shift of the cardiac sympathovagal balance toward sympathetic or parasympathetic predominance, respectively.[2] A previous study has already demonstrated that glucose ingestion stimulates the LF/HF ratio.[5] Nevertheless, the study tested the effect of glucose ingestion on HRV without differentiating the potential effect of glucose per se from the effect of insulin.[5] Because a relationship between insulin action and stimulation of SNS activity has been demonstrated,[1,6-8] a relationship between insulin action and cardiac sympathetic-vagal activity measured as HRV was also hypothesized. We believe that this hypothesis deserves a thorough investigation.

Thus, we attempt herein to answer the following questions: (1) Is there a relationship between insulin action and the LF/HF ratio and, if yes, (2) is the insulin effect dose-dependent?, and (3) Is the insulin effect on HRV mediated by changes in hemodynamic parameters and in the plasma norepinephrine concentration? For this reason, lean and obese subjects underwent a hyperinsulinemic-euglycemic glucose clamp at three different insulin infusion rates while indirect calorimetry and a continuous recording of HRV by the Holter technique were performed. Finally, a hyperinsulinemic glucose clamp was also used along with propranolol infusion in lean subjects.

SUBJECTS AND METHODS

Subjects

Clinical characteristics of the study groups are shown in Table 1. Fifteen lean and 15 obese subjects were studied after an overnight 14-hour fast, and were required to refrain from alcohol intake in the prior 15 days. All subjects were nonsmokers and were recruited among the students of our school of medicine. Each subject was admitted to our department 3 days before each study and ate an isocaloric diet with a caloric distribution of 50% carbohydrate, 30% fat, and 20% protein. Sodium intake was 4.5 to 6 g/d depending on the weight-maintenance caloric requirement. All women were studied in the follicular phase. All subjects were free of any disease, and none were on medication. All were weight-stable for at least 3 months before the study, and none participated in a regular exercise program. All had normal glucose tolerance to a 75-g oral glucose load.[9] Blood pressure was slightly higher in the obese versus the lean group, but it was within the range considered clinically normotensive. All subjects underwent bioimpedance analysis to determine body composition.[10] Waist and hip circumferences were measured in the standing position at the level of the umbilicus and the level of the anterior superior iliac spine, respectively. All subjects provided informed consent to participate in the study, which was approved by the ethics committee of our institution.

Experimental Design

The study protocol was designed to assess in lean and obese subjects the change in HRV with graded infusions of insulin. Each subject was studied on two separate occasions 3 days apart. The order of the studies was assigned at random.

At 7 AM, a catheter was inserted into the right brachial vein for substance infusions. Another catheter was threaded into a wrist vein

From the Department of Geriatric Medicine and Metabolic Diseases, II University of Naples, Naples, Italy.

Submitted July 24, 1998; accepted December 3, 1998.

Address reprint requests to Giuseppe Paolisso, MD, Department of Geriatric Medicine and Metabolic Diseases, Servizio di Astanteria Medica, Piazza Miraglia 2, I-80138 Napoli, Italy.

0026-0495/99/4806-0014$10.00/0

Table 1. Clinical Characteristics of Normal-Weight Healthy Controls and Obese Patients

Characteristic	Controls (n = 15)	Obese Patients (n = 15)	P
Sex ratio (male/female)	9/6	7/8	NS
Age (yr)	33.4 ± 5.2	35.2 ± 4.1	NS
BMI (kg/m²)	23.3 ± 0.7	32.2 ± 3.5	.001
Body fat (%)	23 ± 2	32 ± 3	.001
Fasting plasma glucose (mmol/L)	4.8 ± 0.1	5.1 ± 0.2	NS
2-h plasma glucose (mmol/L)	6.5 ± 0.2	7.1 ± 0.3	NS
Fasting plasma insulin (pmol/L)	75 ± 7.1	92 ± 8.4	.01
Fasting plasma leptin (ng/mL)	11.2 ± 3.1	52.3 ± 8.8	.009
Fasting plasma FFA (μmol/L)	167 ± 43	389 ± 67	.005
Adjusted Rq	0.86 ± 0.02	0.78 ± 0.03	.007
Systolic blood pressure (mm Hg)	115 ± 10.2	125 ± 6.2	NS
Diastolic blood pressure (mm Hg)	70 ± 4.1	75 ± 6.3	NS
Heart rate (beats/min)	70 ± 5.1	70 ± 8.3	NS
TP (ms²)	12,031 ± 895	4,184 ± 292	.006
LF (nu)	67 ± 6.2	24 ± 2.1	.01
HF (nu)	33 ± 1.3	76 ± 5.2	.01
LF/HF ratio	2.1 ± 0.4	0.4 ± 0.09	.005

NOTE. Results are the mean ± SD.

