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Autonomic and Hemodynamic Responses to Insulin in Lean and Obese Humans*

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ABSTRACT

To study the acute effects of insulin on autonomic control of cardiac function, we performed spectral analysis of heart rate variability and measured cardiac dynamics (by two-dimensional echocardiography) in 18 obese (BMI = 35 ± 1 kg·m^{-2}) and 14 lean (BMI = 24 ± 1 kg·m^{-2}) subjects in the basal state and in response to physiological hyperinsulinemia (1 mU·min^{-1}·kg^{-1} insulin clamp). In the lean group, insulin promptly (within 20 min) and consistently depressed spectral powers, both in the low-frequency and high-frequency range. These changes were twice as large as accounted for by the concomitant changes in heart rate (68 ± 2 to 70 ± 2 beats/min). At the end of the 2-h clamp, stroke volume (67 ± 4 to 76 ± 9 ml·min^{-1}) and cardiac output (4.45 ± 0.21 to 5.06 ± 0.55 l·min^{-1}) rose, whereas peripheral vascular resistance fell. The low-to-high frequency ratio increased from 1.7 ± 0.2 to 2.3 ± 0.3 ($P < 0.01$), indicating sympathetic shift of autonomic balance. In the obese group, all basal spectral powers were significantly lower (by 40% on average) than in the lean group, and were further reduced by insulin administration. The low-to-high frequency ratio was higher than in controls at baseline (2.4 ± 0.4, $P < 0.03$), and failed to increase after insulin (2.2 ± 0.3, P = ns). Furthermore, obesity was associated with higher resting stroke volume (89 ± 5 *vs.* 67 ± 4 ml·min^{-1}, $P < 0.01$) and cardiac output (6.01 ± 0.31 *vs.* 4.45 ± 0.21 l·min^{-1}, $P = 0.001$) but lower peripheral vascular resistance (15.1 ± 0.8 *vs.* 19.2 ± 1.1 mmHg·min·L^{-1}, $P = 0.002$), whereas mean arterial blood pressure was similar to control (90 ± 2 *vs.* 86 ± 2 mmHg, P = not significant).

We conclude that physiological hyperinsulinemia causes acute desensitization of sinus node activity to both sympathetic and parasympathetic stimuli, sympathetic shift of autonomic balance, and a high-output, low-resistance hemodynamic state. In the obese, these changes are already present in the basal state, and may therefore be linked with chronic hyperinsulinemia. (*J Clin Endocrinol Metab* **83**: 2084–2090, 1998)

ACUTE insulin administration causes hemodynamic changes even when hypoglycemia is prevented. Thus, a rise in heart rate (1), a small decrease in peripheral vascular resistance (PVR) (2), and an increase in cardiac output (3) have been documented during euglycemic hyperinsulinemia. Moreover, hyperinsulinemia is associated with a dose-dependent increment in the circulating levels of norepinephrine (1), and with a marked stimulation of muscle nerve sympathetic activity (MSNA), as documented by peroneal nerve microneurography (4,5). The latter effect is stronger and more sustained than can be justified by the baroreflex, and, in general, MSNA can be dissociated from small blood pressure fluctuations [*e.g.* during insulin-induced hypoglycemia (6)]. Collectively, these findings have raised the possibility that some acute effects of insulin may be mediated through the central nervous system (7). Experimental support for this notion has been provided by a study in the conscious dog (8), which has shown that selective overinsulinization of the brain during insulin-induced hypoglycemia is associated with a more profound stimulation of hormonal and cardiovascular responses. The aim of the present study was to characterize the central response to insulin by measuring hemodynamics, heart rate variability (HRV), and counterregulatory hormones during physiological hyperinsulinemia with euglycemia.

Obesity is characterized by insulin resistance as well as hemodynamic abnormalities (9). The latter include increased cardiac output in the face of decreased total PVR (TPVR) and expanded blood volume. Whether obesity is also characterized by adrenergic activation has been controversial (10, 11). More recent data obtained in humans by microneurography have been concordant in finding that basal muscle nerve sympathetic activity is raised in direct proportion to body fat (12, 13). Landsberg (7) originally postulated that enhanced autonomic nervous activity in obese subjects is related to the hyperinsulinemia, and that the hypertension associated with obesity may be the maladaptive response to persistent sympathetic excitation. Moreover, the heart is a major target for damage in obesity, as documented by the increased cardiovascular morbidity and mortality that is associated with excess body weight (14, 15). Prolonged stimulation of the adrenergic system may be one of the mechanisms by which cardiovascular risk is enhanced in obesity (16). A further aim of the present study was to test whether the autonomic and hemodynamic responses to insulin are altered in obesity.

