Studying in normal humans has shown that acute physiological increases in plasma insulin in the absence of changes in blood glucose cause opposing sympathetic excitation and vasodilatation in the skeletal muscle vascular bed (2, 3). Furthermore, hyperinsulinemia increases heart rate and cardiac output, which may be secondary to the peripheral vasodilator actions of insulin (2, 3, 6, 28). The insulin-induced increase in heart rate may be caused by increased cardiac sympathetic outflow but also by cardiac vagal withdrawal. A previous study in animals (9) suggested that chronic hyperinsulinemia is accompanied by reduced cardiac vagal activity. The exact mechanisms of insulin-induced increases in heart rate have thus far not been assessed in humans. Spectral analysis of R-R interval (1, 30) and β-adrenergic receptor blockade during a hyperinsulinemic/euglycemic clamp could be used to address this issue.

R-R interval discloses cyclic variations related to the respiratory cycle called respiratory sinus arrhythmia or high-frequency (HF) variability (1, 30). In addition, R-R interval discloses also low-frequency (LF) oscillations, and changes in the amplitude of these cyclic variations provide markers of cardiac vagal and sympathetic modulation (1, 10, 23, 25, 30). In the present study, we performed a hyperinsulinenic/euglycemic clamp in normal human volunteers and measured heart rate, blood pressure, and respiration. R-R interval variability was determined by power spectral analysis.

The insulin-induced changes in HF variability of R-R interval were assessed to test the hypothesis that hyperinsulinemia decreases cardiac vagal modulation. We also administered a continuous intravenous infusion of propranolol, a β-adrenergic receptor blocker, during the hyperinsulinenic/euglycemic clamp to determine if increased cardiac sympathetic outflow contributed to the increase in heart rate induced by insulin.

In addition, animal studies have suggested that insulin may produce nonuniform increases in sympathetic activity (20), and changes in cardiac sympathetic activity during hyperinsulinemia were compared with those present in direct recordings of sympathetic nerve traffic to muscle circulation.

**METHODS**

Subjects. We studied 16 subjects with a mean age of 31 ± 1 (SE) yr (5 males, 11 females) and a mean body mass index of 24 ± 1 kg/m². All subjects were healthy and had blood pressures <130/80 mmHg measured on three different occasions in the sitting position. None was receiving any medication. The study was approved by the Institutional Human Subjects Review Committee, and written informed consent was obtained.

Measurements. Blood pressures were measured with an automatic sphygmomanometer (Lifestat 200; Physio-Control, Redmond, WA). Electrocardiogram and respiration (Biotech and Pneumotrace; Gould Electronics, Valley View, OH) were recorded on all subjects. Forearm blood flow was measured by venous occlusion plethysmography using air-filled latex cuffs. Intraneuronal recordings of muscle sympathetic nerve activity (MSNA) were obtained from leg microneurographic recordings of multunit postganglionic sympathetic fibers, measured in the peroneal nerve posterior to the fibular head (19, 34). We used tungsten microelectrodes (shaft diameter 200 μm, tapering to an uninsulated tip of 1–5 μm). A subcutaneous reference electrode was inserted 2–3 cm away from the recording electrode inserted in the nerve fascicle. The neural signals were amplified, filtered, rectified, and integrated to
obtain a mean voltage display of sympathetic nerve activity.
All nerve recordings met standard criteria (19, 34).

Electrocardiogram, sympathetic neurogram, and respiration
were recorded on a Macintosh Quadra 900 Computer
(Apple Computer, Cupertino, CA) with a MacLab 8/s data
acquisition system (AD Instruments, Milford, MA).

These measurements were also recorded on an IBM
433DX/T computer. All subjects were breathing spontaneously
and were not allowed to speak during the study.

Spectral analysis. Analog-to-digital conversion was
performed online at 1,000 samples/s for the electrocardiogram
and at 200 samples/s for the respiratory signal. Stationary
segments devoid of arrhythmias and artifacts (150–300 s)
were then analyzed offline with a personal computer (IBM
433DX/T).