Abbreviations: NS, not significant; BMI, body mass index; TP, total power.

retrogradely, and the hand was then placed in a heated box (~60°C) for sampling of arterialized blood. After basal measurements, subjects received either square-wave infusions of regular insulin (Humulin R; Eli Lilly, Florence, Italy) at a rate of 0.50, 1, and 2 mU/kg · min (each lasting 90 minutes; insulin study) with euglycemia maintained via the clamp technique,[11] or saline infusion (saline study). In a subset of seven lean subjects, the insulin study was repeated along with propranolol infusion (0.1 mg · kg^{-1} as an intravenous bolus dose followed by continuous intravenous infusion of 0.5 mg · kg^{-1} · min^{-1} throughout the study, Inderal; Zeneca, Milan, Italy; propranolol study). These subjects were chosen because they had anthropometric and metabolic characteristics similar to those of the whole group of lean subjects. The insulin study and insulin propranolol study were always at least 3 days apart. During the saline study, the saline load was matched to the overall volume of saline received during the insulin study without attempting to replace the free water load obligate as 20% dextrose during the hyperinsulinemic-euglycemic glucose clamp. Saline and insulin infusions were of equal length. During each clamp, K_2HPO_4 (0.0038 mEq/kg · min) was infused to prevent hypokalemia and hypophosphatemia. Serum potassium levels were more than 3.8 mEq/L at all times during the hyperinsulinemic glucose clamp. Serum phosphate was unchanged from the basal value throughout the studies. Measurements of insulin-mediated glucose uptake or whole-body glucose disposal ([WBGD] only calculated for 1- and 2-mU/kg · min insulin infusion rate), mean arterial blood pressure (MABP), leg (muscle) blood flow (LBF), leg vascular resistance (LVR), and plasma norepinephrine were obtained in the last 30 minutes (60 to 90 minutes) of each study. Each LBF, LVR, and plasma norepinephrine value represents the mean of at least four determinations made at baseline and in the last 30 minutes at each insulin infusion rate. HRV was also determined in basal conditions and throughout the studies.

Indirect Calorimetry

The respiratory quotient (Rq) and substrate oxidation were calculated from respiratory gas exchange (determined by computerized flow-through canopy gas analyzer system, Deltatrac; Datex, Helsinki, Finland) and urinary nitrogen excretion after correction for changes in the body urea nitrogen pool.[12] At 1 and 2 mU/kg · min insulin infusion, WBGD was assumed to equal insulin-mediated glucose uptake. The rate of nonoxidative glucose metabolism was calculated by subtracting the rate of glucose oxidation from the rate of WBGD at the steady state (last 30 minutes) of the 1- and 2-mU/kg · min insulin infusion.

Cardiovascular Determinations

All cardiovascular measurements were made with subjects under quiet conditions with room temperature maintained at 21°C. Before the procedures, all patients were asked to rest comfortably in bed in the supine position for at least 30 minutes. An effort was made to keep patients unaware of the sampling times to avoid circumstances affecting the heart rate; furthermore, all subjects were accustomed to breathing at a constant rate and were advised to avoid talking during the study.

Baseline blood pressure was recorded by a standard mercury sphygmomanometer (with diastolic blood pressure corresponding to Korotkoff phase V). All determinations were made with the subject at rest after 15 minutes in the supine position on three occasions separated by an interval of 5 minutes; the mean value of three measurements was then calculated and expressed as the baseline arterial blood pressure. Along with the clamp studies, arterial blood pressure and heart rate were determined in real time by a Finapres monitor (Omheda, Englewood, CO). Ambulatory electrocardiographic monitoring was performed with two-channel frequency modulated tape recorders (Remco Italia Cardioline AD 35, recorder model LP103, Milan, Italy). To ensure that variations did not introduce frequency components to the data, after each experiment, the speed of the tape recorder was checked by an expert technician. After accurate skin preparation, electrodes were placed on the chest for bipolar chest leads CM1 (modified V1) on the first channel and CM4 (modified V4) on the second channel. Holter monitoring started 30 minutes after the superficial veins were cannulated. The electrocardiogram was always recorded starting at 8 AM for a 330-minute period (60 minutes for baseline and 270 minutes for glucose clamp). Two experienced investigators analyzed the ambulatory electrocardiographic recording tapes made by the Holter AD35 TOP (Remco Italia Cardioline). An expert cardiologist subsequently checked each QRS complex and triggering R wave. Ectopic beats were corrected for linear interpolation with the adjacent complexes. Electrocardiographic tracings with greater than 1% premature beats were eliminated from the analysis. Power spectral analysis was calculated from a consecutive series of 512 intervals. The power spectral densities were computed by an autoregressive algorithm. Autoregressive spectral analysis was performed after estimation of model coefficients by the Levinson-Durbin algorithm. The model order selection was performed according to the Akaike information criterion[13] and Anderson test.[14] Main spectral components were identified and estimated using the spectral-decomposition algorithm proposed by Johnsen and Andersen, and were then assigned to one of three bands on the basis of their central frequency: very–low-frequency (VLF) band (0 to 0.03 Hz), LF band (0.03 to 0.15 Hz), or HF band (0.15 to 0.45 Hz). Since the physiologic explanation for the VLF component is much less defined and the existence of a specific physiologic process attributable to the heart period change have been strongly questioned,[2] only LF and HF periods were normally considered. LF and HF were always reported in normalized units that represent the relative value of each power component in proportion to the total power minus the VLF component.[2] Normalized units tend to minimize the effect of the changes in total power on the LF and HF components.[2] The LF/HF ratio, an index of sympathovagal balance rather than LF and HF expression of SNS and

parasympathetic nervous system activity per se, was used to calculate and report our data.

Blood flow in the calf was measured with venous occlusion plethysmography (EC 5R; Hokanson, Milan, Italy) using mercury-in-silastic strain gauges. The calf was elevated 10 to 15 cm above the level of the atrium to collapse the veins. Circulation to the foot was arrested by inflating a cuff around the ankles during blood flow determinations, which were performed at 15-second intervals for 5 minutes at baseline and in the last 30 minutes at each insulin infusion rate. LVR was calculated by dividing the MABP (millimeters of mercury) and LBF (liters per minute) and is expressed in arbitrary units.