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Sympathovagal control of cardiac activity was assessed with the use of spectral analysis of HRV. The study of beat-to-beat HRV, modulated by central (vasomotor and respiratory centers) and peripheral oscillators (afferent inputs derived from fluctuations in arterial pressure and respiratory movements), provides quantitative markers of autonomic activity (17). Whereas microneurography only measures regional sympathetic outflow, spectral analysis of spontaneous heart rate oscillations permits an accurate, dynamic, and noninvasive evaluation of sinoatrial node sensitivity to both sympathetic and vagal influences.

Subjects and Methods

Study population

Thirty-two subjects [18 of whom were obese, as defined by a body mass index (BMI) $\geq$ 30 kg·m^{-2}) were studied. All had normal glucose tolerance [on the oral glucose tolerance test (OGTT) by the National Diabetes Data Group criteria (18)] and resting arterial blood pressure levels; none were taking any medications. All subjects had normal liver, renal, and endocrine function tests, and had not lost weight or changed dietary habits during the 6 months preceding the study. Their relevant clinical characteristics are given in Table 1. Another group of 8 healthy subjects (5 female, 3 male, age 37 $\pm$ 3 yr, BMI of 22.0 $\pm$ 0.6 kg·m^{-2}) volunteered for a time-control study (see below). The investigation was approved by the Institutional Review Board of the C.N.R. Institute of Clinical Physiology, and all subjects gave informed consent before the study began.

Experimental protocol

In all study subjects, body composition was evaluated by electrical bioimpedance (19), and the waist and hip circumferences were measured by the same physician. Each subject received an OGTT and a euglycemic insulin clamp on different days approximately 1 week apart. In the time-control experiments, a saline infusion replaced insulin during the clamp study. For the OGTT, 40 g·m^{-2} of glucose was ingested over 5 min, and venous blood was sampled at 30-min intervals for 2 h for plasma glucose measurements. The clamp study, which was carried out after an overnight (12–14 h) fast, consisted of 2 h of euglycemic insulin infusion [at an insulin infusion rate of 7 pmol·min^{-1}·kg^{-1} (= 1 mU$-$min^{-1}·kg^{-1}) (20)]. A polyethylene, 20-gauge catheter was inserted into an antecubital vein for the infusion of glucose and insulin. Another catheter was threaded into a wrist vein retrogradely, and the hand placed in a heated box (~60 C) for the sampling of arterialized blood (21). Following this procedure, the patients rested at least 30 min in the supine position. The following 2 h before the start of insulin infusion constituted the basal period. During the basal period and the insulin clamp, the following data were obtained: a) arterial blood pressure, which was measured by mercury sphygmomanometry at 10-min intervals (in obese individuals a large cuff was used); b) Holter recording of the electrocardiogram (ECG) using a bipolar lead frequency-modulation system (Remco-Cardioline, Milano, Italy) and both an inferior and a precordial lead; c) circulating hormone (CRH, cortisol, GH, PRL, TSH, epinephrine, and norepinephrine) concentrations, which were sampled twice at the end of the 2 h of baseline and twice at the end of the insulin clamp; and d) cardiac output, which was determined noninvasively (in 21 of 32 study subjects) by two-dimensional echocardiography (22) at the end of the basal and clamp periods by the same physician (L.P.). Throughout the study protocol, patency of the sampling catheter was maintained by injecting 1 mL saline after each blood draw (no heparin was used). Furthermore, the blood loss caused by the sampling was replaced by iv saline, whereas the urine loss was empirically replaced by 150 mL of water ingested at the beginning of the basal and the clamp periods. Urine was collected at the end of each study period; in the whole group, urine output averaged 2.0 $\pm$ 0.2 [SEM ml·min^{-1} during the basal period, and 1.7 $\pm$ 0.2 ml·min^{-1} during the clamp; P = not significant (NS) by Wilcoxon's signed-rank test].