The principles of the software for data acquisition and
spectral analysis have been described elsewhere (21, 22, 27).
A discrete Fourier transformation (CARSAN 1.0; Faculty of
Psychology, University of Groningen, Groningen, The Nether-
lands) with a spectral resolution of 0.01 Hz was applied to the
time series of R–R interval. A respirogram synchronized with
the tachogram was also obtained by sampling the signal of
respiratory activity once every cardiac cycle.

Absolute power spectral density functions (ms²) were com-
puted for R–R interval in the 0.02- to 0.50-Hz band (total
variability), in the very-low-frequency (VLF) band (0.02–0.03
Hz), in the LF band (0.04–0.14 Hz), and in the HF band
(0.15–0.35 Hz).

The respirogram underwent a similar analysis to the one
described above.

Hyperinsulinemic/euglycemic clamp. Insulin (Humulin;
Eli Lilly, Indianapolis, IN) diluted in saline with 1 ml of the
subject’s blood was infused intravenously by a digital infusion
pump (Bard Medsystems, North Reading, MA). A hyperinsu-
linemic/euglycemic clamp over 90 min was performed as
previously described (2, 3). Insulin infusion rates were tar-
eted to produce plasma insulin levels of 85 µU/ml. Fasting
plasma glucose levels were maintained by variable 20%
dextrose infusion using an infusion pump (Flo-gard 6200;
Travenol Laboratories, Deerfield, IL).

Plasma glucose and insulin were determined every 5 min.
Plasma glucose was analyzed by a YSI glucose analyzer
(model 27; Yellow Springs Instruments, Yellow Springs, OH).
Plasma insulin levels were determined by radioimmunoassay
(model 27).

Protocol. All studies were started at 7:30 AM after an
overnight fast. Subjects were placed in the supine position
and instrumented for measurement of heart rate, blood
pressure, and respiration. Intravenous catheters were placed
in the right and left antecubital fossae for insulin/glucose
infusion and blood sampling, respectively. The right arm was
instrumented for forearm blood flow measurement. After an
acceptable sympathetic nerve recording site was achieved, 10
min of baseline measurements were obtained before perform-
ning the 90-min hyperinsulinemic/euglycemic clamp.

We repeated 90-min hyperinsulinemic/euglycemic clamps
during a continuous infusion of propranolol in 6 of the 16
normal subjects. We wanted thereby to determine if increased
cardiac sympathetic outflow and/or cardiac vagal withdrawal
contribute to the reduction in R–R interval during hyperinsu-
linemia. Measurements were performed during a 10-min
baseline period before an intravenous injection of 5 mg of
propranolol (26). A continuous infusion of 80 µg/min of
propranolol was then started (26) and continued throughout
the protocol, namely a baseline period of 20 min and a 90-min
hyperinsulinemic/euglycemic clamp.

Vehicle control studies were performed in six normal
subjects (5 males, mean age 28 ± 3 yr, mean body mass index
26 ± 2 kg/m²) in whom 0.2% saline was infused in a
comparable volume to the insulin session.

Data analysis. Measurements were analyzed in a blinded
design fashion during 10 min of baseline recordings and during the
last 15 min of the hyperinsulinemic/euglycemic clamp or
vehicle session. Sympathetic bursts were identified by inspect-
ion of the mean voltage neurogram. MSNA was expressed as
frequency (bursts/min) and as integrated activity (burst
frequency × mean amplitude). The intra- and interobserver
variability in burst identification in our laboratory are 4.3 ±
0.3 and 5.4 ± 0.5%, respectively (4, 19). Forearm blood flow
was expressed as milliliters per minute per 100 milliliters
of forearm volume. Mean arterial pressure was calculated as
diastolic pressure plus one-third pulse pressure. Forearm
vascular resistance was calculated by dividing mean arterial
pressure by flow and is expressed in arbitrary units (AU).

