Impaired baroreflex sensitivity (BRS) is associated with hypertension and cardiovascular risk. Lipid abnormalities accompanying insulin resistance may impair BRS. To test this, nine obese, dyslipidemic hypertensive and seven healthy normotensive individuals were studied. The BRS was measured during a phenylephrine infusion before and after nonesterified fatty acids (NEFAs) and triglycerides were raised for 1 h with an Intralipid and heparin infusion, ie, acute dyslipidemia. The obese group had higher values than lean controls for several components of the insulin resistance syndrome including blood pressure (BP) and heart rate, as well as fasting insulin, triglycerides, and NEFA. The BRS was lower in obese hypertensive subjects than healthy controls at baseline ($P < .001$); BRS declined from $8.3 \pm 0.4$ to $5.2 \pm 0.3$ ($P < .001$) in obese hypertensive subjects and from $15.9 \pm 2.2$ to $7.5 \pm 0.7$ msec/mm Hg ($P = .04$) in controls with acute dyslipidemia. The reduction in BRS correlated with the rise in NEFAs ($r = -0.59, P = .02$) but not triglycerides ($r = -0.07, P = NS$). These observations indicate that elevating NEFAs acutely impairs BRS. The findings suggest that lipid abnormalities in obese hypertensives may contribute to elevated BP and increased cardiovascular events by impairing BRS. Am J Hypertens 2002;15:479–485 © 2002 American Journal of Hypertension, Ltd.

Key Words: Nonesterified, fatty acids, baroreflex, phenylephrine, obesity, hypertension.

A rterial baroreceptors play an important role in modulating BP.1 Hypertension has been associated with impaired baroreflex sensitivity (BRS).1,2 Moreover, abnormal BRS was found in prehypertensive individuals,3 suggesting that baroreceptor dysfunction may participate in the pathogenesis of hypertension. Furthermore, epidemiologic observations suggest that impaired BRS, manifest in part as decreased heart rate variability, is associated with increased cardiovascular risk.4 Thus, abnormalities of arterial BRS are associated with hypertension and cardiovascular events.

Patients with the insulin resistance syndrome, who are often obese, are at increased risk for hypertension and cardiovascular disease. As part of the cardiovascular risk factor cluster, these patients manifest a complex dyslipidemia characterized by increased triglycerides and elevated NEFAs that are resistant to suppression by insulin.5,6 We observed that resistance to insulin’s NEFAs lowering actions correlated positively with BP.5 Furthermore, Pikkujamsa et al have demonstrated that triglycerides are an independent risk factor for decreased heart rate variability.7 Several groups have shown that raising lipids increases BP and heart rate in the short and long term.8,10 However, it is not known whether acute elevations of lipids induce rapid changes in BRS. This study was designed to determine whether raising lipids acutely reduces BRS in subjects with very different levels of insulin action and baroreflex function,11 namely, obese hypertensives with and lean normotensives without evidence of insulin resistance.

Subjects and Methods

Subjects

Two groups of volunteers were recruited by advertisement and paid. The obese hypertensive group included nine patients with BMI $>27$ kg/m$^2$ and an average seated BP $\geq 140/90$ mm Hg measured on at least two separate days off antihypertensive medication. Seven lean normotensive age-, sex-, and ethnicity-matched subjects were recruited.
as controls. The lean subjects had average seated BP of 130/80 measured on at least two separate days and BMI <25 kg/m². Subjects provided written informed consent approved by the Institutional Review Board. Volunteers underwent a medical history, physical examination, and laboratory evaluation to determine eligibility. Dual x-ray absorptiometry (DEXA) scanning was used to assess percent body fat. Obesity was defined as total body fat by DEXA ≥20% for men and ≥25% for women. Lean volunteers were selected if body fat percent by DEXA was <20% and <25% for men and women, respectively. In the hypertensive group, BP medications were discontinued 3 weeks before the study and replaced with felodipine 5 to 10 mg daily for 2 weeks. Felodipine was discontinued 1 week before the study. Hypertensive subjects recorded BP twice daily and were monitored weekly by a physician during the washout and study periods. Subjects avoided all other medications for 2 weeks before the study. They avoided caffeine-containing beverages, food products, and medicines one week before the study and during the study period. All women were studied in the early follicular phase of their menstrual cycle to minimize the potential influence of hormonal variations on BP.