TABLE 1. Clinical and metabolic characteristics of study population

	Lean (n = 14)	Obese (n = 18)
Sex (F/M)	10/4	12/6
Age (yr)	33 $\pm$ 2	39 $\pm$ 2
Body weight (kg)	69 $\pm$ 3	98 $\pm$ 4[a]
BMI (kg·m^{-2})	23.7 $\pm$ 1.0	35.3 $\pm$ 1.0[a]
Waist/hip ratio	0.79 $\pm$ 0.03	0.88 $\pm$ 0.02[a]
Fat mass (%)	29 $\pm$ 2	38 $\pm$ 1[a]
Fasting plasma glucose (mmol/L)	5.0 $\pm$ 0.1	5.2 $\pm$ 0.1
Steady state plasma glucose (mmol/L)	5.0 $\pm$ 0.1	5.3 $\pm$ 0.1
Fasting plasma insulin (pmol/L)	75 $\pm$ 4	109 $\pm$ 8[a]
Steady state plasma insulin (pmol/L)	551 $\pm$ 32	727 $\pm$ 32[a]
M value (μmol·min^{-1}·kg FFM^{-1})	46 $\pm$ 3	36 $\pm$ 3[a]

[a] $P \leq 0.01$ or less for difference between lean and obese subjects.

ECG data processing

The ECG was digitized at 250 samples per sec with a 12-bit per sample precision, and stored in a binary format (2 bytes per sample) on digital tape for further computer analysis. The selected 250 Hz frequency allows detection of R-R oscillations up to 4 msec. The ECG was processed by using extensively tested algorithms (23) to detect the QRS complex and the R-wave reference point by a derivative/amplitude criterion, without interpolation of the original signal. To obtain a spectral representation of R-R, the autoregressive technique was found to be appropriate because of the nondeterministic behavior of the time series (24). The time series were analyzed in consecutive intervals of 256 data points. The intervals were processed by the Levinson-Durbin recursive algorithm (25) to generate the autoregressive coefficients. In the present analysis, the number of coefficients was set at 12. The goodness of the model was tested by evaluating the normality of the distribution of the resulting white noise. For each 256-data point interval, the power spectral function was evaluated, and the most significant spectral components were extracted according to a spectral decomposition algorithm (26, 27).

For the purpose of the present analysis, two major frequency components were considered in the R-R power spectrum: a low-frequency (LF) component (0.03–0.15 Hz, predominantly related to baroreflex control of arterial blood pressure by both sympathetic and vagal activity) and a high-frequency (HF) component (0.15–0.40 Hz, ascribed mostly to respiratory sinus arrhythmia) (28–30). The LF/HF ratio, which is regarded as an index of sympathovagal balance, was also computed from each LF and HF pair. The LF and HF components were expressed in absolute (msec^2) as well as normalized units (*i.e.* as ratios to the total power minus the VLF component). When expressed in normalized units, LF is considered to be a quantitative index of sympathetic activity (31). From each spectrum, the mean R-R interval, the total spectrum power, the power and frequency of each component, and the LF/HF ratio were stored for statistical analysis. The respiratory rate was obtained both from the central frequency of the HF component and by separate spectral analysis of R-wave amplitude variability (the latter is caused by chest and heart movements during respiration) (32).

Analytical procedures

Plasma glucose was measured by the glucose oxidase technique on a Beckman Glucose Analyzer (Beckman, Fullerton, CA). Plasma concentrations of insulin (InsKit, Sorin, Saluggia, Italy) and cortisol (Sorin, Saluggia) were measured by RIA, whereas GH (Hybritech), TSH (Sorin, Saluggia) and PRL (Hybritech) were measured by immunoradiometric assay (IRMA). Plasma catecholamine concentrations were assayed by high-performance liquid chromatography (HLC 725 apparatus) using electrochemical detection (Eurogenetics, Tessenderlo, Belgium). Serum CRH concentrations were measured by RIA, as previously described (33) (courtesy of Dr. F. Petraglia).