Analytical Techniques

Plasma glucose was immediately determined by the glucose oxidase method (Beckman Autoanalyzer; Beckman Instruments, Fullerton, CA). Blood samples for insulin and hematocrit measurements were collected in heparinized tubes. After centrifugation, plasma insulin (Sorin Biomedical, Milan, Italy; coefficient of variation [CV], 3.2% ± 0.2%) and leptin (Linco Research, St Louis, MO; CV, 4.5% ± 0.5%) concentrations were determined by radioimmunoassay. The plasma catecholamine concentration was determined by high-performance liquid chromatography. Urinary nitrogen was determined by the Kjehldahl method.

Statistical Analyses

All results are presented as the mean ± SD. Due to the strong difference in the basal LF/HF ratio between lean and obese subjects, the insulin-mediated increase in the LF/HF ratio was calculated as the Δ with basal values equal to 0. MABP was calculated as diastolic blood pressure plus one third of the systolic-diastolic blood pressure. In correlation analyses, the ΔLF/HF ratio, LBV, LVR, and plasma norepinephrine concentration were calculated as the difference between the baseline value and the mean values for the last 30 minutes at the highest insulin infusion rate. Because the distribution of the frequency domain measures of HRV and plasma insulin and leptin concentrations were extremely skewed, each value was also logarithmically transformed to improve normality for statistical testing and back-transformed for presentation in tables and figures. Student's T test for unpaired data allowed calculation of the difference between the two study groups. Correlations are Pearson product-moment correlations. ANOVA for repeated measurements was performed to calculate time-dependent changes in the ΔLF/HF ratio. Analysis of covariance (ANCOVA) allowed adjustment of the ΔLF/HF ratio for age, gender, body fat, ΔLBF, ΔLVR, and Δplasma norepinephrine, as well as adjustment of the Rq for age, fat mass, and fat-free mass (FFM). For each subject, only adjusted Rq values are reported, and were calculated as the mean value for the group plus the difference between the measured and predicted values for these subjects. Partial correlations allowed a test of the relationship between the ΔLH/HF ratio (as the difference between the basal value and the mean value at the end of the highest insulin infusion rate) and WBGD independently of age, body fat, ΔLVR, ΔLFB, and Δplasma norepinephrine. A P value of .05 was chosen as the level of significance. All calculations were made on an IBM (Portsmouth, England) PC computer with the SOLO software package (BMDP, Cork, Ireland).

RESULTS

Baseline Data

At baseline, lean subjects had a lower body mass index, body fat content, and fasting plasma insulin, leptin, and free fatty acid concentration than obese subjects. The adjusted basal Rq was greater in lean versus obese subjects. Systolic and diastolic blood pressure were slightly but not significantly different between the two groups. The total power and LF were more elevated in lean versus obese subjects, whereas HF was more elevated in obese patients; consequently, lean subjects had a more elevated LF/HF ratio than obese subjects (Table 1). Independently of age and gender, fasting plasma leptin correlated with the basal LF/HF ratio ($r = -.53$, $P < .03$) only in obese subjects. Nevertheless, the correlation was lost after further adjustment for body fat content.

Glucose Clamp Data

In the saline study, saline infusion did not affect any metabolic, cardiovascular, or hemodynamic parameters in lean or obese subjects at any time. Thus, all data are presented comparing the effects of insulin infusion (insulin study) in lean and obese subjects.

Baseline fasting plasma glucose concentrations were not different and stayed within a narrow range (CV, 3.8% ± 0.3% *v* 4.1% ± 0.2%, P = NS) throughout the studies in both groups. Baseline fasting plasma insulin concentrations were lower in lean versus obese subjects (Table 1). Insulin infusion was associated with a progressive increase in plasma insulin without a difference between the two study groups. In these metabolic conditions, insulin-mediated glucose uptake progressively increased in both study groups; nevertheless, lean subjects had better insulin action than obese subjects (Fig 1). Lean subjects had also a more elevated nonoxidative glucose metabolism than obese subjects (26.7 ± 0.8 *v* 12.4 ± 0.6 μmol/kg FFM · min, $P < .01$, and 38.3 ± 0.5 *v* 22.1 ± 0.4 μmol/kg FFM · min, $P < .001$) at 1 and 2 mU/kg · min insulin infusion, respectively. Since the basal LF/HF ratio was significantly different between lean and obese subjects (Table 1), the Δ from the basal value was calculated. Insulin infusion was associated with an increase of the ΔLF/HF ratio in both lean ($P < .001$ for time-dependent changes) and obese ($P < .02$ for time-dependent changes) subjects; however, the extent of insulin-mediated stimulation of the LF/HF ratio was greater in lean versus obese subjects (Fig 1). Insulin infusion did not significantly affect HF values in both lean and obese subjects (data not shown).