**Physiologic Measurements**

Blood pressure was determined by mercury sphygmomanometry. During the screening visit, BP was measured in triplicate after 5 min of rest in the seated position. Mean BP (MBP, mm Hg) was calculated as diastolic BP + [(systolic BP − diastolic BP mm Hg)/3]. The HDI-PulseWave CR-2000 Research CardioVascular Profiling System (Hypertension Diagnostics, Inc., Eagan, MN) was used to determine capacitive compliance (Cᵢ), an estimate of large artery elasticity. This technique, which analyzes the signal-averaged radial arterial waveform based on a modified Windkessel model, correlates well with other measures of vascular compliance.

**Biochemical Measurements**

Blood for NEFAs was promptly added to prechilled Eppendorf tubes containing EDTA and paraoxon to inhibit lipolysis in vitro. The plasma was stored at −70°C before analysis of total plasma NEFAs. Total plasma NEFAs wereanalyzed in triplicate using the commercially available NEFAs-C kit ACS-ACOD Method (Wako Chemicals, Inc., Richmond, VA).

**Protocol**

Each subject reported to the General Clinical Research Center clinical physiology laboratory at 8 AM after an overnight fast. Room temperature was maintained at 75°C F. With the patient supine, intravenous catheters were placed in each antecubital vein. The right arm catheter was used for blood sampling and the left arm catheter for infusions. After a 30-min baseline period, phenylephrine was infused at 0.1 for 10 min and then at 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 μg·kg⁻¹·min⁻¹ for 7 min each. The phenylephrine infusion was discontinued if BP increased by ≥50/30 mm Hg and followed by a 30-min recovery period. BP was measured every 5 minutes during the baseline and recovery periods. During the phenylephrine infusion, BP was measured in the last minute of each dose.

After the 30-min recovery period, 20% Intralipid was infused at 0.8 mL·m⁻²·min⁻¹ combined with heparin at 1000 units·h⁻¹ after a 200 unit heparin bolus. Blood samples were obtained for measurement of plasma NEFAs and triglycerides at baseline and 60 min after starting the intralipid and heparin infusion. After 60 min of the intralipid and heparin, the phenylephrine infusion was repeated, while intralipid and heparin were continued.

During the entire protocol, the HDI/PulseWave CR-2000 system was used to noninvasively estimate large artery elasticity. Blood pressure was measured on the left upper arm while the Arterial PulseWave sensor was stabilized over the right radial artery to obtain the pulse waveform. Blood pressure and arterial waveforms were obtained as follows: 1) three times during the baseline and recovery periods; 2) 30, 45, and 60 min after starting the intralipid and heparin infusion; and 3) during the last minute of each phenylephrine dose.

**Data Analysis**

Data are presented as mean ± 1 SEM. Changes in plasma NEFAs before and after the intralipid and heparin infusion were assessed using two-sided Student paired (same group) and unpaired (between groups) t tests.

The continuous graded phenylephrine infusion was used to assess overall baroreflex activity. The BRS, calculated in the last minute of each phenylephrine dose from BP and heart rate data obtained for each subject, was measured in two ways. First, BRS was determined by the regression line of R-R interval and systolic BP during the phenylephrine infusion. Data obtained from all doses of phenylephrine were used in this analysis. Second, BRS was assessed as the ratio of the change in R-R interval divided by the change in systolic BP (Δmsec/Δmm Hg) from baseline. The lowest phenylephrine doses (0.1 and 0.2 μg·kg⁻¹·min⁻¹) were excluded in the latter analysis of BRS, as these doses do not consistently elicit a pressor response and therefore do not lead to consistent changes in heart rate. The slope of the regression line defined the average BRS during the phenylephrine infusion. Only individual regression lines with a correlation coefficient ≥0.7 were included in the analyses of BRS. The baseline differences in BRS between the obese hypertensive group and the lean normotensive group and the effects of raising lipids with the intralipid and heparin infusion on the BRS within each group were analyzed by two-factor repeated measures analysis of variance.