Data analysis

Fat-free mass (FFM) was calculated as the difference between body weight and fat mass. Whole-body glucose utilization (or the M value) was calculated from the infusion rate of exogenous glucose during the

2nd h of the insulin clamp period, after correction for changes in glucose levels in a distribution volume of 250 ml·kg^{-1}. The M value was normalized by kilogram of FFM (μmol·min^{-1}·kg FFM^{-1}). Mean arterial blood pressure was calculated as the diastolic blood pressure plus one third of the pulse pressure. Cardiac output was estimated by measuring left ventricular outflow tract diameter by two-dimensional echocardiography in the parasternal long axis view and stroke volume by continuous-wave Doppler left ventricular outflow tract samples from the apical long-axis view. TPVR was calculated as the mean blood pressure divided by cardiac output. For statistical analysis, blood pressure values and spectral parameters were averaged over the final 60 min of the basal period and the 2nd hour of insulin administration.

Statistical analysis

All data are given as mean ± SEM. Because of their nonnormal distribution, spectral parameters were transformed into their natural logarithms. Paired means comparison was performed by the Wilcoxon signed-rank test. Two-way ANOVA for repeated measures was used to test for differences between lean and obese subjects and the effect of insulin on the variable in question. Simple and multiple linear regression analysis was carried out by standard techniques. A P value ≤ 0.05 was considered to be statistically significant.

Results

The obese subjects were hyperinsulinemic and insulin resistant, as expected (Table 1). In the whole data set, fasting plasma insulin concentrations were directly related to both BMI (r = 0.56, $P < 0.001$) and the waist/hip ratio (r = 0.43, $P < 0.02$), whereas insulin sensitivity was inversely related to BMI (r = 0.62, $P < 0.0001$) and the waist/hip ratio (r = 0.63, $P < 0.0001$).

Heart rate variability (Table 2)

Preliminary analysis of the data was carried out to assess the influence of physiological determinants such as respiration, age, and heart rate (28–30). The central HF frequency (reflecting the respiratory rate) was stable both before and during insulin administration, in lean as well as obese subjects. Further proof that respiratory activity was unaltered during the clamp was obtained by spectral analysis of R-wave amplitudes, which retrieved mean frequencies [0.27 ± 0.005 Hz (= 16 ± 0.3 respirations per min) in the basal state, and 0.27 ± 0.01 Hz following insulin] that were similar to one another and superimposable on the central HF frequencies. Also the central frequency in the LF range was similar between groups, and was not affected by insulin. Both total spectral power and its components—LF and HF—were an inverse function of age (with r values of 0.41, 0.43, and 0.37, respectively, all $P < 0.05$ or less) and heart rate (r values of 0.43, 0.38, and 0.44, respectively, all $P < 0.05$ or less). In particular, the regressions predict that an increase in age of 10 yr and/or an increase in heart rate of 10 beats/min (bpm) are associated with an approximate 30% decrease in total spectral power.

In the obese group, all baseline spectral powers were found to be markedly reduced (by 40% on average) in comparison with the lean group. On the pooled data, total, LF, and HF power were inversely related to BMI (with r values of 0.42, 0.42, and 0.36, respectively). The relationships of spectral powers with BMI remained statistically significant after adjustment for age and heart rate ($P = 0.003$, $P < 0.03$, and $P < 0.002$ for total, LF, and HF power, respectively, by multiple regression analysis).

In the pooled data, euglycemic hyperinsulinemia reduced the mean R-R interval (by 3% on average, $P = 0.005$), total spectral power (by 21%, $P < 0.001$), and its LF (−18%, $P < 0.01$) and HF component (−22%, $P < 0.01$). These insulin-induced changes in spectral powers were statistically significant in both groups, without difference between obese and lean subjects. The time course of action of insulin on total spectral power was rapid, reaching its maximum at a time (20–40 min) when insulin-stimulated glucose uptake was only approximately 60% of its steady state value (Fig. 1).

In lean subjects, insulin increased normalized LF ($P = 0.01$) and reduced normalized HF ($P = 0.03$); as a consequence, the LF/HF ratio was significantly higher during insulin than at baseline (Fig. 2). In the obese group, the LF/HF ratio was higher than in the lean subjects (by 1.2 units on average, $P < 0.03$ after adjustment by age and heart rate), but failed to be significantly stimulated by insulin (Fig. 2).