Insulin infusion was also associated with changes in hemodynamic parameters. In particular, insulin increased LBF in both lean ($P < .005$ for time-dependent changes) and obese ($P < .05$ for time-dependent changes) subjects. Nevertheless, the extent of the insulin-mediated change in LBF was greater in lean versus obese subjects. MABP showed slight but nonsignificant changes throughout the glucose clamps. LVR significantly declined in lean subjects ($P < .01$ for time-dependent changes), whereas it remained stable in obese subjects. Thus, significant differences between the study groups were found. The basal fasting plasma norepinephrine concentration was slightly but nonsignificantly lower in lean versus obese subjects. Insulin infusion significantly increased plasma norepinephrine in both study groups, but without a difference between lean and obese subjects (Table 2).

Independently of gender, body fat content, Δplasma norepinephrine, ΔLBF, and ΔLVR, only the ΔLF/HF ratio at the end of the fastest insulin infusion rate (0.8 ± 0.2 *v* 0.3 ± 0.2, $P < .04$) was still different between lean and obese subjects.

The ΔLF/HF ratio was significantly correlated with WBGD (calculated at the steady state of the highest insulin infusion

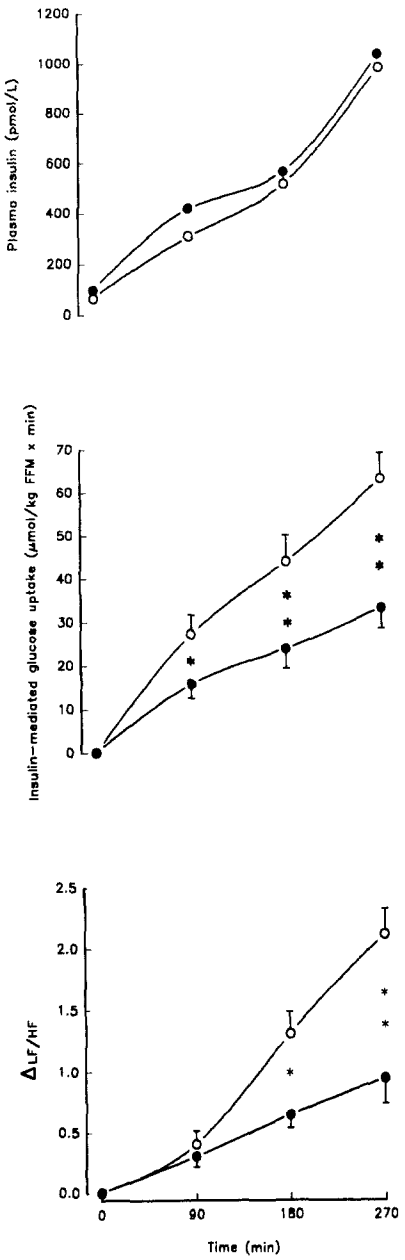

Fig 1. Changes in plasma insulin, insulin-mediated glucose uptake, and ΔLF/HF ratio in lean (n = 15; ○) and obese (n = 15; ●) subjects during graded insulin infusion rates (0.50 mU/kg · min from 0 to 90 minutes, 1 mU/kg · min from 91 to 180 minutes, and 2 mU/kg · min from 181 to 270 minutes. Statistically significant differences between the 2 groups: *$P < .02$, **$P < .001$.

rate) in lean and obese subjects. Such correlations were also independent of age, gender, body fat, ΔLBF, ΔLVR, and Δplasma norepinephrine (Fig 2).

Propranolol Study (n = 7)

Baseline fasting plasma glucose concentrations (4.8 ± 0.3 *v* 5.0 ± 0.3 mmol/L, P = NS) were not different and stayed within a narrow range (CV, 3.8% ± 0.3% *v* 3.9% ± 0.3%, P = NS) in hyperinsulinemia (I) and hyperinsulinemia plus propranolol administration (I + P). Baseline fasting plasma insulin concentrations (61 ± 11 *v* 66 ± 12 pmol/L, P = NS) were similar before I and I + P, respectively. Insulin infusion was associated with a progressive increase in plasma insulin concentrations, which were not different in the two experimental conditions (Fig 3). In these metabolic conditions, insulin-mediated glucose uptake progressively increased in both study groups; nevertheless, better insulin action was found during I versus I + P. The baseline LF/HF ratio (2.1 ± 0.4 *v* 1.7 ± 0.5, P = NS) was not different; insulin infusion was associated with a significant stimulation of the LF/HF ratio in I ($P < .001$ for time-dependent changes *v* baseline) and I + P ($P < .03$ for time-dependent changes *v* baseline). Comparing the two studies, the ΔLF/HF ratio was also more stimulated during I versus I + P (Fig 3).

Hemodynamic data and the plasma norepinephrine concentration before and during I and I + P are reported in Table 3. The baseline LBF, MABP, LVR, and plasma norepinephrine were not different in the two experimental conditions. In the glucose clamp, LBF and plasma norepinephrine increased with I ($P < .001$ for time-dependent changes) and I + P ($P < .05$ for time-dependent changes). Nevertheless, the extent of such change was greater in I versus I + P. MABP increased during I ($P < .01$ for time-dependent changes), but it was unaffected by I + P. Thus, statistically significant differences between I and I + P were found. LVR declined in the I study, whereas it was slightly but nonsignificantly reduced in the I + P study.

In the I + P study, changes in the plasma norepinephrine concentration and ΔLH/HF ratio were poorly correlated ($r = .38$, P = NS).