As the values for Cᵢ were not normally distributed, these data were assessed nonparametrically by the Wilcoxon signed rank test. P values of ≤ .05 were considered...
significant. All analyses were done with SAS version 8.0 software (SAS, Cary, NC).

**Results**

**Characteristics of Study Subjects**

Descriptive characteristics of the obese hypertensive and lean normotensive groups are shown in Table 1. Obese hypertensive subjects had mean values for systolic and diastolic BP in the stage 1 hypertension range as well as increased heart rate compared with normal controls. Obese subjects had higher values than lean controls for insulin, triglyceride, and NEFAs, which are characteristic of the insulin resistance syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obese HTN</th>
<th>Lean NT</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>3/6</td>
<td>3/4</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>39 ± 2</td>
<td>38 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30 ± 1</td>
<td>22 ± 2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Body fat %</td>
<td>30 ± 3</td>
<td>19 ± 2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>144 ± 6</td>
<td>116 ± 6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>96 ± 2</td>
<td>70 ± 3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>113 ± 3</td>
<td>85 ± 4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>68 ± 2</td>
<td>60 ± 3</td>
<td>&lt;.04</td>
</tr>
<tr>
<td>Insulin, µIU/mL</td>
<td>12 ± 3</td>
<td>5 ± 0.9</td>
<td>.05</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>148 ± 14</td>
<td>69 ± 6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>50 ± 3</td>
<td>50 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>NEFAs, µmol/L</td>
<td>612 ± 55</td>
<td>423 ± 64</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

HTN = hypertensive group; NT = normotensive group; NS = not significant; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; HR = heart rate; HDL = high-density lipoprotein; NEFAs = nonesterified fatty acids.

Data are mean ± SEM.

BMI, body fat %, triglycerides, and HDL were determined at screening.

**Raising Lipids With an Intralipid and Heparin Infusion**

After 1 h of the intralipid and heparin infusion, there was a significant rise in the total plasma NEFAs and triglyceride levels in both groups (Fig. 1A and 1B). In obese hypertensive subjects, NEFAs and triglycerides increased by 58% from 612 ± 55 to 973 ± 69 µmol/L (P < .001) and from 148 ± 14 mg/dL to 234 ± 21 mg/dL (P < .001), respectively. In lean normotensive subjects, plasma NEFAs increased from 435 ± 89 µmol/L to 1260 ± 107 µmol/L (P < .001) and triglycerides rose from 69 ± 6 mg/dL to 141 ± 7 mg/dL (P < .001). Plasma NEFAs (P = .03) but not triglycerides (P = .60) increased more in lean normotensive than obese hypertensive subjects.

At the end of the intralipid and heparin infusion, mean BP was not significantly different from baseline in either group. Raising lipids was associated with a marginally significant increase in systolic BP of 3.7% (P = .06) in lean normotensive subjects alone, whereas heart rate increased by 9.5 ± 3.5 beats/min (P = .03) in obese hypertensive subjects only.

**Baroreflex Sensitivity**

In obese hypertensive subjects, the baseline BRS regression line is shifted downward and to the right of that of the lean normotensive controls (Fig. 2A). Within each group, BRS declined acutely with the infusion of intralipid and heparin (Fig. 2A). In other words, at a given level of systolic BP during the phenylephrine infusion, there was a shorter R-R interval after lipids were increased compared with baseline.

The BRS (Δmsec/Δmm Hg) declined from 15.9 ± 2.2 to 7.5 ± 0.7 msec/mm Hg (P = .03) in lean normotensive subjects when plasma lipids were acutely increased (Fig. 2B). Despite their reduced BRS at baseline, obese hypertensive subjects had a further reduction in BRS (from 8.3 ± 0.3 to 5.3 ± 0.3 msec/mm Hg, P = .001) when lipids were raised. In all subjects combined, the increase in NEFAs (r = −0.59, P = .02) but not triglycerides (r = −0.07, P = NS) correlated with the reduction in BRS.