Hemodynamics (Table 3)

In the basal state, the obese subjects had higher cardiac output ($P = 0.001$) and stroke volume ($P < 0.01$), and lower TPVR ($P = 0.002$) in comparison with the lean group. In response to insulin, systolic blood pressure increased only in lean subjects ($P < 0.01$), diastolic blood pressure decreased in both lean ($P < 0.05$) and obese subjects ($P < 0.005$), whereas mean blood pressure only decreased in the obese ($P < 0.01$). Cardiac output increased in both

TABLE 2. Effect of euglycemic hyperinsulinemia on heart rate spectral parameters in lean and obese subjects

	Lean		Obese		P*
	Basal	Insulin	Basal	Insulin	
Mean R-R interval (msec)	853 ± 20	827 ± 21	912 ± 23	883 ± 24	[b]
Total power (msec^2)	3039 ± 449	2497 ± 477	1790 ± 288	1335 ± 261	[a,b]
LF					
Central frequency (hz)	0.096 ± 0.006	0.096 ± 0.006	0.085 ± 0.005	0.078 ± 0.005	NS
Power (msec^2)	1402 ± 214	1257 ± 165	927 ± 147	678 ± 84	[a,b]
Normalized units	48.4 ± 3.3	55.2 ± 3.6	54.5 ± 3.9	58.8 ± 3.9	[b]
HF					
Central frequency (hz)	0.268 ± .006	0.266 ± .006	0.266 ± .006	0.269 ± .031	NS
Power (msec^2)	1399 ± 274	1069 ± 336	745 ± 152	600 ± 200	[a,b]
Normalized units	43.7 ± 3.3	37.6 ± 3.5	38.8 ± 3.6	36.7 ± 3.8	NS

* Two-way ANOVA for repeated measures; [a], $P \leq 0.05$ or less for difference between lean and obese subjects; [b], $P \leq 0.05$ or less for effect of insulin in a paired design; [c], $P \leq 0.05$ interaction group × insulin effect.

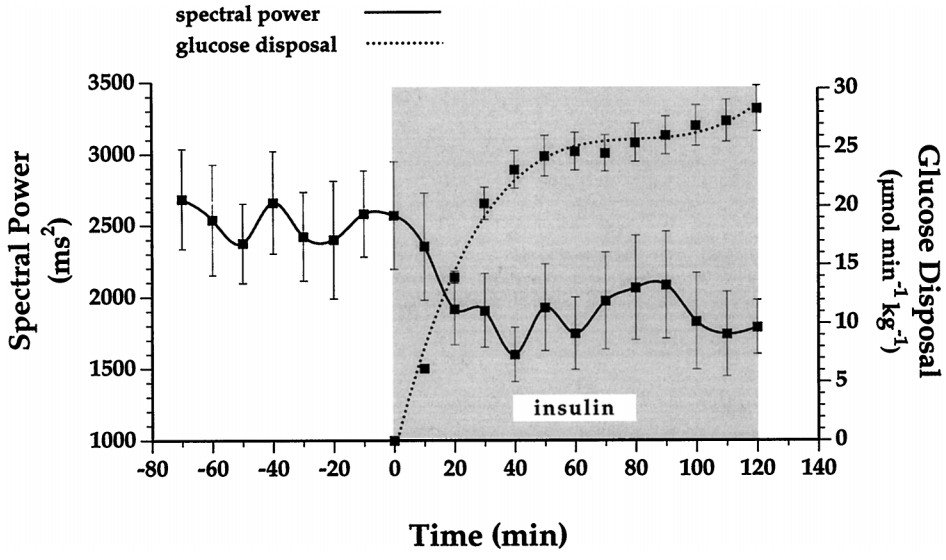

FIG. 1. Time course of total spectral power of heart rate variability and insulin-mediated glucose disposal in healthy subjects.

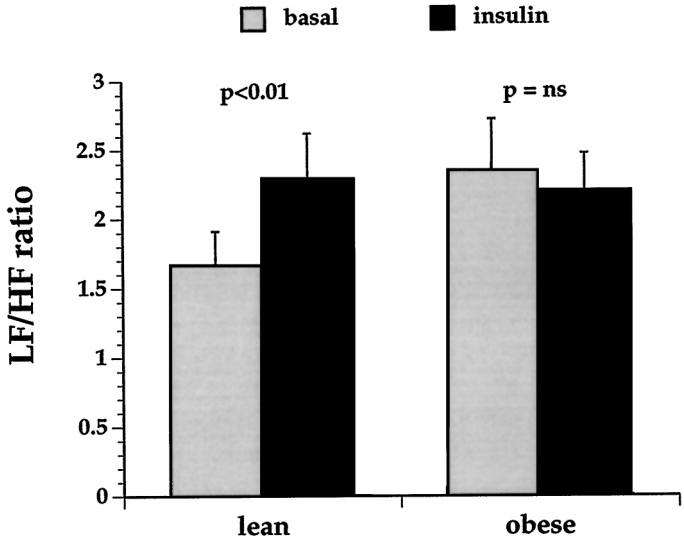

FIG. 2. Changes in LF/HF ratio during euglycemic hyperinsulinemia in lean and obese subjects.