Statistical analysis. Statistical analysis consisted of two-
tailed Wilcoxon tests. Values are expressed as means ± SE.
Significance was assumed at P < 0.05.

RESULTS

Hyperinsulinemic/euglycemic clamp. Fasting plasma
insulin levels averaged 12 ± 2 µU/ml. Steady-state
plasma insulin concentrations were achieved after 30
min of insulin infusion, averaging 84 ± 5 µU/ml.

The baseline plasma glucose level of 85 ± 2 mg/dl did
not change significantly during insulin infusion (89 ± 2
mg/dl at 90 min, P = not significant (NS)). R–R interval
decreased from 1,047 ± 49 to 949 ± 45 ms after 90 min
of insulin infusion (P < 0.001; Fig. 1 and Table 1).
Insulin increased forearm blood flow from 2.1 ± 0.2 to
3.4 ± 0.4 ml·min⁻¹·100 ml⁻¹ (P < 0.01; Fig. 1). Mean
blood pressure was 85 ± 3 mmHg during baseline and
85 ± 3 mmHg after 90 min of insulin infusion. Conse-
sequently, insulin infusion decreased baseline forearm
vascular resistance of 46 ± 5 units to 31 ± 4 units at the
end of the clamp (P < 0.01; Fig. 1). Insulin also caused a
marked increase in mean sympathetic nerve activity
(MSNA) from 208 ± 36 U/min at baseline to 433 ±
62 U/min (25 ± 2 bursts/min) during insulin
(both P < 0.001; Fig. 1).

Insulin decreased the absolute HF variability of R–R
interval (P < 0.01; Fig. 2 and Table 1). This was not due
to a change in the respiratory pattern during insulin,
since respiratory rate was 0.29 ± 0.01 Hz during baseline
and 0.28 ± 0.01 Hz during insulin (P = NS).

Moreover, qualitative assessment of minute ventilation
by the use of the pneumograph suggested that tidal
volume increased during hyperinsulinemia as respira-
tory power increased from 6.5 ± 1.2 AU² during base-
line to 9.6 ± 2.2 AU² during insulin infusion (P < 0.05)
in the absence of changes in respiratory rate. The HF
respiratory variability accounted for 84 ± 1 and 82 ±
3% of the respiratory power during baseline and insulin
infusion, respectively (P = NS). Insulin did not affect
the absolute VLF and LF variabilities of the R–R
interval.

Propranolol. ß-Blockade increased the baseline R–R
interval from 1,103 ± 42 to 1,213 ± 27 ms (P < 0.05,
Table 2). Mean blood pressure and forearm vascular
resistance remained unchanged. Propranolol did not
suppress the decrease in R-R interval induced by insulin. R-R interval decreased from $1,213 \pm 27$ ms during the infusion of propranolol to $1,146 \pm 33$ ms ($P < 0.05$) during the last 15 min of infusion of insulin and propranolol. Mean blood pressure was $81 \pm 2$ mmHg during the infusion of propranolol and $78 \pm 2$ mmHg at the end of the infusion of insulin and propranolol ($P < 0.05$). Forearm vascular resistance was $41 \pm 5$ units during the infusion of propranolol and decreased to $31 \pm 3$ units at the end of the infusion of insulin and propranolol ($P < 0.05$).

Vehicle control studies. R-R interval was $1,072 \pm 65$ ms during baseline and $1,077 \pm 80$ ms during the last 15 min of vehicle infusion ($P = \text{NS}$). Vehicle did not affect forearm blood flow ($2.5 \pm 0.3 \text{ ml/min} \cdot \text{100 ml}$ during baseline and $2.5 \pm 0.3 \text{ ml/min} \cdot \text{100 ml}$ during vehicle, $P = \text{NS}$) and forearm vascular resistance ($38 \pm 6$ units during baseline and $39 \pm 5$ units during vehicle, $P = \text{NS}$). Mean blood pressure was $85 \pm 3$ mmHg during baseline and was $89 \pm 3$ mmHg during the last 15 min of vehicle infusion ($P = 0.03$). However, vehicle did not affect MSNA. MSNA was $269 \pm 42$ U/min (20 $\pm 1$ bursts/min) at baseline and $261 \pm 61$ U/min (16 $\pm 3$ bursts/min) at the end of vehicle infusion ($P = \text{NS}$). R-R interval variability remained unchanged during vehicle infusion. In particular, the absolute HF variability of R-R interval was $1,229 \pm 444$ ms² during baseline and $3,742 \pm 2,901$ ms² during vehicle infusion ($P = \text{NS}$).