**Large Artery Elasticity Index**

Before raising lipids, average values for C1 during the phenylephrine infusion were significantly lower in obese hypertensive subjects at 11.8 ± 1.3 dL/mm Hg than in lean normotensive subjects at 15.6 ± 1.0 dL/mm Hg (P < .0001). During the phenylephrine infusion periods, C1 averaged 15.6 ± 1.0 dL/mm Hg at baseline and declined to 14.1 ± 0.9 dL/mm Hg when NEFAs were raised in the lean normotensive group (P = .02). In contrast, large artery elasticity indices in obese hypertensives were comparable during both phenylephrine infusion periods, despite the change in NEFAs (11.8 ± 1.3 dL/mm Hg vs 11.8 ± 1.5, P = NS).

**Discussion**

The principal finding of this study is that raising plasma lipids systemically with an infusion of Intralipid and heparin acutely reduces BRS assessed by blunting the baroreflex response to the pressor effects of phenylephrine. The acute impairment in BRS in response to elevated lipids occurred in both obese hypertensive subjects with and lean normotensive subjects without evidence for insulin resistance. The acute reduction in BRS correlated with the increase in plasma NEFAs but not triglycerides during the infusion of Intralipid and heparin.

Reduced heart rate variability, which strongly correlates with impairment in BRS, has been associated with hypertension and increased cardiovascular risk. Evidence suggests that lipids modulate cardiovascular autonomic control. Insulin-resistant patients, including obese hypertensive individuals, have several lipid abnormalities. The dyslipidemia of insulin resistance encom-
passes elevated plasma triglyceride and NEFAs concentrations, 17 abnormally large postprandial increases in triglycerides 18 and NEFAs levels and turnover, which are resistant to suppression by insulin. 5,6 These lipid abnormalities may adversely affect baroreceptor function, which would further complicate the cluster of cardiovascular risk factors in these patients. In this study, the short-term infusion of intralipid and heparin was designed to acutely mimic the elevated triglycerides and NEFAs observed among insulin-resistant patients under baseline conditions and, especially, after a high-fat meal.

This is the first study to show that an acute (1-h) elevation of blood lipids impairs BRS in healthy normotensive and obese, insulin-resistant human subjects. Previously, we demonstrated that a 4-h infusion of intralipid and heparin was designed to acutely mimic the elevated triglycerides and NEFAs observed among insulin-resistant patients under baseline conditions and, especially, after a high-fat meal.

The present study confirms our previous impressions and begins to describe the changes in BRS from elevated serum lipids. In the lean normotensive subjects, the rightward shift in the BRS regression line suggests acute BRS resetting under the hyperlipidemic conditions. Potentially, these results indicate that there is an increase in the threshold response to baroreceptor activation. Interestingly, the acute dyslipidemia in lean normotensive subjects reduced BRS to values observed in obese hypertensive subjects at baseline (Fig. 2B). In the obese hypertensive subjects, the further increase in lipids was associated with a flattening of the BRS slope. This phenomenon may indicate that the sensitivity of the carotid baroreceptors to incremental increases in BP is reduced. Further support for the notion that the acute dyslipidemia led to the decline in BRS is provided by a strong inverse correlation between the increase of plasma NEFAs and the decrease in BRS (Fig. 3A).

This study was not designed to define the mechanisms by which the increased lipids acutely impaired BRS. Among the unanswered questions are the effects of individual fatty acids and the potential interaction between elevations of both triglycerides and fatty acids on BRS.

**FIG. 1.** Bar graphs show (A) the average total plasma NEFAs concentration and (B) average triglyceride concentration at baseline and after intralipid and heparin infusion in lean normotensive and obese hypertensive groups. Open bars indicate baseline levels, and solid bars indicate levels after a 1-h infusion of intralipid and heparin (IL/H). *P < .001 baseline v IL/H in the same group. There is a significant difference between the absolute increase in NEFAs (P < .001) but not triglycerides (P = .60) between groups. Data are presented as mean ± SEM. NEFAs = nonesterified fatty acids.
One possible explanation raised by our study is that the rise in lipids may decrease distensibility of large arteries. Because the carotid baroreceptor functions as a mechano-receptor rather than a true pressure sensor, a reduction in large artery distensibility during the infusion of intralipid and heparin could have contributed to the decline in BRS. In lean normotensive individuals, large artery distensibility, represented by C1, declined when lipids were raised and may have contributed to their decline in BRS. However, C1 did not decline in obese hypertensive subjects in response to acute dyslipidemia, yet BRS fell. Therefore, mechanisms in addition to a decline in carotid artery distensibility probably contributed to the fall in BRS during acute dyslipidemia.