DISCUSSION

Our study confirms that insulin shifts cardiac sympathovagal balance toward a sympathetic predominance and demonstrates that (1) the stimulatory effect of insulin is dose-dependent and related to the higher degree of insulin action in lean compared with obese subjects; and (2) the effect of insulin on the LF/HF

Table 2. Hemodynamic Data and Plasma Norepinephrine Concentration in Lean (n = 15) and Obese (n = 15) Groups

Parameter	Baseline	Step I	Step II	Step III
LBF (dL/min)				
Lean	2.3 ± 0.2	3.0 ± 0.2	3.6 ± 0.3	4.0 ± 0.3
Obese	2.1 ± 0.1	2.1 ± 0.2*	2.4 ± 0.4*	2.4 ± 0.1*
NE (nmol/L)				
Lean	1.35 ± 0.17	1.80 ± 0.22	2.15 ± 0.11	2.30 ± 0.14
Obese	1.47 ± 0.11	1.91 ± 0.15	2.25 ± 0.14	2.42 ± 0.11
MABP (mm Hg)				
Lean	85 ± 4	93 ± 3	101 ± 4	102 ± 5
Obese	91 ± 3	97 ± 2	102 ± 4	103 ± 2
LVR (mm Hg · $dL^{-1} \cdot min^{-1}$)				
Lean	37 ± 4	31 ± 3	28 ± 3	25 ± 4
Obese	43 ± 5	46 ± 4*	42 ± 4*	43 ± 5*

NOTE. LVR was calculated by dividing MABP by LBF. Only values for the insulin study are reported. At baseline, no differences between the insulin study and saline study were found. With saline infusion, all values were superimposable to basal values in both groups. Results are the mean ± SD.

Abbreviation: NE, norepinephrine.

*$P < .01$ *v* lean.

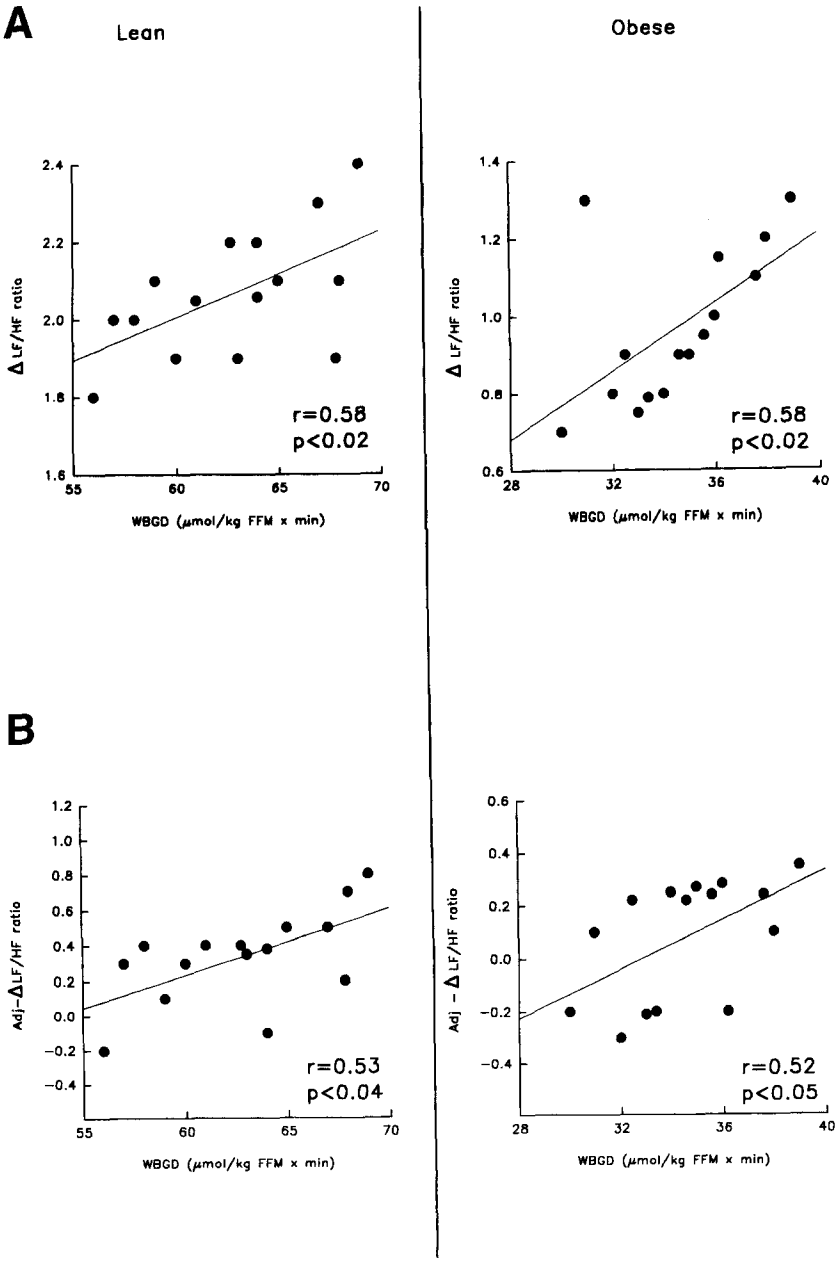

Fig 2. Correlations between the ΔLF/HF ratio and WBGD in lean (n = 15) and obese (n = 15) subjects. Simple (A) and partial (B) correlations are reported. For partial correlations, ΔLF/HF ratio was adjusted for age, gender, body fat, ΔLBF, ΔLVR, and Δplasma norepinephrine.

ratio is only partially dependent on the changes in hemodynamic parameters (LBF and LVR) and in the plasma norepinephrine concentration. Finally, we also confirm that obese subjects have a lower LF/HF ratio than healthy lean subjects.