**DISCUSSION**

In this study, we determined the effects of insulin on the variability of R-R interval. Our study reveals that insulin decreases cardiac vagal tone since the reduction in R-R interval induced by insulin could not be abolished by propranolol. Moreover, insulin decreased also the absolute HF variability of R-R interval but did not affect the absolute LF variability of R-R interval. The HF variability of R-R interval is a marker of cardiac vagal modulation (10, 14, 16, 17, 25) and can be affected by changes in the respiratory-related vagal modulation of R-R interval (breathing pattern; see Refs. 10, 14, 16, 17, 32). However, respiratory rate remained unchanged, and qualitative assessment of respiration suggested that tidal volume increased during hyperinsulinemia, as a possible consequence of increased oxygen consumption during sympathetic hyperactivity (33). Thus our study reveals that reductions in the HF variability in R-R interval were not due to smaller tidal

### Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Insulin</th>
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<tbody>
<tr>
<td>RRI, ms</td>
<td>$1,047 \pm 49$</td>
<td>$949 \pm 45^*$</td>
</tr>
<tr>
<td>Total RRI variability, ms²</td>
<td>$4,005 \pm 921$</td>
<td>$3,033 \pm 726$</td>
</tr>
<tr>
<td>VLF RRI variability, ms²</td>
<td>$547 \pm 103$</td>
<td>$551 \pm 65$</td>
</tr>
<tr>
<td>LF RRI variability, ms²</td>
<td>$1,290 \pm 275$</td>
<td>$1,142 \pm 223$</td>
</tr>
<tr>
<td>HF RRI variability, ms²</td>
<td>$2,110 \pm 598$</td>
<td>$1,261 \pm 494^*$</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>$85 \pm 3$</td>
<td>$85 \pm 3$</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
<td>$2.1 \pm 0.2$</td>
<td>$3.4 \pm 0.4^*$</td>
</tr>
<tr>
<td>FVR, units</td>
<td>$46 \pm 5$</td>
<td>$31 \pm 4^*$</td>
</tr>
<tr>
<td>MSNA, burst/min</td>
<td>$15 \pm 2$</td>
<td>$25 \pm 2^*$</td>
</tr>
<tr>
<td>Amplitude, units</td>
<td>$208 \pm 36$</td>
<td>$433 \pm 76^*$</td>
</tr>
</tbody>
</table>

Data are means $\pm \text{SE}; n = 16$ subjects. RRI, R-R interval; VLF, very low frequency; LF, low frequency; HF, high frequency; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity. *$P < 0.001$ and †$P < 0.01$, insulin vs. baseline.
volumes during hyperinsulinemia (10, 14, 16, 17, 32) and that insulin decreased not only cardiac vagal tone but also cardiac vagal modulation.

Previous studies on heart rate variability during hyperinsulinemia (7, 11) neither recorded MSNA nor administered a β-adrenergic receptor blocker during hyperinsulinemic/euglycemic clamps. In contrast, our study reveals that propranolol does not abolish the decrease in R-R interval induced by insulin and that insulin increases heart rate only by 6 beats/min at a time when sympathetic drive to muscle circulation is increased by more than twofold. Thus our results suggest that a reduction in cardiac vagal tone contributed at least in part to the small increase in heart rate during hyperinsulinemia and that insulin produces differential increases in sympathetic activity in humans. These results support previous observations in animals in which insulin increased lumbar but not renal sympathetic nerve activity (20) and in humans in which insulin increased sympathetic nerve activity to muscle but not to skin (8). This finding could also explain why heart rate remained unchanged during hyperinsulinemia in the study of Bellavere et al. (7) and increased only by two beats per minute in the study of Emdin et al. (11).