groups as a result of increments in both heart rate and stroke volume, whereas TPVR decreased significantly. None of the latter changes were different between lean and obese individuals.

In the whole population, insulin induced a significant rise in circulating CRH (1.2 ± 0.4 *vs.* 0.2 ± 0.1 pmol/L, basal *vs.* insulin, $P = 0.02$), norepinephrine (162 ± 11 *vs.* 191 ± 9 pg/mL, $P < 0.0004$), PRL (4.62 ± 0.34 *vs.* 5.21 ± 0.40 ng/mL, $P < 0.007$), and cortisol concentrations (68 ± 6 *vs.* 86 ± 8 pmol/L, $P < 0.04$), whereas serum TSH decreased (1.51 ± 0.17 *vs.* 1.15 ± 0.13 μU/mL, $P < 0.01$). GH fell somewhat, but the change did not reach statistical significance. Plasma epinephrine did not change significantly. None of these insulin-induced hormonal changes were different between lean and obese subjects

In the time-control (saline infusion) experiments, plasma glucose and insulin concentrations declined slightly, and GH rose significantly as fasting progressed; none of the other hormones nor any hemodynamic or spectral parameter showed any significant change (Table 4).

Discussion

Effect of insulin on heart rate variability

In nonobese subjects, insulin exerted two distinct actions on HRV. First, total spectral power was markedly reduced during euglycemic hyperinsulinemia (Table 2). This change was at least twice as large as that predicted (9%) from the concomitant increase in heart rate; therefore, it represents a direct effect of insulin. Its time course was somewhat more rapid than the stimulation of whole-body glucose uptake (Fig. 1), and was unrelated to the level of insulin sensitivity. More specifically, the insulin-induced decrease in total spectral power resulted from a reduction in both the LF and HF component. This finding demonstrates that acute hyperinsulinemia attenuates the variability of sinoatrial node activity in response to both sympathetic and parasympathetic influences. The mechanism of this generalized desensitization is not known but may be linked with insulin-induced stimulation of Na^+-K^+ exchange and the attendant membrane hyperpolarization (34). In cultured baroreceptor neurons, activation of Na^+-K^+ exchange reduces baroreceptor excitability through hyperpolarization (35, 36). In dogs with experimental heart failure, inhibition of the Na^+ pump by ouabain restores baroreceptor sensitivity (37). We have previously shown that in human forearm skeletal muscle insulin effectively stimulates K^+ uptake in a manner that is inhibited by ouabain and independent of forearm glucose uptake (38). Similarly, in the current studies desensitization of sinoatrial node activity and stimulation of peripheral glucose metabolism were parallel but unrelated consequences of systemic insulinization. Thus, in addition to paracrine agents (39), insulin is a previously unrecognized factor modulating arterial baroreflex sensitivity. Of interest in this regard is the circumstance that *in vitro* insulin can induce membrane hyperpolarization also in central neurons (40).

The second specific action of insulin was to augment the fraction of total power in the LF range (*i.e.* the normalized LF index) as well as the ratio of LF/HF power (the LF/HF index,

TABLE 3. Effect of euglycemic hyperinsulinemia on hemodynamic parameters in lean and obese subjects