The SNS is an important regulatory mechanism in both metabolic and cardiovascular functions. HRV analysis might be a useful alternative method to investigate autonomic nervous system activity at the cardiac level. In fact, the LF/HF ratio is considered to mirror cardiac sympathovagal balance.[2-4] It has previously been shown that glucose ingestion enhances the LF/HF ratio.[5] Whether the stimulatory effect of glucose on the LF/HF ratio is due to glucose per se or is mediated by an increase in plasma insulin remains undetermined. In our study, the glucose clamp technique allowed maintenance of the fasting plasma glucose concentration within a narrow range despite a remarkable increase in plasma insulin. In these metabolic conditions, changes in plasma insulin rather than glucose stimulate the LF/HF ratio and thus produce a shift of the cardiac autonomic nervous system activity toward sympathetic predominance. Such results are in agreement with previous data highlighting the stimulatory role of insulin on SNS activity. In animals, Rappaport et al[15] used 2-deoxyglucose, an inhibitor of intracellular glucose metabolism, to evaluate the relationship between the glucose load and SNS activity independently of intracellular glucose metabolism. The conclusion of the study was that long-term ingestion of 2-deoxyglucose elicits a SNS activity (evaluated as increased adrenaline urinary excretion and adrenal medullar adrenaline depletion) not associated with intracellular glucose metabolism. In human subjects, Rowe et al[16] provided evidence for a stimulation of SNS activity with the

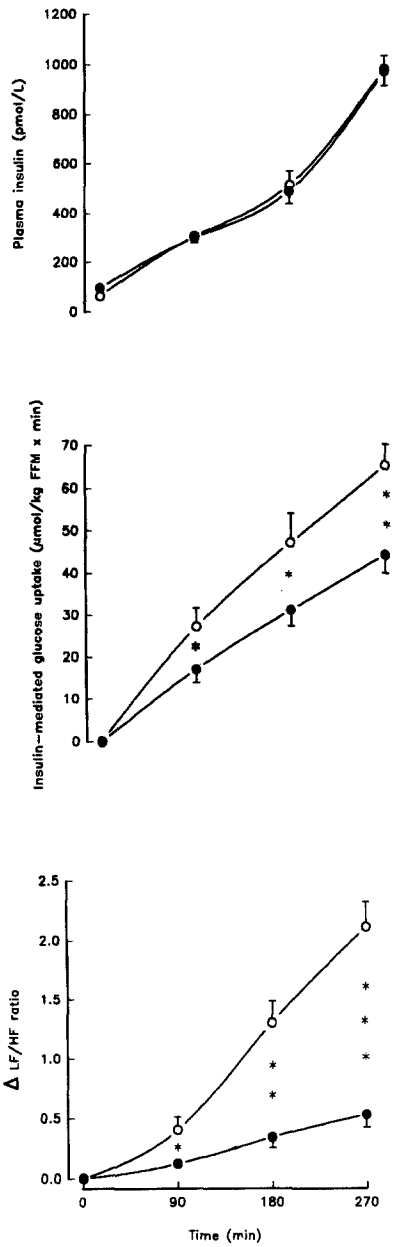

Fig 3. Changes in plasma insulin, insulin-mediated glucose uptake, and ΔLF/HF ratio during insulin (○) and insulin + propranolol (●) infusion in lean subjects (n = 7). In both studies, graded insulin infusion rates were used (0.50 mU/kg · min from 0 to 90 minutes, 1 mU/kg · min from 91 to 180 minutes, and 2 mU/kg · min from 181 to 270 minutes). Propranolol was infused at 0.1 mg · kg^{-1} as an intravenous bolus dose followed by a continuous intravenous infusion of 0.5 mg · kg^{-1} · min^{-1} throughout the study. Statistically significant differences between the 2 groups: *$P < .01$, **$P < .001$.

euglycemic-hyperinsulinemic clamp. In particular, hyperinsulinemia was associated with a significant increase in the plasma adrenaline concentration[16]; the investigators suggested that insulin may stimulate SNS activity by increasing glucose metabolism in critical central neurons. Berne et al[17] studied changes in SNS activity by MSNA following carbohydrate feeding. The study showed a similar stimulatory effect of oral D-glucose and D-xylose on MSNA,[17] thus excluding the possibility that intracellular glucose metabolism plays a regulatory role in SNS activity. Fructose ingestion, which increases carbohydrate oxidation as much as glucose ingestion but does not cause hyperinsulinemia, does not increase SNS activity in muscle.[17] In lean healthy subjects, hyperinsulinemia-induced stimulation of skeletal muscle blood flow is accompanied by a marked stimulation of sympathetic neural outflow, which in turn is responsible for norepinephrine release.[18] Most recently, an inverse relationship has also been demonstrated between the fasting plasma insulin concentration, a proxy of insulin resistance,[19] and the basal LF/HF ratio in non–insulin-dependent diabetics and nondiabetics.[20] Furthermore, insulin infusion has been shown to stimulate the sympathetic component of cardiac sympathovagal balance in the offspring of non–insulin-dependent diabetic parents with insulin resistance.[21] However, in this latter study, only one dose of insulin was used and no hemodynamic parameters were recorded.