As an alternative or adjunctive possibility, an elevation of plasma lipids may acutely change baroreceptor function by altering the electrophysical properties of the receptors via enhancement of Na+/H+ pump activity. Some fatty acids

FIG. 2. A and B): Line graph shows relationship between the RR interval (msec) relative to the systolic BP (mm Hg) during the graded phenylephrine infusion at baseline (closed circle) and after 1 h of intralipid and heparin infusion in both groups (closed square). The baseline BRS (msec/mm Hg) is significantly less in the obese hypertensive compared with the lean normotensive group (P < .0001). After the 1-h intralipid and heparin infusion, the BRS is significantly reduced in the lean normotensive and obese hypertensive groups (P = .0008 and P = .0009, respectively). Data were analyzed by repeated measures ANOVA. B) Bar graph demonstrates the change in BRS (Δmsec/Δmm Hg) in the lean normotensive and obese hypertensive groups before (open bars) and after intralipid and heparin infusion (solid bars). Data are presented as mean ± SEM. *P = .03; #P = .001. BP = blood pressure; BRS = baroreflex sensitivity; other abbreviation as in Fig. 1.
could stimulate pump activity directly, or indirectly by activation of protein kinase C, phospholipase A2, or incorporation into the cell membrane. The enhanced sodium transport could increase the threshold pressure necessary to excite the baroreceptor, thereby reducing BRS.

The methodology used to assess baroreflex responses has several limitations. First, only a portion of the baroreflex curve has been analyzed. The effect of acute hyperlipidemia on BRS would be more fully characterized by assessing the heart rate response to the depressor effects of nitroprusside as well as the pressor actions of phenylephrine. Second, noninvasive measurements of blood pressure are not as accurate as direct, intra-arterial recordings. However, the increased accuracy of intra-arterial recordings and use of the ramp method would probably result in more profound differences in BRS from the elevation of fatty acids, ie, the lower signal-to-noise ratio of noninvasive BP measurements does not create but, rather, masks true differences. Third, heparin, which was used to activate vascular lipoprotein lipase and to accelerate the hydrolysis of fatty acids from triglycerides, may have effects that could influence BRS. However, heparin concentrations probably did not exceed −0.05 mg · mL⁻¹ (0.3 U · mL⁻¹). At this concentration, heparin is unlikely to alter signal transduction or endothelial NO production, which could potentially affect BRS. Fourth, Intralipid is a soybean emulsion comprised principally of linoleic and oleic acids esterified to glycerol. We and others have shown that these fatty acids affect signal transduction processes of vascular smooth muscle and endothelial cells in vitro. Because individual fatty acids are not approved by the FDA for human studies except in trace amounts, we did not examine the effects of individual NEFA on BRS. Fifth, carotid artery distensibility was not directly measured. Determination of large artery elasticity extrapolated from the radial pulse waveform may not detect small changes in the carotid artery distensibility that could alter BRS. Sixth, our studies were intentionally limited to obese hypertensive and lean normotensive subjects with widely disparate levels of insulin action and BRS. Subsequent studies in lean hypertensive and obese normotensive subjects,

**FIG. 3.** A) Line graph shows relationship between the change in total NEFAs concentrations (Δµmol/L) and the change in BRS (Δmsec/Δmm Hg) for all subjects (r = −0.59, P = .02). B) Line graph shows there is no relationship between the change in triglyceride level (Δmg/dL) and the change in BRS (Δmsec/Δmm Hg) (r = −0.07, P = NS) Abbreviations as in Figs. 1 and 2.
who manifest intermediate levels of impairment in insulin action and BRS, will help to fully characterize the impact of blood pressure, insulin resistance, and obesity on BRS.

Despite the limitations, this study raises the possibility that the dyslipidemia accompanying insulin resistance, which includes elevated plasma NEFAs, participates in the pathogenesis of hypertension and increased cardiovascular risk by adverse effects on baroreflex sensitivity.

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References