	Lean		Obese		P*
	Basal	Insulin	Basal	Insulin	
Systolic blood pressure (mmHg)	111 ± 2	117 ± 2	115 ± 2	113 ± 2	[c]
Diastolic blood pressure (mmHg)	74 ± 2	72 ± 2	77 ± 2	73 ± 2	[b]
Mean blood pressure (mmHg)	86 ± 2	87 ± 2	90 ± 2	86 ± 2	[c]
Pulse pressure (mmHg)	37 ± 2	45 ± 2	37 ± 2	40 ± 1	[b]
Heart rate (bpm)	68 ± 2	70 ± 2	65 ± 2	68 ± 2	[b]
Cardiac output ($L \cdot min^{-1}$)	4.45 ± 0.21	5.06 ± 0.55	6.01 ± 0.31	6.95 ± 0.30	[a,b]
Stroke volume ($mL \cdot min^{-1}$)	67 ± 4	76 ± 9	89 ± 5	99 ± 4	[a,b]
TPVR ($mmHg \cdot min \cdot L^{-1}$)	19.2 ± 1.1	18.4 ± 2.1	15.1 ± 0.8	12.8 ± 0.7	[a,b]

* Two-way ANOVA for repeated measures; [a], $P \leq 0.05$ or less for difference between lean and obese subjects; [b], $P \leq 0.05$ or less for effect of insulin in a paired design; [c], $P \leq 0.05$ for interaction group × insulin effect.

TABLE 4. Hormonal, hemodynamic, and heart rate spectral parameters during saline infusion

	Basal	Saline	p^a
Plasma glucose (mmol/L)	5.1 ± 0.1	4.9 ± 0.1	0.0001
Plasma insulin (pmol/L)	56 ± 4	48 ± 3	0.01
Plasma norepinephrine (pg/mL)	112 ± 15	119 ± 16	NS
Plasma cortisol (pmol/L)	72 ± 9	57 ± 5	NS
Serum GH (ng/mL)	0.40 ± 0.1	0.80 ± 0.10	0.06
Serum PRL (ng/mL)	4.9 ± 0.4	4.9 ± 0.8	NS
Serum TSH (μU/mL)	0.9 ± 0.2	0.9 ± 0.2	NS
Systolic blood pressure (mmHg)	107 ± 3	108 ± 2	NS
Diastolic blood pressure (mmHg)	71 ± 2	72 ± 2	NS
Mean blood pressure (mmHg)	82.7 ± 2.3	84.3 ± 1.9	NS
Heart rate (bpm)	64.7 ± 2.6	62.3 ± 1.9	NS
Mean R-R interval (msec)	879 ± 10	870 ± 26	NS
Total power ($msec^2$)	3237 ± 1042	3153 ± 1060	NS
LF ($msec^2$)	1379 ± 325	1444 ± 289	NS
HF ($msec^2$)	1709 ± 694	1606 ± 829	NS

[a] Wilcoxon signed-rank test.

Fig. 2); the latter change was caused by a simultaneous increase in normalized LF and decrease in normalized HF (Table 2). Together, the normalized LF and the LF/HF ratio reflect the strength of the sympathetic tone in relation to the vagal tone (41). Thus, insulin acutely alters sympathovagal control of cardiac activity both by enhancing sympathetic outflow and by withdrawing vagal tone. The observed increases in heart rate and circulating norepinephrine levels are coherent with sympathetic activation. The fact that both arms of the autonomic nervous system were affected in a reciprocal fashion supports the interpretation that peripheral inputs (*i.e.* fluctuations in blood pressure) are substantially integrated by direct central influences.

Effect of insulin on hemodynamics

The systemic hemodynamic response to insulin consisted of small reductions in TPVR and diastolic blood pressure, and an increase in heart rate and cardiac output. The increase in cardiac output is similar in size to that measured by Baron and Brechtel (42) in clamp experiments in healthy volunteers with the use of a dye dilution technique. The decrease in TPVR has been attributed to limb vasodilatation (2, 42), mediated by the release of nitric oxide (3). An additional mechanism may be a contraction in blood volume, which we have recently reported to occur under experimental conditions similar to those of the present study (43). The increase in stroke volume, reflecting increased myocardial contractility, is further proof that sympathetic outflow to the heart was enhanced.

Effect of insulin on neurohormones

Insulin administration led to a rise in arterial norepinephrine, but not epinephrine, levels, as found by others (1). This was accompanied by increased cortisol [also previously reported during euglycemic hyperinsulinemia (44, 45)] and PRL, but decreased TSH and, to a lesser extent, GH concentrations. This pattern of hormonal responses is compatible with a moderate stress reaction (46). More specifically, these changes are the predicted consequence of an acute stimulation of the release of CRH, which exerts stimulatory influences on ACTH and SRIH, and inhibitory influences on GnRH, GHRH, and TRH (47). In agreement with this prediction, serum CRH levels rose detectably after insulin. Spillover from the hypothalamic-pituitary portal circulation into the systemic circulation is conceivable, because plasma CRH has been documented to increase after insulin-induced hypoglycemia (48, 49). This neurohormonal response thus indicates that circulating insulin acts directly on the brain also in the absence of hypoglycemia, thereby extending to humans the observations of Davis *et al.* (8) in the dog.