Insulin sympathoexcitatory effects are mediated at least in part by a central neural action and possibly by baroreflex mechanisms secondary to peripheral vasodilatation (28). Increased sympathetic drive during hyperinsulinemic-euglycemic clamps is primarily targeted at the skeletal muscle vasculature. In addition, there is evidence that insulin selectively stimulates blood flow and decreases vascular resistance in skeletal muscle, chiefly by potentiation of endothelium-dependent vas-

<table>
<thead>
<tr>
<th>Table 2. Group data measurements of RRI, MBP, FBF, and FVR during baseline, propranolol infusion, and infusion of both propranolol and insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RRI, ms</strong></td>
</tr>
<tr>
<td>1,103 ± 42</td>
</tr>
<tr>
<td><strong>MBP, mmHg</strong></td>
</tr>
<tr>
<td><strong>FBF, ml · min⁻¹ · 100 ml⁻¹</strong></td>
</tr>
<tr>
<td><strong>FVR, units</strong></td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 subjects. *P < 0.05 vs. baseline. †P < 0.05 vs. propranolol alone.
dilatation and attenuation of sympathetic vasoconstriction (28). Accordingly, blood pressure did not change during hyperinsulinemia in our study as a result of opposing pressor and depressor actions of insulin in the skeletal vasculature. We also reported similar small changes in blood pressure during hyperglycemic/euglycemic clamps in two previous studies (2,3). Regionally nonuniform increases in sympathetic drive opposing the vasodilatory action of insulin (2,3,28,29) could explain why more than twofold increases in sympathetic nerve activity to muscle circulation were not accompanied by the expected increases in blood pressure.

Perspectives

The continuing difficulty with research on acute effects of increases in insulin on autonomic control is to establish relevance to the chronic hyperinsulinemia of obesity (33). There are, however, several studies that also suggest that cardiac vagal outflow is decreased, in the absence of a heightened cardiac sympathetic outflow, during chronic hyperinsulinemia. First, there is recent evidence of nonuniform sympathetic activation in obese subjects in whom cardiac sympathetic activity, directly assessed by norepinephrine spillover techniques, is depressed (33). Second, sequential parasympathetic blockade with atropine and sympathetic blockade with esmolol have revealed that weight gain impairs more cardiac parasympathetic control than cardiac sympathetic control in nonobese and obese subjects (5). Third, similar direct evidence of increased heart rate as a result of cardiac parasympathetic withdrawal rather than increased sympathetic stimulation was also found in obese dogs (31). Last, two other studies in which heart rate variability provided indirect measures of cardiac autonomic control also concluded that heart rate increases as a result of parasympathetic withdrawal when body weight increases (15,24).

Thus our data may provide a mechanistic explanation for previous observations of cardiac parasympathetic withdrawal in the absence of cardiac sympathetic activation in obese hyperinsulinemic subjects. The exact mechanisms by which obesity may have differential effects on cardiac sympathetic and vagal control are unknown. It is believed that central nervous mechanisms for sympathetic activation in obesity may reside in the hypothalamus (33), with neuropeptide Y being an important neurotransmitter (33,13) that also possesses vagal inhibitory capacities (36).

Finally, there is indirect evidence that decreased cardiac vagal activity precedes onsets of ventricular tachycardia (12), and it is therefore tempting to speculate that insulin-induced cardiac vagal withdrawal might contribute to the increased cardiovascular mortality of obesity.

In conclusion, this study suggests that acute physiological elevations in plasma insulin decrease cardiac vagal tone and modulation and that insulin produces regionally nonuniform increases in muscle and cardiac sympathetic activity in humans.

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