Interestingly, our study also demonstrates that the effect of insulin on the LF/HF ratio was only partially dependent on the effect of insulin on LBF, LVR, and plasma norepinephrine. In fact, we performed a dose-dependent study measuring the effect of graded insulin infusion on changes in hemodynamic parameters, plasma norepinephrine, and HRV. As expected, insulin-mediated stimulation of the LF/HF ratio paralleled the insulin-mediated changes in hemodynamic parameters and plasma norepinephrine concentrations. Thus, one could suggest that the effect of insulin on HRV is mainly mediated by the changes in LBF, LVR, and plasma norepinephrine and is therefore dependent on baroreflex activity. Nevertheless, with insulin infused at the highest rate (2 mU/kg · min), the stimulatory effect of insulin on HRV was found to be independent of the changes in hemodynamic parameters and plasma norepinephrine. One could argue that an insulin infusion rate of 2 mU/kg · min produced a supraphysiologic plasma insulin concentration; indeed, such concentrations frequently occur in the postprandial

Table 3. Hemodynamic Data and Plasma Norepinephrine Concentration During Glucose Clamp Without and With Propranolol Administration in Lean Subjects (n = 7)

Parameter	Baseline	Step I	Step II	Step III
LBF (dL/min)				
I	2.3 ± 0.2	3.0 ± 0.2	3.6 ± 0.3	4.0 ± 0.3
I + P	2.2 ± 0.3	2.3 ± 0.3*	2.6 ± 0.4*	2.9 ± 0.3*
NE (nmol/L)				
I	1.35 ± 0.17	1.80 ± 0.22	2.15 ± 0.11	2.30 ± 0.14
I + P	1.18 ± 0.21	1.88 ± 0.23	2.20 ± 0.20	2.33 ± 0.17
MABP (mm Hg/L)				
I	85 ± 4	93 ± 3	101 ± 4	102 ± 5
I + P	85 ± 3	85 ± 4	88 ± 3*	90 ± 4*
LVR (mm Hg · dL^{-1} · min^{-1})				
I	37 ± 4	31 ± 3	28 ± 3	25 ± 4
I + P	38 ± 3	37 ± 5	33 ± 6*	31 ± 3*

NOTE. Results are the mean ± SD. P was infused at 0.1 mg · kg^{-1} as an intravenous bolus dose followed by a continuous intravenous infusion of 0.5 mg · kg^{-1} · min^{-1} throughout the study.

Abbreviations: I, hyperinsulinemia; P, propranolol.

*$P < .01$, I *vl* + P.

state or after an oral glucose load in obese subjects. Additional evidence comes from the propranolol study. Propranolol is a nonselective β-adrenergic antagonist with no intrinsic sympathetic activity. With propranolol infusion, insulin-mediated glucose uptake was decreased by 27%, a value not different from that reported previously.[22] The propranolol-mediated decline in insulin-mediated glucose uptake parallels the decline in insulin-mediated stimulation of the LF/HF ratio, with the two phenomena being correlated. Since propranolol infusion counteracts the interference of insulin on hemodynamic parameters, at least at the dose used in the present study,[23-24] the stimulatory role of insulin on the LF/HF ratio should also be due to other pathophysiologic pathways. A direct stimulatory effect of insulin on the central nervous system[25] cannot be ruled out. In fact, it has been shown that insulin acts on ventromedial hypothalamic cells,[2,25] and such an effect might explain the complex metabolic and cardiovascular interrelations among an elevated body weight gain, SNS overactivity, and hypertension. Thus, one cannot exclude the possibility that the stimulatory effect of insulin on the LF/HF ratio might be part of the stimulatory effect of insulin on ventromedial hypothalamic cells. In obese patients, we found a lower stimulatory response of HRV components in response to insulin infusion. Such results might be due to an impaired insulin-mediated change in LBF and LVR, a result in agreement with previous studies,[26-27] and to the presence of insulin resistance. The main role of insulin resistance as a determinant of impaired insulin-mediated stimulation of the LF/HF is also in agreement with the hypothesis that in insulin-resistant subjects a lower stimulation of the SNS may also be responsible for an exaggerated weight gain.[7]

An indirect finding of our study is the positive correlation between the fasting level of plasma leptin, the product of the ob gene, and the baseline LF/HF ratio. This finding seems to support the evidence that obesity is associated with SNS overactivity.[8]

With regard to substrate oxidation, the lack of significant correlation between the LF/HF ratio and insulin-stimulated nonoxidative glucose metabolism seems to support the hypothesis that the relationship between SNS activity and insulin action does not affect the intracellular metabolic fate of glucose.

Our study may be similar to others.[28-29] Nevertheless, several differences exist between our study and previous studies: (1) in our study, a dose-effect curve between the plasma insulin concentration and changes in HRV in both lean and obese subjects was constructed; (2) we attempted to differentiate between a central and/or cardiac effect of insulin by using propranolol infusion; and (3) in the study by Bellavere et al,[28] only a very small group of subjects were investigated. Such differences may thus account for the discrepancy in the results. In fact, the insulin-mediated increase in the LF/HF ratio was related to HF inhibition in one study[27] and to LF stimulation in our study.