Taken together, the changes in systemic hemodynamics, autonomic activity, and neurohormones support the conclusion that the cardiac effects of insulin *per se*—consisting of increased contractility and heart rate, and reduced rate variability—are not only the reflex response to effects on the peripheral vasculature, but are part of a stress reaction directly elicited by insulin in the central nervous system. In support of this interpretation, recent evidence (50) has shown that in normal subjects 48 h of low-dose dexamethazone pretreatment abolished the increase in muscle sympathetic nerve activity, the rise in circulating norepinephrine, and the calf vasodilatation measured during euglycemic hyperinsulinemia, similar to the current experiments. As dexamethazone is a potent inhibitor of CRH release (47), a role for CRH as central transducer of the stress response to insulin appears likely.

Influence of obesity

Basal spectral powers were all lower (by 40%) in the obese group, the reduction being proportional to BMI. Thus, independently of the effect of age on HRV (51), obesity is a state of reduced sensitivity of the sinoatrial node to both sympa-

thetic and vagal influences. Before insulin administration, the LF/HF ratio was slightly increased in obese subjects, suggesting mild sympathetic overactivity. This finding is in agreement with the microneurographic data of Vollenweider *et al*. (52), who reported significantly increased MSNA in a small group of obese patients in the basal, resting state. Although peroneal microneurography records sympathetic traffic to the skeletal muscles of the lower limb, whereas spectral analysis of heart rate explores an integrated sympathovagal function, a recent study has shown that the spectral parameters of both activities (including the LF/HF ratio) are strongly correlated with one another over a range of heart rates (55–81 bpm) (53). Following insulin, the LF/HF ratio failed to increase in the obese group (Fig. 2); the difference from the lean group was not dependent on the respective changes in heart rate. This result suggests an inherent inability of insulin to shift the autonomic control of heart rate in obesity. In accord with these spectral data, Vollenweider *et al*. (52) found that in obese individuals basal MSNA fails to be stimulated by insulin. Also coherent with our result is the observation by Grassi *et al*. (13), that in obese subjects the changes in MSNA elicited through pharmacological modulation of blood pressure are blunted in comparison with those of lean subjects. Because MSNA is sensitive to low doses of insulin (52), it has been hypothesized that the chronic hyperinsulinemia of the obese may be the signal that causes attenuation of baroreflex responses and prevents enhancement of sympathetic tone by an acute insulin increment.

In the fasting state, stroke volume was higher, and PVR was lower, in the obese than the lean group. This high-output, low-resistance hemodynamic pattern, which is characteristic of normotensive obesity (9), is similar to that induced by insulin administration in the whole group (Table 3). Thus, the obese basal state reproduces some of the systemic hemodynamic effects of acute insulin administration. Notably, however, the hemodynamic (heart rate, cardiac output, and PVR) and hormonal (in particular, plasma norepinephrine levels) responses to acute insulin administration were preserved in the obese group. Thus, in obesity the stress response evoked by a standardized insulin stimulus differs from the normal response in that it does not include sympathovagal modulation of heart rate (the LF/HF ratio in the current studies) or skeletal muscle sympathetic traffic [microneurographic data (52)]. This finding resonates with the results of Gudbjörnsdottir *et al*. (54), who reported increased renal plasma flow and muscle sympathetic activity but unchanged renal and total body norepinephrine spillover in obese men with hypertension.

In summary, obesity is a state of chronic desensitization and impaired autonomic modulation of sinoatrial activity. As such, it recapitulates some features of the hemodynamic and heart rate response to acute insulin administration, suggesting a role for chronic hyperinsulinemia. These altered responses may be a substrate for both the increase in ischemic heart disease (55) and the higher incidence of arrhythmias and sudden death (56) that have been observed in obese subjects, and may be amenable to treatment through reduction of hyperinsulinemia.

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