A potential limitation of our study may be the length of each insulin infusion rate. In fact, a previous study described insulin to activate intracellular processes with a delay in obese compared with lean subjects.[30] Thus, one could argue that a different response in the ΔLF/HF ratio to insulin infusion between lean and obese subjects might be due to the activation time. However, taking into account the insulin infusion rates and the peripheral dose attained, as well as the length of infusion, such a possibility is very unlikely.

In conclusion, our study demonstrates that insulin stimulates HRV components in both lean and obese patients and thus produces a shift of the cardiac autonomic nervous system activity toward sympathetic predominance. Such changes are only partially dependent on changes in LBF and LVR.

REFERENCES

1. Revean G, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities—The role of insulin resistance and the sympathoadrenal system. N Engl J Med 334:374-381, 1996
2. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability. Standards of measurements, physiological interpretation, and clinical use. Eur Heart J 17:354-381, 1996
3. Malliani A, Pagani M, Lombardi F, et al: Cardiovascular neural regulation explored in the frequency domain. Circulation 84:1482-1492, 1991
4. Malliani A, Lombardi F, Pagani M: Power spectral analysis of heart rate variability: A tool to explore neural regulatory mechanisms. Br Heart J 71:1-4, 1994
5. Paolisso G, Manzella D, Ferrara N, et al: Glucose ingestion affects cardiac ANS in healthy subjects with different amounts of body fat. Am J Physiol 273:E471-E478, 1997
6. Landsberg L: Insulin resistance, energy balance and sympathetic nervous system activity. Clin Exp Hypertens A12:817-830, 1990
7. Spraul M, Ravussin E, Fontvieille AM, et al: Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. J Clin Invest 92:1730-1735, 1993
8. Grassi G, Seravalle G, Cattaneo BM, et al: Sympathetic activation in obese normotensive subjects. Hypertension 25:560-563, 1997
9. World Health Organization Study Group: Diabetes mellitus. World Health Organ Tech Rep Ser 727:9-17, 1985
10. Deurenberg P, Van der Koij E, Evers P, et al: Assessment of body composition by bioelectrical impedance in a population aged >60 yrs. Am J Clin Nutr 51:3-6, 1990
11. De Fronzo RA, Tobin JD, Anderson R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979
12. Ferrannini E: The theoretical basis of indirect calorimetry: A review. Metabolism 37:287-301, 1988
13. Kay SM: Modern Spectral Estimation—Theory and Application. Englewood Cliffs, NJ, Prentice-Hall, 1977, pp 221-237
14. Johnsen SJ, Andersen N: On power estimation in maximum entropy spectral analysis. Geophysics 43:681-690, 1978
15. Rappaport EB, Young JB, Landsberg L: Effects of 2-deoxy-D-glucose on the cardiac sympathetic nerves and the adrenal medullary response. Endocrinology 110:650-656, 1982
16. Rowe JW, Young JB, Minaker KL, et al: Effect of insulin and glucose infusion on sympathetic nervous activity in normal man. Diabetes 30:219-222, 1982
17. Berne C, Fagius J, Niklasson F: Sympathetic response to oral carbohydrate administration. Evidence from microelectrode nerve recordings. J Clin Invest 84:1403-1409, 1989
18. Vollenweider P, Tappy L, Randin D, et al: Differential effects of

hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 92:147-154, 1993

19. De Fronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and cardiovascular disease. Diabetes Care 14:173-194, 1991

20. Tory JP, Niskanen LK, Mantysaari MJ, et al: Do high proinsulin and C-peptide levels play a role in autonomic nervous dysfunction? Power spectral analysis in patients with non–insulin dependent diabetes and non-diabetic patients. Circulation 96:1185-1191, 1997

21. Laitinen T, Vauhkonen I, Niskanen L, et al: Heart rate variability during euglycemic hyperinsulinemic clamp in offspring of NIDDM patients and control subjects, in IDF Meeting Abstract Book. Helsinki, Finland, 1997, p A577 (abstr)

22. Lithell H, Pollare T, Vesseby B: Metabolic effects of pindolol and propranolol in a double blind cross-over study in hypertensive patients. Blood Press 1:92-101, 1992

23. Creager MA, Liang CS, Coffman JD: Beta-adrenergic mediated vasodilator response to insulin in the human forearm. J Pharmacol Exp Ther 235:709-714, 1985

24. Van Veen S, Chang PC: Prostaglandin and nitric oxide mediate insulin-induced vasodilation in the human forearm. Cardiovasc Res 34:223-229, 1997

25. Landsberg L, Young JB: Insulin-mediated glucose metabolism in the relationship between dietary intake and sympathetic nervous system activity. Int J Obes 9:63-68, 1985 (suppl 2)

26. Laakso M, Edelman SV, Brechtel G, et al: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J Clin Invest 85:1844-1852, 1990

27. Vollenweider P, Randin D, Tappy L, et al: Impaired insulin-induced sympathetic neural activation and vasodilation in skeletal muscle in obese humans. J Clin Invest 93:2365-2371, 1994

28. Bellavere A, Cacciatori V, Moghetti P, et al: Acute effect of insulin on autonomic regulation of the cardiovascular system: A study by heart rate spectral analysis. Diabet Med 13:709-714, 1997

29. Muscelli E, Emdin M, Natali A, et al: Autonomic and hemodynamic responses to insulin in lean and obese humans. J Clin Endocrinol Metab 83:2084-2090, 1998

30. Prager R, Wallace P, Olefsky JM: In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 78:472-481, 1986